Surgical site infections in Benin: what about hand- hygiene practices and antibiotic resistance?

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“It only takes as long to wash your hands well as it takes twice to sing Happy Birthday “

Ignaz Semmelweis

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ABBREVIATIONS

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ABHR	Alcohol based hand rub
ABR:	Antibiotic resistance `
AMR :	Antimicrobial resistance
ARG	Antibiotic resistance gene
DNA	Desoxyribonucleic acid
E.coli	*Escherichia coli*
EPC	Enterobacterales producing carbapenemases
ESBL	Extended-spectrum beta-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HAIs:	Healthcare associated infections
HH	Hand hygiene
LMIC	Low-and middle-income country
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MLST	Multi-Locus Sequence Type
MDR	Multidrug resistance
MSSA	Methicillin-susceptible *S.aureus*
MALDI-TOF	Matrix-assisted laser desorption ionization time of flight
MLST :	Multi-locus sequence type
OXA	Oxacillinase
PBP	Penicillin-binding protein

ABBREVIATIONS

PCR	Polymerase chain reaction
PMQR	Plasmid mediated quinolone resistance
SAP:	Surgical antimicrobial prophylaxis
SCCmec	Staphylococcus cassette chromosome mec
SSI:	Surgical site infection
WHO	World Health Organization
WGS	Whole- Genome Sequencing

1 GENERAL INTRODUCTION

1.1 GENERAL INTRODUCTION

Healthcare-associated infections (HAIs) are a major global public health problem, increasing the transmission of drug-resistant infections[1]. The term HAIs include infections developed in any health care facility where patients obtain healthcare (long–term care, family medicine clinics, home care and ambulatory care) [2]. Surgical site infections (SSIs) are among the most common HAIs and are reported to be the third most common nosocomial infection preceded only by urinary tract infections and pneumonias [3]. In a recent study, the World Health Organization (WHO) shows that SSIs remains the most surveyed and common HAI in low-income and middle-income countries and concern up to two-third of patients who have undergone a surgical intervention [4]. Worldwide, SSIs appear in 2%-20% of patients after intervention, and the rate vary according to the local resources, the environment in which interventions are performed and the surgery risk factors [5]. Rates are described to be higher in low middle-income countries (LMICs) because of precariousness of health facilities resources. A 2016 report by the WHO highlighted the fact that the risk of SSI was significantly higher in Sub-Saharan environment [4]. SSIs increase mortality and morbidity rate as well as the increasing of the use of broad-spectrum antibiotics and consequently the emergence of multidrug-resistant bacteria (MDR)[6].

Antimicrobial resistance (AMR) is a complex phenomenon resulting from the natural evolution of microbes [7]. AMR kills and increase the costs of our healthcare, damage economies and threatens health security. According to the

data by Centers for Diseases Control and prevention, over 2.8 million antibiotic resistant infections occur in the United States each year, and more than 35.000 people die because of complications caused by resistance to antibiotics [8]. The problem of AMR is deeply worrying and constitute a global challenge in LMICs where we suffer from a lack of biological and epidemiological data, a lower level of awareness and the absence of national or local developed guidelines [7]. Moreover, the self-medication, poor drug quality, purchasing antibiotics without prescription of a physician or other qualified health professional and the scarcity of laboratory with good equipment to perform antibiogram are other reasons that complicate the situation [9]. The causes of antibiotic resistance are complex, and AMR constitute the greatest global public health challenges of our time. AMR is well-established with a link between various niches and three domains: human, animal, and environmental health; this is the reason why it is necessary to mention the "One health approach" to combat this global health threat [10].

Hand hygiene and specifically the use of alcoholic hand rub solution remains the most important measures to prevent the SSIs and the transmission of MDR bacteria in hospitals, but implementation is still a challenge particularly in LMICs where the environmental and resources infrastructures are lacking [11,12]. They are several studies that showed that compliance with guidelines in hygienic hand disinfection is less than optimal in surgery services [13–15]. Numerous studies highlighted the cornerstone role of healthcare workers (HCWs) hands in the propagation of bacteria within the healthcare environment and consequently the patient's security [16]. HCW's compliance with hand hygiene guidelines has generally been determined using self-

reporting, observational, or interventional approaches. An important disadvantage of direct observation is that data collected may be biased by observer effects also known as "Hawthorne effects"[17]. For these reasons, data collected using qualitative methods may be valuable, as interviews can provide rich data that better explore the issues under examination, such as HCW's compliance with HH guidelines. In combating the spread of antimicrobial resistance, hand hygiene is at the center of effective infection prevention and control (IPC).

Reliable estimates of the global burden of HAIs are hampered by a scarcity of data that adequately describes endemic infections at the national and regional levels, particularly in LMICs. Very few countries have national surveillance systems for HAIs.[18]. In Benin, the first one nationwide survey about prevalence of HAIs infections and MDR bacteria was conducted in 2012 and showed an overall prevalence of 19.1% [18]. In this study, resistance of leading nosocomial bacteria to antibiotics included methicillin resistant *staphylococcus aureus* (MRSA) and extended spectrum beta lactamases (ESBL) producing Enterobacterales was respectively 52.5% and 67.6% [18]. Piecemeal studies have been carried out in the country, but without molecular epidemiology of MDR bacteria. Before this thesis, nothing was known either about hand hygiene practices in Benin hospitals, neither about the infection control policies there.

Due to this lack of data, a grant by ARES (Académie de la Recherche pour l'Enseignement Supérieur) in 2018 made it possible to introduce the Multidisciplinary Strategy for Prevention and Infection Controle (MUSTPIC) project in six public hospitals in Benin (https://uclouvain.be/en/research-

institutes/ldri/must-pic.html.Two departments were involved. As gastrointestinal interventions were common in the six hospitals , the first was gastrointestinal surgery. The second was cesareans, which had been free of charge since 2009, together with a kit containing antibiotics such as ampicillin and gentamicin [19].

The overall aim of MUSTPIC project was to reduce HAIs by improving hand hygiene and the rational use of antibiotics. The activities proposed for this project included this thesis, which focuses on hand hygiene, the microbiological aspects of surgical site infections, and measures for their prevention. Globally, they are two different steps in this project: the observation or preliminary phasis (observation of hand hygiene, antibiotic prophylaxis in surgery), and the intervention phasis with implementation of guidelines and training in hand hygiene. Between the two phasis they are technical approach with the sampling of all patients who presented SSIs during the period. The Figure 1 is an overview of MUSTPIC project and the 3 PhD thesis of the project.

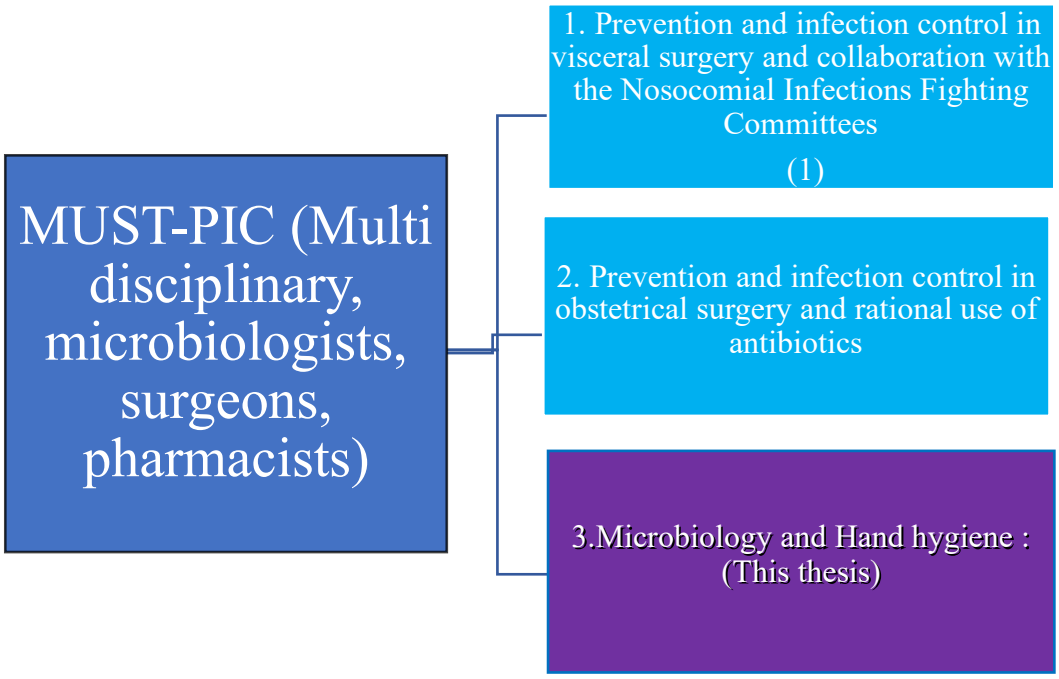


Figure 1. Overview of the MUSTPIC project with part 3 dedicated to this thesis.

Table 1. List of activities of MUSTPIC project (2018-2022)

Research team (2018-2022)	**Activities**	**Published article or in preparation (in relation with activities)**
Researcher 1	-Audit of surgical antimicrobial prophylaxis in gastro-intestinal surgery -Audit of "CLIN" activities -Interviews of CLIN team members to describe problem for the success of WHO multimodal strategy.	1. Audit of antibiotic prophylaxis practice in gastrointestinal surgery in 5 hospitals in southern Benin. 2. Audit of Nosocomial infection control's committee operation and internal policy on hospital hygiene and use of antibiotics
Researcher 2	-Audit of surgical antimicrobial prophylaxis (SAP) in maternity wards -quality of antibiotics used for the SAP in hospitals	1.Antibiotic usage in patients having undergone caesarean [20] 2.Healthcare professional's knowledge and beliefs of antibiotic prophylaxis in caesarean section; a mixed methods study[21]
Researcher 3 (Hand hygiene and bacteriology: this thesis)	-Hand hygiene observation -interviews of HCWs regarding HH improvements -Microbiology of SSIs -Molecular aspects of MDR detected	1. Hand hygiene in surgery in Benin: opportunities and challenges [21] 2. Results of antibiogram of causative bacteria isolated o SSIs [22] 3. Molecular analysis of *Escherichia coli* producing extended spectrum beta-lactamases [23] 4. Case report of XDR *Acinetobacter baumannii* [24] 5. Hand hygiene interviews helping to understand HCWs behavior regarding the HH practices.
Interventions based on all audit activities and results: global surgery guidelines for rational use of antibiotics in Benin Feedback and training sessions in hospitals		

This introduction is divided into three sections.1) a definition of surgical site infections, the etiology of bacteria isolated on them, the risk factors and causes of SSIs, 2) antimicrobial resistance and molecular aspects of the multidrug resistant bacteria of interest such as extended-spectrum beta-lactamases and carbapenems producing organisms and 3) the concept of hand hygiene including determinants of HH compliance and “My five moments for hand hygiene”.

1.2 SURGICAL SITE INFECTIONS

Definition, historical background, classification, and risk factors

Etiology and microorganisms responsible

The core components for effective infection and Prevention control program

Organization of an SSIs control program

The details of SSI surveillance

1.2.1 Definition, historical background, classification, and risk factors

Surgical site infections (SSI) have replaced previously used term surgical wound infection[22]. Surgical site infections are the third most cited healthcare associated infections (after pneumoniae and urinary tract infection) and one of the frequent causes of post-operative morbidity [23]. *SSI occur at the incision site and/or deeper underlying tissue spaces and organs within 30 days of a surgical procedure (or within 90 days for surgeries involving implantation of prosthetic material*) [24]. SSIs are estimated to add 11 more days to a patient's hospital length of stay and are one of the most reason unscheduled readmissions that occur during or after an intervention [24,25]. According to the World Health Organization (WHO), SSIs are the most frequently reported HAI in LMICs , but their burden remain less investigated compared to high income countries because of difficulty to gather reliable data [5]. Amongst 100 hospitalized patients, seven in developed and 15 in developing countries will develop at least one HAI [26].

Hippocrates commented on wound healing said –"*if the pus is white and not heinous, the health shall come; but if it is ichorous and muddy, the death will ensue*'. It was not until the 19th century that a breakthrough took place that eradicated the term pus laudable from the medical literature [27]. It was then that a Hungarian obstetrician Ignaz Philipp Semmelweis (1818-1865) proposed to physicians to wash their hands in chlorinated water before examining patients, and this fact was followed by a drastic decrease in mortality [28].

The CDC definition thus describes three levels of SSIs as follow [22]:

- Superficial incisional, affecting the skin and subcutaneous tissue. These infections might show localized signs such as redness, pain, heat, or swelling at the site of the incision or by the drainage of pus.

- Deep incisional, affecting the fascial and muscle layers. These infections can be detected by the presence of pus or abscess, fever with tenderness of the wound, or a separation of the edges of the incision exposing the deeper tissues.

- Organ or space infection, which involves any part of the anatomy (organs or spaces) other than the incision that is opened or manipulated during the surgical procedure, for example, a joint or the peritoneum. These infections can be suspected by the drainage of pus, or the formation of an abscess detected by histopathological.

  Contamination classes according to Altemeier: this classification grades (I-IV) allow surgeons to promptly identify the patients at risk of surgical site infection that is resume as follows [29]:

- Class I/Clean surgery (SSI risk <1%) :The surgical procedure involves a normally sterile area of the body with skin normally intact. Moreover, the procedure does not involves opening of the gastro-intestinal, respiratory, Genito-urinary, or Oro-pharyngeal tract.

- Class II/Clean-Contaminated surgery (SSI risk 2-5%): the procedure involves opening the gastro-intestinal, respiratory, Genito-urinary, or Oro-pharyngeal

tract under tightly controlled technical conditions and in the absence of abnormal contamination.

- Class III/Contaminated surgery (SSI risk 5-10%): Massive surgical-site soiling by gastro-intestinal lumen contents, opening of the genito-urinary or biliary tract infection. Recent open traumatic wounds.

- Class IV/Dirty-infected surgery (SSI risk > 10%) : Surgical procedure involving a body site that contains pus, foreign bodies, or feces. Traumatic wounds created more than 4 hours earlier This definition suggests the presence of microorganisms responsible for SSI in the surgical-site before the operation.

  Patients who are less fit, those who have a great length of hospital stay and are undergoing longer and more complex interventions are more susceptible to present surgical site infections [30,31]. A statistically significant association was found between surgical antimicrobial prophylaxis (SAP) and increased risk of SSI [32]. The SAP remains important factor for reduction the rate of SSIs when its correctly practice according to the criteria such as duration, dose, timing, indication and the choice of antibiotics. Indeed, the objective of SAP is to deliver adequate antibiotic exposure throughout the surgical site to inhibit the bacteria commonly associated with SSI for a procedure [32]. Antimicrobial prophylaxis with cefazolin is a proposed choice because this antibiotic has excellent activity against common Gram-positive and certain Gram-negative aerobes, and correct tissue penetration [33].

The table 2 summarizes different risk factors depending on the patients and surgery. The risk factor depends on the type of surgery (caesarean, appendicitis) but some factors remain the same and are described frequently worldwide: the young maternal age for caesarean section and the inappropriate timing or dosing of antibiotic prophylaxis[34].

Table 2: Main patient-related and surgery-dependent factors that may influence the risk of surgical site infections[34] .

PATIENT DEPENDENT	Age, nutritional status, diabetes, smoking, obesity, concomitant infection, impaired immunity, colonization by resistant bacteria, length of hospitalization.
SURGERY DEPENDENT	Skin disinfection, antibiotic prophylaxis, operating room air conditioning, improper instrument sterilization, insufficient hemostasis preoperative shaving, duration of operation, , dead space.

1.2.2 Etiology and microorganisms responsible

The bacteria commonly described in SSIs are broadly divided into aerobic and facultative bacteria in one group and anaerobic bacteria in the other, into Gram positive and Gram-negative bacteria. It is important to recognize that most infections occurring in surgical patients are caused by endogenous bacteria and that the bacteria isolated depend on the procedure [35]. It is helpful to know the normal microbial flora of the anatomical parts of body because this details directly helps SAP and suspect the origin of plausible infections. Consequently, the knowledge of different causative bacteria is helpful in laboratory because it takes up to 72 hours for a final culture to give the identification of bacteria, however the Gram staining and biochemical tests are sufficient to give earlier response about the causative bacteria.

The most isolated bacteria are *S. aureus*, coagulase-negative *staphylococci, enterococcus spp.* and *Escherichia coli*. This observation of high number of MDR is due to the widespread use of broad-spectrum antibiotics. Another reason is the fact that certain organisms remote from the operative site, particularly in patients undergoing insertion of a prosthesis or other implant. For example, in Uganda in 2019 Gram-negative bacteria were the most isolated with the dominant being *Klebsiella pneumoniae* [36] while in Nepal in the same year, *S.aureus* and *E. coli* were the most isolates in wound infections [37]. According to the studies realized by the European Centers for Diseases Prevention and Control (ECDC), *Staphylococcus aureus* has become the most common cause of SSI in the recent year [38]. In the nationwide prevalence study realized in 2012 in Benin, the most isolated bacteria on SSIs were respectively: *S. aureus , E. coli, P. aeruginos*a and *Enterococcus spp* [39]. The following table showed the common bacteria isolated according to the type of surgery.

Table 3: Summarize of bacteria commonly isolated associated with type of surgery[34].

Type of surgery	Common bacteria
Obstetrics and gynecological	Gram-negative bacteria, enterococci, anaerobes
Gastroduodenal	Gram-negative bacteria, enterococci, anaerobes
Colorectal	Gram-negative bacteria and anaerobes
Biliary tract	Gram-negative bacteria and anaerobes
Appendicectomy	Gram-negative bacteria and anaerobes

Due to the unavailability of proper and adequate consumable, isolation of anaerobic bacteria in our laboratories in Benin constitutes an important limitation. Although they can survive for variable periods while exposed to oxygen, they require an anaerobic environment for growth and invasion and for elaboration of the toxins that account for their dramatic virulence in infections. Their isolation in laboratories is laborious and necessitate additional technique for creation of anaerobic environment. We can cite for example the use of the 16rRNA gene sequencing for detection in developing countries or special culture media which support the growth of anaerobes [40]

1.2.3 The core components for effective infection and Prevention control program

WHO established based on the development of evidence-based recommendations the core components of infection prevention and control programs that should be implemented in acute health care facilities and at the national level [41]. For combatting HAIs and AMR, the WHO recommend implementing the IPC program with the dedicated and trained team. IPC programs represent one component of quality service in hospitals. Nevertheless, among 133 respondent countries, only 54 had a national IPC program in place and only fewer reported to have these program in all tertiary hospitals [41]. The IPC guidelines are an important framework which help to support the performance of good practices in the implementation of IPC in hospitals. For a successful implementations the following details must be highlighted:

- IPC education and training: amongst documents established related to IPC national strategies or action plans only few addressed specialized training of IPC professionals.
- HAI surveillance: core component of national and facility IPC programs nevertheless in Africa, the poor laboratory capacity in term of equipment's and qualified human resources as well as data management constitute the barriers of surveillance.
- Multimodal strategies with three or usually five several components as for example the change of system, education, and training of HCWs, monitoring of practices, reminders in workplace and culture change
- Audit of IPC practices

- Workload staffing and bed occupancy
- Built environment materials and equipment's

The following figure is a resume of the core components of IPC programs

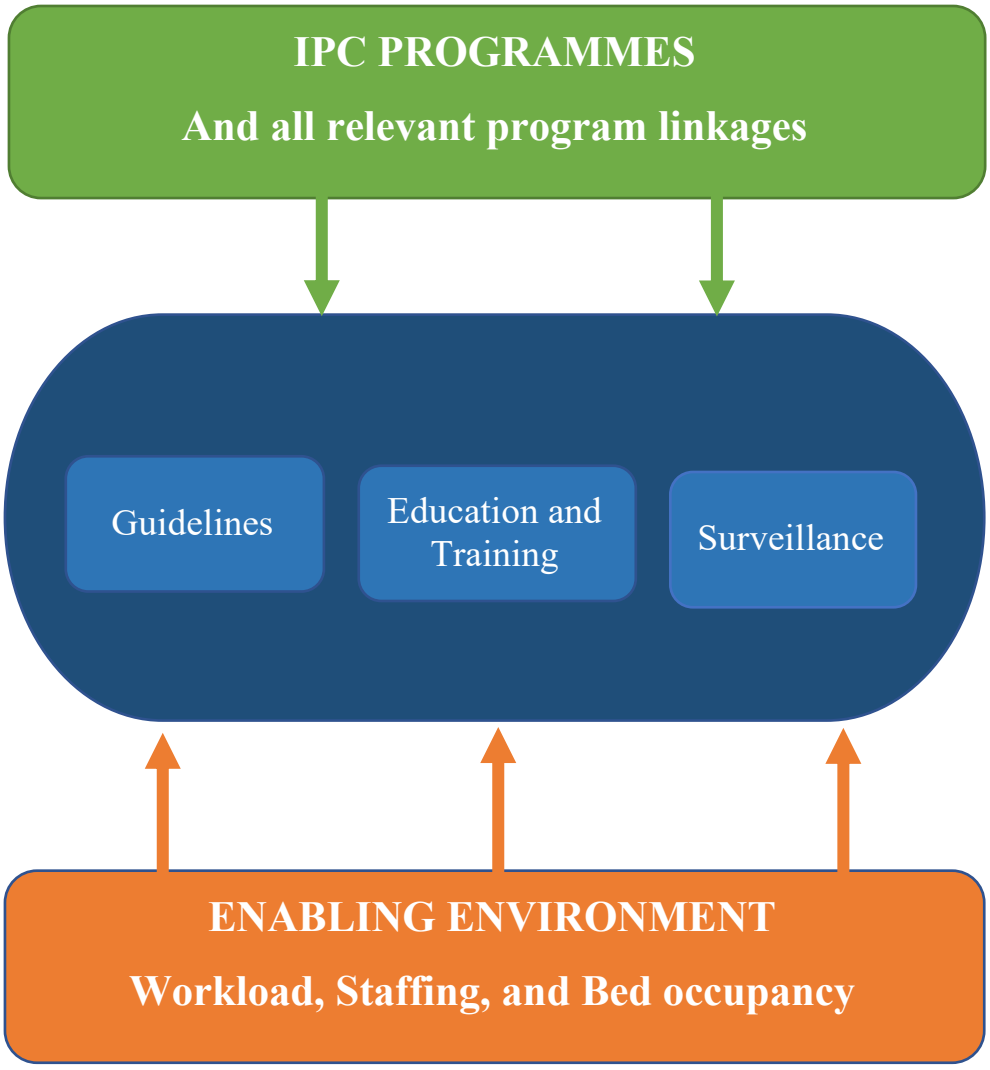


Figure 2: Representation of the core component of IPC program [41]

1.2.4 Organization of an SSIs control program

The surveillance of HAIs and particularly SSIs is one of the most important component of infection control program. The surveillance of SSIs includes different steps such as definition, detection, report, and interpretation of SSIs. This process is a challenge particularly in LMICs countries [42]. The goal of surveillance is firstly the collection of data on SSI rates to evaluate the magnitude and impact of the problem in healthcare setting. Secondly, data obtained will be analyzed with careful interpretation. Finally surveillance data allows through previously analyses, identification of improvement actions and conducting of sustainable interventions. SSI surveillance is an important phasis to detect and understand how the problem is high and remain one of the components of effective IPC recommended by the WHO [43]. Active surveillance and post discharge follow-up of patients are reliable and concise method to determine the incidence of SSIs but are expensive particularly in LMICs where the patient's security is not always a priority [43,44]. In our setting where follow-up visits are not sub-optimal, the incidence of SSI is probably underestimated.

In Benin, we started with the Glass (Global Antimicrobial Resistance and Use surveillance System) with implementation of surveillance site in six public hospitals. The first step concerned the training of actors in laboratories regarding identification and antibiogram of bacteria of interest.

To reduce the incidence of SSIs, evidence-based surgical care bundles have been implemented as part of SSI prevention policies [45,46].

1.3 ANTIBIOTIC RESISTANCE AND MOLECULAR EPIDEMIOLOGY

Antibiotic resistance: What why where and how prevent?

Mechanisms of acquired resistance of bacteria

Laboratory detection of extended-spectrum beta lactamases, carbapenem-resistant organisms (CRO and methicillin resistant Staphylococcus aureus (MRSA)

Methicillin resistant staphylococcus aureus (MRSA): antimicrobial resistance genes and virulence factors

1.3.1 Antibiotic resistance: What why where and how prevent?

Antibiotic resistance is known to be a public health concern of the 21st century [47]. This antibiotic resistance constitutes a normal phenomenon on the evolution of bacteria and was recognized to exist before the introduction of first antibiotic [48]. When bacteria are resistant to antibiotics, it develops the ability to growth or survive in the presence of sufficient concentration of antibiotic that usually provoke inhibition or death of bacteria of the same species [48]. Resistance to antibiotics occurs when the concentration required to inhibit or kill bacteria exceeds the minimum achievable in a patient [49]. The definition of susceptibility and resistance was based on the pharmacodynamics and pharmacokinetics characteristics of antibiotics. When bacteria are categorized as susceptible, they are a good chance of treating infection, but with resistant bacteria, good antibiotics for treatment are challenging practicians particularly in the LMICs where the last line antibiotics are often unavailable.

We describe another type of resistance define as natural resistance. Natural resistance of bacteria could be intrinsic and exist in all bacteria of the species or induced i.e. the genes naturally exist in the bacteria but will be expressed after exposure to antibiotic [50,51]. The mechanisms or resistance that underline this intrinsic resistance are the natural activity of efflux pumps and the reduction of permeability of the outer membrane [50,51].

Table 4: example of bacteria presented intrinsic resistance [52].

Organisms	Intrinsic resistance
Gram positive	aztreonam
Gram negative	glycopeptides and lipopeptides
Escherichia coli	macrolides
Klebsiella pneumoniae	ampicillin, ticarcillin
Pseudomonas aeruginosa	Sulfonamides, ampicillin, 1st, and 2nd generation cephalosporins, chloramphenicol and tetracycline

Antimicrobial resistance causes an increasing rate of mortality, morbidity and supplementary costs for patients and healthcare settings. The burden of AMR in LMICs is higher than developing countries because of the unavailability of last line antibiotics, the self-medication practice, the hygienic conditions, the massive imprudent use of antibiotics both in communities and clinical settings, the possibility to take this drugs over the counter and without doctor's prescription and the lack of quality of control of all antibiotics available in the country [53,54]. The following table presents the factors and stakeholders that contribute to the problem of AMR in Benin.

Table 5: Factors and stakeholders contributing to the problem of antimicrobial resistance [55–58]

Factors	Contribution/examples
Poor quality of antibiotics	Absence of quality control of all antibiotics circulate in the country, sometimes the poor-quality drugs are circulated in unformal market, and later producing sub-inhibitory concentration in vivo and consequently selection of resistant strains.
Lack of national regulation	Non availability of AMR action plans and strict regulations about distribution of antibiotics on counter. Since 2020, they are strong recommendation of government which require pharmacists not to sell antibiotics without medical prescriptions.
Prescribers	Antibiotics sellers usually have little knowledge of the require dosage indication and contraindications of antibiotics even if they are medical doctors as the basically training on antibiotics remains insufficient.
Patients	Self-medication and non-observance of treatment (dosage and duration)
Animal industry and environment	The use of antibiotics in environment and livestock can favor the survival and dissemination of resistant strains and can be transferred to humans through the consumption food or habits in environment. “The one health” challenge must be promoted and explain to population and health care providers.

1.3.2 Mechanisms of acquired resistance of bacteria

Classically the three main antimicrobial resistance mechanisms are : (1) the modification /inactivation of the antibiotic; (2) alteration/change in the target site of antibiotic; and (4) active drug efflux [59].

1.3.2.1 Modification of target molecules

Antibiotics might target multiple components of bacterial cell. The variation or acquisition changes in the target site of antibiotics will prevent their binding and therefore induce resistance. The target site changes are often the consequences of spontaneous mutation of gene on the chromosome [60]. As antibiotic interaction with the target site is specific, important effect on antibiotic binding would be appear although the alteration of the target molecule is minor or insignificant. The resistance by modification of enzyme is described in both Gram negative and positive bacteria.

i) An alteration in the 30s or 50s subunit of the ribosome affects the protein synthesis ..

ii) Alteration in Penicillin-binding-proteins (PBP): Gram positive bacteria for example, usually resist to Beta-lactam drugs by alteration in the structure and/ or number of penicillin-binding-proteins (PBP). The PBP is a transpeptidase that prevent the cross-linking of the glycan chains in bacteria cell wall [61]. The minor or major change (increase or decrease number of PBP) in the wall cell provoke a change of their structure and impacts the amount of drug that can totally or partially bind to that target [49]. The typical example is the methicillin resistant *Staphylococcus aureus* (MRSA). MRSA

is conferred by the *mecA* gene, which transform PBP on PBP2A with a reduction of affinity for beta-lactam activity [62].

iii) Alteration of cell wall precursors: Glycopeptides (vancomycin and teicoplanin) that remain the last line therapeutic option of the MRSA treatment act in the cell wall synthesis and particularly D-alanyl-D-alanine residues of peptidoglycan precursors [63].The strains that show resistance to vancomycin but remain sensitive to teicoplanin are namely Van B and Van C but the Van A-type resistance is observed with resistance to teicoplanin and vancomycin [64].

iv) Alteration of DNA gyrase or topoisomerase IV: the quinolones target synthesis of nucleic acid and specifically their resistance mechanisms depend on the bacteria, for Gram negative quinolones act by inhibition in DNA gyrase while in Gram positive resistance is via the topoisomerase IV. These mutations cause structural change of gyrase and topoisomerase IV and consequently a decrease of affinity of these antibiotics with target sites [65,66]. The mechanism of resistance impact 2 previously enzymes DNA gyrase (genes gyrA and Par C) and topoisomerase IV (genes Par C and E) [52].

1.3.2.2 Efflux pumps

Efflux pumps are a kind of membrane proteins that expulse antibiotics from the cell and maintain their low-intracellular concentrations [63]. At the same moment, when antibiotics are entering in the cells, efflux mechanisms are pumping them out before their reach their targets. The pumps are located to the cytoplasmic membrane while porins were found in the outer membrane.

Except polymyxin, all classes of antibiotics are susceptible to activation of efflux pumps system [67]. These pumps can transport and get out a wide range of antibiotics such as macrolides, tetracyclines and fluoroquinolones. This fact explains implication of efflux system in the multidrug resistance physiology [60].

1.3.2.3 Antibiotic inactivation

Three main enzymes inactivate antibiotics: beta-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferases (AACs).

- *Beta-lactamases*

  Beta-lactamases inactive by hydrolyzation almost all beta-lactams that have ester and amide bond for example Penicillin, cephalosporins, monobactams and carbapenems by attacking their beta-lactam rings [68]. Beta lactamases are the most important enzymes and are classified according to two main classification systems. First the Ambler classification is based on the structure of amino acid sequence and separates beta-lactamases into 4 groups (A, B, C, and D) [69]. On the other hand, the Bush-Jacoby classification was based on biochemical function on these enzymes mainly on substrate specificity [70]. To date, more than 1,000 different beta-lactamases have been described (www.lahey.org/studies) and more continue to be reported, ten times increase since 1990 [71]. Beta lactamases of classes A, C, and D use a serine as an enzyme active center, whereas beta-lactamases of class B use the metal zinc. Beta-lactamases that degrades carbapenems have been identified in all classes A to D.

According to the Ambler classification which is the most used in literature we described.

i) Class A beta-lactamases : also named and referred as penicillinase, this class is susceptible to clavulanic acid. This class include enzyme which have little or no activity against cephalosporin. TEM and SHV were progenitors of extended spectrum beta-lactamases (ESBls). ESBLs are a group of enzymes which allow resistance to penicillin , third generation cephalosporins, aztreonam, cefamandole, cefoperazone, but are susceptible to methoxy-cephalosporins (cephamycins) and carbapenems [72].After first penicillinases and ESBLs, they are many mutations of TEM , SHV, ESBLs, such as TEM-3, SHV-2, and CTX-M, which hydrolyze cefotaxime with susceptibility to clavulanic acid. The TEM, SHV and CTX-M type enzymes are derived from plasmid and consequently are easily transmitted among different bacterial species [73].

ii) Class B beta-lactamases: these enzymes have on their active center zinc or heavy metals and are metallo-beta-lactamases (MBLs). As metal is located on enzymatic active center, their activity is inhibited by chelating agents such as ethylenediaminetetraacetic acid (EDTA). These classes of enzymes are resistant to inactivation by clavulanate, aztreonam and carbapenems. the support of transmission of these enzymes could be plasmid, transposon, integron or other mobile genetic elements. Based on amino acid sequence they are 3 subclasses of MBLs (B1, B2, B3). To date the carbapenem resistant metallo-beta-lactamases imipenemase (IMP), Verona integrated-encoded MBL (VIM), New Delhi MBL (NDM), Sao Paulo MBL (SPM), Germany

imipenemase (GIM) and Florence imipenemase (FIM) were described [74–77].

iii) Class C beta-lactamases: Also named cephalosporinases, this class hydrolyzes cephalosporins. The class C include extended spectrum cephalosporins unlike the Class A beta-lactamases and we observe resistance to all beta-lactams except carbapenems [78]. These enzymes are not inhibited by clavulanate.

iv) Class D beta-lactamases: namely OXA enzymes, this class is constituted by oxacillin hydrolyzing enzymes described mostly in *Enterobacterales* and *Pseudomonas*. OXA enzymes with carbapenem-hydrolyzing activity have mainly described on chromosome in *Acinetobacter baumannii* strains, however OXA-23 and OXA-48 have been reported on plasmids [79]. These enzymes are poorly inhibited by clavulanic acid or EDTA. OXA enzyme type present a weak activity against carbapenems and was firstly described in organisms such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* and more frequently in *Enterobacterales* [80,81]. Generally, OXA-48-type producers show difficulty to be identified because of their point mutant analogues with ESBLs and consequently their prevalence rate are probably underestimate [82].

- *Aminoglycoside modifying enzymes (AME's)*

  Aminoglycosides are specifically inhibited by specific enzymes: phosphoryl-transferases, nucleotidyl-transferases or adenylyl-transferases and aminoglycosides acetyl transferases (AACs) [60]. The aminoglycoside N-acetyltransferases belong to the GCN5-related N-acetyltransferase superfamily[83]. AAC (6)' enzymes are subdividing into two groups

according to the profile of aminoglycosides that they inhibited. Amongst these enzymes,

importance that was described in large variety of Enterobacterales [83]. The high dissemination of these enzymes amongst bacteria is due to the fact that the aac (6')-Ib gene is often present in integron, transposons, plasmids, genomic island, and other genetic structures [83].

- *Chloramphenicol-acetyl-transferases*
  Chloramphenicol acetyltransferases (CAT) catalyzes the acetyl-CoA dependent acetylation of chloramphenicol, resulting in the inactivation of the drugs chloramphenicol, thiamphenicol and azidamfenicol [84]. Gram-negative and some Gram-positive bacteria strains are resistant to chloramphenicol.

1.3.3 Laboratory detection of extended-spectrum beta lactamases, carbapenem-resistant organisms (CRO and methicillin resistant Staphylococcus aureus (MRSA)

For the detection of ESBLs producing *Enterobacterales,* laboratories used the Kirby- Bauer Disc diffusion methods with cefotaxime 30ug and ceftazidime 30 ug with and without clavulanic acid. ESBL positive isolates show an increase of 5mm zone of inhibition with clavulanic acid as compared to the zone size for antibiotics alone [85].
They are another confirmation tests such as the Vitek2 ESBL panel, the minimal inhibitory concentration-strip. They are also screening agar plates,

chromogenic medium (ChromID) that demonstrate high sensitivity for ESBLs [86].

Table 6: Resume of phenotypic and molecular methods commonly used.

Conventional methods	**Non-conventional methods**	**Microfluidic technologies**
➢ Phenotypic • Manual (LMICs (e.g. agar dilution, disk diffusion and broth microdilution) Automated methods (e.g. VITEK 2 compact, Phoenix Beckton Dickinson) ➢ Molecular based	➢ Genome Sequencing and metagenomics • Pyrosequencing • Whole genome sequencing • Nanopore MALDI-TOF mass spectrometry	➢ Colorimetric-based ➢ PH-based ➢ Point of care (POC) and single-cell molecule

- *Phenotype-Based methods:*

  In many African countries, the disc diffusion method remains the reference but shown variability in ability to detect underlying mechanism or resistance. Some laboratories as yours, complete by the modified Hodge test (MHT) [87], however this test is recommended by the CLSI in 2009 [88], the test is not specific for the detection of certain types of carbapenemases. They are phenotypic tests such as Carba Nordmann-Poirel [89], with technique require no expertise, are reproducible, inexpensive, and easily adapted in laboratory with poor resources and molecular equipment's .

  Different methods exist for the detection of MRSA in laboratories: the cefoxitin disc diffusion test that is most reliable than traditionally Oxacillin

disc diffusion test, minimum inhibitory concentration (MIC) of cefoxitin as determined by MIC test strip and currently the detection of chromogenic specific agar for MRSA [90].

- *Genotype-Based Techniques*

  Molecular techniques represent the best way for the detection of carbapenemases, ESBLs and MRSA. Polymerase chain reaction present excellent sensitivity and specificity, more than phenotypic methods and MHT [91]. Theses molecular methods are labor-intensive, time consuming, cost of consumables and reagents and sometimes necessitate lot of spaces in laboratory for installation particularly in African countries. Some authors described the use of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a new tool to rapidly detect carbapenem producing organisms in fresh blood cultures [92]. They are another molecular methods such as the multilocus sequence typing , pulsed field gel electrophoresis, amplified fragment length polymorphism and multiple locus variable-number tandem repeat analysis [93]. The whole genome sequencing (WGS) has already use in developing countries and is laborious and time-consuming. However, WGS provide interesting and useful information, their implementation in African laboratories is not always optimal when we often do not correctly perform phenotypic identification of bacteria.

  The molecular analysis of MRSA strains included PCR which is considered as the gold standard [94]. Pulsed-field gel electrophoresis (PGFE), multilocus sequence typing (MLST) and the determination of sequence polymorphism

of the *staphylococcus* protein A (*spa)* that remains the most popular MRSA typing[95].

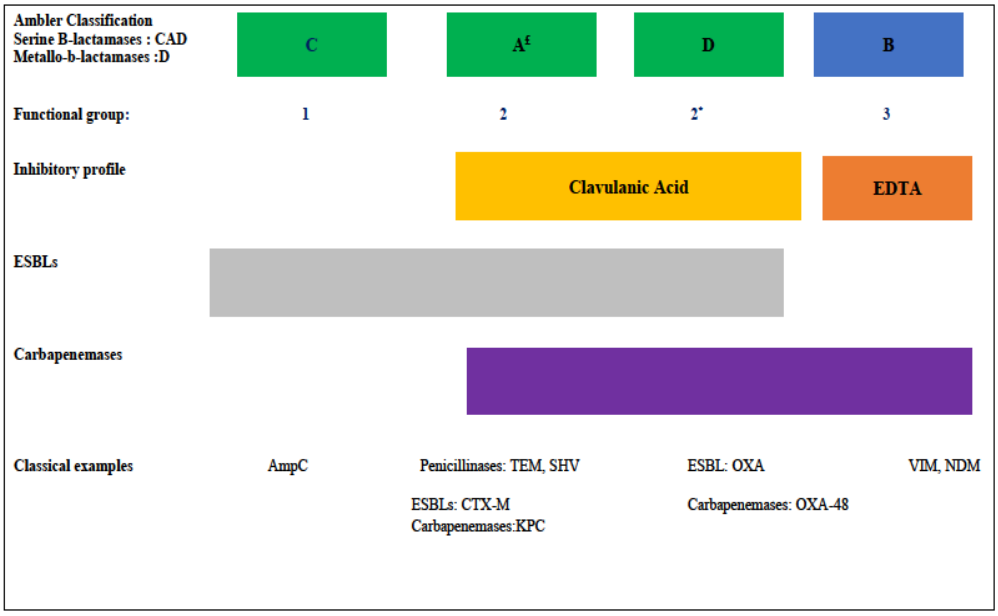


Figure 3: Schematic representation of beta-lactamases follow the Ambler classification and correlation with the Bush-Jacobi functional classification [96] £Class A enzymes are the most diverse and include penicillinases, ESBLs and carbapenems *Ambler class D enzymes belong to the functional group/subgroup 2d.

1.3.4 Methicillin resistant staphylococcus aureus (MRSA): antimicrobial resistance genes and virulence factors

Staphylococcus aureus represent a frequent cause of infections in hospital and community settings. In samples collected by MUSTPIC project *S. aureus* is the second common isolates in gastrointestinal surgery but the top one in maternity. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of leading bacteria involved in hospital-acquired infections [97]. The resistance is due to acquisition of SCCmec element, which carries *mecA*, the gene that is the consequence of mutation of penicillin-binding protein (PBP). The SCCmec element contains resistance genes not only to all beta-lactams antibiotics but also aminoglycosides and glycopeptides [98]. The mortality rate is higher with patients infected with resistant strains. They are several extracellular cytotoxins produced by S. *aureus* virulence that explains high mortality caused by skin and soft tissue infections and involved in the pathogenesis. The toxins of interest include Panton-valentine leucocidin toxins (PVL), exfoliative toxins (*eta* and e*tb*), arginine catabolic mobile element (ACME, *arcA*), beta-hemolysin (*hlb),* toxic shock syndrome toxin-1 (TSST-1) and accessory gene regulator (*agr*) and alpha-hemolysin (*hla)* [99]. The most important toxins detected in our isolates are follows:

- *The Panton-Valentine leucocidin (PVL) is a multicomponent protein cytotoxin which belongs to the pore-forming toxin family that forms an octameric pore in the affected membranes of human polymorphonuclear neutrophils (PMNs), macrophages and monocytes* [100]. PVL is a pore-forming toxin encoded by *luk*F-PV and *luk*S-PV and can be present in both methicillin-susceptible *S. aureus* (MSSA) and MRSA [101].

- The toxic shock syndrome toxin (TSST-1) is a protein causing a releasing of tumor necrosis factor-alpha , non-specific T-cell proliferation and interleukin production, all enzymes that complicate variety of diseases. The presence of this protein can provoke within 24 hours without adequate treatment a lethal shock [102].
- The heptameric beta-barrel forming alpha toxin (*hla)* that is involve in the necrosis of epidermidis and dermis layers as well as contributes to the severity of USA300 skin infections [103]. Many studies described the role and importance of hla in the occurrence of dermo necrosis during skin infections. Strains of MRSA that carry *tst* genes are most described and known to be associated to the wound and blood infections [104].

1.4 HAND HYGIENE

The role of hands in germ transmission

Relationship between hand hygiene and healthcare associated infections

Hand hygiene

The WHO five moments

Compliance with hand hygiene best practices

Hand-hygiene practice among healthcare workers and factors influencing adherence to recommended HH practices

The Theoretical Domain Framework

1.4.1 The role of hands in germ transmission

Hand hygiene constitutes the starting point for the prevention of cross transmission in hospitals [105]. Bacteria described on the hands could be divided into two types, namely transient and resident. Although the count of transient and resident flora varies between individuals, it is frequently constant for any given individual. Resident flora has two main protective roles: microbial antagonism and competition for nutrients in the ecosystem. Transient flora, which colonizes the superficial layers of the skin, is more amenable to removal by hand hygiene [106]. Resident flora contains strains such as coagulase-negative *staphylococci,* while Corynebacterium spp., Micrococcus spp. and Bacillus spp. were found to be elements of transient flora and consequently represent pathogenic microorganisms [107]. Transient microorganisms do not usually multiply on the skin, but they survive and sporadically multiply on skin surface. The transient organisms often contaminated the hands of HCWs during their daily tasks or surfaces adjacent to patients [107].

SSIs can be caused either by microorganisms transmitted from other patients or HCWs or from surrounding environment (exogenous).

The microorganisms commonly found on the hands in clinical settings (including vancomycin resistant *Enterococcus*, Candida spp. and multidrug resistant bacteria) can survive for as long as 150 hours [108]. The skin epithelial cells containing viable microorganisms which are shed from normal skin with ability to contaminate the gowns, bed linen, bedside furniture and other objects that surround the patient [108,109]. Hand carriage of resistant pathogens has increasingly been demonstrated to be associated with cross transmission and nosocomial infections [110]. The higher rates of hand

contamination are reported from critical care area such as intensive care units and surgery departments and the HCWs often struggle under the heavy workload forgetting to wash their hands[111]. The following scheme is a synthesis of five steps of cross transmission during the care.

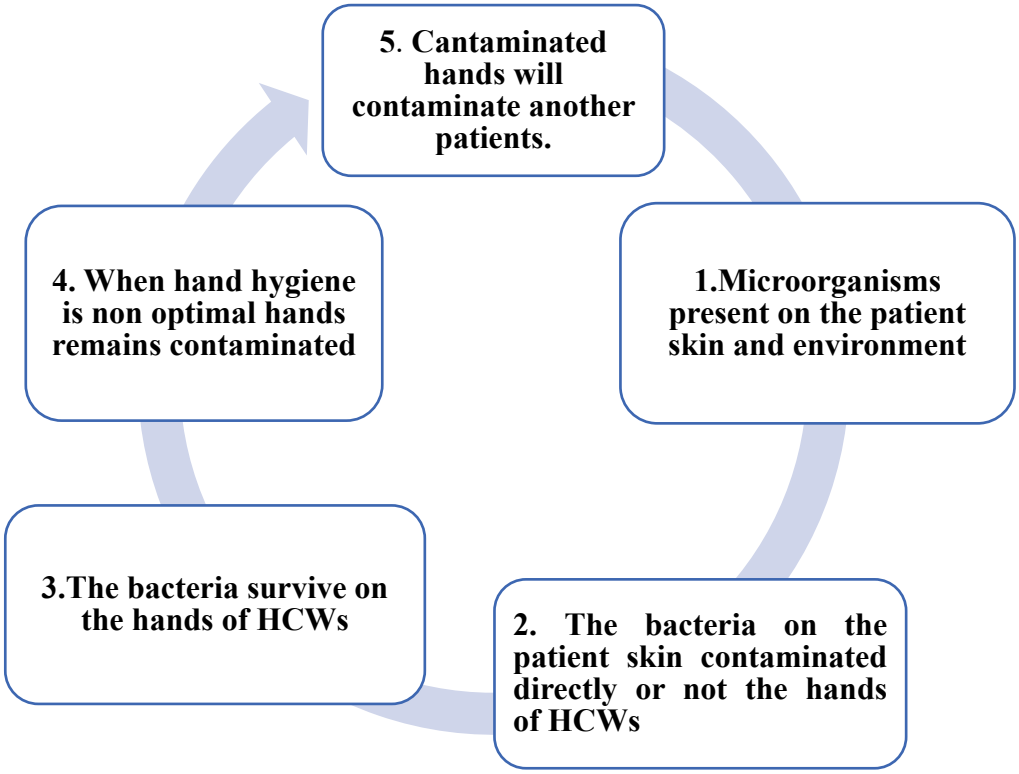


Figure 4: Steps of cross transmission during the care delivery.

1.4.2 Relationship between hand hygiene and healthcare associated infections

The role of hand hygiene in the prevention of cross transmission of bacteria during care is well established and more than evidence since recent years [112]. Compliance of healthcare workers with best practices varies between settings and countries but is usually low and insufficient to ensure patient safety. Hand hygiene practices among HCWs were first recognized as an effective tool to reduce maternal mortality in the late 1840's in a Vienna hospital by Dr. Ignaz Semmelweis [113]. Since then, the importance of HH in the reduction of HAIs number has been highlighted in many studies and systematic reviews. Most studies showed the relation between improved HH and the reduction of HAIs and cross-transmission [112,114,115].

Strong evidence highlighted the fact that hand hygiene was used as the main intervention and a significant improvement in HH compliance and/or increased alcohol-based-hand rub (ABHR) consumption. These improvements were associated with substantial decreases in multidrug resistant organisms. To be efficient hand hygiene should be practice according to a certain prerequisites such as:

- Absence of ring and bracelets; the skin underneath rings have more microorganisms and this number increases with the number of rings worn [116].
- Fingernails, nail technology and nail polish because that region contains more bacteria which are largely inaccessible during hand hygiene practices comparatively to the nail without polish [117]. Long nails should be avoided when we give care in hospitals because they are also more likely to tear the gloves.

Alcohol-based antiseptic hand rub (ABHR) is one of the most important components of hand hygiene programs and these programs have been reported to decrease the rate of SSIs and the emergence of multidrug-resistant bacteria in hospitals [118]. The advantages of using alcohol in LMICs are that it requires less time, irritates hands less often than does hand washing, and removes bacteria with more efficacity. WHO and CDC recommendations for ABHR application time is between 20 to 30 seconds or about 20 seconds [119]. In the "Ward Gel" quasi-experimental study realized in 2017 in Uganda for example, author's improve hand-hygiene compliance by providing training and ABHR. The availability of ABHR is crucial in the system change and the implementation of WHO multimodal strategy and impressive improvement in HH is possible through frequent provision of ABHR [120]. ***In this study, ABHR locally made from sugar cane with recommended concentration of Ethanol according to the standards*** [121]. In summary, improvement of HH practice among HCWs has been found to be associated to a decrease of the rate of SSIs, however this promotion will not be efficient without the culture of use of ABHR in LMICs countries.

1.4.3 Hand hygiene

Many observational studies showed that the hand hygiene compliance among healthcare workers is non-optimal [12]. Several barriers to appropriate hand hygiene have been described among which the forgetfulness, ignorance of guidelines, high workload and insufficient time, lack of infrastructure and resources particularly in LMICs [11,13,14,122].

1.4.4 The "WHO five moments"

The standard recognition of indications for HH during patient care is a fundamental step for monitoring and performance feedback. A human factors approach inspired an iterative process at the University of Geneva Hospitals with input from specialists from different countries and an international expert panel of the WHO First Global Patient Safety Challenge. It resulted in the innovative design of the concept of "My five moments for Hand hygiene", which proposed a unified vision for HCWs, trainers and observers to minimize inter-individual variation and lead to an increasing the effectiveness of hand hygiene practices [123]. The concept does not in any way reduce the need for HH. It is a tool to identify moments when HH must be performed, as well as to distinguish those when it is not useful. (Figure 5)

Hand hygiene indications are closely related to the patient zone concept. The patient zone includes the patient, the area around the patient, the area around the patient, and some surfaces and items that are temporarily and exclusively dedicated to him /her. The My five Moments image has become a trademark for hand hygiene promotion and has undergone unprecedented viral spread worldwide [123].



Figure 5: *The WHO five moments. World Health Organization. WHO guidelines on hand hygiene in healthcare. WHO Press, Geneva, Switzerland 2009.*

1.4.5 Compliance with hand hygiene best practices

Hand hygiene is a general term referring to any action of hand cleansing. Hand-hygiene during healthcare delivery should be practiced either by handwashing or by hand rubbing at specific moments described by the WHO when the risk of transmission occurs. These moments have been identified according to the available scientific evidence and are reflected in specific «opportunities" for hand hygiene opportunity for hand-hygiene is a moment

during healthcare activities when HH is necessary to avoid the germ transmission by hands [119]. This term is very close to the concept of HH 'indication', which is the reason that explain a HH action. An opportunity is identified by the presence of one or more indications. The non-compliance with hand hygiene explains the transmission of pathogens and the development of HAIs.

To minimize differences in the way they are understood and applied by HCWs trainers and observers of HH practices hand hygiene indications should be universally understandable. If one step of intervention after the direct observation is the feedback to HCWs about their practices, it is essential that observers have a clear understanding of the correct indications of HH. Compliance with HH is calculated as the ratio of the number of performed actions to the number of opportunities. In addition to compliance with recommended indications, it is important to consider the appropriateness of the technique used for handwashing or hand rubbing. The WHO recommendations include the execution of specific movements to cover all hand surfaces while respecting recommended time duration of 20-30 s and 40-60 s for hand rubbing and washing, respectively [124]. Direct observation remains a cornerstone in the analysis of HH compliance however, authors recommended that at least five opportunities should be observed to determine individual optimal HH compliance and give feedback [125].

1.4.6 Hand-hygiene practice among healthcare workers and factors influencing adherence to recommended HH practices

"*Hand hygiene is repetitive and sometimes could result in the formation of habits, and habits consequently may be change or influence behavior "*[126]. Understanding HH behavior regarding hand hygiene practices is the first essential point that conduct to the interventions in healthcare [127]. According to the WHO, based on observational studies conducted in hospitals, HCWs performed hand hygiene on average from 5 to as many as 42 times per shift and 1.7-15.2 times per hour [127]. The frequency of HH episodes varies according to the setting where observations were be performed. For example the number of opportunities varies between hospital wards, so the number of opportunities is higher in intensive care unit than in pediatric ward [127].

Factors influencing hand hygiene practices have been summarized by the WHO hand hygiene in healthcare, which classified these factors into three groups [128]:

i)Observed risk factors for non-compliance: (being doctors rather than nurse, wearing gloves, working during weekend and in intensive care units, automated sinks)

ii) self-reported factors for non-compliance, (lack of infrastructure and resources, no role models from colleagues, forgetfulness) and

iii) perceived barriers to appropriate hand hygiene (lack of institutional safety climate) .

Pittet D. and Boyce J.M. [128] also classified factors associated with non-compliance with hand hygiene into three level.

i. Individual level; including lack of education/ experience, lack of knowledge of guidelines, being a refractory non-complier

ii. Group level; including lack of education/ lack of performance feedback on hand hygiene adherence, high workload, downsizing/ understaffing, lack of encouragement/ role model from key staff

iii. Institutional level; lack of existing guidelines (written), lack of available/ suitable hand-hygiene agents, lack of skin care promotion/ agent, lack of hand hygiene facilities, lack of culture/ tradition of compliance, lack of administration leadership/ sanction/ rewarding/ support.

In Benin, before MUSTPIC project, nothing was known about the HH compliance and attitudes of HCWs about hand hygiene.

1.4.7 The Theoretical Domain Framework

Behavioral theories have been applied for a better understanding of reasons that explain the non-optimal compliance amongst HCWs in hospitals, however few studies come from LMICs. The theoretical domain framework (TDF) is a theory of behavior change, which provides a useful conceptual basis for exploring and understanding the behavior change process and its effect on the HCW's behavior[129]. This TDF constitute the first step before implementation of WHO multimodal strategy. After the observational study it is important to discuss with HCWs to understand the reasons which explain the non-optimal compliance and help for improving compliance. Moreover, before intervention, factors associated with non-compliance in HH behavior should be elucidated. The TDF consists of fourteen domains which cover different aspects of behavior. These domains are summarized in the following table.

Table 7: Domains in theoretical domain frameworks , from Cane et al, 2012 [130]

Theoretical Domain	**Definition**
Knowledge	An awareness of the existence of something
Skills	An ability of proficiency acquired through practice
Social/ Professional Role and Identity	A coherent set of behaviors and displayed personal qualities of an individual in a social or work setting
Beliefs about capabilities	Acceptance of truth, reality or validity about an ability, talent, or facility that person can put to constructive use
Optimism	The confidence that things will happen for the best or that desired goals will be attained
Beliefs about consequences	Acceptance of truth, reality, or validity about outcomes of behavior in each situation
Reinforcement	Increasing of probability of response by arranging a dependent relationship, or contingency, between the response and a given stimulus
Intentions	A conscious decision to perform a behavior or a resolve to act in a certain way

Goals	Mental representations of outcomes or end states that an individual wants to achieve
Memory, attention, and decision processes	The ability to retain information, focus selectively on aspects of the environment and choose between two or more alternatives
Environmental context and resource	Any circumstance of a person's situation or environment that discourages or encourages the development of skills and abilities, independence, social competence, and adaptative behavior
Social influences	Those interpersonal processes that can cause individuals to change their thoughts, feelings, or behaviors
Emotion	A complex reaction pattern, involving experiential, behavioral and physiological elements, by which the individual attempts to deal with a personally significant matter or event
Behavioral regulation	Anything aimed at managing or changing objectively observed or measured actions

Most of this research has used interviews and focus group to provide details of factors related to behavior change. In recent year there has been a lot of interest in using the TDF for changing clinical practice, as it represents a

unifying theoretical framework to identify systematically the key aspects behavioral change including intrapersonal (individual), interpersonal, institutional and community fact [131–134].

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2 AIM AND OBJECTIVES

RESEARCH AIM

The main causes of antibiotic resistance are the non-adherence to hand hygiene rules and the irrational use of antibiotics. Beside confirming the causative agents isolated in surgical site infections in Benin, the general objective of this thesis is to contribute to improvement of hospital hygiene management and rational use of antibiotics in surgical services (obstetrical and gastrointestinal). The work described in this thesis focused on the rational use of antibiotics to decrease the rate of antimicrobial resistance. To that end, three major questions for research were raised:

1. **What is the Hand hygiene practices among healthcare workers in hospitals in the South of Benin?**

2. **Which bacteria are commonly isolated on surgical site infections in Benin per ward (gastrointestinal surgery or maternity)? Based on etiological bacteria, we described the molecular characterization of resistant bacteria and analyzed the phylogenomic link between isolates.**

3. **Which interventions for the prevention of SSI could be implemented based on the first results of previous chapter (hand hygiene observation and interviews, etiological bacteria involved in SSI) and on other results of the project?**

The thesis is divided into three parts: the first one describes hand hygiene practices, the second is a result of antibiogram and molecular analysis of bacteria involving in SSIs and the third part is a resume of first actions for infection control in hospitals.

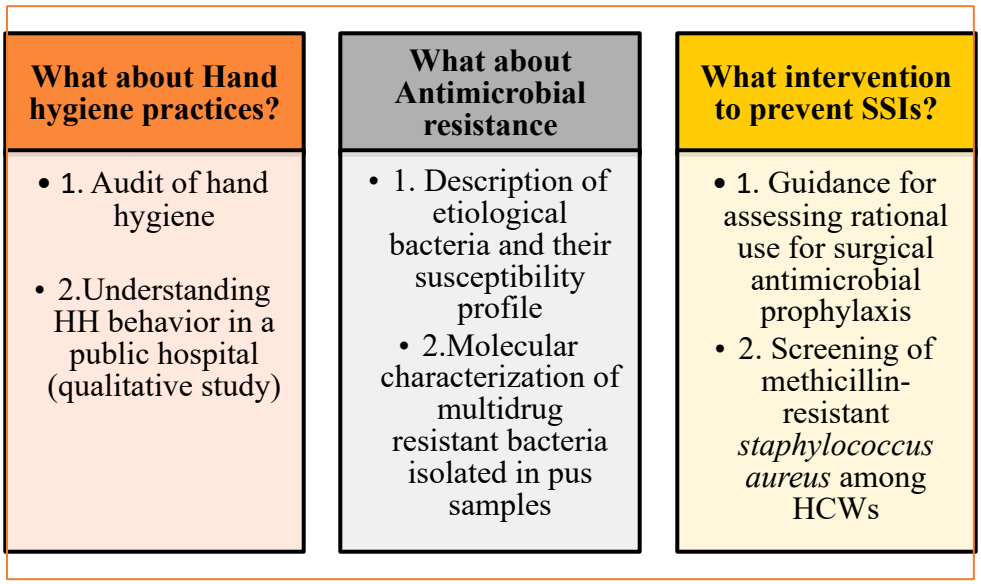


Figure 6: Study framework of the thesis: "Surgical site infections in Benin: what about hand hygiene practices and antimicrobial resistance?"

3 RESULTS

1.1 CHAPTER I : HAND HYGIENE PRACTICES AMONG HEALTHCARE WORKERS: THE FIRST STEP IN TACKLING ANTIMICROBIAL RESISTANCE

Chapter I at the glance

What is already known about this chapter?

- The compliance of HH among healthcare workers is relatively low in developing countries.
- HH compliance seems better with nurses than doctors in many studies
- In developing countries the healthcare settings are often deficient, and resources and infrastructures lack
- Although the direct observation of HCWs is the most reliable method for measuring the rate of adherence to HH, the "Hawthorne effect" must be reduced
- The qualitative study design is important to explore and understand the behavioral determinant of HH compliance among HCWs.

What this chapter adds?

- Through this study, the interviewed HCWs presented some perspectives for improving the adherence to HH recommendations:
    - Highlighting or design a model who will remind to colleagues to wash their hands
    - Promote the training of patients who could help HCWs to improve their compliance
- In our hospitals, the use of water remains common while the availability of ABHR is non optimal .
- The duration of HH techniques was not respected.

Hand hygiene in surgery in Benin: opportunities and challenges

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Antimicrobial Resistance and Infection Control

RESEARCH Open Access

Hand hygiene in surgery in Benin: opportunities and challenges

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Abstract

Background: Hand Hygiene (HH) has been described as the cornerstone and starting point in all infection control. Compliance to HH is a fundamental quality indicator. The aim of this study was to investigate the HH compliance among Health-care Workers (HCWs) in Benin surgical care units.

Methods: A multicenter prospective observational study was conducted for two months. The World Health Organization (WHO) Hand Hygiene Observation Tool was used in obstetric and gastrointestinal surgery through six public hospitals in Benin. HH compliance was calculated by dividing the number of times HH was performed by the total number of opportunities. HH technique and duration were also observed.

Results: A total of 1315 HH opportunities were identified during observation period. Overall, the compliance rate was 33.3% (438/1315), without significant difference between professional categories (nurses =34.2%; auxiliaries =32.7%; and physicians =32.4%; $p = 0.705$). However, compliance rates differed ($p < 0.001$) between obstetric (49.4%) and gastrointestinal surgery (24.3%). Generally, HCWs were more compliant after body fluid exposure (54.5%) and after touching patient (37.5%), but less before patient contact (25.9%) and after touching patient surroundings (29.1%). HCWs were more likely to use soap and water (72.1%) compared to the alcohol based hand rub solution (27.9%). For all of the WHO five moments, hand washing was the most preferred action. For instance, hand rub only was observed 3.9% after body fluid exposure and 16.3% before aseptic action compared to hand washing at 50.6 and 16.7% respectively. Duration of HH performance was not correctly adhered to 94% of alcohol hand rub cases (mean duration 9 ± 6 s instead of 20 to 30 s) and 99.5% of hand washing cases (10 ± 7 s instead of the recommended 40 to 60 s). Of the 432 HCWs observed, 77.3% followed HH prerequisites (i.e. no artificial fingernails, no jewellery). We also noted a lack of permanent hand hygiene infrastructures such as sink, soap, towels and clean water.

Conclusion: Compliance in surgery was found to be low in Benin hospitals. They missed two opportunities out of three to apply HH and when HH was applied, technique and duration were not appropriate. HH practices should be a priority to improve patient safety in Benin.

Keywords: Hand hygiene, Alcohol based hand rub, Hand washing, Surgery, Observation

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Background

Healthcare-Associated Infections (HAIs) are a major problem for patient safety. They are related to adverse events such as longer hospitalizations, increased antimicrobial resistance, morbidity and mortality, economic and psychological consequences [1]. In developed countries, HAIs affect 5–15% of hospitalized patients and can reach 9–37% of those admitted to intensive care units [2]. The situation is more dire in low-income countries where health care systems are often less developed [3, 4]. HAIs prevention must be a priority as decreasing their incidence [1, 3].

The hands of Healthcare Workers (HCWs) are the most common vehicle for the transmission of microorganisms from patient to patient within the healthcare environment. For instance, *Klebsiella Sp*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and Gram-negative bacilli are some of the organisms that are likely to be found on HCWs hands [2]. Hand hygiene (HH; i.e. washing hands with soap and water, or disinfection using alcohol based hand rub solution: ABHR) is essential to prevent and control HAIs as it reduces microbial colonization and direct transmission from patient to patient [5, 6]. However, compliance with HH among HCWs is frequently reported as poor and is usually estimated as less than 50% [7]. Average compliance with HH recommendations varies between hospital wards, among professional categories, working conditions as well as according to the definitions used in different studies [8–10].

To promote HH, World Health Organization (WHO) has published "My five moments for hand hygiene", describing the HH opportunities, which are the moments during healthcare activities when HH is necessary to interrupt germ transmission by hands: Moment 1- before touching a patient, Moment 2- before clean/ aseptic procedure, Moment 3- after body fluid exposure risk, Moment 4- after touching a patient and Moment 5- after touching patient surroundings [8, 11]. The compliance to HH is the proportion of times that HCWs perform HH of all five observed opportunities [12]. The WHO and the Centers for Disease Control and Prevention (CDC) recommend that HCWs wash their hands with soap and water, when visible soiling is present for 40 to 60 s. When hands are not visibly soiled, alcohol based hand rub (ABHR) for 20 to 30 s is recommended [13].

Despite the evidence of benefits of good HH practices and the relative cost-effectiveness and simplicity of this procedure, compliance remains a challenge, and more so in developing countries [2, 14]. Different factors have been described to influence HH compliance. For instance, the lack of appropriate infrastructure, the cultural backgrounds and even religious beliefs can play an important role in hindering good practices [15]. The professional category such as doctors, nurses and situations requiring wearing of gowns and gloves can also determine poor HH compliance [2].

Even tool in obstetric and gastrointestinal surgery exist in Benin, this study is to the best of our knowledge, the first to document the utilization of the WHO HH observation method [16]. Therefore, the aim of the study was to assess the rate of HH compliance among HCWs using the WHO My five moments for hand hygiene tool in obstetric and gastrointestinal surgery.

Methods

Design and setting

Direct observation of HCWs during patient care activity by trained and validated observers is recognized as the gold standard for hand hygiene monitoring [12]. We conducted this prospective observational study for 2 months (June–July 2018), at six hospitals in the south of Benin to allow enough time for the collection of 200 opportunities according to the sample size recommended by the WHO.

These hospitals belonged to intermediate (i.e. first reference in the department; Centre hospitalier Universitaire Départemental de l'Ouémé Plateau) and central (i.e. tertiary care; Centre Hospitalier Universitaire Hubert Koutoukou de Maga CNHU, Centre Hospitalier Universitaire de la Mère et de l'Enfant CHUMEL, Bethesda, Centre Hospitalier de zone Suru Lere and Centre Hospitalier Universitaire de Zone d'Abomey-Calavi) level of health care in Benin. Observations were conducted in two selected settings: obstetric (delivery room and ward) and gastrointestinal surgery (operating room and ward).

Participants

All HCWs (physicians, nurses-auxiliaries, and other HCWs) present in the room were eligible for observation if they provided care to patients. Observations were conducted unobtrusively during routine patient care, mostly during the day but also during the night. HCWs were unaware of being observed to minimize "Hawthorne effect". Additionally, observers did not provide details of the study procedures for HCWs.

Outcomes

We used the previously validated WHO "My five moments for hand hygiene" observation tool to collect HH opportunities and calculate the primary outcome: HH compliance. The term "opportunity" for HH is defined as a "moment during healthcare activities when HH is necessary to interrupt germ transmission by hands" [14].

Each application of alcohol hand rub or hand washing with soap and water was regarded as a complied HH opportunity. For instance, any HCW who decontaminated their hands immediately after a contact with a patient

and then directly attended another patient without touching any object (for example no touching of door handles or any other patients) was considered to have complied with HH practices in relation to the second patient [8, 14].

Secondary outcomes included determinants of compliance, the observation of HCWs adherence to basic HH parameters such as short clean nails and white tips < 3 mm long, use of nail polish (bare nails), absence of jewellery (rings, bracelets and watches) and the duration of HH.

Variables and data collection

To gather the required information, we developed a 3-part paper observation grid including: (A) adherence to basic HH parameters, (B) "My 5 moments for HH" tool and (C) techniques and duration of HH. Pre-test observation was done out of the study period for 2 days with 2 voluntary nurses in each hospital to calibrate this observation grid.

The observer team was composed of eleven medical students. The author and a specialist of hygiene and infection control, working in one of the six hospitals, previously trained the students. Each observer was allocated to one hospital and one setting (obstetric section or gastrointestinal surgery). Before the beginning of the data collection, observers underwent 1) a 3-day coaching on HH and the use of the two grids, and 2) a 1-week training in each hospital to familiarize themselves with the infrastructure, the procedures, local HH policy (use of clean water, soap and alcohol) and the personnel.

During the training, we used the WHO training film which describe possible situations during observations sessions. Each observer completed the observational form separately while observing the same HCW and the same care sequence. Results were then compared and discordant notifications were discussed. This process was repeated until concordance is reached in terms of the number of hand hygiene opportunities and hand hygiene actions that occurred. During the study period, observer team received periodic reinforcement of education (twice per month) focusing on technical issues to conduct observations according to Sax H. method [12].

Trained observers completed one observation grid per observation session. The observation session is a period of 20 ± 10 min in a care setting [14]. HCWs could be observed during several sessions. Each HCW was observed for a maximum of four HH opportunities during the observed care session according to WHO recommendations [1].

In addition, we used monthly ward infrastructure survey to collect data about infrastructure at ward level. This grid is divided into 28 indicators with questions about resources and facilities for HH practices such as: sink, water and alcohol, presence or absence of reminders in workplace. At the end of each month, a grid was completed by observers and senior nurses of each hospital. **Statistics.**

The data were initially recorded on paper and then entered directly into Epidata 3.1. Analysis was done using SPSS version 22 software. Analyses of all variables were conducted overall, and the results presented by frequencies and percentages. Compliance was calculated by dividing the numbers of times hand hygiene was performed by the total number of opportunities.

The chi square test was used to test associations between variables if the expected frequency was above 5. If the expected frequency below 5, Fisher's exact test was used. In all analyses, p value ≤0.05 was considered to be statistically significant.

Results

General characteristics

For 60 days, we observed 432 HCWs around six hospitals (57.9% women versus 42.1% males). Most of them were nurses (55.6%) and auxiliaries (25.7%), followed by physicians (13.4%) and others 5.3% (i.e. technicians, anesthetists and student physicians or nurses). Table 1 shows the characteristics of observed HCWs.

Hand hygiene compliance and its determinants

One thousand three hundred fifteen HH opportunities were observed. The overall HH compliance was 33.3% [30.8-CI-35.8], with no significant difference between professional categories. Indeed, compliance was 32.4% among physicians, 34.2% among nurses and 25.6% for other HCWs ($p = 0.705$). The compliance was higher among females than males. (40.5% vs. 26.4% $p < 0.001$).

In maternity, HH compliance reached 49.4% [44.9-CI-53.9] while surgery services scored 24.3% [21.4-CI-27.2] ($p < 0.001$). HH compliance before patient contact was 25.2, 33.0% before a clean or aseptic procedure, 37.5% after patient contact 29.1% after contact with patient surroundings and 54.5% after body fluid exposure. The compliance rate did not significantly differ according to the shift (39.4% in the afternoon, 32.4% in the morning, and 50.0% in the night $p = 0.113$) (Table 2).

Hand hygiene technique and duration

When HH was applied, technique and duration were often not appropriate. Hand washing (HW) remains the most preferred action. Among 438 HH actions, HW was performed in 316 cases (72.1%) while ABHR was performed in 122 cases (27.9%). 0% used both technics ABHR and soap and water. After body fluid exposure when hands were visibly soiled, hand washing was performed in 50.6% of cases while ABHR was for 3.9%.

Table 1 Characteristics of observed HCWs

Characteristics	Hosp A	Hosp B	Hosp C	Hosp D	Hosp E	Hosp F	Total
Professional category							
Physicians n (%)	26 (17.5)	1 (2.9)	6 (9.8)	15 (17.6)	4 (7.4)	6 (12.2)	58 (13.4)
Nurses and auxiliaries n (%)	117 (78.5)	33 (97.1)	42 (68.9)	67 (78.8)	50 (92.6)	42 (85.7)	351 (81.3)
Others n (%)	6 (4.0)	0 (0.0)	13 (21.3)	3 (3.6)	0 (0.0)	1 (2.0)	23 (5.3)
Total n (%)	149 (100.0)	34 (100.0)	61 (100.0)	85 (100.0)	54 (100.0)	49 (100.0)	432 (100.0)

Hosp F=Intermediate level, and the others = central level. A:CNHU-HKM, B:BETHESDA C: CHU-SURU-LERE, D: CHUZ ABOMEY-CALAVI, E: CHUMEL, F: CHUZ PORTO-NOVO.

Before aseptic action and for other moments hand washing was always the first action of compliant HCWs through six hospitals (Fig. 1).

Hand hygiene duration was almost never fully observed by HCWs.(< 6.0% for ABHR and 0.5% for hand washing). The median duration for hand washing was 10 ± 7 s (instead of 40 to 60 s) and 9 ± 6 s (instead of 20 to 30 s) for ABHR (Table 3).

Adherence to basic hygiene parameters

The adherence to basic hygiene parameters regarding nails and jewellery among HCWs was higher than the HH compliance.

In total, more than 77% ($n = 334$) had their hands free of jewellery and 91% ($n = 393$) of them had natural short and without polish fingernails. Only 1.8% ($n = 8$) of healthcare professionals had nails extensions.

Table 2 Hand hygiene compliance among HCWs in Benin

Characteristics	Hand Hygiene opportunities (n)	Hand Hygiene actions (n°)	% Compliance	*P* value
Overall Wards	1315	438	33.3	
Surgery visceral	841	204	24.3	$P < 0.001$
Maternity	474	234	49.4	
Professional category				
Physician	139	45	32.4	$P = .705$
Nurses	752	257	34.2	
Auxiliary nurse	385	126	32.7	
Others	39	10	25.6	
Sex				
Male	671	177	26.4	$P < 0.001$
Female	644	261	40.5	
Shift				
Morning	1161	376	32.4	$P = .113$
Afternoon	142	56	39.4	
Night	12	6	50.0	
WHO five moments				
Moment 1	393	99	25.2	$P < 0.001$
Moment 2	209	69	33.0	
Moment 3	77	42	54.5	
Moment 4	509	191	37.5	
Moment 5	127	37	29.1	
Care room				
Bandage area	571	267	46.8	$P < 0.001$
Hospitalization	691	139	20.1	
Others	53	32	60.4	

Five moments: 1-before touching patient, 2-Before clean/aseptic procedure, 3-after body fluid exposure risk, 4-after touching a patient, 5-after touching patient surroundings.

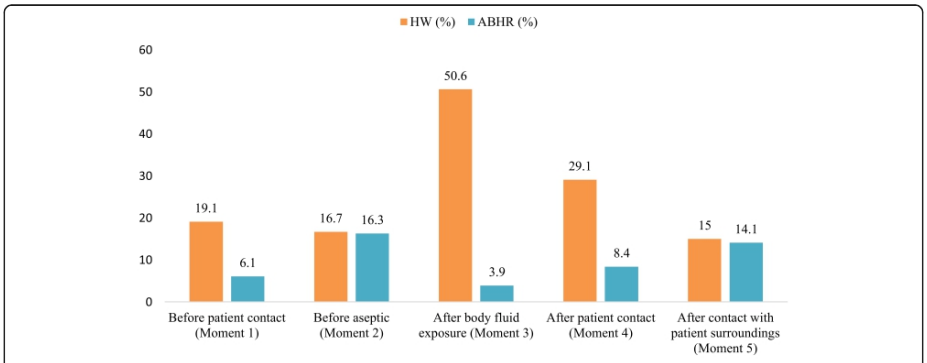


Fig. 1 Hand hygiene actions by indications among HCWs (Benin 2018)

Hand hygiene infrastructures

Clean water and alcohol based hand rub was only available half of time in all of six hospitals, sinks were often inconveniently located: out of the patient room or the point of care. None of the hospitals provided single-use towels. Only two hospitals displayed WHO posters explaining correct use of hand rub and handwashing technique. Observed hospitals did not organize training sessions about HH for HCWs. (Table 4) summarizes HH infrastructures for six hospitals.

Discussion

Hand hygiene is one of the foremost techniques to reduce HAIs [17]. However, overall HH compliance was 33.3% in this observational study. The behavior of HCWs showed a tendency towards self-protection, that is, with a higher compliance rate after body fluid exposure risk and after patient contact than before touching patient. The adherence to HH parameters regarding hair, nails and the use of jewellery was better than over all compliance. HH infrastructures were deficient or inconstantly present at the point of care. This multicenter observational study is the first in Benin which assessed HH compliance according to WHO recommendations. However, this also makes it difficult to compare our results with prior findings.

Table 3 Hand hygiene duration among compliant HCWs in June–July 2018

Hand Hygiene Duration	Frequency (n)	Percentage (%)
Wash duration in seconds (s)	Median washing (10 ± 7)	
Less than 40	199	99.5
More than 40	1	0.5
Alcohol rub duration (s)	Median ABHR (9 ± 6)	
Less than 20	78	94.0
More than 20	5	6.0

Overall HH compliance was lower (33.3%) than findings of authors from Nigeria (65.3%), Brazil (46.7%) and Saudi Arabia (50.3%) [18]. Hand hygiene compliance seems better in maternity care room. The possible reason might due to the possible regular availability of water and washing agents in maternity compared to the gastrointestinal surgery. Luangasanatip et al. found in a systematic review and network meta-analysis of 41 hand hygiene studies that baseline compliance with HH among HCWs was on average only 38.7% [19] . A 24 h observational study in Ethiopia showed lower (22.0%) compliance rates than in our study but they collected less opportunities than in our study [4]. In Benin, the WHO's multimodal hand hygiene improvement strategy is not implemented yet [20]. Many studies suggest that HH intervention such as the multimodal strategy of WHO has a great potential to improve HCW compliance [18, 21].

The compliance was low across all professional categories. However, nurses tend to be more compliant than auxiliary nurses and physicians ($P = 0.705$). This observation is concordant with the findings of other studies [22, 23]. In the literature, compliance with HH among nurses are better than doctors [24, 25]. In a multicenter study done in 5 countries (Costa Rica, Italy, Mali, Pakistan and Saudi Arabia), physicians had the lowest and the nurses had the highest compliance except for Mali [8]. The lower compliance among physicians could be explained by the absence of a team leader acting as a role model for other colleagues [26]. Medical students reported feeling strongly influenced by negative role models thereby abstaining from compliance with HH guidelines [27]. Erasmus et al. showed that physicians seem to be skeptical or less convinced than other

Table 4 Hand hygiene infrastructures in six hospitals, 2018

Characteristics	Hosp A	Hosp B	Hosp C	Hosp D	Hosp E	Hosp F
Is Running water available	A	I	I	I	I	I
Is water visibly clean	Yes	Yes	Yes	Yes	Yes	Yes
Kind of taps available	Hand- Operated	Hand-operated	Hand-operated	Hand-operated	Hand-operated	Hand-operated
Are disposable towels available at all sinks	No	No	No	No	No	No
Is soap available at all sinks	I	Yes	Yes	Yes	Yes	I
Is an ABHR available (%)	100	50	50	100	50	50
Are posters illustrating HH technique beside each sink	No	No	Yes	No	Yes	No
Is any other type of reminder on HH available on this ward?	No	No	No	No	No	No
Are audits on HH compliance periodically performed?	No	No	No	No	No	No

I Intermittently = Unavailability half of time. *ABHR* Alcohol based Hand Rub, *A* Always

professional categories about the effectiveness of HH to reduce HAI or to limit the spread of antimicrobial resistance and improve patient safety [27, 28]. Some studies even suggested turning nurses into ambassadors of hand hygiene programs [29, 30]. In most of the cases, nurses are aware of the rationale for hand hygiene procedures and represent a large working group that performs the greatest amount of direct patient care. If nurse behavior can change, the impact on the healthcare system will be significant [31]. Clearly, there is a need to develop strategies to improve physician's compliance [17]. In our setting for instance the best way will to implement in physicians 7 years cursus, hand hygiene courses. No study has been undertaken in Benin about the importance of including HH program in the curriculum. But it has been done elsewhere. For instance Chakravarthy et al. found that "the knowledge levels of medical students about HH is far below expectations; the administrators should take upon themselves to include this topic in the educational curriculum" [32].

The compliance was low before patient contact (25.2%) and after contact with patient surroundings (29.1%). Dancer et al. argue that environmental cleaning needs to be improved generally and specifically at hand touch sites [33]. Hand touch sites with the highest risk to patients are those which are next to the patient, for example, bedrails, lockers, over beds tables and door handles [33, 34]. Carling et al. found that more than half of the inanimate objects such as those previously listed were not microbiologically clean when screened [35]. By contrast, HCWs were more compliant after body fluid exposure and after patient contact ($p < 0.001$). This finding is in line with an observational study in Kuwaiti hospitals that suggested that HCWs perform HH for their own protection, rather than to protect their patients [36]. Self-protection tendency has been identified in multiple studies [36, 37].

Despite recommendation of the Centers for Disease Control (CDC) and WHO guidelines on HH in healthcare to use ABHR solution as the preferred means for routine hand hygiene [21, 38], its use remains poor. Among 438 HH actions, only 122 (27.9%) used alcohol based hand rub solution while 316 (72.1%) performed hand washing. This finding could be explained by lack of knowledge concerning its benefits, and also lack of infrastructure in our hospitals. Moreover, the few HCWs who washed their hands, did so inefficiently. The median time for hand rubbing was 9 s and only 6% respected the correct time. There was no gain in reducing bacterial counts from hand rubbing longer than 30 but a 30 s application is usually sufficient [39]. By contrast, the median time for hand washing was 10 s and 0.5% respected the correct duration. Reported reasons for not respecting HH duration and technique include too many opportunities per hour, or simply ignorance of guidelines or forgetfulness [7, 29, 40].

Unfortunately 22.7% of the HCWs still use long natural, polished or artificial fingernails when caring patients. This is strongly discouraged by the WHO because the majority of flora on the hands are found under and around the fingernails [13]. Artificial and long polished nail harbor harmful bacteria and studies described difficulty in their elimination with cleaning. Alcohol-based hand rub solution cleared pathogens from both artificial and natural nails better than antimicrobial soap [41, 42]. However, jewellery must be kept to a minimum. Jeweled rings or rings with stones should not be worn, a plain wedding ring is permitted [43].

There are serious barriers to compliance in the observed hospitals. For instance, the compliance rate was particularly low (20% vs 46,8%; $p < 0.01$) in hospitalization rooms compared to the bandage room where we can intermittently find sinks and other infrastructures. By contrast, in all of 6 hospitals, supplies were not placed at key location throughout patient room. Absence or irregularity of HH supplies

can sometimes discourage personal to wash their hands when indicated [23]. Limited access to hand hygiene supplies such as soap or alcohol hand rub solutions are challenges compliance [23]. Cantrell et al. found a positive correlation between compliance rates and the presence of a sink in the patient's room [44]. In addition, there were no poster and other visual reminders for HH at work in some hospitals which may help increase compliance. The present audit is the first one in all of these hospitals, making it very important for future hospital planning, management and patient safety. Successful promotion of hand hygiene strategies (e.g., making hand hygiene products available at the point of care, allocate special budget for HH, improvement of HH education among HCWs) should be considered in all of these hospitals.

Strengths and limitations

The main strength of this study is the settings: it is the first study to document the utilization of the WHO HH observational method. Moreover, it included two wards in six different hospitals. Despite the limited numbers of observations during the night shift and weekends and the fact that data collection did not record the use of gloves, this study provides HCWs with reliable information about HH practice in Benin. The present study was conducted using direct observation method, the most reliable method for measuring the rate of adherence to HH [45]. Observers were trained and used validated data collection forms. A potential bias associated with observation is called the "Hawthorne effect". HCWs have opportunities to change their behavior when they know that they are being observed [46]. So, they were not aware they were being observed to diminish this effect.

This study was the first step of MUltidisciplinary STrategy for Prevention and Infection Control (MUST-PIC) project. This project aims to increase HCWs compliance by implementation of WHO Multimodal Strategy which includes 5 keys components: system changes, HCWs training and education, evaluation and feedback, reminders in the workplace, and promotion of an institutional safety climate. Next steps include a qualitative assessment of factors underlying HH compliance with interviews among HCWs.

Conclusion

This study showed a poor HH compliance among different HCWs in surgery in Benin. As HH is an influential and cost-effective way of reducing HAIs, it should become an educational priority in Benin health care settings with the promotion of the WHO multimodal hand hygiene strategy: "Hand care is safer care". Access to HH resources should be emphasized as an integral part of HH improvement strategy.

Abbreviations

HCWs: Health care workers; WHO: World health organization; HAIs: Healthcare associated infections; HH: Hand hygiene; HHO: Hand hygiene opportunities; ABHR: Alcohol based hand rub; CI: Confidence interval; HW: Hand washing

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Authors' contributions

CLY: Study design, facilitation of data collection, data analysis and drafting the manuscript, OD and AS: Conception of the idea, Study design, revising the manuscript, AMD and AF: supervision of observation team, revising the manuscript, DC: Hand Hygiene observation grid and manuscript revising, EM: statistical analysis and revising the manuscript All authors read and approved the last version.

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Availability of data and materials

The data supporting the results of this study are included within the article and its additional files.

Ethics approval and consent to participate

The study protocol was approved by the ethical committee of Medical Science of Medicine Faculty (FSS). Acceptance letter and convention letter was obtained from each hospital director. Information on the study was explained to the participants, including procedures, potential risks and benefits of the study. Confidentiality was maintained at all levels of the study by avoiding use of name and other identifiers.

Consent for publication

Not-applicable.

Competing interests

The authors declare there are no conflict of interests.

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Understanding Hand Hygiene Behavior in a Public Hospital in Benin Using the Theoretical Domain Frameworks: The First Step for Designing Appropriate Interventions

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Abstract: Background: Hand Hygiene (HH) is widely recognized to be one of the most successful and cost-effective measures for reducing the incidence of healthcare-associated infections (HAIs). The hand hygiene behavior of hospital healthcare workers (HCWs) is not well-documented in Benin. Therefore, Theoretical Domains Framework (TDF) was used to identify the behavioral determinants that may impact HCWs' hand-hygiene compliance in a public hospital. Methods: A qualitative design comprising face-to-face semi-structured interviews with nine HCWs. The interviews included questions on transmission of infections, hand-hygiene practices, problems with their implementation; and ways to improve hand hygiene compliance. Two pharmacists independently coded interviews into behavioral domains using the TDF and then subdivided them into several themes. Interview transcripts were analyzed following 3-steps approach: coding, generation of specific beliefs, and identification of relevant domains. Results: Almost all interviewees have cited the environmental context and resources (such as lack of water) as a barrier to HH practice. They also believed that role models had a significant impact on the good practices of others HCWs. Fortunately ,they were confident of their capabilities to perform appropriate HH behaviors. The majority (7/9) reported having the necessary knowledge and skills and believed they could carry out appropriate HH behavior. In all cases, the participants were motivated to carry out HH behavior, and it was recognized that HH remains the cornerstone to reduce health care associated infections. Conclusion: This study identified several behavioral constructs aligned with the TDF that can be targeted and help for the development of new hand-hygiene interventions. These may increase the likelihood of a successful intervention, thereby improving HH compliance and patient safety, especially in hospitals.

Keywords: hand hygiene compliance; healthcare workers; implementation science; theoretical domains framework; qualitative interviews; Benin

1. Background

Evidence shows that effective and consistent hand-hygiene practices have proven their effectiveness as simple but important measures for prevention of the healthcare-associated infections (HAIs) [1]. Sustained improvements in hand-hygiene practices were therefore a challenge for the World Health Organization's (WHO), whose intention was to help in decreasing of the international burden of HAIs [2]. However, the compliance of healthcare workers (HCWs) with these practices is poor [3], as recently shown in Benin [4].

HCWs' compliance with hand hygiene guidelines has generally been determined using self-reporting, observational, or interventional approaches. The common barriers described with these methods were: the sex (being female), being a physician rather than a nurse, working in an intensive care unit, working during weekdays rather than the weekend, lack of time and organizational support [5]. Nevertheless, direct observation studies may be biased by observer effects (also known as Hawthorne effects) [6]. Similarly, data collection using self-recall method may suffer from memory-recall bias [7]. For these reasons, data collected using qualitative methods may be valuable interviews can provide rich data that better explore the issues under examination, such as HCWs' compliance with HH guidelines [6]. Qualitative studies are recognized as making deeper contributions to evidence-based practice and health services-research [8]. Moreover, interviews can help researchers to access the thoughts and feelings of research participants.

Various factors have been associated with poor HH practices in healthcare settings, including a lack of infrastructures (number and location) and limited behavioral change interventions [9]. Indeed, to successfully design a hand-hygiene promotion intervention, we first need to understand the determinants of current behaviors informed by a theory of behavioral change. The reasons underlying HCWs' low levels of hand-hygiene compliance in resource-limited settings are rarely investigated, and little is known about current beliefs either on the advantages of infection control strategies, or on behavioral enablers for infection control improvement.

Different theories of behavior change exist, but with similar and overlapping constructs [10]. The diversity and number of these theories have been identified as possible reasons why theoretical approaches are not used in the design of interventions. The (TDF) was developed to provide a structure to support the application of theoretical approaches to interventions with the goal of contributing to the change of behavior. The TDF is a well-validated, predefined, consensus-based, theoretical framework for supporting the implementation of healthcare guidelines and consist of 14 domains as follows: knowledge, skills, social professional role and identity, beliefs about capabilities, optimism, beliefs about consequences, reinforcement, intention, goals, memory attention and decision processes, environmental context and resources, social influences, emotion, and behavioral regulation. [11,12]. By providing a common language for characterizing contexts [13], targeted problems and interventions in generalizable terms, it also guides the adaptation of implementation strategies [14].

Therefore, the purpose of the study was firstly to use the TDF as an analytical framework to gain a global understanding of HH behavior in public hospital in Benin through semi-structured interviews with healthcare workers. Secondly, according to the analysis of these interviews, suggestions for the types of interventions that may be effective in improving the HH compliance of HCWs will be briefly described. In this way, we propose to diagnose the barriers and enablers to HH compliance in hospital, and thereby prescribe appropriate interventions to address their local challenges in a targeted way.

2. Methods

2.1. Study Design

This descriptive qualitative study used semi-structured interviews to explore HH compliance based on participants' first-hand accounts of their experiences. This study was

part of the MUSTPIC (Multidisciplinary Strategy for Prevention and Infection Control) study that assessed hand hygiene practices and promote rational use of antibiotics in surgery services in Benin. They are different activities among which we observed hand hygiene practices among healthcare workers, analyze the quality of antibiotics used for prophylaxis and described the epidemiology of multidrug resistant bacteria involved in surgical site infections[15]. We report here qualitative results based semi-structured interviews.

2.2. Setting

The study was conducted between February and March 2019. Participants were HCWs at a 36-bed confessional hospital directed by clergymen in Cotonou. This hospital has the cheapest consultation fees of public and private hospitals in Benin. Based on our purposive sampling method, we sought to include caregivers working at critical point of care, such as surgery. To ensure a maximum level of participation, the interviews took place on the surgical ward at places and times convenient to the interviewees.

2.3. Participants

Eligible participants were physicians, nurses, surgeons, and cleaning staff in the surgery ward. In surgery department we have count 23 healthcare workers for all specialties as follows: 5 cleaning staff members, 10 nurses, 4 surgeons and 4 physicians. All were aged 18 or older, were in regular physical contact with patients, and would not be on vacation or an extended period of leave when the interviews were conducted. This study is integrated on MUSTPIC project which aimed to describe hand hygiene compliance and understand the perceptions of HCWs about hand hygiene as well as the perspectives for improvement.

2.4. Data Collection

The interviews were carried out by three facilitators (2 medical psychologists) who did not know the participants they interviewed, while a second one took notes and made audio recordings. Interviews were conducted at a place and time chosen by the participants. These interviews were download by Olympus DSS player software to laptop to be analyzed. Then, interviews were transcribed verbatim. The question grid included open-ended questions, which allowed new ideas and questions to arise out of the participants' responses. Each session began with a small introduction in which a participant met the researchers, was given a brief description of the study's goals, and an assurance of confidentiality. Before beginning each session, each participant provided oral informed consent. No monetary incentive was offered.

2.5. Data Analysis

To ensure rigor and trustworthiness of the data, content analysis of the interview transcript was performed by 2 researchers (CY, and AA) who did not take part in recruitment or not conducting the interviews. No software was used to support analysis. Analysis was performed through annotated copies of the interviews. After coding the interviews independently, the two coders gathered, compared their codes, and resolves disagreements through discussion to achieve consensus. Following completion of analysis, exemplar quotes for each domains were chosen by consensus between the two researchers.

The data was analyzed on the basis of the TDF, which we had chosen because it integrates behavior-change theories and uses tools that support the implementation of behavior-change interventions [16]. The TDF framework was initially developed for implementation research to identify influences on health professional behavior related to implementation of evidence-based recommendations. A synthesis of 33 theories of behavior and behavior changes clustered into 14 (originally 12) domains [17] , the TDF is a

theoretical framework rather than a theory and it provides a theoretical lens through which to view the cognitive, affective, social, and environmental influences on behavior [18]. The 14 domains provide a complete coverage of the potential multi-level determinants of health-related behavior and guide the use of broad prompts that enable interviewees to consider a wide range of possibilities without asking leading questions[19]. As a reminder: domains are simplified groupings of constructs from multiple theories, and constructs are the components of a theory used to explain behavior [16].

The recordings were verified by the interviewer prior to analysis, which was facilitated using Microsoft Word and consisted of the two following steps:

Coding. To facilitate analysis and ensure consistency in coding, the transcripts were coded into TDF domains by two team members trained in qualitative analysis using thematic content analysis. Beforehand, however, a coding scheme was determined by consensus by two team members who had read the first 2 transcripts. This scheme comprised codes provided definitions and examples of the codes and provided examples of quotes that were covered by the codes. The code resulting from this process was then used to analyze remaining transcripts.

Generation of specific beliefs**.** A specific belief is a collection of participant responses regarding theme that suggests a problem and/or influence on the target behavior. For each utterance (i.e., for each coded interview quote), specific beliefs were generated in TDF domains by one team member and double-checked for accuracy by a second team member. Beliefs statements were initially written to be specific to each code; later, similar belief statements were merged to form the themes.

3. Results

3.1. Characteristics of Interviewees

A total of nine participants (five male and four female) were recruited for the interviews; their characteristics are summarized in Table 1.

The time they had worked on the surgical ward ranged from 4 to 11 years. The interviews duration ranged from 45 to 60 min. The analysis identified 11 theoretical domains. (Table 2).

Table 1. Characteristics of interviewees.

Interviewees	Number (n)
Gender	
Male	05
Female	04
Staff profession	
Surgeons	02
Physicians	02
Nurses	03
Cleaning staff	02

We identified eight behavioral themes that influenced participants' HH compliance. These themes [20] were mapped against the theoretical domains (Table 2). Further analysis allowed them to be classified either as enablers of correct HH or as barriers against it. Four enablers and four barriers were identified: As enablers the concerned domains were (a) their beliefs about their socio-professional role as HCWs, (b) their knowledge of hand hygiene, (c) their social influences, and (d) beliefs about the negative consequences of poor HH.

The identified barriers were : (a) Memory attention and decision process (i.e., participants' understanding of the importance of being a role model to their colleagues), (b) behavioral regulation, (c) environmental resources (such as a shortage of supplies and the unavailability of clean water) and (d) participants' beliefs about negative consequences

[16] to themselves (such as skin irritation). Almost half of the responses on the environmental resources theme were related to the unavailability of clean water. The remaining half were attributed to the bad quality of the only water that was available. As a barrier to HH, damage to skin [16]was the predominant concern of half of the participants; it had been coded to the theme of beliefs about negative consequences to self.

3.2. Enablers Factors

Social Professional Role and Identity

An important enabler of optimal HH compliance was the professional responsibility [20] some physicians felt towards protecting they needed to treat patients from infections which would be the exact opposite of the initial intention. For example, one physician said, "HCWs care about HH in hospital, healthcare is a primordial gesture" (interview 2).

Other's participants said that protecting themselves and their family had a greater priority than protecting their patients. Protection for themselves and their family was reported to be strong motivators of HH compliance: *"The aim of hand-washing is to protect the patients, but mostly to protect myself and my family as well"* (interview 4, surgeon).

Some participants believed that, as much as needing to refresh HH practice and usefulness and educational strategies, the role of a model was important to reminding colleagues about the WHO's five moments as followed: a) before touching a patient, b) before clean/aseptic procedure, c) after body fluid exposure, d) after touching a patient and e) after touching patient surroundings [1]. Some interviewees appear to copy the HH behavior of the physicians they see at work, often resulting in poor HH habits that will, in turn , be copied by future healthcare workers (HCWs). Interviewees insisted on patients' sensibilization as they can also prevent the transmission of HAIs *"It was necessary to raise awareness and put posters at the water points. At the very least, these attract visitors' attention, and make them look"* (interview 3, nurse). Posters are one of the solutions our participants proposed.

3.3. Knowledge

Knowledge of hand hygiene procedures was an important enabler of optimal HH compliance among interviewees[16]. However, despite the knowledge of procedures and the evidence linking HH to healthcare-associated infections, HH technics were not always performed correctly. They were aware of the advantages of good HH and recognized that they were sometimes superficial during their daily tasks. As a surgeon noted, *"when I wash my hands, I create a kind of wall between myself and the patient. So bacteria are on one side of the wall, and the patient is on the other side"* (Interview 4, surgeon).

"You cannot touch a suppurated wound and only rub after your hands afterwards. Normally you must wash your hands beforehand" (interview 4, surgeon)

Although most participants have sufficient knowledge about the practice of HH, some professional groups needed to improve their practices of the proper technique, and to use it at the right time. As one participant noted, *"I wash my hands every time, but I'm not sure I always do it correctly?"* (Interview 3, nurse).

Some participants highlighted the important problem of patient empowerment in the hospital's HH practices, and that patients and visitors had also contributed to improvements in HH: *"While staff working in the hospital are aware of HH guidelines, those who come occasionally have no idea about them, which is ignorance"* (interview 2, physician).

One important point is knowledge of the benefits of handwashing. Medical personnel are aware of its advantages, and often say that it is this knowledge that causes them to be compliant. *"It's because I'm a health worker that I know all the benefits of handwashing"* (interview 1, nurse).

3.4. Social Influences

Participants indicated that their colleagues were supportive about HH practices, and that they could count on each other to improve HH, although one nurse confided to us that when she respected properly hand hygiene guidelines all the time, she was treated like a "figure of fun" by other nurses. On the other hand, HH practices sometimes became an obsession for HCWs. Since – as stated above – they were conscious of the consequences of not respecting it, they could not prevent themselves from practicing it. *"I already know that germs are always present in the hospital, and that's how it is in my head"* (interview 1, nurse).

3.5. Beliefs about the Negative Consequences of Poor Hand Hygiene

All the participants expressed a belief that lack of HH contributes to increasing of infection and poor patient outcomes: "*The lack of HH could harm somebody and provoke supplementary costs for patients […] when infections appear, we use higher doses of antibiotics.* (Interview 3, nurse).

HCWs also recognized the impact of their own forgetfulness and negligence during their daily tasks. "*When it is respected correctly, hand hygiene reduces most germs, and avoids healthcare-associated infections"* (interview 3, nurse).

They were also aware that forgetfulness and negligence could be a source of illnesses they themselves could contract – another reason to pay attention to it: *"[HH] is a good thing: it prevents disease"* (interview 5, cleaning staff).

3.6. Barriers to Proper Hand Hygiene

3.6.1. Memory Attention and Decision Process

Most participants (6/9) expressed a strong belief that the basic approach of education, reminders and training had had a considerable effect. But the content, focus, the frequency, and modes of delivery needed to be modulated and refreshed. One participant talked about the important role played by the model of prevention and control of infection in the hospital. He had to remind other colleagues of the WHO five moments and the importance of HH: "*Sometimes, I would like to talk about ignorance, it is ignorance for the visitors, we should put on all waters source reminders and HH posters"* (interview 3, nurse).

Although, the other members of team could be counted on give reminders and improve compliance, they were sometimes simply inattentive and forget to wash their hands. "*To be honest, if I want to go to the patients, I may sometimes forget to wash my hands, but when I come back, I remember to wash them "* (interview 3, nurse).

In hospitals, time is an important consideration. Staff are sometimes overwhelmed and do not take the time to sanitize their hands properly when they should. "*If I didn't have time to go out to sanitize them during the operation, I will do it before I finish with the patient at the end of the operation"* (interview 1, nurse).

3.6.2. Behavioral Regulation

All interviewees believed that periodic or continuous training by hospital authorities should be promoted at specific times and should be contain posters and specific hand hygiene techniques. A member of the cleaning staff believed that "*one of the most important things we've done was the training and awareness-raising"* (interview 5, cleaner). Nonetheless, some of (3/9) participants stated that they had never been trained in HH.

According to our participants, hand hygiene was above all a question of nail hygiene, and that, to meet hospital standards, cutting and/or properly cleaning your nails was therefore an essential part of complete hand hygiene. Usually, most of them agreed with the following statement*: the nails hide germs inside for those that let the nails grow and do not maintain them" (interview 1, nurse).* Nurses, particularly the nurse in interview 1, were more likely to describe HH as habitual behavior and recognized that they had received at least one training program on it. This point is illustrated by the following quote: *"Although we*

receive hand-hygiene training all of the time, we also need to know the practical aspects, not just the theoretical ones." (Interviews 2).

3.6.3. Environmental Context and Resources

When participants were asked about what most influenced their HH practice, they referred to two things: organizational culture and the availability of resources. System constraints were consistently identified as important barriers to HH compliance. If HH resources were easily available within the clinical environment, they triggered hand hygiene. *"If the sinks were better located, I would perform HH better"* (interview 8, surgeon).

The lack of alcohol-based hand rub (ABHR) and sinks in consultation rooms, the chaotic context was all reported to be barriers to good hand-hygiene compliance. The availability of soap and ABHR only one time out of two was a barrier to the convenient use of soap or alcohol for hand hygiene. "*The ABHR is not compulsory – I mean, it's not always available"* (interview 4, surgeon).

Some interviewees described more undesirable quality of water on the hospital and said they were afraid to use it to wash their hands. *"We're in Benin here, in Cotonou. Haven't you ever seen dirty tap water?"*(interview 3, nurse). Due to that fear, they brought their own water: " *I don't entrust my health to the hospital; if I'm not well, I can't..., I can buy water outside, and keep that to wash my hands with"* (interview 1, nurse).

The hospital also lacked taps whose locations were inconvenient: "I think there are taps, but there aren't many of them, and they're not located properly (interview 1, nurse). "Sometimes the water or the tap doesn't work in surgical area, and surgeons are forced to wait, or find water in other services" (interview 5, cleaning staff). As well as the lack of taps or their poor localization, there was another obstacle to compliance: water cuts. "Sometimes the water is turned off. At your home, do you turn off the water?" (Interview 8, surgeon).

Some participants – particularly cleaning staff – highlighted the fact that they were forced to make water provision in reservoir (see interviews 5 and 8).

3.7. Beliefs about Negatives Consequences of Practicing HH

Some interviewees have challenged us about how dry they hands became because of HH practice in work. They believed that the consequences on the quality of their skins constituted a sort of barrier to their hand hygiene on a daily basis. As you can see. The "belief about negatives consequences" can be benefit on one hand and a barrier on the other hand.

Table 2. Resume of TDF and illustrative excerpts.

Theoretical Domains Frameworks	Themes Aligned	Illustrative Excerpts
Knowledge	• Awareness of HH guidelines and advantages • Knowledge of HH policy and procedures • Awareness of evidence linking HH to healthcare-associated infections • Good understanding of advantages of HH	"*When I wash my hands, I create a kind of wall between 2 patients. So bacteria are on one side and patient on the other side of wall*" (interview 4, surgeon).
Skills	• Completing HH training by technical aspect • Frequent repetition of HH training by themselves	"*We need a training more and more, moreover and these trainings sessions need to contain more than theoretical information – the HH technic is also important*" (Interview 3, nurse).

	• Infection control education and communication • Regarding HH as a skill	
Social/ professional role and identity	• HH should be completed correctly by all caregivers • HH should be performed not only by caregivers but also patients and visitors • Importance of patients' role in avoiding the transmission of infection	*"Importance of hygiene of the nurses who are also in first contact with the patients, and the hygiene of the nursing staff, doctors and all those who pass by"* (Interview 2, physician)
Beliefs about capabilities	• Confidence that HCWs will follow HH guidelines if HH infrastructures are improved • Being confident and positive about improving HH compliance	*"This hospital can be better if they give us resources and infrastructures"* (Interview 2, physician). *"It would be a lie if I said that this hospital was totally clean. I can't declare that the level of cleanness is high. I give it five out of ten – the hospital is not totally clean"* (Interview 7, cleaner)
Beliefs about consequences	• Performing HH reduces the rates of healthcare associated infections • Performing HH damages my hands, particularly ABHR, which makes them dry	*"Sometimes I wash my hands, but without efficacity because we have doubt about the quality of water. Efficacity of HH depends on technique too"* (Interview 1, nurse).
Motivations and goals	• HH is always a necessity • The importance of HH in self-protection • HH knowledge and training are a necessity	*"Proper hand hygiene is effective at protecting my family, my colleagues and patient lives, so it firstly to protect my own self"* (Interview 9)
Memory attention and decision process	• HH posters are useful for my HH daily practice • Practicing HH is a habit	*"We would do better with a model, someone who reminds us of the HH practices and guidelines"* (Interview 8, surgeon).
Environmental context and resources	• Easy access to hand-hygiene products makes it easier to practice HH • The quality of water is very important for HH to be effective • Sometimes HH is not effective and suffers from the lack of materials	*"The difficulty is the great lack of infrastructure in our hospital. And sometimes the quality of water is also doubtful"* (Interview 1, nurse) *"It's difficult to measure the quality of hand washing… they just wash their hands, and think it's clean enough"* (Interview 3, nurse) *"if the sink would be better located, I will perform better hand-hygiene action"* (Interview 2, physician)
Social influence	• The model can influence HH practice • Leadership and role models on the ward can help us to improve HH compliance.	*"We can count on the team to improve HH compliance – they will remind their colleagues to wash their hands"* (Interview 4,surgeon)
Emotion	• The feeling of dryness and irritability of skin caused by recurrent washing	

	• A kind of security procured by hand washing	*"I prefer washing with soap to the alcohol use because of dryness that it occurs"* (Interview 4, surgeon)
Behavioral regulation	• Education of patients and continue frequently the training of HCWs • Information and sensibilization • Ability to prioritize and organize goals • Hand-hygiene audits	*"Education, information and consciousness-raising concerns to all of us :patients, HCWs and visitors"* (Interview 8, surgeon).

4. Discussion

This qualitative study explored determinants of successful HH practice in healthcare professionals in Benin. Our participants identified many elements that commonly affect the compliance of their colleagues around the world. Our study is the first to use the Theoretical Domain Framework to systematically understand enablers and barriers of HH in Benin. To identify the specific determinants of performance of HH, and improve it, behavioral research into HH is needed to design tailored interventions. Indeed, these factors – which range from personal beliefs to organizational and social contexts – are potential targets for behavior-change interventions that guide improvements of HH compliance. Using TDF, we identified 11 domains representing potential barriers to, or enablers of HCWs' hand-hygiene practice. At the heart of our findings were six domains that were the most likely impact adherence to HH guidelines: (1) environmental context and resources, (2) social/professional role and identity, (3) knowledge, (4) beliefs about consequences, (5) behavioral regulation and (6) memory attention and decision process.

We found that, as key enablers of optimal HH practice, participant's knowledge and skills were interconnected. Some participants reporting having received some instructions on HH during their professional or induction training. As knowledge of HH is often reported to be one of the most important determinants of actual HH behavior [21,22], training is an important factor in behavioral improvement. All the interviewees in our study did indeed report that their beliefs in the "knowledge" domain had a big effect on their HH behavior. They were aware of the importance of HH in reducing healthcare-associated infections and recognized the benefits of good HH adherence. However, even though training and education have been claimed to play an important role in improving HH compliance and are supported elsewhere as a pivotal influencer [23,24], some interviewees noted the theoretical aspects of various HH courses. They suggested that, until high compliance was reached, hospital administrators should continue providing HH training based on observation and immediate feedback. There was also a need to change this training model based on a structured behavior modification program.

While most of the HCWs defined hands as the major vehicle for the transmission of infections, the two cleaners we interviewed were still uncertain about when to perform HH. Their assessment of the need to perform HH was influenced by the concepts of "cleanliness" and "dirtiness," which they perceived or sensed emotionally, feelings of "dirtiness" being evoked particularly by intimate contact with patients and bodily fluids [25]. In interviews, they also made a distinction between something that was clean and something that was sterile. Even if appearance is already a good indicator of cleanliness, it cannot always show whether something is dirty. The infrequent cleaning and poor ward hygiene was highlighted in many other studies in low middle-income countries where HCWs emphasized the influence of resource constraints and needed inputs[26,27]. They also pointed something they thought important about HH in the hand itself: the nails. For them, nails were a good vector of infections. For HH to be performed properly, they proposed that the nails should be cut or cleaned with special attention.

Another most cited barriers to good HH adherence were environmental context and resources (mainly lack of time and accessibility of products). When the workload was

high, HCWs' compliance was affected by the ease or difficulty of access to HH products. In our institution, alcohol-based products are located one case out of three[4].. Some authors showed that the conviction that HH required relatively little effort was consistently associated with good adherence [28]. Access but also the proximity of resources was discussed, by sax[28]. Thus, for example, some interviewees (5/9) needed the waters points to be located on each floor of the hospital.

Role models remains a significant key for good practice of the HCWs interviewed. The potential for senior doctors or infection prevention and control (IPC) members as role models has been frequently identified in the literature [29,30]. Not only do behavioral models consider role models to be a significant part of the decision-making process [31], but compliance has also been found to be greater in those who perceive themselves to be role models [32]. In contrast, Lankford et al. demonstrated that HCWs were negatively influenced about HH if they were in room with a peer or higher-ranking person who did not perform it [33]. Even if these findings suggest that HH behaviors can be affected by peer or role-model HH compliance, group compliance with HH procedures may be negatively influenced by learned behaviors or time constraints [33].

Participant's understanding and abilities about improving HH compliance were consistent with their professional responsibilities. Another enabler of optimal HH compliance was the professional responsibility; some physicians were concerned about the protection of patients from infections. The influence of the professional group of a HCWs is also important. While all physicians in our study reported to correctly perform HH, recent studies indicates that physicians are often excluded from studies of HH compliance, and showed underperformance compared to their colleagues in nursing and allied health professions [29]. Moreover, in our setting, hand hygiene topic is not included in training curriculum. Protecting themselves and not taking infections home to their family were reported to be strong enabler to effective HH. As HCWs consider themselves as an important intermediary between patients and especially their illnesses, they see themselves as a good vector of transmission from patient A to patient B. To evaluate explanations for non-compliance to HH best practices in real-time, Fuller and colleagues used a codebook based on the TDF [34] and reported that 44% of explanations for non-compliance with HH could be mapped to attention memory, and decision-making. In our study, this domain represents the third most common barrier.

As part of an overall strategy for facilitating patients' active patients 'active involvement in their healthcare management, it has also been suggested that patients and visitors be empowered to remind staff to wash their hands [35,36], even though patient empowerment has more commonly been used in relation to chronic disease management than in acute care settings [37]. In Australia, authors have actively promoted empowerment patients' and visitors through HH campaigns such as "it's OK to ask," or by reminding hospital staff to wash their hands [38]. However, while some clinical managers were supportive of trialing patient empowerment to remind staff to wash their hands, they also identified several potential barriers to implementing this strategy.

All in all, HCWs' compliance with HH continues to be a major challenge. Ongoing education and information are necessary for HCWs, patients and visitors alike. Our own HCWs' key recommendations for supporting HH compliance focused on (a) the availability of environmental resources such as hand-care products and facilities for performing HH in convenient locations; (b) awareness-raising (patients and HCWs); and (c) training in the practical aspects of HH.

The strengths of this study lie first in the use of the TDF – to help in the establishment of the process of implementation [39]. Secondly, by interviewing physicians and nurses, we gained insight into the perspectives of the key groups responsible for most inpatient medical services in the hospital. A potential weakness is the risk of response bias by participants who could have been influenced by a desire to provide the "correct response". Another limitation is the relatively low number of interviewees. However, even though our interviewees differed about their roles and specialties, their responses to the question

of HH tended to converge, indicating that the behavioral determinants were similar. We also had the opinion of other workers such as cleaners. Even though, at first glance, they did not have the same knowledge of the pathologies, or the risks related to non-compliance, their opinions were like those of the other interviewees. To avoid the biases related to objectivity that may be present in these types of studies, our study was also carried out independently by two investigators who then pooled their work. This qualitative study represents a backbone for further interventions in hospitals in Benin.

5. Conclusions

Best practice for improving hand hygiene in hospitals has not yet been established, and compliance in developing countries remains non optimal. Although direct observation is considered as the "gold standard" method for the measurement of HH compliance, it provides little insight into why a particular behavior does or does not occur. Viewed from the perspective of HCWs, several suggestions can be made for strengthening hospital hand-hygiene strategies. The first involves organizational support and leadership, which should include role modelling. The second involves ongoing education of HCWs, visitor and patients on the importance of good HH compliance. This study is the first step that come before implementation of a solid system change in our healthcare setting.

Author Contributions: C.L.Y., A.A. and O.D. were responsible for conceiving of the focus and design of the study, for completing all data collection, analysis, and interpretation. R.H. was involved in the guide of interviews. C.B. trained anthropologists and conducted preparation of material and data collection. A.S., O.D. R.H., C.B. and F.M.D. provided comments and suggestions that were incorporated into revision. All of authors have read and approved the final version of the manuscript

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Informed Consent Statement: The participants provided their written informed consent to participate in this study. Ethical approval for this project (012-19/UAC/FSS/CER-SS, Benin) was granted by the Health Faculty's ethical review board. All data were analyzed and presented anonymously.

Data Availability Statement: The data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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1.2 CHAPTER II : ANTIMICROBIAL RESISTANCE IN HOSPITALIZED SURGICAL PATIENTS : PHENOTYPIC AND MOLECULAR RESULTS

Chapter II at the glance

What is already known about this subject?

- Antimicrobial resistance remains a major public health particularly in developing countries where the hygienic conditions and good equipment's are deficient
- The quality of antibiotics and availability of last resort antibiotics for MDR treatment are a challenge in LMICs
- The misuse and irrational use of antibiotics, and the lack of antimicrobial stewardship in Benin remain a matter of concern for health authorities.
- The detection of molecular mechanisms of resistance is fundamental for treatment of patients particularly in the case of carbapenemases producing bacteria.

What this chapter add?

- No isolates showed a wild-type susceptible phenotype
- Alarming rate of MDR (90.8%) in SSIs
- This is the first results of antibiogram for isolates through six public hospitals
- Sincere collaboration between clinicians and the laboratory
- Although the phylogenomic investigation did not reveal evidence of hospital acquired transmission, we found two similar strains collected from patients in different hospitals.

Antimicrobial resistance in hospitalized surgical patients : a silently emerging public health concern in Benin.

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Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Benin

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Abstract

Background: Surgical site infections are related to high morbidity, mortality and healthcare costs. Because the emergence of multidrug-resistant bacteria in hospitals is becoming a worldwide challenge for surgeons who treat healthcare-associated infections, we wished to identify the causative agents involved in these infections and the rate of multidrug-resistant bacteria in six public hospitals in Benin.

Methods: Using standard microbiological procedures, we processed pus specimens collected from obstetrics and gastrointestinal surgery wards. Mass spectrometry (MALDI-TOF) was used for confirmation. For the antibiotic susceptibility test, we first used the Kirby-Bauer disk diffusion method. The secondary test (by microdilution) used the Beckton Dickinson Phoenix automated system (Becton Dickinson Diagnostic, USA).

Results: We included 304 patients, whose median length of stay was 9 days. A total of 259 wound swabs (85.2%) had positive aerobic bacterial growth. In obstetrics, *S. aureus* (28.5%, n = 42) was the most common isolate. In contrast, Gram-negative bacteria (GNB) were predominant in gastrointestinal surgery, the most dominant being *E.coli* (38.4%, n = 31). Overall, 90.8% (n = 208) of aerobic bacteria were multidrug resistant. Two-thirds of *S. aureus* (65.3%, n = 32) were methicillin-resistant *Staphylococcus aureus* (MRSA), three of which carried both MRSA and induced clindamycin resistance (ICR). GNB showed high resistance to ceftazidime, ceftriaxone and cefepime. Extended-spectrum beta-lactamases were presented by 69.4% *of E.coli* (n = 43/62) and 83.3% of *K. pneumoniae* (n = 25/30). Overall, twelve Gram-negative bacteria (5.24%) showed resistance to at least one carbapenem. No isolates showed a wild-type susceptible phenotype.

Conclusion: This study shows the alarming prevalence of multidrug-resistant organisms from surgical site infections in Benin hospitals. To reduce the spread of such bacteria in Benin, periodic surveillance of surgical site infections and strict adherence to good hand-hygiene practice are essential.

Keywords: ESBL, Methicillin-resistant *staphylococcus aureus*, Surgical site infection, Multidrug-resistant organisms

Background

Surgical site infections (SSIs) are infections of an incision, organ or space that occur after surgery [1]. In the United States, SSIs are the third most common hospital-acquired infections, accounting for 38% of all nosocomial infections according to the National Nosocomial Infection Surveillance System of the Centers for Disease Control (CDC's) [2]. In countries with limited

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resources, SSIs are the most common in the overall patient population, affecting up to 66% of patients who have had an operation—nine times more than in industrialized countries [3]. SSIs increase the length of postoperative hospital stay, the cost of healthcare, and the rate of hospital readmissions [4].

The common bacterial pathogens isolated from SSIs are *Staphylococcus aureus, coagulase negative Staphylococcus* (CoNS), *Acinetobacter* spp., *Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., and anaerobes such as *Clostridium* spp., and *Peptostreptococcus* spp. [5, 6].The acquisition of antibiotic resistance mechanisms by these bacterial strains has highlighted challenges for the management of SSIs around the world. These challenges have been made even greater by methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamases (ESBL) producing Enterobacterales, and the involvement of polymicrobial flora and fungi [7, 8]. ESBL have been reported most often in *Escherichia coli* and *Klebsiella* spp., but also in other bacterial species such as *Pseudomonas aeruginosa,* and *Enterobacter cloacae* [9].

The battle between bacteria and their susceptibility to drugs remains difficult for researchers and clinicians, and also for drug companies that seek effective drugs. SSIs by resistant drug bacteria are also becoming a serious concern in developing countries such as Benin, due mainly to crowded hospitlal environments, the irrational prescription of antimicrobial agents, and poor infection prevention and control programs [10]. Due to these inadequate SSI-surveillance programs, healthcare centers are unable to obtain proper updates on bacteria that are resistant to antimicrobial drugs [11]. The correct antibiotics are expensive and hard to access [12], and the use of broad-spectrum antibiotics is common.

In Benin, most clinical laboratories are not equipped with testing facilities that can detect multidrug-resistant bacteria [13]. Although the treatment process and infection-control measures depend on appropriate knowledge not only of updated antimicrobial therapy, but particularly of pathogens and their resistance, [14] these laboratories have little data on these pathogens' patterns of antimicrobial resistance. Identifying a bacteria and determining its susceptibility pattern therefore benefits patients, and also helps clinicians select chemotherapies that avoid the emergence of multidrug resistance organisms in hospital [15]. To generate locally applicable data and guide empirical therapy in areas where culture and drug-susceptibility testing facilities are scarce, this study thus sought to determine the magnitude of multidrug-resistant bacteria identified from SSIs.

Methods and materials

Study design and setting

This cross-sectional study was designed and conducted explicitly to determine the bacteriological profile of aerobes isolated from SSIs. These isolates were identified, and antimicrobial susceptibility testing was performed, in order to analyze the prevalence of multidrug-resistant organisms (MDRO) and particular phenotypes: MRSA, ESBL, and carbapenemase producing bacteria (CPO).

Patients who consented to participate in the study were included between January 2019 and January 2020. Patients who had had initial surgery in another hospital or ward (internal medicine or emergency), or had had prior to antibiotic treatment, and those who did not volunteer to participate were excluded from the study. We included the obstretrics wards (particularly ceasarean sections) and gastrointestinal wards at six public hospitals in Benin: *Bethesda, Centre Hospitalier Universitaire de Zone de Suru Lere, Centre National Hospitalier Université Hubert Koutoukou Maga* (CNHU-HKM), *Centre Hospitalier Universitaire de la Mère et L'enfant* (CHUMEL), *Centre Hospitalier Universitaire Départemental Ouéme/Plateau* (CHUDOP) and *Centre Hospitalier Universitaire de Zone d'Abomey Calavi.* Our choice of wards lay in the facts that caesarean sections are one of the most frequent surgical procedures worldwide [16], and that gastrointestinal section is available in these six public hospitals. All participating hospitals were located in the south of Benin, thereby allowing daily transport from each hospital to the CNHU-HKM laboratory, where all wound swabs collected were analyzed. All results were confirmed in the laboratory of the *Cliniques Universitaires Saint-Luc-UCLouvain* (Brussels, Belgium).

Sampling and collection method

The case definitions and clinical criteria of SSIs (superficial incisional SSI, deep incisional SSI, and organ/space SSI) were taken from the guidelines of the Centers for Disease Control and Prevention on the prevention of SSI [2]. A preliminary step in the project consisted of training nurses in the sampling technique. These nurses then collected all the samples per hospital. On a day when a clinical SSI was detected, one swab was collected aseptically from each infected patient using a sterile cotton swab. Each sample was labeled with the date of sample collection, the collection method, and the patient's details. The swab was immediately dipped into a sterile tube with transport medium (Amies, Beckton Dickinson) and delivered to the bacteriology laboratory at CNHU-HKM. Coagulase-negative *staphylococcus aureus* were considered as pathogens only when isolated in two consecutive sampling swabs.

Socio-demographics and clinical data were obtained from the patients' files and by physical examination using a structured questionnaire. The following data were collected: ward to which admitted, age, gender, history of illness, and antibiotics used during and after surgery. Before the actual data was collected, a pretest of the data collection instrument was conducted to ensure the appropriateness of the questionnaire. If necessary, modifications were made. Data collection was supervised daily by the research team.

Processing of samples

Macroscopic and microscopic examination of samples

All the specimens were visually examined for consistency, color, turbidity, and the presence or absence of blood. Gram staining of each specimen was performed [17].

Culture of specimens and isolation of bacteria

Bacterial identification was performed according to the guidelines for microbiological methods of the European Committee of Antimicrobial Susceptibility Testing guidelines [18]. Cultures were incubated for a total of 48 h (if there was no growth at 24 h) at 37 °C in aerobic atmosphere, and then examined for microbial growth. Identification of Gram-positive bacteria was done using Gram staining, and the catalase test, coagulase test, DNase test and Pastorex staphylococci plus test (Pastorex, staph plus Biorad). Gram-negative strains were identified using various biochemical tests: oxidase, and characteristics of the Analytical Profile Index (API 20E, Biomerieux, Lyon) such as the Voges Proskauer (VP) test, indole test and citrate utilization.

All identifications were confirmed in Belgium by using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry (Brucker Daltonics, Bremen, Germany). Due to budgetary constraints and a lack of laboratory facilities, we were unable to investigate anaerobic bacteria.

Antimicrobial susceptibility test (AST)

Antimicrobial susceptibility testing was performed for all isolates according to the modified Kirby Bauer disk diffusion technique as described in the European Committee on Antimicrobial Susceptibility Testing guidelines [18]. Antibiotics were purchased from BioRad (Marnes-la-Coquette, France) and included for *S. aureus:* ampicillin (10 µg), cefotaxime (30 gµ), cefoxitin (30 µg), gentamicin (10 µg),amikacin (30 µg), ciprofloxacin (5 µg), trimethoprim + sulfamethoxazole (25 gµ), tetracycline (30 gµ), and chloramphenicol (30 gµ). The lactose fermenters bacteria were tested for ampicillin (10 µg), piperacillin (100 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftriaxone (30 gµ), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 gµ), ciprofloxacin (5 µg), trimethoprim + sulfamethoxazole (25 gµ), imipenem (10 µg), and meropenem (10 µg). For lactose non-fermenters bacteria, the disks used included ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), ciprofloxacin and meropenem (10 gµ). After 24 h of incubation at 37 °C, the inhibition zones were measured and the results were analyzed.

Phenotypic test for multidrug-resistant bacteria, extended-spectrum beta-lactamases, inducible clindamycin resistant (ICR) in S. aureus, and carbapenemases production

Multidrug resistance was defined as resistance to three or more antimicrobial classes [19]. The presence of ESBL was detected using the double-disk synergy test (DDST) between clavulanate and third-generation cephalosporins and/or aztreonam [20]. Methicillin-resistant *S. aureus* isolates were detected using the cefoxitin disk (30 gμ) method. The diameter of the zone of inhibition for cefoxitin was < 21 mm [21]. Similarly, inducible macrolide-lincosamide streptogramin-B (iMLS$_B$) resistance was detected in *S. aureus* with the D-test disk method using clindamycin (2 ug) and erythromycin (15 ug) on MHA plates. After overnight incubation, isolates with a flattened zone of inhibition adjacent to the erythromycin disk (referred to as a "D" zone) were considered to exhibit inducible clindamycin resistance [22]. The presence of resistance to at least one carbapenem was checked with the RESIST-3O.K.N.ICT (Coris Bioconcept, Gembloux, Belgium), which detects OXA 48, KPC and NDM carbapenems. The final results of the ICT test were read when they became positive, at the latest after 15 min. All ESBL, MRSA and CPO strains were confirmed and characterized by whole-genome sequencing.

Quality control

Standard operating procedures (SOPs) were strictly followed during all bacteriological procedures, starting from sample collection, isolation, identification and antibiotic susceptibility testing. All culture media were prepared according to the manufacturers' directions, and were checked for their sterility and performance. Two international control bacteria strains, *E. coli ATCC 25922* and S. *aureus ATCC 25923*, were used as reference strains for quality control of the antimicrobial susceptibility and biochemical tests. The same strain of *E.coli* was also considered as a negative control during the screening and phenotypic tests of ESBL-producing lactose fermenters bacteria. In Belgium, the same strain of *E.coli* was also considered as a control for mass spectrometry (MALDI-TOF). For transportation only, we used swabs with transport medium (Amies, Beckton Dickinson).

Data analysis and statistical tests

Data was entered in Epi-data version 3.1, transferred to Statistical Package for Social Sciences (SPSS) software version 25, and Microsoft Excel software for analysis. Quantitative variables were expressed as median with interquartile range (IQR). A P-value less than 0.05 was considered statistically significant.

Results

Socio-demographic and clinical characteristics

A total of 304 wound swabs were collected from 174 patients with clinical signs of surgical site infections. Obstetrics patients [n = 148; median age 29 years (24-IQR-34)] represented 195 swabs (64.1%). The median length of stay in obstetrics and gastrointestinal surgery was 9 days (6-IQR-14). In gastrointestinal surgery, the patients' ages ranged from 18 to 76 years, with a median age of 35 years (25-IQR 50). A majority of the patients were female (80.3%). Emergency surgery was the most common type of surgery (82.6%).

Surgical antimicrobial prophylaxis

Of the samples collected, 172/304 (56.6%) originated from patients who had received preoperative antimicrobial prophylaxis for more than 24 h after surgery. Monotherapy with ceftriaxone was administered to the highest number of patients 57/172 (33%), ampicillin was administered to 23/172 (13%) and amoxicillin/clavulanic acid was administered to 17/172 (9.9%). The most prescribed regimen among the combination regimens was ceftriaxone + metronidazole 35/172 (20%). In both obstetrics and gastrointestinal surgery, there was no difference between the antimicrobial classes used before and after surgery.

Bacterial etiologic agents isolated per ward

Among the 304 wound-swab cultures, 259 (85.2%) were positive for aerobic bacterial growth. Forty-five (n = 45,14.8%) yielded negative results, while 85 (27.9%) were excluded because they presented more than two germs and were considered to be polymicrobial. Of the 174 remaining samples, 55 (31.6%) yielded polymorph flora with two different bacteria, while 119 (68.4%) yielded a single isolate. Altogether, 229 isolates were identified. While Gram-positive microorganisms represented 21.4% (n = 49) of isolates, 78.6% (n = 180) were Gram-negative. Whereas *Staphylococcus aureus* (28.5%, n = 42), *Pseudomonas.aeruginosa* (21.6%, n = 32) and *Escherichia. coli* (20.9%, n = 31) were the most frequent in the obstetrics ward, *Escherichia. coli* (38.4%, n = 31), *Klebsiella pneumoniae* (21.0%, n = 17) and *Enterobacter cloacae* (12.3%, n = 10) were the most prevalent in gastrointestinal surgery (Figs. 1 and 2).

The genus was confirmed of all the bacteria we identified. No discrepancy appeared with *Staphylococcus aureus*. Two discrepancies each were noted with negative Gram bacteria: *Klebsiella pneumoniae* (API) versus *Klebsiella varicola* (MALDI-TOF), and *Pseudomonas aeruginosa* (API) versus *Pseudomonas mendocina (*MALDI-TOF).

Drug resistance patterns of the isolates to different classes of antibiotics

Staphylococcus aureus

All Gram-positive organisms were *S. aureus*. Almost all *S. aureus* isolates were resistant to penicillin (98%, n = 48/49), and 32 isolates (65.3%, n = 32/49) were resistant to methicillin (MRSA phenotype). Cefoxitin is considered to be a marker of the MRSA phenotype, and we observed the same rate of resistance in all beta-lactams classes. Resistance to cotrimoxazole was found in 10.2% of isolates, to gentamicin in 38.8%, and to ciprofloxacin in 36.7%. Ninety-eight percent of isolates were susceptible to clindamycin and no resistance to vancomycin was observed (Fig. 3).

Lactose fermenters bacteria

While all lactose fermenters bacteria (LFB) showed resistance to many of the antibiotics tested, amikacin and imipenem remained the most active (95.9% and 99.2%, respectively). However, there was also considerable resistance to other aminoglycosides (61.5% of LFB were resistant to gentamicin). Almost all LFB (99.2%) showed resistance to ampicillin. Three-quarters (75.4%) were resistant to ceftriaxone, 76.2% to cefotaxime and 73.8% to cefepime. Resistance to quinolones reached 68.9% for ciprofloxacin. There was also a high level of resistance to cotrimoxazole (83.6%). We noted 6.6% of LFB that were resistant to at least ertapenem (Fig. 4).

Lactose non-fermenters bacteria

The lactose non-fermenters bacteria (LNFB) (*Acinetobacter baumannii* n = 20 and *P. aeruginosa* n = 38*)* showed low resistance to ciprofloxacin (20.7%), ceftazidime (20.7%), gentamicin (31.0%) and piperacillin/tazobactam (34.5%). Unfortunately, 8.6% of LNFB were resistant to imipenem and 10.3% of LNFB were resistant meropenem (Fig. 5).

Prevalence of ESBL and carbapenem-resistant isolates of gram negative rods

Forty-three of the 62 isolates of *E.coli* (69.4%) were ESBL producers, as were 25 of the 30 (83.3%) *K. pneumoniae* isolates, and 16 of the 21 *E. cloacae* (76.2%) isolates. Five isolates (8%) of *E. coli* and three *E. cloacae* (14%) were resistant to at least one of the carbapenems

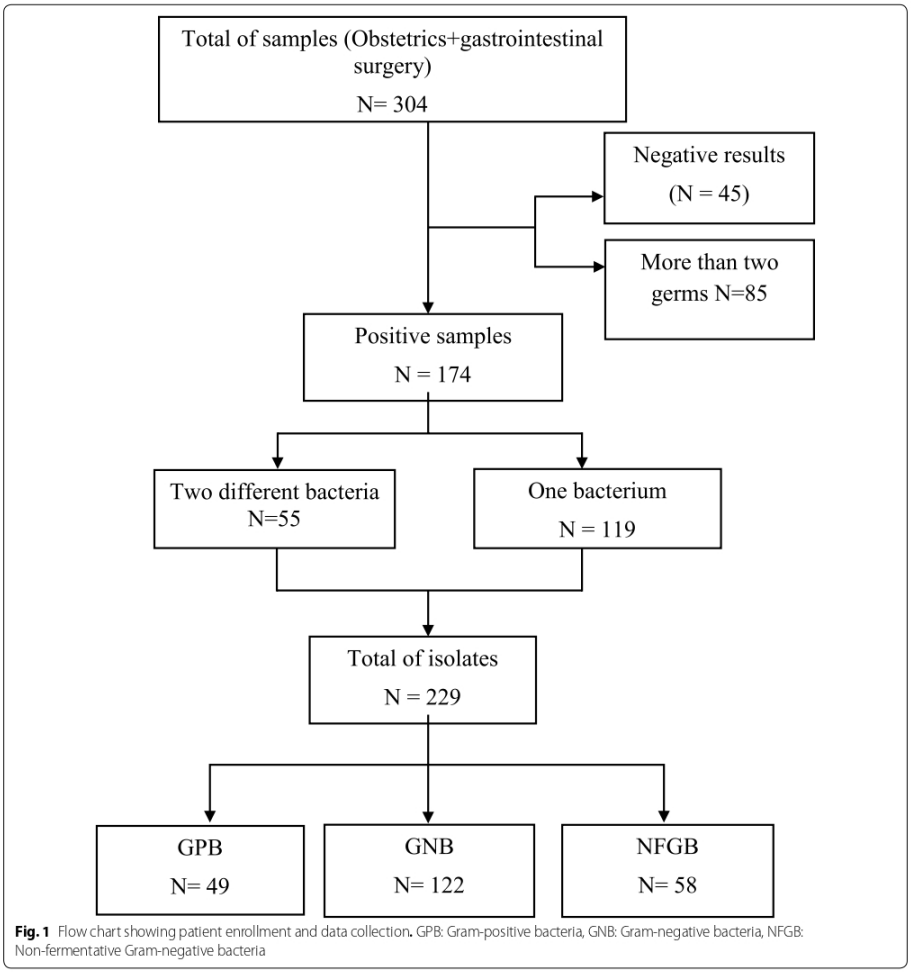


Fig. 1 Flow chart showing patient enrollment and data collection. GPB: Gram-positive bacteria, GNB: Gram-negative bacteria, NFGB: Non-fermentative Gram-negative bacteria

tested (imipenem, meropenem and ertapenem). For all of the ESBL strains (84), the percentage of co-resistance was 85.7% (72/84) for quinolones, 65.5% (55/84) for aminoglycosides, and 67.8% (57/84) for trimethoprim-sulfamethoxazole. Three isolates of *P.aeruginosa* (8%) and one of *A. baumannii* (5%) showed resistance to carbapenems. With the RESIST -3 O.K.N.ICT test, we detected New Delhi-Metallo-beta-lactamase (NDM) in *A.baumannii* and VIM in *P.aeruginosa*. According to the PCR, the ESBL isolates harbored mainly Cefotaximases-Munich (CTX-M) enzymes. The molecular description of these genes will be reported soon.

Multidrug resistance pattern of bacterial isolates

As well as majorities of *E.coli* (93.5%) and *E. cloacae* (95.2%), 69.4% of *S. aureus* strains were resistant to more

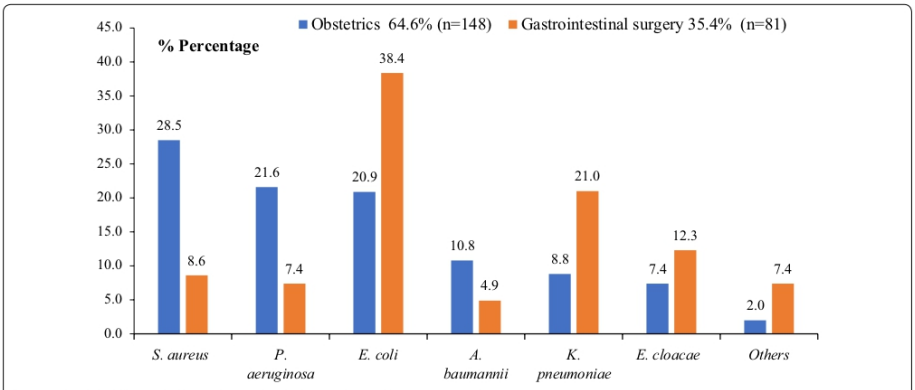


Fig. 2 Proportion of bacterial species per ward: Obstetrics and Gastrointestinal

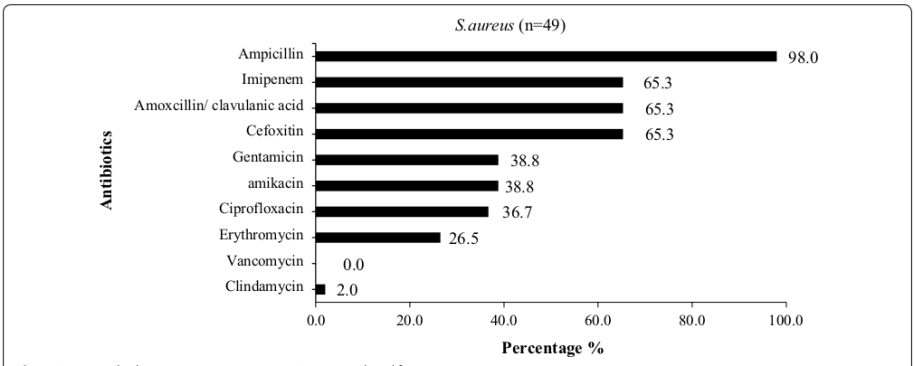


Fig. 3 Antimicrobialresistance pattern among *S.aureus* isolated from pus specimens

than five antimicrobial classes. All *P. aeruginosa* and *A. baumannii* strains were also resistant to more than five classes of antibiotic (Table 1).

Discussion

This study provides insight into the causative bacteria and sensitivity profiles of SSIs in six hospitals in Benin. Overall, 90.8% of aerobic bacteria were resistant to more than five antimicrobial classes—an MDRO rate that was higher than those described in Ethiopia and Uganda [23, 24].

Three main factors may have contributed to these high rates of MDRO. The first is likely to have been associated with the country's overall lack of antimicrobial resistance surveillance and stewardship programs. There is enough evidence to indicate that, by improving the use of antibiotics, such programs help both to understand the pattern of resistance and to prevent the development of antibiotic resistance [6]. The second reason might be associated with the lack of comprehensive national policies on antibiotics use. Instead, it is common practice in Benin to buy antibiotics—including large-spectrum ones—without prescription from private drug vendors and pharmacies. The third reason may be due to the lack of diagnosic laboratory services before the administration of antibiotics by clinicians

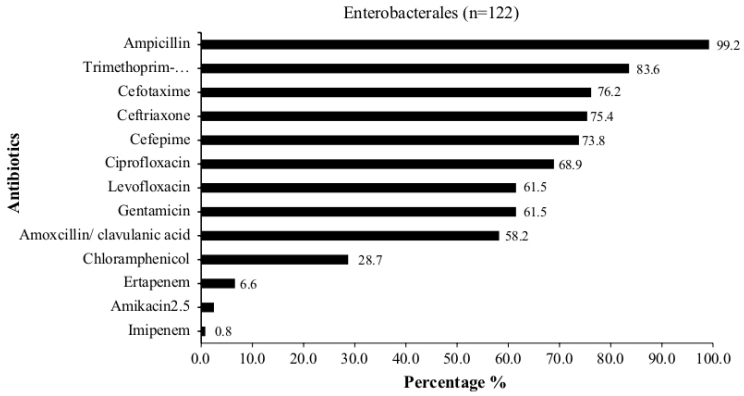


Fig. 4 Antimicrobialresistance pattern among Enterobacterales isolated in pus

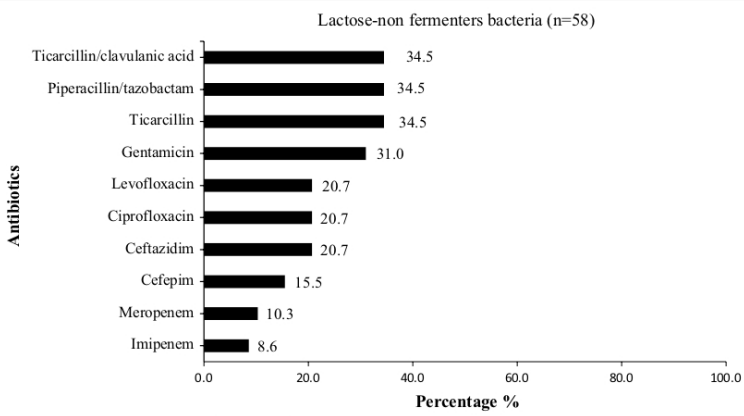


Fig. 5 Antimicrobial resistance pattern among lactose non-fermenters bacteria

who do not have an antibiogram or evidence of the causative agents.

The most common isolates in obstetrics were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This finding is in line with other studies that described S. *aureus* to be associated with SSIs in obstetric wards [25–27]; *S. aureus* is considered to be a commensal organism of the skin and can easily contaminate a wound [28]. The high frequency of *P. aeruginosa* could be explained by the fact that this bacteria is intrinsically resistant to ceftriaxone, which is currently the drug most commonly used for prophylaxis in Benin.

According to the AST results, almost all *S. aureus* isolates (98%) were resistant to penicillin. While a similarly high resistance of *S. aureus* to penicillin was also reported in Uganda and Nepal [23, 26], resistance to ampicillin was observed in only 4% of isolates in India [29]. Such variations in the susceptibility pattern may be attributed to differences in the rational use

Table 1 Multiple drug resistance patterns of the isolated bacteria

Isolates	Total (N)	R2 (%)	R3 (%)	R4 (%)	≥ R5 (%)
E. coli	62	0 (0)	1(1.6)	3 (4.8)	58 (93.5)
S. aureus	49	7 (14.3)	6 (12.2)	2 (9.1)	34 (69.4)
P. aeruginosa	38	0 (0)	0 (0)	0 (0)	38 (100)
K. pneumoniae	30	0 (0)	0 (0)	1(3.4)	29 (96.6)
E. cloacae	21	0 (0)	1(4.8)	0 (0)	20 (95.2)
A baumannii	20	0 (0)	0 (0)	0 (0)	20 (100)
Others	9	0 (0)	0 (0)	0 (0%)	9 (100)
Total	229	7 (3.1)	8 (3.5)	6 (2.6)	208 (90.8)

R2–R5 number of antibiotics class to which an isolate was resistant

of antibiotics. In Benin, ampicillin has also been widely used as a prophylaxis after ceftriaxone.

Two-thirds of our isolates (65.3%) were resistant to cefoxitin and were reported as MRSA species. Cefoxitin has been reported as a surrogate marker for the detection of methicillin resistance. The cefoxitin zone diameter remains an interpretive criterion for the prediction of mecA-mediated resistance [30]. Upreti and Shrestha in Nepal found the same rate of MRSA [31, 32]. In a retrospective single center study conducted in 2016 by Mercy Ship during surgical outreaches in six sub-Saharan African countries (Benin, Togo, Liberia, Madagascar, Congo and Sierra-leone), Lai PS et al. found the highest rates of MRSA in Benin (34.6%) and Congo (31.9%), and the lowest rate in Togo (14.3%) and Madagascar (14.5%) [33]. The difference in the rates of isolation of MRSA between studies may have been due not only to differences in the levels of inappropriate use of antibiotics, but also to the effectiveness of hygiene programs. In a previous study [34], we found hand-hygiene compliance among Benin healthcare providers to be only 33.3%. The treatment of infections caused by MRSA may also require the use of reserve drugs such as glycopeptids or lincosamides. However, the fact that we observed no resistance to vancomycin in our study can be explained by the fact that this antibiotic was not available in Benin.

E. coli, K. pneumoniae and *E. cloacae* were the commonest isolates in gastrointestinal surgery in our study. The predominance of *E. coli* has been reported in some other recent studies [35–37]. In Morocco and Uganda, the authors showed *K. pneumoniae* to be the predominant Gram-negative bacteria [23, 36]. This predominance could be attributed to their diverse habitats (which includes inanimate surfaces in hospitals), their multidrug resistance pattern, and possible contamination from the intestinal tract during surgery.

The bacteria most frequently involved in SSIs change from time to time and also vary with hospital settings. Our finding that GNB showed high resistance to ceftriaxone, ceftazidime, cefepime, and cotrimoxazole is in agreement with various studies worldwide [38–41]. The high rate of bacterial resistance to ceftriaxone is likely due to frequent use of this antibiotic in and outside hospitals. Our finding that almost all *P. aeruginosa* and *A. baumannii* were sensitive to amikacin and had relatively moderate resistance to cefepime (15.5%), ceftazidime (20.7%), and ciprofloxacin (20.7%) are similar to those in studies in Nepal and India [31, 32], which observed moderate resistance to ciprofloxacin (6.2% to 24%). High sensitivity to imipenem and amikacin may be due to the limited exposure of these drugs to the prescription antibiotics that are relatively more expensive and not constently available in Benin. For instance, amikacin is available for one case out of two; clinicians are forced to buy it from neighboring countries such as Nigeria and Togo.

The emergence of ESBL-producing Gram-negative rods has attracted increasing concern in the developing world [12]. The majority of LFB isolates in our study were ESBL producers. Upreti et al. reported that 25% of *E. coli* and 40% of *K. pneumoniae* are ESBL producers [32]. In 2016, Benin was found to have the highest rate of third-generation cephalosporin-resistant LFB of six sub-Saharan African countries [33]. Almost all ESBL producers showed co-resistance to other class of antibiotics, such as aminoglycosides (65.5%), quinolones (85.7%), and trimethoprim-sulfamethoxazole (67.8%). This high co-resistance may be due to the occurrence of gene-encoding resistance to aminoglycosides, trimethoprim-sulfamethoxazole and quinolones on the same plasmid that encode ESBL production [42].

Although patients in the present study received prophylactic antimicrobials such as ceftriaxone and ampicillin prior to surgery, the antibiogram results showed that the isolated organisms were resistant to these antimicrobial agents. Ceftriaxone is also known to favor the emergence of ESBL. This high antibiotic resistance implies that if immediate action is not taken, recommended antibiotics such as cefazolin may be rendered useless.

In our study, 10.3% of *P.aeruginosa* and *A. baumannii* showed resistance to meropenem and constituted pan-drug resistance bacteria according to the Magiorakos classification [19]. This resistance was due to mechanisms that are often expressed in hospital-acquired strains of *Acinetobacter* and *Pseudomonas*, such as beta-lactamases, alterations in cell-wall channel (porins), and efflux pumps. Due to the unavailability of an effective last therapeutic option such as ceftazidime-avibactam, the increasing rate of carbapenemase-producing organisms in this study is of great concern [43].

To the best of our knowledge, our report is the first from Benin on the rate of MDRO. Like other LMICs,

Benin does not have a strongly regulated antibiotic-prescription system, which makes it particularly easy to misuse antibiotics. Our findings thus constitute an urgent call for monitoring and optimizing antimicrobial use there. Our first recommendation is for a multidisciplinary approach to the management of SSIs that involves clinicians, pharmacists, microbiologists and infection-control specialists. Second, strengthening laboratory services at the local and national levels would ensure effective surveillance of antimicrobial resistance. Finally, to minimize the spread of MDRO, we recommend strict adherence to good infection-prevention control practices, particularly hand hygiene and the disinfection of inanimate surfaces.

This study is part of the Multidisciplinary Strategy for Prevention and Infection Control in Benin (MUSTPIC). One challenge facing this strategy is the question of how, on the basis of our findings, national guidelines may be formulated for the correct application of SAP in our hospitals.

Limitations of this study

While the strength of this study lies in its prospective nature, a limitation should also be noted. As our study did not isolate strict anaerobes, the number of bacterial isolates that were reported negative may have been underestimated. Relevant additional information would be produced by further studies. Molecular characterization of MDRO would have generated more useful epidemiological results.

Conclusion

This study helps quantify the extent of drug-resistant bacteria in surgical site infections in Benin. As two-thirds of isolates were producers of ESBL and MRSA, this prevalence particularly concerns GNB and *S. aureus*. None of the isolates showed a wild-type susceptible phenotype. With regard to reducing the spread of multidrug-resistant bacteria in Benin, these findings represent an urgent call for the judicious use of antibiotics, for strict adherence to good hand-hygiene practices, and for the provision of antibiotics with high activity. Even though research on AMR in Benin is still at a very early stage, it is essential to establish surveillance programs that reduce the burden of surgical site infections.

Abbreviations

AMR: Antimicrobial resistance; AST: Antimicrobial susceptibility test; ATCC: American type culture collection; ESBL: Extended-spectrum beta-lactamase; MDRO: Multi-drug resistance organisms; MRSA: Methicillin resistant *Staphylococcus aureus*; WHO: World health organization; LFB: Lactose fermenters bacteria; LNFB: Lactose non-fermenters bacteria; HAIs: Healthcare associated infections; SSI: Surgical site infection; SOPs: Standard operative procedures; SPSS: Statistical package for social sciences; LMIC: Low middle-income country.

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Authors' contributions

First author: CLY is the primary author, who designed the study, performed laboratory investigations, and prepared the manuscript. DA helped for arrangements during the laboratory process and supervised the analyses in the CNHU laboratory. OD and AS designed the study and revised the complete manuscript for submission. AK, HRV and FVB revised the complete manuscript for submission. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are avalaible from the corresponding author.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the Health Faculty (FSS, Benin) under reference number: 012-19/UAC/FSS/CER-SS. Informed written consent was also obtained from participants and /or guardians after explanation of the objective of the study. For better patient management, all the laboratory tests were done free of charge and their results were communicated to the treating physicians as early as possible.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflicts of interests associated with the publication of this paper.

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CASE REPORT Open Access

First detection of a plasmid-encoded New-Delhi metallo-beta-lactamase-1 (NDM-1) producing *Acinetobacter baumannii* using whole genome sequencing, isolated in a clinical setting in Benin

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Abstract

Background: Carbapenem-resistant *Acinetobacter baumannii* is considered a top priority pathogen by the World Health Organization for combatting increasing antibiotic resistance and development of new drugs. Since it was originally reported in *Klebsiella pneumoniae* in 2009, the quick spread of the bla_{NDM-1} gene encoding a New-Delhi metallo-beta-lactamase-1 (NDM-1) is increasingly recognized as a serious threat. This gene is usually carried by large plasmids and has already been documented in diverse bacterial species, including *A. baumannii*. Here, we report the first detection of a NDM-1-producing *A. baumannii* strain isolated in Benin.

Case presentation: A 31-year-old woman was admitted to a surgical unit with a diagnosis of post-cesarean hematoma. An extensively-drug resistant *A. baumannii* strain solely susceptible to amikacin, colistin and ciprofloxacin, and resistant to several other antibiotics including ceftazidime, imipenem, meropenem, gentamicin, tobramycin, ceftazidime/avibactam, and sulfamethoxazole-trimethoprim, was isolated from the wound. Production of NDM-1 was demonstrated by immunochromatographic testing. Whole genome sequencing of the isolate confirmed the presence of bla_{NDM-1}, but also antibiotic resistance genes against multiple beta-lactamases and other classes of antibiotics, in addition to several virulence genes. Moreover, the bla_{NDM-1} gene was found to be present in a Tn125 transposon integrated on a plasmid.

Conclusions: The discovery of this extensively-drug resistant *A. baumannii* strain carrying bla_{NDM-1} in Benin is worrying, especially because of its high potential risk of horizontal gene transfer due to being integrated into a transposon located on a plasmid. Strict control and prevention measures should be taken, once NDM-1 positive *A. baumannii* has been identified to prevent transfer of this resistance gene to other Enterobacterales. Capacity building is required by

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governmental agencies to provide suitable antibiotic treatment options and strategies, in combination with strengthening laboratory services for detection and surveillance of this pathogen.

Keywords: *Acinetobacter baumannii*, New-Delhi metallo-beta-lactamase, Whole-genome sequencing, Benin

Background

Acinetobacter baumannii is an opportunistic nosocomial pathogen responsible for a broad range of infections [1]. Nosocomial isolates of this bacterium are often resistant to almost all currently available antibiotics. Some striking features of this bacterium, such as its ability to cause opportunistic infections, to develop antimicrobial resistance and to survive under adverse environmental conditions, have contributed to its wide dissemination [1]. The global spread of carbapenem-resistant *A. baumannii* has been observed and is considered a sentinel event of emerging antimicrobial resistance [2].

Carbapenem resistance mechanisms in *A. baumannii* are more commonly mediated by carbapenem-hydrolyzing class D beta-lactamases and less often by class B metallo-beta-lactamases [3]. Gram-negative bacteria with the New-Delhi metallo-beta-lactamase type-1 (NDM-1), encoded by the $bla_{\text{NDM-1}}$ gene, utilize at least one zinc atom at the active site to facilitate hydrolysis of a broad variety of beta-lactams and carbapenems [4]. The initial case detection of NDM-1 production was reported in 2009 in a clinical urinary isolate of *Klebsiella pneumoniae* from a 59-year-old man who returned to Sweden after hospitalization in India [5]. Afterwards, the genetic context of $bla_{\text{NDM-1}}$ was reported in 2011 in a clinical *A. baumannii* isolate discovered in a German hospital [6]. The $bla_{\text{NDM-1}}$ gene was integrated on a new transposon structure (Tn125) flanked by two insertion elements [6]. Subsequently, Tn125-harboring $bla_{\text{NDM-1}}$ was reported on chromosomes in several multiple-resistant *Acinetobacter* spp. isolates throughout Europe [7]. Moreover, a $bla_{\text{NDM-1}}$ bearing plasmid, pNDM-BJ01, was reported in 2012, isolated from a clinical *Acinetobacter lwoffii* strain in China [8]. This plasmid carried a complete Tn125 transposon and showed high horizontal transferability. Since then, several NDM-1 positive plasmids have been isolated from *Acinetobacter* spp. in China with similar structures to pNDM-BJ01 [9]. To date, NDM carbapenems have been reported in most regions around the world owing to the rapid dissemination of the gene between members of the Enterobacterales and *Acinetobacter* spp. in human and environmental isolates [1]. So far, only limited reports exist on NDM-1 producing *A. baumannii* in Africa, and those published are mostly from northern or southern African countries [10, 11]. Here, we describe the first case of an extensively drug-resistant *A. baumannii* strain producing NDM-1- in Benin.

Case presentation

A 31-year-old woman was admitted to a surgical unit at a public hospital in Benin on March 06, 2019. She had undergone a ceasarean in a different hospital and presented hematoma, but previous treatment information and conditions were not recorded upon admission. During laparotomy, surgical antimicrobial prophylaxis was administered as intravenous ceftriaxone (1 g), and after intervention, empirical antibiotic therapy consisting of intravenous imipenem (500 mg every 8 h) was initiated for one week. On the tenth day, the patient's clinical condition worsened and she developed fever (38 °C) and wound suppuration. Preliminary investigation revealed that the patient had no previous history of travel or hospitalization abroad. Intensive programs of environmental cleaning and strict contact isolation precautions were applied. However, following the initial treatment of 500 mg imipenem per 8 h, the patient preferred to continue with unspecified indigenous treatment due to lack of financial support. As she was no longer in the hospital, the clinical outcome is unknown.

The culture of a pus swab revealed Gram-negative coccobacilli that were glucose-non-fermentative, non-motile, and oxidase-negative. Biochemical identification was performed with the Analytical Profile Index (API 20E, Biomérieux, France) and results were confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Antimicrobial susceptibility testing was assessed using the modified Kirby-Bauer disc diffusion method and confirmation was done by the microbroth dilution method. The interpretation breakpoints were based on the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/ast_of_bacteria/). Except for amikacin, colistin and ciprofloxacin, the isolate was resistant to all tested antimicrobial agents with the following Minimum Inhibitory Concentration (MIC) values: ceftazidime (> 16 mg/l), imipenem and meropenem (> 16 mg/l), gentamicin and tobramycin (> 8 mg/l), ceftazidime/avibactam (> 16/4), sulfamethoxazole-trimethoprim (> 8/152). The isolate was therefore considered as extensively drug resistant (XDR) [4]. Additionally, to confirm the resistance pattern, we used the multiplex lateral flow immunochromatographic test, the RESIST-3 O.K.N. ICT (Coris Bioconcept, Gembloux, Belgium), which confirmed the presence of NDM.

Whole-genome sequencing (WGS) was subsequently performed for detection and characterization of resistance genes, using DNA from a single-colony isolate employing the EZ1 advanced XL biorobot and the tissue DNA kit (Qiagen, Hilden, Germany) with the bacterial card, according to the manufacturer's instructions. A standard Nextera XT library (Illumina, San Diego, USA) was constructed (Nextera XT DNA library preparation kit, Illumina, San Diego, USA) and subsequently sequenced on an Illumina MiSeq instrument with a 250-bp paired-end protocol (MiSeq v3 chemistry, Illumina, San Diego, USA) according to the manufacturer's instructions. Data was analyzed as follows. First, reads were trimmed with Trimmomatic 0.36 [12] with the settings 'NexteraPE-PE.fa:2:30:10', 'LEADING:10', 'TRAILING:10', 'SLIDINGWINDOW:4:20', and 'MINLEN:40'. Processed reads were then assembled de novo using SPAdes 3.13.0 [13] with the 'careful' option enabled and the 'cov-cutoff' parameter set to 10. Contigs smaller than 1000 bases were removed with seqtk seq 1.2 (https://github.com/lh3/seqtk) using the '-L' option. Assembly statistics were determined using Quast 4.4 with default settings [14]. Genome annotations were created using Prokka 1.13 [15] (Table 1).

The NCBI National Database of Antibiotic Resistant Organisms (NDARO) [16], Virulence Factor (full) database (VFDB) [17], and PlasmidFinder database, were used for genotypic detection of genes encoding antimicrobials, virulence factors, and plasmid replicons, respectively, using SRST2 0.2.0 [18] with default settings. The XDR status of the isolate was confirmed by harboring several resistance genes against aminoglycosides (*aph(6)-Id, aph(3")-Ib, ant(3")-IIa,* and *aac(3)-IId*), beta-lactamases ($bla_{\text{NDM-1}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-558}}$, $bla_{\text{ADC-166}}$), macrolide-lincosamide-streptogramin B (*msr(E)*), macrolide (*mph(E)*), sulfonamide (*sul2*), tetracycline (*tet(39)*), and bleomycin (*ble*). Additionally, 67 loci encoding different virulence factors were detected including genes related to biofilm formation such as *ompA*, *bfmS*, *csuE*, and a K1 capsular polysaccharide (ABK1) (see Additional file 1). No plasmid replicons from the PlasmidFinder database were detected.

Sequence typing was performed with the corresponding regular multi-locus sequence typing (MLST) schemes from Oxford University and Institut Pasteur [19], as described in Bogaerts et al. [20]. MLST analysis detected sequence type 836 (Oxford University scheme) and 388 (Institut Pasteur scheme). No isolates were present for the former, but the latter returned two isolates from Taiwan (from 2012 and 2013) and a single isolate from Norway (year unknown) with the same sequence type. The sample was screened for the presence of the Tn125 transposon by mapping (trimmed) reads against its reference sequence (NCBI KF702386.1) using Bowtie2 2.3.0 [21] with the '–sensitive' setting enabled. In total, 99.55% of the Tn125 transposon reference length of 10,624 bp was covered by at least one read, with three breakpoints however present at ~ 7.7 kb, 8.5 kb, and 9.2 kb, indicating some minor rearrangements. The median depth of coverage of the Tn125 transposon was 98.20× (compared to 83.0X for the whole genome). The alignment and annotation for the Tn125 transposon are visually represented in Fig. 1. An additional de novo assembly was then performed using plasmidSpades 3.13.0 with the '-plasmid' and '-careful' options enabled [22] to reconstruct putative plasmids. This resulted in 21 contigs, with the $bla_{\text{NDM-1}}$ gene located near the center of the largest contig (88,063 bp) and its surrounding region of 7626 bp aligned to the Tn125 transposon with over 99% sequence identity. Outside of this region, no alignments were found between this putative plasmid contig and the pNDM-BJ01 (NCBI NC_019268.1) plasmid. Additional screening of the putative plasmid contig against the Plasmid Database (PLSDB) online platform [23] (v2020_03_04) with mash was therefore performed and provided four matches: a large plasmid of 78,125 bp (NCBI CP038501.1) that was found in *A. baumannii*, and three smaller ones (NCBI JQ739158.1, KF220658.1, KR059864.1) with sizes 4797, 1634 and 7865 bp, respectively. Alignments with BLAST

Table 1 Genome assembly, and putative plasmid, statistics

	Cumulative length (bp)	Nb. of contigs	GG-content (%)	Nb. of coding sequences	Nb. of tRNAs	NCBI accession
Genome assembly	4,082,860	39	38.92	3838	64	JABBFT000000000
Putative plasmid	88,063	1	42.13	101	0	JABBFT000000000 (Sequence ID: Sciensano:GLGAKIBC_35)

This table contains statistics for the de novo assembly and annotation of the genome and putative plasmid. The number of coding sequences and tRNAs were extracted from the Prokka annotations. The genome and putative plasmid sequences were uploaded to NCBI as a single submission, with the putative plasmid annotated as 'Sciensano:GLGAKIBC_35'

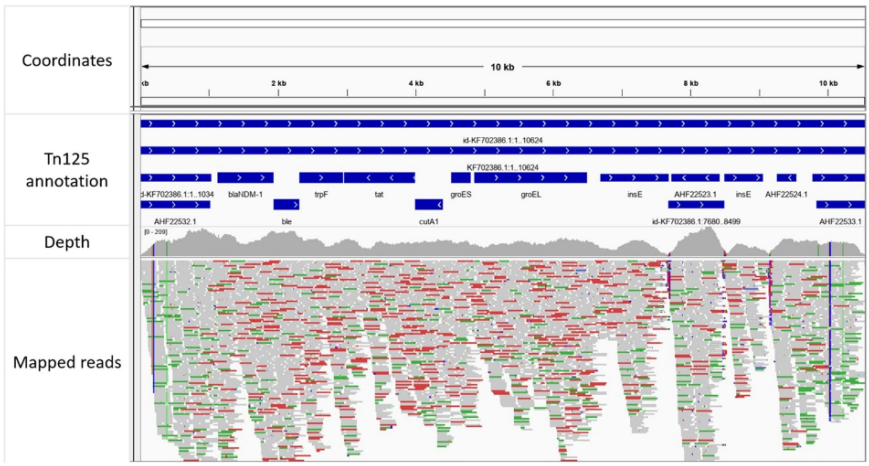


Fig. 1 Detection of the Tn125 transposon carrying the bla_{NDM-1} gene. The top half of the figure contains the genomic coordinates and annotation of the Tn125 transposon sequence retrieved from NCBI (KF702386.1). The lower half of the figure contains the sequencing depth and reads mapped to the corresponding regions. Visualization created with IGV [29]

showed that the majority of the putative plasmid contig carrying bla_{NDM-1} aligned to regions on the larger CP038501.1 plasmid (albeit with several rearrangements), except for the region containing the sequence that aligned to the Tn125 transposon as illustrated in Fig. 2. The three smaller plasmid matches all corresponded to parts of the Tn125 transposon (results not shown).

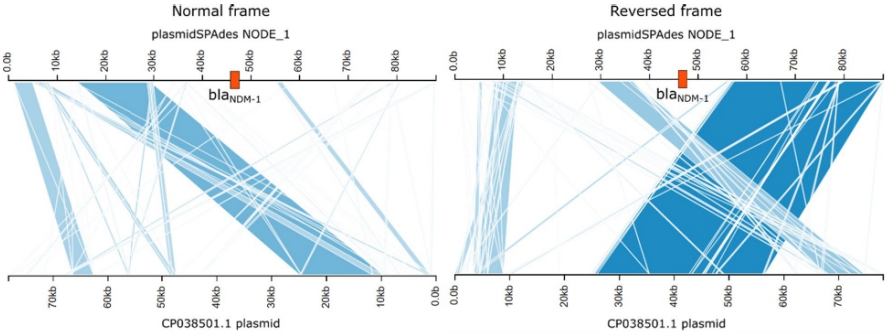


Fig. 2 Alignment of the putative plasmid containing bla_{NDM-1} against the CP038501.1 plasmid. The figure illustrates the putative plasmid generated with plasmidSPAdes aligned to the CP038501.1 plasmid that was detected as best match with PLSDB. The location of bla_{NDM-1} on the putative plasmid is indicated in orange. High-quality alignments between the putative plasmid and CP038501.1 are indicated in blue (with darker blue representing higher BLAST bit scores). The left and right plots illustrate the alignments in normal frame (plus strand/plus strand) and reverse frame (plus strand/minus strand), respectively. Visualization created with Kablammo [30]

Discussion and conclusion

To the best of our knowledge, we present the first description of a NDM-1 producing *A. baumannii* isolated in Benin. In particular, we detected the Tn125 transposon that harbors the $bla_{\text{NDM-1}}$ gene on a contig of 88,063 bp. Because this contig was generated with plasmidSpades and a search against PLSDB demonstrated high similarity to the CP038501.1 plasmid previously reported in *A. baumannii* but not carrying the $bla_{\text{NDM-1}}$ gene, our results indicate that most likely a plasmid similar to CP038501.1 obtained the $bla_{\text{NDM-1}}$ gene through the integration of the Tn125 transposon. In particular, the horizontal transfer capacity of this transposon in relationship with genes encoding NDM has been previously demonstrated within *Acinetobacter* and also other bacteria [24, 25]. Moreover, the strain showed an XDR pattern with resistance against several other antibiotics confirmed both by phenotypical testing and the detection of several other resistance genes, thereby limiting therapeutic options. Accordingly, *A. baumannii* clinical isolates usually exhibit multidrug resistance phenotypes, facilitating their persistence in hospital settings [26]. The ease of availability of antibiotics is probably one of the biggest contributors to antibiotics resistance. Especially in developing countries, there is little regulation on the retail of pharmaceuticals. Moreover, the strain harbored several virulence genes related to biofilm formation. Biofilm formation in *A. baumannii* has been suggested to decrease the diffusion of drugs through the bacterial cells leading to multidrug resistance and also aids the strong survival ability *of A. baumannii* in harsh environments [26].

Low- and middle-income countries suffer a lack of infrastructure and resources to monitor AMR and perform optimal antibiotic treatment. For instance, cefazolin is the recommended antibiotic for surgical antimicrobial prophylaxis (SAP), but unfortunately this antibiotic is unavailable in Benin. The use of broad spectrum cephalosporins such as ceftriaxone in SAP is even more likely to induce resistance than cefazolin and other widely used surgical prophylactic drugs. Treatment options are limited for patients infected with XDR strains, increasing the severity of such infections. In this case, the XDR strain was only susceptible to amikacin, ciprofloxacin and colistin. Amikacin is however only available for one case out of two in Benin. Clinicians are often forced to buy it from neighboring countries such as Nigeria or Togo. Novel treatments effective against NDM-producing *A. baumannii* isolates are limited, and reduced activity was observed for strains that produced particular types of other beta-lactamases such as oxacillinases [27]. Moreover, Benin does not have the necessary quality assurance mechanisms to ensure that antibiotics being supplied are of high quality. Knowledge of the occurrence of NDM-1 producing bacteria may encourage pharmaceutical companies and the Ministry of Health to facilitate the provision of 'last-resort' antibiotics. Innovative therapeutic strategies such as bacteriophage therapy and monoclonal antibodies, or soon-to-be commercially available antibiotics such as plazomicin or cefiderocol, should also be considered for future use [27].

The threat of increasing antibiotics resistance is not limited to *A. baumannii*. For instance, methicillin-resistant *Staphylococcus aureus* and extended-spectrum beta-lactamase producing organisms are also frequently detected in certain public teaching hospitals. Our laboratories need to be better resourced so that they can deliver antibiotics susceptibility information. This includes equipment, consumable resources and their supply chain, as well as development of the necessary human resources to perform tests, store, curate and disseminate data [28]. Unfortunately, phenotypic detection of carbapenemase-producing bacteria is not routinely performed. The immunochromatographic RESIST-3 O.K.N. ICT test that can detect the presence of NDM enzymes, represents a cost-effective alternative to costlier and less widely available characterization methods that rely on molecular amplification.

In conclusion, the isolation of this XDR *A. baumannii* strain containing genes encoding NDM-1 and other beta-lactamases is worrying. The presence of $bla_{\text{NDM-1}}$ located on the Tn125 transposon integrated in a plasmid represents a serious threat because of its potentially high horizontal transferability. Carbapenemase-producing XDR bacteria can be fatal in resource limited countries where therapeutic antibiotic options are restricted. Through this study, we want to alarm the official and governmental agencies to contribute to strengthening laboratory services and equipment at both the local and national level, and urge them to make arrangements for the provision of drugs such as avibactam combinations in Benin. Additionally, further efforts to characterize the molecular structure of this plasmid will aid to assess its transferability and hence risk of spread in the overall population, as well as benefit developing targeted detection methods for surveillance at the population level.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-020-00411-w.

Additional file 1. Alignment results for all detected antimicrobial resistance gene (NCBI-AMR).

Additional file 2. Virulence factor database (VFDB) of *A.baumannii* NDM-1.

Abbreviations
EUCAST: European Committee on Antimicrobial Susceptibility Testing; MDR: Multidrug-resistant; NDM-1: New-Delhi metallo-beta-lactamase-1; VFDB: Virulence factor database; ST: Sequence type; XDR: Extensively drug-resistant; PLSDB: Plasmid database.

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Author's contributions
CY, OD, FVB, DA and AS conceived and designed the study. BB, KV, NR, SDK and KM designed and performed the WGS and bioinformatics analysis. CY, BB and KV wrote the original draft of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Generated WGS data in this study have been submitted to NCBI SRA as BioProject PRJNA624101. The Additional file 2 consists out of detailed alignment results for all detected antimicrobial resistance ('gene_detection-NCBI_AMR.zip') and virulence factor genes ('gene_detection-VFDB_full.zip').

Ethics approval and consent to participate
The study was approved by the institutional review board of the health faculty (FSS Benin): 012-19/UAC/FSS/CER-SS.

Consent for publication
Written informed consent was taken from the patient for publication of this case report and any accompanying images.

Competing interests
The authors declare that they have no competing interests.

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Whole-Genome Sequencing-Based antimicrobial resistance characterization and phylogenomic investigation of 19 multidrug-resistant and extended spectrum beta-lactamase-positive *Escherichia coli* strains collected from hospital patients in Benin in 2019

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Whole-Genome Sequencing-Based Antimicrobial Resistance Characterization and Phylogenomic Investigation of 19 Multidrug-Resistant and Extended-Spectrum Beta-Lactamase-Positive *Escherichia coli* Strains Collected From Hospital Patients in Benin in 2019

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The increasing worldwide prevalence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* constitutes a serious threat to global public health. Surgical site infections are associated with high morbidity and mortality rates in developing countries, fueled by the limited availability of effective antibiotics. We used whole-genome sequencing (WGS) to evaluate antimicrobial resistance and the phylogenomic relationships of 19 ESBL-positive *E. coli* isolates collected from surgical site infections in patients across public hospitals in Benin in 2019. Isolates were identified by MALDI-TOF mass spectrometry and phenotypically tested for susceptibility to 16 antibiotics. Core-genome multi-locus sequence typing and single-nucleotide polymorphism-based phylogenomic methods were used to investigate the relatedness between samples. The broader phylogenetic context was characterized through the inclusion of publicly available genome data. Among the 19 isolates, 13 different sequence types (STs) were observed, including ST131 ($n = 2$),

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ST38 ($n = 2$), ST410 ($n = 2$), ST405 ($n = 2$), ST617 ($n = 2$), and ST1193 ($n = 2$). The $bla_{CTX\text{-}M\text{-}15}$ gene encoding ESBL resistance was found in 15 isolates (78.9%), as well as other genes associated with ESBL, such as $bla_{OXA\text{-}1}$ ($n = 14$) and $bla_{TEM\text{-}1}$ ($n = 9$). Additionally, we frequently observed genes encoding resistance against aminoglycosides [*aac-(6')-Ib-cr*, $n = 14$], quinolones (*qnrS1*, $n = 4$), tetracyclines [*tet(B)*, $n = 14$], sulfonamides (*sul2*, $n = 14$), and trimethoprim (*dfrA17*, $n = 13$). Nonsynonymous chromosomal mutations in the housekeeping genes *parC* and *gyrA* associated with resistance to fluoroquinolones were also detected in multiple isolates. Although the phylogenomic investigation did not reveal evidence of hospital-acquired transmissions, we observed two very similar strains collected from patients in different hospitals. By characterizing a set of multidrug-resistant isolates collected from a largely unexplored environment, this study highlights the added value for WGS as an effective early warning system for emerging pathogens and antimicrobial resistance.

Keywords: *Escherichia coli*, whole-genome sequencing, extended-spectrum beta-lactamases, antimicrobial resistance, Benin

INTRODUCTION

Multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing bacteria pose a growing clinical and public health risk (Pitout and Laupland, 2008). In 2017, the World Health Organization (WHO) listed ESBL-producing *Enterobacterales*, including *E. coli*, as pathogens of critical priority for research and development of antibiotics (Asokan et al., 2019). ESBLs confer resistance to penicillins; to first-, second-, third-, and fourth-generation cephalosporins; and to aztreonam (but not to the cephamycins or carbapenems; Brolund, 2014). ESBL genes are commonly observed in association with genes that confer resistance to other classes of antibiotics, resulting in multidrug resistance (Harbarth et al., 2015). Additionally, they are easily transferred between bacteria through horizontal gene transfer (Brolund, 2014).

Two classification systems for beta-lactamases are currently in use. The most widely used is the Ambler molecular classification (Hall and Barlow, 2005), which defines four molecular classes of beta-lactamases based on amino-acid homology (A, B, C, and D). The most prevalent ESBLs belong to class A, which includes the Cefotaximase-Munich (CTX-M), Temoneria (TEM), and Sulfhydryl Variable (SHV) families.

Hospital-acquired infections of ESBL-producing *Enterobacterales* were first described in 1983 (Bradford, 2001), where resistance arose from point mutations in plasmid-mediated genes encoding beta-lactamase enzymes. These enzymes include TEM-1, TEM-2, and SHV-1, as well as CTX-M, which later became predominant worldwide (Hall and Barlow, 2005). Studies have shown that CTX-M-15-producing *E. coli* comprises one of the most prevalent ESBL-producing *Enterobacterales* (Cantón et al., 2012; Harbarth et al., 2015; Nwafia et al., 2019) and that the global dissemination of ESBL-producing *E. coli* is associated with specific clones mainly assigned to sequence types 131 (ST131) and 405 (ST405; Naseer and Sundsfjord, 2011). As these resistant strains are associated with higher mortality rates, extended hospital stays, and higher health costs, the burden of these infections is enormous.

Since only a few molecular typing studies have examined *E. coli* related infections in hospitals in low- and middle-income countries (LMICs), such as Benin, limited information is available on the subtypes that cause infections and their transmission dynamics in LMICs. Genotypic antimicrobial resistance evaluation has been performed mainly by polymerase chain reaction (PCR), which often fails to capture the full complexity of antimicrobial genetic structures (Anago et al., 2015; Koudokpon et al., 2018). In recent years, whole-genome sequencing (WGS) has increasingly been used to examine drug-resistant commensal and pathogenic *E. coli*, providing more complete insight into the genetic structures associated with the evolution of multidrug resistance (MDR) and transmission dynamics (Punina et al., 2015). In Benin, this information is currently lacking, as WGS-based studies are rarely performed. Therefore, we performed WGS on 19 ESBL-positive strains of clinical *E. coli* isolated from surgical site infections from patients in four public hospitals and characterized their phenotypic and genotypic AMR profiles, and phylogenomic diversity.

MATERIALS AND METHODS

Study Overview and Ethics Consent

This study was part of a larger project (Multidisciplinary Strategy for Prevention and Infection Control: MUSTPIC) conducted from April 2018 to January 2020 to explore etiological bacteria involved in surgical site infections in six public hospitals in Benin. Overall, 229 isolates were collected in the context of this project. This study was approved by the Ethics Committee of the Faculty of Health Sciences (FSS, Benin) under reference number: 012-19/UAC/FSS/CER-SS. Written informed consent was obtained from each participant before enrolment into the study.

Sample Collection and Species Identification

Samples were originally collected as wound swabs between January 2019 and January 2020 from patients that had consented to participate. We included the obstetric (particularly caesarean sections) and gastrointestinal wards at four of these six public hospitals in Benin. To maintain confidentiality, each of these hospitals was randomly assigned a letter A-D. These wards were chosen because caesarean sections are one of the most common surgical procedures, and gastrointestinal wards were present in all the hospitals. All participating hospitals are located in the south of Benin, thereby allowing daily transport from each hospital to the CNHU-HKM laboratory, where all wound swabs were initially collected and analyzed. All identifications were confirmed in Belgium using Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry (Brucker daltonics, Bremen, Germany) employing a threshold of ≥2.0. Out of 229 samples, 49 were identified as *Staphylococcus aureus* and 180 as belonging to the *Enterobacterales*, of which 62 samples were identified as *E. coli*.

Antimicrobial Susceptibility Testing

The 62 isolates identified as *E. coli* were tested for susceptibility toward 16 antimicrobials using the modified Kirby-Bauer disk diffusion method according to the EUCAST guidelines (EUCAST, 2015). *Escherichia coli* strain ATCC 25922 was used for quality control. The following antimicrobial disks (Bio-Rad, Marnes-la-coquette, France) were used amikacin (30 µg), amoxicillin-clavulanic acid (20/10 µg), ampicillin (10 µg), cefepime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), imipenem (10 µg), levofloxacin (5 µg), meropenem (10 µg), piperacillin (100 µg), tobramycin (10 µg), and trimethoprim + sulfamethoxazole (25 µg). Isolates that were resistant to at least three different classes of antimicrobials were considered as MDR (Magiorakos et al., 2012). Antimicrobial classes for 15 of the 16 tested antibiotics were assigned based on the ResFinder classification.[1] Levofloxacin was missing from the aforementioned classification and manually assigned to fluoroquinolones. The ESBL phenotype was identified by the double-disk synergy method on Mueller Hinton agar using ceftazidime and ceftriaxone placed at 20 mm apart from a disk containing amoxicillin and clavulanic acid. A clear-cut enhancement of the inhibition in front of either ceftazidime and/or ceftriaxone disks toward the clavulanic acid-containing disk (also called "champagne-cork" or "keyhole") was interpreted as positive for ESBL production. Additionally, isolates were tested for the presence of the beta-lactamase *ampC* phenotype using the cefoxitin-cloxacillin disk diffusion test, as described previously (Thean et al., 2009). Out of the 62 *E. coli* isolates, 43 exhibited ESBL-positive phenotypes. A subset of 19 isolates was then selected for WGS analysis based on their phenotypic resistance out of these ESBL-positive phenotypes in order to increase the chance of inferring transmission dynamics for this subset of strains, while also trying to retain samples highly resistant to different antibiotics and originating from the four different hospitals. An overview of the selected isolates is provided in **Table 1**.

[1]https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/antibiotic_classes.txt

Whole-Genome Sequencing

DNA extraction for selected isolates was done by using the Qiagen universal Biorobot (Limburg, Netherlands) according to the manufacturer's instructions. Isolate sequencing libraries were created using Nextera XT DNA library preparation (Illumina, San Diego, CA) according to the manufacturer's instructions, and subsequently underwent Illumina sequencing using the MiSeq V3 chemistry (Illumina, San Diego, CA) for production of 2 × 250 bp paired-end reads. All sequencing data have been submitted to SRA (Leinonen et al., 2011) under BioProject PRJNA701417, and individual accession numbers are provided in **Supplementary Table S1**.

WGS-Based Isolate Characterization

All WGS reads were trimmed and *de novo* assembled using SPAdes (Bankevich et al., 2012) as described in Bogaerts et al. (2021). Detection of AMR genes was performed as described in Bogaerts et al. (2019) using the sequences from the NCBI NDARO database (downloaded on March 27, 2020; Feldgarden et al., 2019). Hits with less than 90% sequence identity or less than 90% target coverage were filtered out. A local installation of PointFinder (checked out from BitBucket on February 27, 2019; Zankari et al., 2017) was used to detect mutations associated with AMR. Phenotypic resistance was predicted when at least one gene or mutation was detected with resistance to the corresponding antibiotic(s). Isolates were then typed using the methodology described in Bogaerts et al. (2019) using the classic MLST and cgMLST schemes from EnteroBase (Zhou et al., 2020), downloaded on the September 6, 2020. The putative genetic origin (i.e., chromosomal or plasmidic) of the detected resistance genes was evaluated by classifying the corresponding contigs using the "mob-recon" command from MOB-suite 3.0.0 with default settings (Robertson and Nash, 2018).

The samples were screened for contaminants using Kraken 2 2.0.7 (Wood and Salzberg, 2014) with default parameters and an in-house database containing all NCBI RefSeq Genome entries with the "Complete Genome" assembly level (database accessed February 18, 2019; O'Leary et al., 2016) accession prefixes NC, NW, AC, NG, NT, NS, and NZ of the following taxonomic groups: archaea, bacteria, fungi, human, protozoa, and viruses. Samples for which Kraken 2 classified more than 5% of reads to a species other than *E. coli* were considered contaminated. For these samples, reads classified as the contaminant species (up to the genus level) were removed before the *de novo* assembly. All subsequent analyses were performed on these cleaned assemblies.

Comparison of Phenotypic and Genotypic Predicted AMR Susceptibility

Correspondence with observed phenotypic resistance was evaluated using the following definitions: true positive (TP)

TABLE 1 | Overview of the 19 samples sequenced in this study.

Sample name	Hospital	Ward	Collection date	Surgical intervention	Patient age	Patient sex
s_12116	D	GI	October 15, 2019	Peritonitis	18	Female
s_12117	D	GI	October 15, 2019	Peritonitis	18	Male
s_12155	C	M	October 16, 2019	Caesarean	26	Female
s_12301	C	M	October 20, 2019	Caesarean	35	Female
s_12414	B	GI	October 22, 2019	NA	36	Male
s_12479	C	M	October 23, 2019	Caesarean	22	Female
s_12480	D	M	October 23, 2019	Caesarean	34	Female
s_12845	D	GI	October 31, 2019	Appendicitis	18	Male
s_12849	D	GI	October 31, 2019	Appendicitis	21	Male
s_13022	D	GI	November 08, 2019	Evisceration	55	Female
s_13150	A	GI	November 08, 2019	Peritonitis	40	Female
s_13959	A	GI	November 26, 2019	Appendicitis	52	Female
s_13987	B	GI	November 26, 2019	Appendicitis	23	Female
s_3117	A	M	November 12, 2019	Caesarean	32	Female
s_316	D	GI	November 28, 2019	Appendicitis	23	Male
s_317	D	GI	June 06, 2019	Appendicitis	33	Male
s_4294	D	GI	June 04, 2019	NA	36	Male
s_6558	D	GI	May 30, 2019	NA	35	Male
s_90	A	M	November 28, 2019	Caesarean	28	Female

M, maternal; GI, gastro-internal; and NA, Not available.

and false negative (FN) as cases with phenotypic resistance to an antibiotic where the WGS-based workflow predicted resistance and susceptibility, respectively, and true negative (TN) and false positive (FP) as cases with phenotypic susceptibility to an antibiotic where the WGS-based workflow predicted susceptibility and resistance, respectively. This comparison was limited to six of the tested antibiotics for which the NDARO database or PointFinder databases contained at least one entry. For the remaining 10 antibiotics, the WGS workflow could only perform predictions at the level of the parent class since the database only contains information up to this level. For example, the NDARO database contains genes annotated as associated with resistance to cephalosporins, but it does not provide information on resistance to specific members of this class, such as cefepime or cefotaxime.

Phylogenomic Analysis

The relatedness between samples from this study was first determined by constructing phylogenies based on cgMLST results. Allele matrices were filtered by removing samples with less than 90% of loci detected and afterward removing loci detected in less than 90% of samples. Minimum spanning trees (MSTs) were then constructed from the filtered allele matrices using GrapeTree 2.2 (Zhou et al., 2017) with the "method" option set to "MSTreeV2." The phylogeny was visualized and annotated in the web-based iTOL platform (Letunic and Bork, 2019). The broader phylogenomic context of samples was also investigated by including all assemblies assigned to any of the STs detected in our samples collected after 2018 in Africa or Europe, retrieved from EnteroBase on January 19, 2021. Initially, only samples from Africa were collected, but since only very few samples matched these criteria, the dataset was extended with samples from Europe. Separate MSTs were then constructed for the six STs with at least two samples from this study (ST38, ST131, ST405, ST410, ST617, and ST1193) using the same methodology as described above. Additionally, Single-nucleotide polymorphism (SNP) addresses were determined using SnapperDB 1.0.6 (Ashton et al., 2015) and PHEnix v1.4.1,[2] as described previously (Nouws et al., 2020), for all newly sequenced samples and genomes retrieved from EnteroBase with available WGS Illumina data. An additional filtering step was introduced to remove SNPs located in regions of the reference genomes flagged as prophages by PHAST (Zhou et al., 2011) with default settings. The SNP address is a strain level 7-digit nomenclature based on the number of pairwise SNP differences. Each digit represents the cluster membership for the given number of SNP differences, starting (right to left) with 0 (i.e., no SNP differences) to 5, 10, 25, 50, 100, and 250. Isolates sharing the same cluster digit differ by fewer than the corresponding number of SNPs (Ashton et al., 2015). An example of the SNP address methodology is provided in **Supplementary Figures S2–S3**. The minimum required average mapping depth was lowered to 25x to ensure all samples from this study could be analyzed. Reference genomes from the corresponding ST were selected randomly from all genomes available in EnteroBase (**Supplementary Table S2**) for the respective STs but only considering complete genomes. Sequences with a "plasmid" annotation in the header were removed from the reference genomes before running the SNP typing analysis. AMR prediction for public genomes was performed as described above (phenotypic AMR susceptibility information was not available for these genomes).

[2]https://github.com/phe-bioinformatics/PHEnix

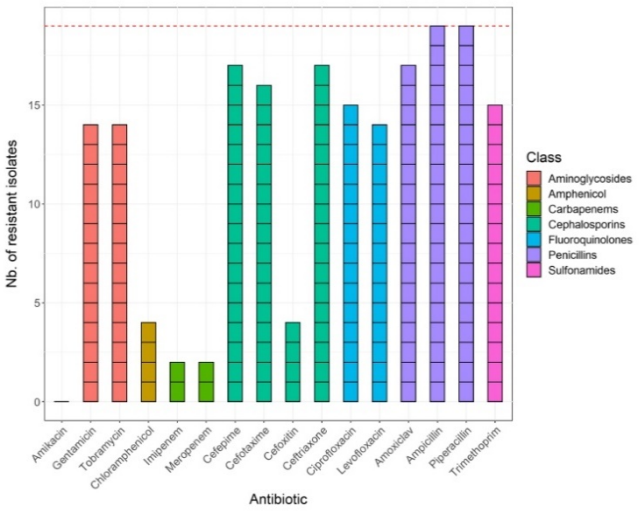


FIGURE 1 | Results of phenotypic antimicrobial susceptibility testing of the 19 sequenced *E. coli* isolates. Bars are colored according to the corresponding class of antibiotics. The red horizontal line corresponds to the total number of isolates ($n = 19$). Trimethoprim + sulfamethoxazole was abbreviated to trimethoprim and amoxicillin + clavulanic acid to amoxiclav.

RESULTS

Phenotypic Antimicrobial Susceptibility Testing

An overview of AMR susceptibility testing results of the 19 selected ESBL-positive isolates is provided in **Figure 1**. All 19 isolates exhibited resistance to ampicillin and piperacillin. Most samples also displayed low susceptibility to amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftriaxone, ciprofloxacin, gentamycin, levofloxacin, tobramycin, and trimethoprim + sulfamethoxazole. Four isolates showed resistance to cefoxitin and chloramphenicol, while two isolates showed resistance to imipenem and meropenem. None of the tested isolates exhibited resistance to amikacin.

Isolate Characterization Based on WGS Data

Overviews of read trimming and *de novo* assembly statistics for the sequenced isolates are provided in **Supplementary Tables S3** and **S4**, respectively. Assembly statistics indicated generally high quality, with a median total assembly length of 5,064,374 bp and N50 of 168,657 bp. Samples s_316 and s_4294 were outliers with a larger total assembly length (9,568,828 bp and 8,564,805 bp, respectively) and smaller N50 (40,329 bp and 69,875 bp, respectively). Kraken 2 analysis indicated that these samples also contained reads from *Acinetobacter* besides *E. coli*, likely explaining their increased cumulative assembly lengths and lower N50. Notwithstanding, only a minor fraction of the total reads was identified as *Acinetobacter*, indicating that enough *E. coli* reads were present in both samples to be retained for further analysis. Reads classified as *Acinetobacter* were therefore removed before performing a new *de novo* assembly, which was used for further analysis for these two samples. We performed additional analysis to confirm that the contamination indicated by Kraken 2 in these two samples was not due to a plasmid, but rather due to the presence of an *Acinetobacter baumannii* strain, for which results are provided in the **Supplementary Material**. The 19 samples were classified into 13 different STs: ST10 (n: 1), ST38 (n: 2), ST127 (n: 1), ST131 (n: 2), ST167 (n: 1), ST354 (n: 1), ST405 (n: 2), ST410 (n: 2), ST569 (n: 1), ST617 (n: 2), ST648 (n: 1), ST1193 (n: 2), and ST2659 (n: 1), as indicated in **Supplementary Table S5**.

Genotypic WGS-Based AMR Characterization

We detected several beta-lactam resistance genes across our samples, including $bla_{\text{CTX-M-15}}$, $bla_{\text{CMY-42}}$, bla_{EC}, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-181}}$, and $bla_{\text{TEM-1}}$. We also detected the *aph(3″)-Ib* (68.4%, n = 13) and *aph(6)-Id* (73.7%, n = 14) genes associated with resistance

to streptomycin, in the large majority of samples, as well as the *aac(6′)-Ib-cr* gene (73.7%, n = 14), responsible for a reduction in ciprofloxacin activity. Four different fluoroquinolone resistance determinants were observed [*qnrS1*, *qepA8*, *qepA4*, and *aac(6′)-Ib-cr*]. Other AMR genes that were detected were associated with trimethoprim resistance (*dfrA8*, *dfrA12*, *dfrA14*, and *dfrA17*), macrolide resistance *mph(A)*, sulphonamide resistance (*sul1*, *sul2*), tetracycline resistance *tet(A)* and *tet(B)*, and phenicol resistance (*catA1*). Additionally, 16 isolates carried at least one nonsynonymous mutation in the housekeeping genes *parC* S80I, *gyrA* S83L, *gyrA* D87N, and *parE* S458A, which are associated with resistance to fluoroquinolones.

A complete overview of the detected AMR genes, point mutations, and predicted phenotypes with WGS is provided in **Supplementary Table S6**. The majority of resistance genes was located on contigs with a plasmid origin predicted by MOB-suite, as indicated in **Supplementary Table S7**. Resistance was most commonly predicted for cephalosporins (all 19 samples), tetracycline ($n = 18$), and quinolones ($n = 17$).

Comparison of Phenotypic and Genotypically Predicted AMR Susceptibility

Full results for comparing genotypically predicted and observed phenotypic AMR profiles are provided in **Supplementary Table S8**. Out of 62 resistant phenotypes, 60 were predicted correctly (i.e., TP). Sample s_12117 was incorrectly predicted as sensitive to gentamicin, and sample s_4294 to tobramycin. While sample s_4294 was flagged as contaminated by our Kraken 2 analysis, we confirmed that these mismatches were not caused by the additional filtering step because no associated genes were presented in unfiltered assemblies (results not shown). Out of 52 susceptible phenotypes, 34 were predicted correctly (i.e., TN). The large majority of the 18 FP was limited to amikacin ($n = 14$) and caused by the detection of the *aac(6′)-Ib* gene in susceptible samples. The presence of this gene likely does not suffice for phenotypic resistance at the tested dosage (see also section "Discussion"). Similarly, for resistance to ciprofloxacin, the two FP samples, s_13022 and s_90, only carried the *gyrA* S83L mutation, while TP samples also carried multiple other mutations, including *gyrA* D87N, *parC* S80I, and *parE* L416F. Combining the predictions across all samples resulted in WGS-based prediction accuracy of 82.5%, precision of 76.9%, sensitivity of 96.8%, and specificity of 65.4%. When discarding the results for amikacin, the accuracy increased to 93.6%, precision to 93.8%, sensitivity to 96.8%, and specificity to 87.5%.

Phylogenomic Analysis

More than 90% of cgMLST loci were detected in all samples from this study. The resulting MST is shown in **Figure 2**. Generally, considerable phylogenetic differences were observed, with large branch lengths between isolates assigned to different STs. Six groups of two isolates with the same sequence type were found, i.e., ST38, ST131, ST405, ST410, ST617, and ST1193. Samples assigned to ST38 and ST617 differed by 190 and 34 cgMLST alleles, respectively. These relatively large genetic differences indicate that epidemiological links between these cases are unlikely, and the corresponding phylogenies are therefore not discussed in the main manuscript, but a description of the phylogenomic context for these samples can be found in the **Supplementary Material**. Results for the isolates assigned to the other four STs are described in detail below, in order of decreasing phylogenomic similarity based on cgMLST distance (i.e., cluster with most closely related samples from Benin first). SNP distance matrices for the isolates included in the SNP analysis are provided in **Supplementary Tables S9–S12**.

ST405 Cluster

The phylogeny for this ST was constructed using 24 additional genomes retrieved from EnteroBase that matched the criteria listed in the Material and Methods. No additional samples from Africa were available. The MST based on cgMLST for this ST is shown in **Figure 3A**, along with their corresponding SNP addresses. The two samples from this study in this cluster, s_317 and s_3117, were identical based on both cgMLST and SNP typing and exhibited identical phenotypic and genotypic AMR profiles. These results indicate that both isolates are identical, despite being obtained from different patients at different hospitals with 16 days in between. Phenotypic testing of these samples revealed resistance to 8 of the 16 tested antibiotics. The most similar isolate from EnteroBase was ESC_RB8807_AS, collected in Switzerland in 2019, which differed by three cgMLST loci, but WGS read data were not available for this sample rendering it impossible to investigate relatedness based on SNPs.

ST131 Cluster

The phylogeny for this ST was constructed using an additional 74 genomes retrieved from EnteroBase, which only contained two additional samples from Africa. The MST based on cgMLST and SNP addresses for this ST are shown in **Figure 3B**. The two samples from this study, s_13987 and s_13959, differed by only three cgMLST loci and had identical phenotypic and similar genotypic AMR profiles (the beta-lactamase gene bla_{TEM} was present in only s_13959) but still differed with between 100 and 250 SNPs according to their SNP address. A point source outbreak is therefore unlikely with this amount of observed variation, despite their close time of isolation (both samples were obtained on the same day). Additionally, both samples were obtained in different hospitals. Compared to the samples retrieved from EnteroBase, ESC_JB0483AA_AS isolated in Spain in 2018 was the most closely related isolate but still differed by 47 cgMLST loci. Predicted AMR susceptibility to different antibiotics was widespread in this cluster but did not correlate to the typology of the tree.

ST1193 Cluster

The phylogeny for this ST was constructed using six additional samples retrieved from EnteroBase. No additional samples from Africa were available. The MST based on cgMLST and SNP addresses for this ST are shown in **Figure 3C**. The two samples

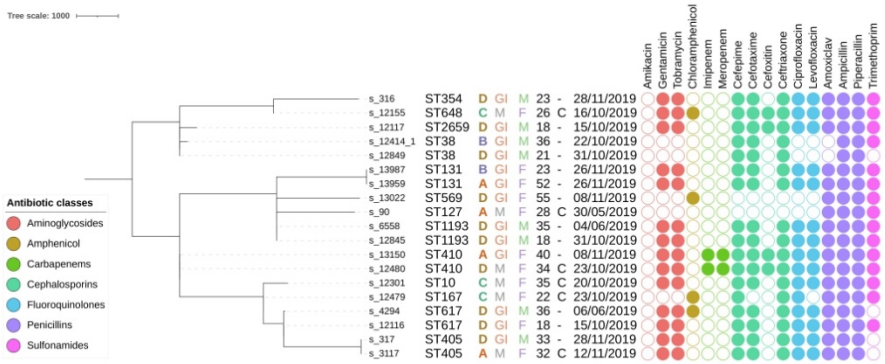


FIGURE 2 | Minimum spanning tree containing all *E. coli* samples sequenced in this study based on cgMLST results. The scale bar expresses the number of allele differences. Annotations are (from left to right) sample name, sequence type (based on classical MLST), hospital letter code, hospital ward, patient gender, patient age, surgical intervention, isolation date, and phenotypically determined AMR susceptibility (a filled circle indicates phenotypic resistance to the corresponding antibiotic). Circles are colored according to the class of the antibiotic as indicated in the legend in the bottom left corner. Abbreviations: maternal (M), gastrointestinal (GI), male (M), female (F), and caesarian (C).

from this study, s_6558 and s_12845, differed by five cgMLST alleles and between 50 and 100 SNPs and exhibited identical phenotypic and genotypic AMR profiles. Both samples were obtained from the same hospital with several months in between (as seen in ST617, described in the **Supplementary Results**). Similar to the two samples from the ST131 cluster, a point source outbreak appears therefore unlikely.

ST410 Cluster

The phylogeny for this ST was constructed using 23 additional samples retrieved from EnteroBase. Only one additional sample from Africa was available. The MST based on cgMLST and SNP addresses for this ST are shown in **Figure 3D**. The two samples from this study, s_12480 and s_13150, differed by 15 cgMLST alleles and between 50 and 100 SNPs. Compared to the samples from this study assigned to other STs, the number of allele differences was quite large compared to the number of SNP differences. Additional screening against inter- and intraspecies contamination did not reveal issues with either sample (results not shown). Both samples exhibited identical phenotypic and genotypic AMR profiles, which both indicated very high rates of resistance. Given the sizable genomic distance between both samples, and the fact they were collected in different hospitals, an epidemiological link between both samples appears unlikely. The only publicly available sample from Africa for this cluster, ESC_FB9431AA_AS, was collected in Nigeria (a neighboring country of Benin) in 2018 but was quite distant from the samples from Benin. The most similar sample from EnteroBase was ESC_FB0885AA_AS, which differed 13 alleles to s_12480 and 12 alleles to s_13150. Since no read data were available for this sample, the SNP address could not be determined.

DISCUSSION

To the best of our knowledge, this is the first study that employs WGS to investigate AMR profiles and phylogenomic relatedness among clinical *E. coli* isolated in Benin.

All of the 19 selected *E. coli* isolates showed phenotypic resistance to at least four of the 16 phenotypically tested antibiotics, and 14 samples showed resistance to 10 or more. These observations are in line with our previous study conducted in 2019 in the same hospitals, in which we reported 208 MDR-positive samples on a total of 229 aerobic bacteria (90.8%; Yehouenou et al., 2020). High resistance rates were observed in all isolates, especially to aminoglycosides, cephalosporins, penicillins, quinolones, and trimethoprim + sulfamethoxazole. Two isolates were also resistant to carbapenems, which was also observed recently in Nigeria (Aworh et al., 2021), and four were resistant to amphenicol. The high degree of AMR in our study is not surprising, as these antibiotics (ceftriaxone, ciprofloxacin, and beta-lactamases) are easily accessible and commonly used in Benin for therapeutic purposes and very few antimicrobial stewardship programs are in place.

The $bla_{\text{CTX-M-15}}$ gene is the most frequently reported gene that encodes for CTX-M enzymes in ESBLs (Zhao and Hu, 2013) and was observed in 15 isolates (78.9%) in this study. Four of those isolates also harbored *ampC* genes (bla_{CMY}) and other beta-lactamase genes ($bla_{\text{TEM-1}}$). AmpC beta-lactamases are cephalosporinases that are poorly inhibited by clavulanic acid. The co-occurrence of multiple AmpC beta-lactamases was previously reported in *E. coli* in Tunisia (Chérif et al., 2016) and various other studies, especially in isolates collected in African countries (Ribeiro et al., 2016; Sonda et al., 2018; Irenge et al., 2019; Jesumirhewe et al., 2020). These findings suggest that genes

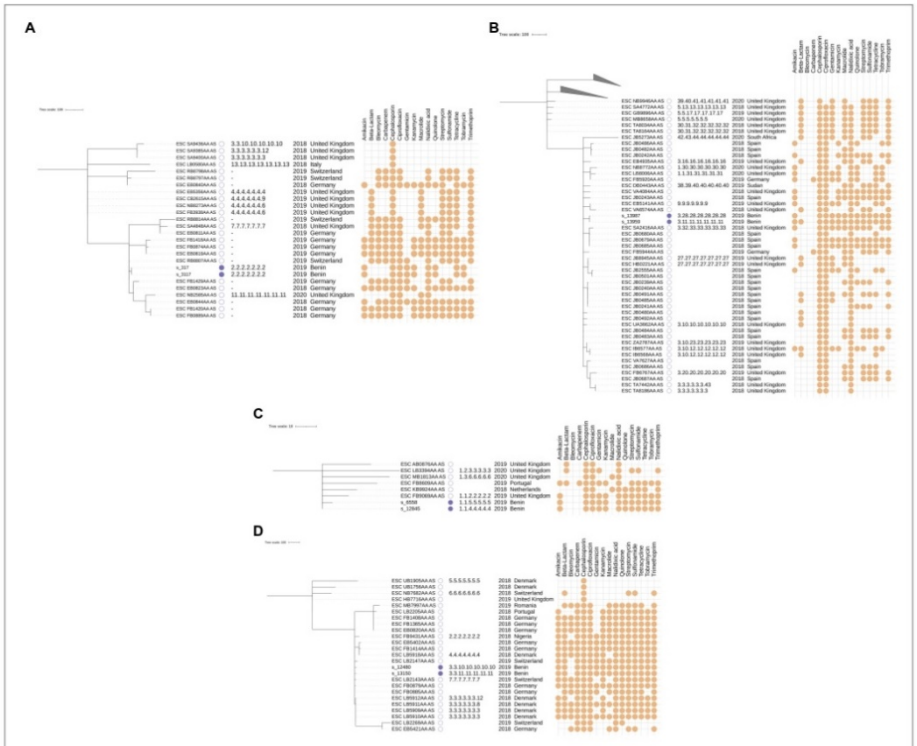


FIGURE 3 | cgMLST-based minimum spanning trees annotated with SNP typing results for samples classified as ST405 **(A)**, ST131 **(B)**, ST1193 **(C)**, and ST410 **(D)**. The scale bar and branch lengths are expressed as the number of allele differences. Annotations are (from left to right) as: sample name, sample origin (this study with a filled blue box or EnteroBase with an empty box), SNP address, isolation year, isolation country, and predicted AMR susceptibility based on genotypic AMR prediction. Samples with missing SNP addresses did not have publicly available Illumina paired-end data or were filtered out because their coverage was too low. Predicted AMR susceptibility is shown for the 16 antibiotics that were most often predicted (full results are provided in **Supplementary Table S5**). The top two clades in subfigure **B** were collapsed for visual clarity and contain 18 and 9 isolates, respectively.

from the CTX-M family are currently replacing SHV and TEM in *Enterobacterales*, as reported previously (Camacho et al., 2009).

In our study, 78.9% of ESBL-producing strains ($n = 15/19$) exhibited phenotypic resistance to quinolones, consistent with the previously reported association between ESBL production and quinolone resistance in *Enterobacterales* (Robicsek et al., 2006). This association might be explained by co-selection between ESBL and other AMR genes when exposed to various antimicrobials (Robicsek et al., 2006; Crémet et al., 2011). The *aac(6′)-Ib-cr* was observed frequently and was present in 14 strains. This gene has spread rapidly among *Enterobacterales*, and although conferring only low-level resistance, it may create an environment that facilitates the selection of highly resistant determinants, especially in organisms harboring other quinolone resistance determinants (Roy et al., 2021).

Out of 62 resistant phenotypes, 60 were correctly genotypically predicted, leading to an accuracy of 82.5%, which is considerably lower than other studies (Kozyreva et al., 2017; Bogaerts et al., 2021). Performance was mainly impacted by lower specificity due to predicted resistances supported only by a single genomic feature, which might not have been sufficient for resistance at the tested dosage. For example, samples s_13022 and s_90 carried the *gyrA* p.S83L mutation, associated with fluoroquinolone resistance, but still were susceptible to the antibiotic. Other samples,

such as s_12116 or s_12117, did exhibit phenotypic resistance but also carried three additional mutations associated with resistance to ciprofloxacin. We assume that a similar effect caused the FP predictions for amikacin. This hypothesis is also supported by the fact that our predictions for this antibiotic matched with the results of the NCBI AMRFinderPlus (Feldgarden et al., 2019) and online ResFinder (Zankari et al., 2012) tools (unpublished results). Accuracy increased to 93.6% when the results for amikacin were removed, in line with the performance obtained in the aforementioned studies.

We used MOB-suite to determine the genetic origin of the detected resistance genes as they are known to be frequently or almost exclusively plasmid-encoded (Brolund, 2014), associated with a higher risk of spreading. This analysis confirmed that most of the detected AMR genes were found on contigs predicted to be plasmid-encoded. However, this analysis might have suffered from lower accuracy since only short-read data were available. In future investigations, employing long-read sequencing might therefore be a promising alternative because longs reads can substantially facilitate predicting the genomic origin of detected AMR genes (Lemon et al., 2017; Berbers et al., 2020).

We observed 13 different sequence types with six STs (ST131, ST617, ST38, ST1193, ST410, and ST405) occurring twice, consistent with earlier observations in Tanzania where the same six STs were observed in a collection of 38 *E. coli* samples (Sonda et al., 2018). Resistance to cephalosporins and ciprofloxacin has often been reported in strains classified as ST131 (Cagnacci et al., 2008; Aibinu et al., 2012). The presence of other genetic lineages different from ST131 shows the potential for genetic diversification and emergence of new epidemic strains (Xu et al., 2011). In particular, the association of these clones with quinolone resistance, by carrying the often plasmid-encoded *qnr*, or, *aac-(6′)-Ib-cr* genes, is very worrisome due to its potential for further dissemination.

Although some STs (e.g., ST410) were found to contain more AMR determinants than other STs (e.g., ST38), AMR profiles were generally not correlated with the phylogeny (see **Figure 3**), suggesting that horizontal transfer of AMR genes is common, as also shown in numerous other studies on *E. coli* (Rodriguez-Villalobos et al., 2005; Upreti et al., 2018; Nwafia et al., 2019). Nevertheless, some genes have been reported to be associated with particular STs, such as the $bla_{\text{CTX-M-15}}$ gene, which is often present in the ST131 and ST410 isolates (Tegha et al., 2021), as also observed in this study (**Supplementary Table S6**).

We observed a generally large diversity among the analyzed *E. coli* strains circulating in the hospitals in Benin. The large phylogenomic differences between the isolates suggest that the infections were caused by multiple generally not very closely related *E. coli* strains, the notable exceptions being samples s_317 and s_3117, which were completely identical. Within individual hospitals, the two most closely related samples differed by at least five cgMLST alleles, i.e., samples s_12845 and s_6558 that were both isolated in hospital D. This diversity was also reflected in the number of observed STs with most samples belonging to different STs and only six STs that contained two isolates. Investigation of the samples assigned to the same STs indicated at least one ST in which both samples were identical (ST405); two STs with samples that were relatively closely related based on cgMLST, with 15 and 5 cgMLST alleles difference, but still differed by between 50 and 100 SNPs (ST410 and ST1193, respectively); and one ST with samples that were closely related based on cgMLST but still differed by between 100 and 250 SNPs (ST131). The sequenced isolates did therefore not indicate a pattern of hospital-acquired and transmitted infections, since they were either identical but isolated in different hospitals (ST405); relatively related but isolated in different hospitals (ST410); or their overall number of SNPs differed by at least 100 (ST38, ST131, ST617, and ST1193). It should be highlighted that these observations do not imply that no hospital-acquired infections and transmissions took place in Benin in 2019, which would be highly unlikely, given that multiple studies have shown that such transmissions constitute a major problem for nosocomial *E. coli* (Peleg and Hooper, 2010; Matta et al., 2018). Most likely, we did not observe such infections and transmissions because not enough samples were isolated and sequenced. More WGS-based screening is required to fully capture the transmission dynamics of the circulating strains. Nevertheless, we did observe a pattern suggestive of sporadic introductions of diverse strains into hospitals from the community, which corroborates results obtained in Tanzania by Sonda et al. in 2018, who observed 21 different sequence types in a collection of 38 clinical *E. coli*, the most common of which were ST131 and ST10 (Sonda et al., 2018). Jesumirhewe et al. observed ST131, ST405, and ST410 in a collection of 17 clinical *E. coli* isolated in Nigeria (Jesumirhewe et al., 2020). ST131 is the dominant international clinical clone, and ST405 was previously associated with the carriage of ESBLs (Aibinu et al., 2012). ST410 has been reported worldwide in extra-intestinal strains associated with resistance to fluoroquinolones, third-generation cephalosporins, and carbapenems (Roer et al., 2018).

Although a few samples in our study were related to isolates sampled in Europe in the background collection retrieved from EnteroBase, only distant phylogenetic relationships could be observed for most of them. This indicates that the background collection is missing isolates to provide the proper phylogenomic context for the samples in our study. This can be explained by the lack of available WGS data from Africa, which accounted for only a very minor fraction of the total dataset (with only two isolates for ST131 and one for ST410 being available). Consequently, the gap in available data and surveillance for Benin and Africa renders it impossible to study the history of the transmission and spread of these *E. coli* isolates within Benin and in a broader African context, and to investigate the relatively close relationship of some samples with European samples. To better understand the spread and dispersion of AMR and pathogenic *E. coli* in Africa, more extensive monitoring in this region, including by WGS, is required.

CONCLUSION

We report the first WGS-based analysis of AMR and phylogenomics relatedness of 19 clinical ESBL-positive samples collected from Benin in 2019. Although our study was limited by the relatively low number of sequenced samples, this provides a first genomic resource for Benin. In particular, the observed high levels of *E. coli* diversity regarding their antimicrobial resistance genes and sequence types underline the necessity for concerted efforts to routinely screen more bacterial isolates of clinical importance, even in resource-limited settings, such as Benin. Increased WGS-based surveillance will aid to fill the gaps in observed genotypes and help improve our understanding of the transmission dynamics and the prevalence of AMR genes in *E. coli* within Benin, and by extension, Africa. The information generated in this study not only provides updates on AMR at the hospital level but can also serve as a basis in formulating pragmatic antimicrobial stewardship programs and infection control initiatives, and accentuates the need for the competent authorities to provide more resources to study drug-resistant infections circulating in Benin by means of WGS.

DATA AVAILABILITY STATEMENT

The datasets supporting the conclusions of this study have been deposited in the NCBI SRA under BioProject PRJNA633966. Metadata and individual accession numbers are available in the **Supplementary Material**.

AUTHOR CONTRIBUTIONS

CY, OD, FD, DA, and AS conceived and designed the study. BB, KV, NR, SK, and KM designed and performed the WGS and bioinformatics analysis. CY, ET, BB, and KV wrote the original draft of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.752883/full#supplementary-material

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Carbapenem-Resistant Organisms Isolated in Surgical Site Infections in Benin: A Public Health Problem

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Abstract: An alarming worldwide increase in antimicrobial resistance is complicating the management of surgical site infections (SSIs), especially in low-middle income countries. The main objective of this study was to describe the pattern of carbapenem-resistant bacteria in hospitalized patients and to highlight the challenge of their detection in Benin. We collected pus samples from patients suspected to have SSIs in hospitals. After bacterial identification by MALDI-TOF mass spectrometry, antimicrobial susceptibility was performed according to the Kirby–Bauer method. Carbapenem-resistant strains were characterized using, successively, the Modified Hodge Test (MHT), the RESIST-5 O.K.N.V.I: a multiplex lateral flow and finally the polymerase chain reaction. Six isolates were resistant to three tested carbapenems and almost all antibiotics we tested but remained susceptible to amikacin. Four (66.7%) of them harbored some ESBL genes ($bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}1}$). The MHT was positive for *Carbapenems* but not for *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. As surgical antimicrobial prophylaxis, five of the six patients received ceftriaxone. The following carbapenems genes were identified: $bla_{OXA\text{-}48}$ (33.3%, n = 2), bla_{NDM} (33.3%, n = 2) and bla_{VIM} (33.3%, n = 2). These findings indicate a need for local and national antimicrobial resistance surveillance and the strengthening of antimicrobial stewardship programs in the country.

Keywords: carbapenem-resistant organisms; OXA-48; NDM; VIM; surgical site infections; Benin

1. Introduction

Surgical site infections (SSIs) are one of the most frequent types of healthcare-associated infections (HAIs). They can significantly increase both clinical and economic burdens [1], which could be explained by the direct costs of prolonged hospitalization and the increasing rates of antimicrobial resistance [2]. Of particular concern to surgeons are extended-spectrum beta-lactamases (ESBL) and carbapenem resistance in Gram-negative organisms [3]. This is especially the case in resource-limited countries, where constrained health infrastructures and limited antibiotic options mean that their spread would have devasting consequences. Due to the lack of new alternative treatment options, carbapenem resistance in Gram-negative organisms—especially *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*—has thus become a matter of concern worldwide [4].

Resistance to carbapenems can be caused by the production of carbapenemases (class A: *Klebsiella pneumoniae* carbapenems (KPC); class B: metallo-beta-lactamases (NDM, VIM IMP-types); and Ambler class D: oxacillinases (OXA-48)). Their spread is mainly due to plasmids acquisition or other mobile elements, to permeability alterations caused by the loss of porins, and to an overexpressed efflux system [5]. The most critical mechanism underlying resistance to carbapenems is the production of carbapenemases: an enzyme that can hydrolyze almost all beta-lactam antibiotics [6]. Another concern is the broad-spectrum resistance of *P. aeruginosa*, which is mainly due to a combination of four factors: (1) low outer-membrane permeability; (2) the presence of the inducible AmpC chromosomal beta-lactamase; (3) the synergistic action of several multidrug efflux systems; and (4) the presence of transferable resistance determinants, particularly carbapenem-hydrolyzing enzymes [7–9].

The accurate and timely detection of carbapenem-resistant *Enterobacterales (CRE)*, especially carbapenemases-producing *Enterobacterales (CPE)*, is important for the prevention and treatment of such infections [10]. However, despite the therapeutic implications of knowing whether an organism produces carbapenemases [11], most clinical microbiology laboratories still do not yet characterize the mechanism behind carbapenem resistance. Although antimicrobial susceptibility testing (AST) results alone are often enough to support the selection of appropriate antibiotic therapy, it seems necessary to identify the carbapenemases mechanism, particularly when access to AST for newer agents is limited. This is the case with antibiotics such as ceftazidime-avibactam or meropenem-vaborbactam, which are active against some carbapenemases (such as *Klebsiella pneumoniae* carbapenems (KPCs)), but not against others (such as metallo-beta-lactamases {MBLs}, including the New Delhi metallo-beta-lactamases {NDMs}) [12].

Although the spread of carbapenem-producing *Enterobacterales* is now being reported at alarming levels worldwide, very little data are available for Africa, particularly West Africa [11]. In Benin, for example, the lack of a national antimicrobial surveillance system and the resulting shortage of quality data make it difficult to evaluate carbapenem resistance [13]. Given these considerations and the lack of information globally, the purpose of this study was to determine the patterns of carbapenem-non-susceptible organisms isolated in surgical site infections in Benin and to summarize the challenges of their detection.

2. Materials and Methods

2.1. Ethics Statement

This study was part of a larger project entitled "Multidisciplinary Strategy for Prevention and Infection Control" for which ethical approval was obtained from the Faculty of Health Sciences (Cotonou, Benin) under reference number: 012-19/UAC/FSS/CER-SS. Written informed consent was sought from each participant before enrolment in the study.

2.2. Study Design, Bacterial Isolation and Culture

A descriptive cross-sectional study was designed and carried out to determine the epidemiology of surgical site infections in Benin. This study is part of a larger project (Multidisciplinary Strategy for Prevention and Infection Control: MUSTPIC) that was conducted from April 2018 to January 2020 to explore the etiological bacteria involved in surgical site infections in Benin. For one year in this period (January 2019 to January 2020), we included two wards (maternity and gastrointestinal) at six public hospitals. These wards were chosen for two reasons: 1) cesarean sections are one of the most common surgical procedures in Benin, and 2) all hospitals have a gastrointestinal ward. During the study period, all patients $\geq$ 18 years of age and presenting SSIs according to the Centers for Diseases Control and Prevention (CDC) criteria [14] were offered inclusion in the study. The eligible patients who fulfilled the inclusion criteria were identified by a trained nurse's team and were subject to wound sampling according to the standard operating procedures (SOPs). Sociodemographic and clinical data were collected by using a standardized questionnaire. A total of 304 wound swabs were collected from 174 patients with clinical signs of surgical

infections. Gram-negative bacteria identification was performed according to the guidelines for microbiological methods of the European Committee of Antimicrobial Susceptibility Testing guidelines [15]. We used various biochemical tests: oxidase and characteristics of the Analytical Profile Index (API 20E, Biomerieux, Lyon) such as the Voges Proskauer (VP) test, indole test and citrate utilization. Cultures were incubated for a total of 48 h (if there was no growth at 24 h) at 37 °C in an aerobic atmosphere, and then examined for microbial growth. All identifications were confirmed in Belgium (Cliniques universitaires Saint-luc) using MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry (Brucker Daltonics, Bremen, Germany), employing a threshold of ≥2.0. For this study, only Gram-negative bacteria were concerned. The Gram-positive bacteria will be analyzed and published elsewhere. Figure 1 summarizes the selected CRO amongst carbapenem non-susceptible isolates.

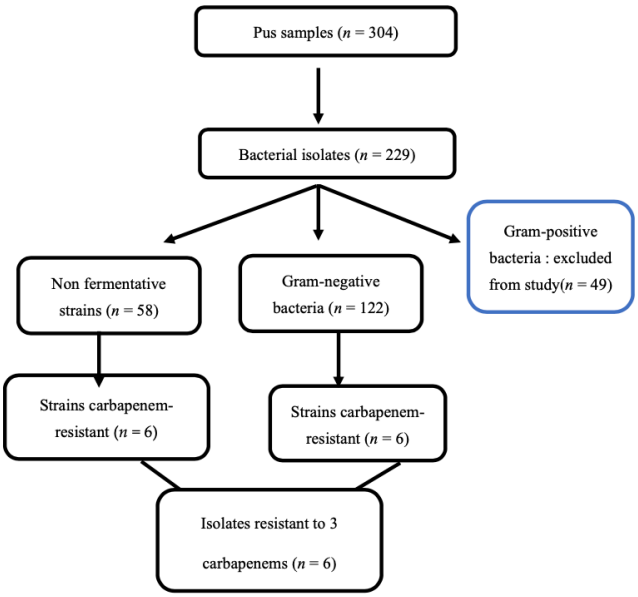


Figure 1. Flowchart of study showing selected carbapenem non-susceptible isolates.

2.3. Antimicrobial Susceptibility Testing (AST)

The following antibiotics (Biorad, Marnes-la-Coquette, France) were tested for *Enterobacterales:* ampicillin (10 µg), cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), trimethoprim + sulfamethoxazole (25 µg), cefoxitin (30 µg), amikacin (30 µg), gentamycin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cefotaxime (30 µg), piperacillin (100 µg), tobramycin (10 µg), imipenem (10 µg) and chloramphenicol (30 µg). For non-fermenting bacteria, we also included ceftriaxone (30 µg), ceftazidime (30 µg), piperacillin + tazobactam (110 µg) and ticarcillin + clavulanic acid (85 µg). The results were interpreted according to the latest EUCAST breakpoints (www.eucast.org/clinical_breakpoints (accessed on 25 July 2022)) guidelines. In brief, after 24 h of incubation at 35 °C, the inhibition zones were measured, and the results were analyzed and interpreted. Isolates that were resistant to at least three different antimicrobial classes were considered as multidrug resistant bacteria [16].

2.4. Phenotypic Confirmation Test for Carbapenemases Production

Detection was completed using a Modified Hodge Test (MHT): an obsolete test in which an overnight culture suspension of *Escherichia coli* ATCC *(American Type Culture Collection)* 25,922 adjusted to 0.5 McFarland standard was inoculated on an MH agar plate and allowed to dry for 3–5 min. After drying, a 10-µg imipenem disk (Biorad, Marnes-la-Coquette, France) was placed at the center of the plate, and the test strain was streaked in a straight line from the edge of the disk to the periphery of the plate. The plate was incubated aerobically overnight at 35 °C. The isolate was considered as positive for the production of carbapenemases if, within the inhibition zone of the imipenem susceptibility disc, there was a clover-leaf type indentation at the intersection of a tested organism and the *E. coli* 25,922 [17]. CLSI recommended, since 2020, the use of CarbaNP, mCIM and eCIM tests [18].

A second confirmation of carbapenemases production was performed in Belgium by using the RESIST-5 O.K.N.V.I [19]: a multiplex lateral flow assay for the identification of five common domestic carbapenemases (OXA-48, KPC, NDM, VIM and IMP) genes.

2.5. Quality Control

Standard operating procedures (SOPs) were strictly followed during all analytical procedures, starting from sample collection, isolation, identification and antibiotic susceptibility testing. All culture media were prepared according to the manufacturers' instructions and were checked for their sterility and performance. As reference strains for quality control of the antimicrobial susceptibility and biochemical tests, we used two international control bacteria strains: *E. coli* ATCC 25,922 and *S. aureus* ATCC 25923.

2.6. Genotyping of ESBL and Carbapenemases-Producing Organisms

A single colony was suspended in 200 µL of distilled water. Before extraction, 10 µL of a McFarland equivalence turbidity standard 3.0 of *A. baumannii* ATCC 19,606 was added to the suspension as an internal extraction control. The samples then boiled at 95 °C for 10 min on a heating block. The 25 µL amplification mixture was then prepared containing 2 µL of DNA extract; 2 × 12.5 µL of master mix multiplex PCR kit (Qiagen Benelux, Antwerp, Belgium); and 200 µM of each primer. The carbapenem-resistant genes (bla_{OXA-48}, bla_{NDM}, bla_{VIM}) were detected using an in-house multiplex PCR set up in a microbiology laboratory (Cliniques universitaires Saint-Luc Brussels, Belgium) according to the procedure published by Bogaerts et al. [20]. Table 1 summarizes different primers and probes used for the multiplex PCR in the laboratory.

Table 1. List of primers and probes used for the in-house multiplex PCR.

Target Gene	Primer Name	Sequence (5′-3′)	Amplicon Size
NDM	NDM-1R NDM-1F-FAM	CAT-TAG-CCG-CTG-CAT-TGA-T ACT-TGG-CCT-TGC-TGT-CCT-T	596 bp
VIM	VIM437R VIM437R-FAM	ATT-CAG-CCA-GAT-CGG-CAT-C TGT-CCG-TGA-TGG-TGA-TGA-GT	435 bp
OXA-48	OXA-48 R OXA-48F-FAM	CAT-CCT-TAA-CCA-CGC-CCA-AAT-C TGT-CCG-TGA-TGG-TGA-TGA-GT	265 bp
CTX-M1	CTXM1R CTXM1F-FAM	AGC-TTA-TTC-ATC-GCC-ACG-TT AAA-AAT-CAC-TGC-GYC-AGT-TC	412 bp
OXA-1	OXA30R OXA30F-FAM	TAA-ACC-CTT-CAA-ACC-ATC-CGT TGG-AAC-AGC-AAT-CAT-ACA-CCA	388 bp
TEM	TEM500R TEM500F-FAM	CGG-GAG-GGC-TTA-CCA-TCT-GGC CAA-CTC-GGT-CGC-CGC-ATA-CAC-TA	501 bp

3. Results

3.1. Identification and Antimicrobial Susceptibility Results

Among 304 pus samples, we identified 229 strains. While Gram-positive bacteria represented 21.4% (n = 49) of isolates, 78.6% (n = 180) were Gram-negative. Altogether, twelve isolates were resistant to at least one tested carbapenem, and six were resistant to the three tested carbapenems (meropenem, ertapenem and imipenem). These isolates were

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	NDM-1F-FAM	ACT-TGG-CCT-TGC-TGT-CCT-T	
VIM	VIM437R	ATT-CAG-CCA-GAT-CGG-CAT-C	435 bp
	VIM437R-FAM	TGT-CCG-TGA-TGG-TGA-TGA-GT	
OXA-48	OXA-48 R	CAT-CCT-TAA-CCA-CGC-CCA-AAT-C	265 bp
	OXA-48F-FAM	TGT-CCG-TGA-TGG-TGA-TGA-GT	
CTX-M1	CTXM1R	AGC-TTA-TTC-ATC-GCC-ACG-TT	412 bp
	CTXM1F-FAM	AAA-AAT-CAC-TGC-GYC-AGT-TC	
OXA-1	OXA30R	TAA-ACC-CTT-CAA-ACC-ATC-CGT	388 bp
	OXA30F-FAM	TGG-AAC-AGC-AAT-CAT-ACA-CCA	
TEM	TEM500R	CGG-GAG-GGC-TTA-CCA-TCT-GGC	501 bp
	TEM500F-FAM	CAA-CTC-GGT-CGC-CGC-ATA-CAC-TA	

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as follows: *Escherichia coli* (*n* = 2), *Pseudomonas aeruginosa* (*n* = 1), *Pseudomonas mendocina* (*n* = 1), *Enterobacter cloacae* (*n* = 1) and *Acinetobacter baumannii* (*n* = 1). Isolates shared a multidrug-resistant phenotype. They were resistant to carbapenems and almost all beta-lactam antibiotics including broad-spectrum cephalosporins, amoxicillin/clavulanic acid and fluoroquinolones, but only susceptible to amikacin. Figure 1 shows the flowchart of the study.

3.2. Results of Modified Hodge Test (MHT) and RESIST-5 O.K.N.V.I

The MHT was positive for *Enterobacterales* (*E. coli* and *E. cloacae*) only. The test was not performed for non-fermentative bacteria.

The multiplex lateral flow immunochromatographic test showed positive tests for OXA-48, NDM and VIM carbapenems.

3.3. Molecular Detection of ESBL and Carbapenemases Genes

Amongst 12 isolates that presented at least resistance to one carbapenem, six were phenotypically confirmed as ESBL and carbapenemases producers' organisms and carried at least one carbapenemase gene. Four isolates (2 strains of *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*) co-harbored ESBL and carbapenemases genes. The two *Enterobacterales* (*E. coli* and *E. cloacae*) carried three ESBL genes ($bla_{TEM,}$ bla_{OXA} and bla_{CTX-M}). The non-fermentative bacteria (*Pseudomonas* and *Acinetobacter*) encoded metallo-beta-lactamases and, respectively, VIM and NDM. Except for two isolates, the four others showed at least two beta-lactamases genes. The following tables successively showed the socio-demographic characteristics of included patients (Table 2) and the molecular results of carbapenem resistant strains and their antimicrobial susceptibility (Table 3).

Table 2. Socio-demographic characteristics of included patients.

Samples	Age/Gender	Surgical Antimicrobial Prophylaxis (SAP)	Length of Stay (Days)	Procedure
13150	40/F	ceftriaxone	16	peritonitis
12480	34/F	ceftriaxone	28	cesarean
8062	49/M	ceftriaxone	14	peritonitis
6672	28/F	amoxicillin/clavulanic acid	14	cesarean
5823	35/F	ceftriaxone	14	cesarean
3159	31/F	ceftriaxone	10	cesarean

F: female; M: male.

Table 3. Antimicrobial susceptibility test and PCR results for six isolates resistant to three tested carbapenems.

	13150	12480	8062	6672	5823	3159
MHT	+	+	+	*NT*	*NT*	*NT*
Beta-lactamases	$CTXM_1$,OXA_1 TEM, OXA_{48}	$CTXM_1$,OXA_1 TEM,OXA_{48}	$CTXM_1$,OXA_1-TEM,NDM	$CTXM_1$,OXA_1 VIM	VIM	NDM
Name of isolates	*E. coli*	*E. coli*	*E. cloacae*	*P. aeruginosa*	*P. mendocina*	*A. baumannii*
Antibiotics						
Ticarcillin	R	R	R	R	R	R
Piperacillin	R	R	R	R	R	R
Ticarcillin/clavulanic acid	R	R	R	R	R	R
Piperacillin/tazobactam	R	R	R	R	R	R
Ceftazidime	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	R
Cefepime	R	R	R	R	R	R
Imipenem	R	R	R	R	R	R
Ertapenem	R	R	R	R	R	R
Meropenem	R	R	R	R	R	R
Aztreonam	R	R	R	R	R	R
Gentamicin	R	R	R	R	R	R
Tobramycin	R	R	R	R	R	R
Amikacin	S	S	S	S	S	S
Ciprofloxacin	R	R	R	S	R	R
Levofloxacin	R	R	R	S	R	R
Trimethoprim-sulfamethoxazole	R	R	R	R	R	R

Abbreviation: MHT: Modified Hodge test; NT: not tested and +: positive results.

4. Discussion

Due to treatment failure and the limitations of alternative therapeutic options, the emergence and dissemination of carbapenem-resistant Gram-negative bacteria remain a challenge for clinicians/healthcare infrastructures. In the present study, we determined the patterns of carbapenem-resistant isolates recovered from pus samples in Benin and summarized the challenges of detecting them in the laboratory. All of the six isolates were resistant to multiple classes of the antibiotics tested, typically to ertapenem, imipenem, meropenem, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole and piperacillin-tazobactam. Although Loqman S. et al. reported a similar antibiotic susceptibility profile in Morocco [21], Mathlouthi et al. [22] observed low resistance to imipenem in Tunisian and Libyan hospitals. Such a contradiction outlines the need for a national surveillance system in Africa to study routinely MDR strains. In a study conducted in 2014 with pediatric patients, the authors showed resistance to co-amoxiclav, cefotaxime, cefuroxime, ceftazidime and cefixime and aztreonam, which are in accordance with our results [23]. In 2020, in Alexandria, in a subset of 65 isolates provided from surgical site infection, half of the Gram-negative bacteria, mainly *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*, were resistant to carbapenems [24]. The resulted epidemiological data should help healthcare services as well as clinicians to prevent surgical site infections.

OXA-48 was detected in two strains of *Escherichia coli*. Initially and most frequently, the blaOXA-48 genes were identified in *K. pneumoniae* strains. However, other carbapenems resistance species have recently been recognized as OXA-48 producers [22], including *Enterobacter* spp., *Klebsiella. oxytoca*, *Escherichia. coli* and *Citrobacter. freundii*. OXA-48 and OXA-48-like enzymes are one of the most common CPE enzymes detected worldwide [25]. The family takes its name from the CPE enzyme first identified—OXA-48—and includes several sequence variants that are transmissible via plasmids [26]. OXA-48 carbapenemases are of major concern due to both the detecting issues and to their association with treatment failure [26]. While glycopeptides and daptomycin are still reliable alternatives to methicillin-resistant *staphylococcus aureus*, treatment options are dramatically limited in carbapenemases-producing Gram-negative bacilli. Carbapenemases-producing Gram-negative are particularly resistant to all or nearly all beta-lactams, and often carry other genes that encode resistance mechanisms to fluoroquinolones and/or aminoglycosides [27].

Due to its capacity for colonizing environmental surfaces and spreading within the hospital, carbapenem-resistant *A. baumannii* imposes a high healthcare burden. It is intrinsically resistant to several classes of antibiotic agent, and few treatment options are available [28]. In our subset of studied MDR strains, we identified an NDM-producing *Acinetobater baumannii*, showing the importance of timely identification [28]. In Mulago Hospital, Kampala (Uganda), 2.7% of *A. baumannii* isolates were carbapenem resistant [29]. At Kenyatta National Hospital in Nairobi (Kenya), 85% of *A. baumannii* were MDR [30]. According to the review published in 2020 by Kindu M. et al. VIM carbapenemases were highly prevalent carbapenemase types among *P. aeruginosa* isolates in Africa [31]. As VIM enzymes are one of the most widespread metallo-beta-lactamases that are commonly associated with class 1 integrons or even plasmids, they contribute to the global spread of this resistance mechanism.

To our knowledge, we detected in this study the first case of VIM-producing *P. mendocina* in Benin. Few cases of human infection by *P.mendocina* have been reported since its first description in 1970 by Palleroni et al. after it had been found in water and soil samples collected in Mendoza province in Argentina [32]. More recently, authors in Argentina also reported two cases of VIM-producing *P. mendocina* infections in two hospitalized severely burned patients [33]. As greater understanding would result from studying the distribution of this enzyme in the *pseudomonas* species, further investigations are needed, not only to confirm the dissemination of carbapenemases-producing bacilli in Benin, but also to boost the establishment of an active national surveillance program.

In a context in which the prevalence of carbapenemases is increasing, the early identification of CPE and differentiation between CPE and non-CPE lead not only to improved

clinical outcomes, but also to time and cost savings [34]. Rather than using PCR-based methods, which are cost-intensive, time consuming and cannot detect novel unidentified genes, phenotypic methods seem effective and reliable for routine clinical use, particularly in developing countries. Even though the overnight incubation of the Modified Hodge Test (MHT) increased turnaround time, the test was low-cost and needed no special reagent or equipment. However, even though the MHT was recommended by CLSI in 2009, some authors do not recommend it [35]: MHT is not specific for the detection of all carbapenemases enzymes, most significantly with isolates showing weak positive results and AmpC producers [36]. It also has low sensitivity for certain types of carbapenemases (MBL and some OXA types), and lacks specificity, since ESBL and AmpC producers with porin mutations can give false-positive results [37]. Bonnin et al. used the MHT on 19 carbapenemases-producing isolates and found negative results for all the NDM-producing isolates tested, and only weak positive results for the VIM, IMP and OXA-type producers [38]. Hence, all negative MHT should be cautiously interpreted, based on the epidemiological and local data. This test was recommended by CLSI a long time before, and now is an obsolete method. Nevertheless, in our laboratory this test continues to be performed in the routine. Moreover, imipenem was the most resistant of the three tested carbapenems in this study, and this fact could be explained by the overexpression of the efflux pump and loss of OprD porin that are the most common mechanisms of carbapenem resistance in non-fermentative bacteria, especially *P. aeruginosa.* The phenotypic resistance observed may be attributed to the presence of the broad specificity of drug efflux pumps [39].

The aim of surgical antimicrobial prophylaxis (SAP) is to prevent SSIs by administering an antibiotic that targets the microbes most likely to contaminate the surgical site [27]. It should be noted that employing the antibiotic with the narrowest possible spectrum for the shortest possible period, while achieving adequate and timeous tissue levels for the duration of the surgery, reduces SSIs and adverse effects as well as microbial resistance [40,41]. We observed that the most prescribed antibiotics for prophylaxis were third-generation cephalosporins (Table 2), which were not required for prophylaxis [42] as they have less activity against staphylococcal infections, have high rates of resistance development and impose a higher financial burden on patients than cefazolin [43]. In addition, the emergence of extended-spectrum beta-lactamase-producing microorganisms is due to the overuse of third-generation cephalosporins [44]. Although cefazolin is not available in Benin, the appropriate use of SAP is a proven strategy for reducing surgical site infections, and consequently antimicrobial resistance. Our findings also highlight the importance of implementing the antimicrobial stewardship program, of continued medical education and of making the recommended antibiotics available.

The high rate of antimicrobial resistance in developing countries is related, among other things, to limited antibiotic options: a problem that is compounded by the fact that few antibiotic alternatives are currently in the pipeline. Partly because development of new antimicrobial drugs is not financially attractive [45], the development of AMR is exacerbated by the poor availability, affordability, accessibility, and quality of antibiotic agents. In addition, self-treatment with antibiotic agents is common in low-middle income countries, where antibiotics continue to be dispensed without a doctor's prescription [46], even though the health authorities do not officially allow their sale if they have not been prescribed by a doctor. The factors associated with AMR also include a lack of data on local antibiotic agents, and fear of treatment failure. In Benin, policies to regulate antibiotic use are insufficient and poorly enforced. As the unregulated use of antibiotic agents in LMICs makes them easily available over the counter, their improper uses contribute to the risk of AMR in the country [47]. Moreover, there is a lack of national guidelines for the practice of SAP in hospitals.

This study highlights once again the importance of early detection of CRO. The Modified Hodge Test is obsolete and should be replaced by recommended tests in our laboratory [18]. This should improve the treatment of patients. The practice of SAP is non-optimal because of the unavailability of national guidelines in surgeries departments, and

the lack of a national program for antimicrobial stewardship [48]. The project establishes the first guidelines for the rational use of antibiotics. This guideline should be promoted in all hospitals.

We understand and acknowledge the limitations of the present study. Firstly, the analysis was performed on a small subset of isolates, which may limit the generalization of the findings. Secondly, further molecular investigation using more robust whole-genome approaches should help lead to a better understanding of the dynamics of transmission of carbapenem-producing organisms.

5. Conclusions

In this study, all isolates were resistant to all tested antibiotics, except amikacin. Ceftriaxone was the most used antibiotic in surgery. The carbapenem resistant strains harbored at least two beta-lactamases genes. The rapid dissemination of carbapenem-resistant strains represents a major therapeutic and epidemiological threat that requires regular surveillance studies and the implementation of strict hygiene procedures, especially in Benin hospitals, where adherence to internationally accepted infection control policies seem not optimal. Moreover, the promotion of accurate and reliable phenotypic tests should be established to improve the detection of CRO in our laboratory in Benin.

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1.3 CHAPTER III : INTERVENTIONS FOR SURGICAL SITE INFECTION PREVENTION

Chapter III at the glance

What is already known about this chapter?

- Antibiotic stewardship programs have been playing an increasing role in patient care and hospital settings
- Implementation of antimicrobial stewardship programs (ASPs) in Africa is a challenge
- ASPs help in improvement of compliance with antibiotic guidelines and reducing the rate of antibiotic resistance
- They are seven core elements associated with ASPs: Leadership commitment, education, antimicrobial expertise, accountability (antimicrobial champion), reporting (audit and feedback), tracking (monitoring of antibiotic consumption) and action (multidisciplinary collaboration).
- In Benin, the practice of surgical antimicrobial prophylaxis was not well achieved and not respected local guidelines
- As hand hygiene compliance (33.3%) is relatively low amongst healthcare workers, we observed also relatively high rate of methicillin resistant *staphylococcus aureus* (65.3%) in isolates

What this chapter add?

- Based on results of the project, we proposed a surgery guidelines for the rational use of antibiotics

- This guidelines propose a combination of antibiotics according to the results of antibiogram of isolates and their availability in the country.
- They are the first activities of SSI prevention such as the feedback of audits (hand hygiene and antimicrobial prophylaxis)
- Despite controverses, the screening of MRSA was realized and can constitute the baseline for future actions
- We detected three dominant clones (ST152, ST121 and ST8) from patients and HCWs with presence of leucocidin of Panton-valentine (luk-F-PV) in all isolates and the toxic syndrome toxin1(TSST-1) genes were detected in 29.6% of isolates.

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IN PREPARATION

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ABSTRACT

Background:

Improving surgical antibiotic prophylaxis (SAP) practices for the reduction of surgical site infections (SSIs) remains challenging in low- and middle-income countries, especially in the absence of local guidelines. We aimed to implement guidelines for the rational use of antibiotics in surgical prophylaxis in gastrointestinal and obstetric surgeries wards in Benin.<

Methods:

The Delphi's study involved two rounds of consensus building between February and April 2021. The expert panel included 14 experts from Benin and Belgium: microbiologists, pharmacists, surgeons, gynecologists, hygienists, and infectious diseases specialists. Guidelines on surgical antimicrobial prophylaxis were developed using recent results on the antimicrobial susceptibility of bacteria isolated in surgical site infections from six hospitals and the availability of antibiotics in Benin. In addition, some recommendations about the treatment of SSIs were included: specific cases of gastrointestinal interventions, and simple or complicated cases of caesarean section. The guidelines are included in a booklet providing general information about antibiotics and antimicrobial resistance. The panel used a four-level Likert-type scale to record agreement or disagreement with antibiotics schemes for prophylaxis and SSIs treatment. Consensus was established at 70% of agreement by experts or an upper quartile ≥3.25 for each item.

Results:

The national guideline panel reached a consensus on eleven essential combinations of antibiotics for SAP and SSIs treatment. Evaluation of the first round showed that > 90% consensus had been reached on all the statements on the regimen that should be applied for surgical antimicrobial prophylaxis and SSIs treatment. Experts suggested minor modifications to the wording and the content of the booklet. A second version of the booklet including the guidelines was sent to the experts for final approval.

Conclusions: Through validation of the contents based on experts' consensus, it was possible to agree on an essential approach to improving the practices of surgical antimicrobial prophylaxis in hospitals in Benin. As valuable feedback was provided by the multidisciplinary team, this consensus guideline will help to standardize the use of antibiotics in surgical wards.

Keywords: Delphi Process - surgical site infection - surgical antimicrobial prophylaxis - antibiotics guideline - Benin.

1 INTRODUCTION

Antimicrobial resistance (AMR) is currently one of the greatest health threats worldwide[1–3] In recent years, several national and international projects and initiatives have been developed to improve its prevention through strategies for strengthening health systems and surveillance, reducing antibiotics misuse, and improving AMR prevention[4,5]. Due not only to the dramatic increase in AMR in many regions but also to the paucity of new drug development, it is more important than ever in all areas of medicine to use appropriate antibiotics in ways that are both prudent and controlled. It is estimated that antibiotics are prescribed unnecessarily in nearly fifty percent of cases – an irrational use of antibiotics that is a key contributor to AMR[6]. Given the well-established relationship between antibiotic use and the development of resistance, the World Health Organization (WHO), other and international and professional organizations have advocated the development of antimicrobial stewardship programs to improve antibiotic use[7].

Surgical site infection (SSI) is defined as occurring in the superficial, deep, or organ space around the surgical site within 30 days of the surgical procedure, or within 1 year of implants [8]. The role of surgical antibiotic prophylaxis (SAP) is to deliver adequate antibiotic exposure throughout the surgical site to cover the commonly isolated organisms[9]. SAP consists of initiating a very brief course of antibiotics shortly before the start of operative procedures[9] The narrowest possible antibiotic spectrums such as first-generation cephalosporins (cefazolin) are common antibiotics used due to their spectrum of activity and extensive experience in SAP[10,11]. Nevertheless, in Benin, the recommended cefazolin is not available.

Findings from countries including Benin have shown that poor guideline compliance leads to the inappropriate use of SAP[12,13]. In Benin, no national guidelines for SAP have been agreed. Moreover, there is no consensus on the recommendations of SAP used in the operating room.

Since 2018, visceral surgery and obstetric wards in six Beninese hospitals have been running the Multidisciplinary Strategy for Prevention and Infection Control (MUSTPIC) project, which aims to improve hand hygiene and the rational use of antibiotics in surgical services. An important step in the project was to evaluate surgical antibiotic prophylaxis, which in many countries is known to be based not on national guidelines but the overall knowledge of medical teams[14]. The obstetric (caesarean sections) and gastrointestinal wards were chosen for this, as surgical procedures performed there are the most common in the hospitals in question. We observed in hospitals the practices of SAP in gastrointestinal and obstetric wards according to

five conventional criteria: indication, choice of molecule, dose administered, timing and duration of administration. In the maternity ward, three (molecule, dose and duration) of the five criteria were less appropriately achieved and only 31.2% of women received optimal antibiotic prophylaxis for cesarean section[15] However, for caesarean sections, these hospitals have a specific kit containing ampicillin, metronidazole, and gentamicin that is available to patients, free of charge. Despite the use of SAP, the MUSTPIC team observed that almost all the bacteria isolated in maternity and gastrointestinal wards when SSIs appeared were resistant to ampicillin[16]

The principal objective of this study, which is part of the implementation section of the MUSTPIC project is the use of a modified Delphi approach to establish an antibiotic guideline for surgery departments in Benin. This guideline was specifically intended to provide partner hospitals and health authorities with validated recommendations that could serve as a basis for rational antibiotic use in surgical antimicrobial prophylaxis and treatment of surgical site infections.

2 METHODS

2.1 GUIDELINES DEVELOPMENT

The guidelines were drawn up by a team of researchers (PhD students, pharmacists, specialists in antibiotic utilization, hygienist, microbiologist). The guidelines are gathered in a booklet structured in 3 sections : 1) general information about antibiotics (pharmacology, good practice of SAP, rational use of antibiotics, drug-drug interactions, allergies, pregnancy and lactation, availability of antibiotics in Benin), antimicrobial resistance (mechanisms of resistance, multi-drug resistance) and hospital-acquired infections, 2) six recommendation statements about SAP in obstetrics and gastrointestinal surgery, 3) two recommendation statements for SSIs treatment in maternity and in gastrointestinal surgery (first and second choices), 4) links to international guidelines about antibiotic treatment.

The statements for SAP were based on four criteria as follow: mapping of the germs isolated from surgical site infections in the six participating hospitals; the sensitivity of these germs; international recommendations; and the antibiotics available and accessible in Benin [16,17]. The recommendations about the treatment of SSIs were based on literature.

The questionnaire was composed of the eight statements to rate, accompanied with possibility to comment each statement, and of three opened questions about the first and fourth sections of the booklet.

The Figure 1 is a schematic steps leading to the validation of the guideline.

2.2 DELPHI METHOD

When guidelines are insufficient or unavailable, the rigorous application of scientific research techniques for achieving consensus allows for experts to validate combination of antibiotics proposed for the SAP and the SSIs treatment [18,19]

Developed mainly by Dalkey and Helmer [20] Delphi technique is an accepted method that enables a group of experts to achieve consensus and give their opinions on real-world concerning a certain topic area[19] . Experts are defined as those with a good practical, political, legal, or administrative knowledge of a specific subject, who also have sufficient

legitimacy to express an opinion that is representative of the group of actors to which they belong[21]

In our case, we adopted a modified Delphi method consisting of two rounds of email questionnaires with a final validation. We chose the modified Delphi technique in preference to other methods of consensus such as the nominal group technique (also known as the expert panel). There were two reasons for this. First, it is flexible and allows a greater number of experts to participate. Second, even though it allows for a certain degree of interaction between experts, it is also especially useful when face-to-face discussion is not feasible, as in our own situation, especially because of Covid 19 outbreak that not allowed gathering all experts.

To facilitate international participation, the panelists had the possibility to directly send their feedback by e-mail. Within 15 days after each round, panelists were informed of the results obtained and of the individual comments made. So as to not be influenced by others during the process, they were kept unaware of each other's identities and opinions[22]. As well as allowing the experts to reconsider their points of view, this mode of debate allows contrasting opinions to emerge and converge, thereby making it possible to achieve the greatest possible consensus.

2.3 RECRUITMENT OF EXPERT PANEL

Since the Delphi technique was first defined, it has been recommended that experts should be extremely knowledgeable in this specific topic on "antibiotics, antimicrobial resistance, and surgical antimicrobial prophylaxis". The researchers identified a list of potential national and international experts for the Delphi panel, defining experts as individuals who had expertise related to the concepts, activities, and topic in question, i.e., antibiotic utilization and antimicrobial resistance. Twenty-one experts were contacted via e-mail to participate in the Delphi study in January 2021. Participants were not offered any financial compensation or other kinds of incentive. As this was a convenience sample, we had first obtained their consent to participate in validation.

Fourteen experts accepted to participate. A validation committee was then formed, made up of representatives from each of the included hospitals and other experts: experts in infection prevention and control, microbiology, infectiology, surgery, hospital hygiene, pharmacology, gynecology, and clinical pharmacy.

2.4 DATA COLLECTION AND ANALYSIS

The expert panelists were asked to rate combinations of antibiotics for each section. For this purpose, the question was "what is your professional view on the proposal concerning the treatment of surgical site infections or on the new proposal regarding surgical antibiotic prophylaxis?». In line with previous Delphi studies, each panelist used a 4-point Likert scale to indicate their level of agreement with each statement (4 = strongly agree, 3 = agree, 2 = Disagree, 1= strongly disagree).

The median response value and interquartile ranges were calculated per statement. Statements were then rejected or included based on a priori consensus rules:

- An upper quartile ≥ 3.25 or 70% of respondents answering Strongly agree or agree, indicated consensus amongst panelists, and the statement was accepted (consensus "in")
- A lower quartile ≤ 1.75 indicated disagreement, and the statement was rejected (consensus "out")
- If the interquartile range between 1.75 and 3.25 this indicated a lack of agreement between panelists and the need for further review of the statement (no consensus). In such cases, the statements were reviewed by the research team, and either revised and included in the next round or rejected based on the panelists' additional comments.

After each statement, panelists were also able to add any observations they deemed necessary and to use an open text box to propose helpful actions and strategies that had not been covered in the questionnaire[23]

In round 1, participants had an opportunity to suggest additional statements for inclusion in the questionnaire and the final version.

Round 1 took place between 15 and 28 February 2021. Between 29 February and 7 March, the research team added all their comments and corrections to the statements and other sections of the guidelines. To maximize the response rate, up to two e-mail reminders per participant were sent per round.

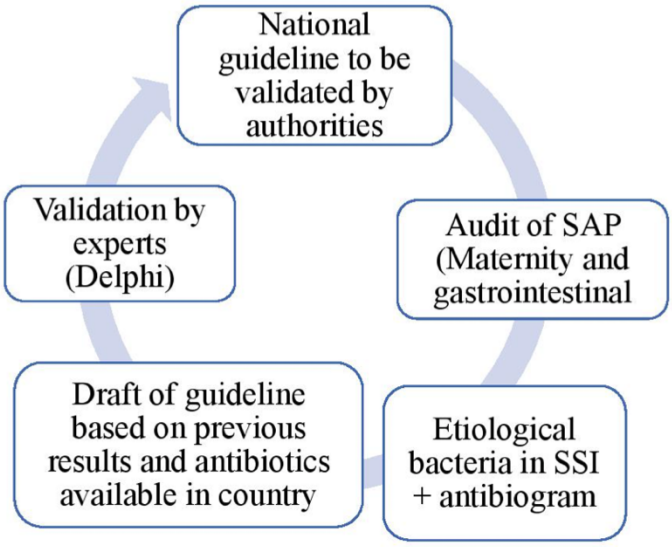


Figure 1: Successive steps leading to the validation of MUSTPIC guideline

2.5 ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The MUSTPIC project was approved by the Institutional review Board of the health Faculty (FSS, Benin) under reference number: **012-19/UAC/FSS/CER-SS**

3 RESULTS

The overview of Delphi results is show in Figure 2. Consensus was achieved for all statements in the first round.

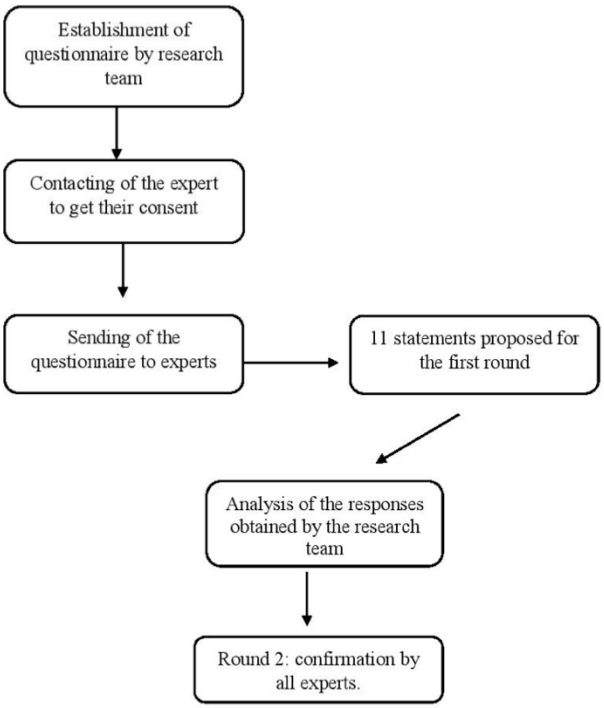


Figure 2: Flow diagram illustrating the first survey round of the Delphi study. All statements were resolved after this round.

Table 1. Demographic characteristics of Delphi participants.

Experts	Benin	Belgium
Gynecologists	2	
Surgeons	4	
Infectious disease specialist	2	
Pharmacologist	1	1
Pharmacist		2
Anesthetist	1	
Infection control specialist		1

The response rate to the first-round was 100% (14/14). Consensus on the choice of antibiotics used in prophylaxis in the gastrointestinal and caesarean section was over 70% for all eleven statements. To improve the guidelines, some experts provided feedback in open-ended questions. Between round 1 and the final reformulation , these observations were considered, and the paragraphs concerned were included as appropriate. These observations were related not to the content but to the form and order of chapters in the guidelines. A second version of the guidelines was then sent to the participants. As all statements were validated, the last consultation meeting did not take place. The resulting guidelines can be found in the online supplementary material.

4 DISCUSSION

The primary objective of this study was to identify the best association of antibiotics for preventing and treating surgical site infections for clinical practice in Benin. This guideline represents the last step of the process that consisted first of the observation of the practice of SAP in hospitals, secondly the results of antibiogram of bacteria isolated in SSIs in the same hospitals and finally the availability of antibiotics in Benin.

Antimicrobial resistance remains a global threat affecting all healthcare systems and especially the LMICs where they are a lack of last-line antibiotics to treat infections[24]. Moreover, another challenge for LMICs is the lack of national guidelines for the practice of SAP and infection treatment in hospitals[25,26]. An international project gathered surgeons from 57 LMICs, including Benin, to discuss and prioritize topics for future global surgery guidelines for the top ranked topic. The development of Global surgery guidelines for the prevention of surgical-site infection appeared as the top priority[27]. The guidelines developed here meet that need. To our knowledge, the resulting guidelines are the first in the field of antibiotic resistance and consumption in the country. In a setting characterized by incomplete knowledge of guidelines regarding the SAP and the treatment of SSIs, the Delphi approach proved to be an important tool for synthesizing the cumulative experience of an array of subject-matter experts[28]

Earlier in the MUSTPIC project, we described after the audit of SAP, the susceptibility profile of bacteria isolated in surgical site infections in hospitals. From our data, almost bacteria were resistant to ampicillin, and two-thirds of bacteria were resistant to the second and third generations of cephalosporin[16]. New antibiotic combinations were proposed according to the ecology of the bacteria in the hospitals. In maternity services, for instance, the existing kit for caesarean contains the following antibiotics: ampicillin, gentamicin, and metronidazole [29]. However, in our study we demonstrated that ampicillin remains one of the less effective antibiotics[16]. As cefazolin is not available in Benin, we proposed cefuroxime for non-complicate cases of caesarean. The use of broad spectrum cephalosporins such as ceftriaxone in SAP is even more likely to induce resistance than cefazolin and other second generation of cephalosporins[30,31].

Based on the solid evidence-based data provided by the results of the previous studies of MUSTPIC [16,32–34], all items have reached consensus and were validated after the first round. This could be explained by the fact that all the experts included in the validation had

been aware of the importance of SAP. Although the relatively minor changes (supplementary material with the summary of responses of experts) to the combinations of antibiotics resulting from the Delphi process provided reassurance that no extensive modifications of the antibiotics chosen for SSIs were required, the participation of a higher number of experts would have provided greater assurance that each of the items had been assessed reliably.

Despite the high degree of freedom regarding the number of participants, the literature reports a median number similar to that described in our study [35,36]. When considering similar methodologies, the number of rounds performed during the Delphi process tends to vary. While some studies report only one round, generally when a "real-time" Delphi approach is conducted, most studies report two or three rounds, with two being more prevalent [37] Even though the single round in our study was consistent with the literature, it was nonetheless a limitation, as a higher number of rounds may have brought a higher consensus rate on the combination antibiotics and on other comments on antibiotics using for the SAP. To help reduce potential bias, all participants were blinded to who participated in the expert panel as individual correspondence was sent, which can reduce the effects of dominant individuals, and also manipulation or coercion into conformity with certain viewpoints[37,38] This study has another strength: the heterogeneity of the panel, and the many years of experience it represented, both characteristic of the range of stakeholders with an interest in the outcomes of this study

In the period between 4 and 14 July 2021, training on antibiotic prophylaxis was conducted by the researcher's team online and/or face-to-face at each hospital according to the agendas of the members of CLINs (Comité de Lutte contre les Infections Nosocomiales, i.e., the Beninese committee for preventing nosocomial infections). Each participant received a personal booklet with the guidelines. The members of CLINs were in turn invited to implement and disseminate the guidelines in their hospitals through training of the appropriate staff.

This guideline will be promoted and disseminated within the competent authorities and partner hospitals. This activity provided a blueprint for setting up an infection-control policy and for updating the guidelines according to the local epidemiology of hospitals.

The future steps of the MUSTPIC project are to continue sharing the guidelines first to the included hospitals and then to all the relevant departments of the health ministry. The researcher team is committed to explain the importance of these guidelines and their correct use to healthcare professionals in the surgery services. Finally, we will promote its use in other hospitals that were not involved in the initial project. In the next project, we will pilot the audit

of use of guidelines in hospitals to assess the usability and operability and if necessary, improve this first edition.

5 CONCLUSION

The aim of the guideline was to provide to healthcare workers standardized instructions for performing surgical antibiotic prophylaxis correctly in Benin. The validation of the statements of this guide will certainly contribute to a great improvement in the use of antibiotics in hospitals in Benin.

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AUTHORS' CONTRIBUTIONS

OD and FMD conceived and developed the study protocol. CY and AA collected expert panel data. AA analyzed the results, which were then interpreted by all authors. All authors contributed to data interpretation. CY, AD and AF drafted the article. All authors read, amended, and approved the final manuscript.

FUNDING

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AVAILABILITY AND DATA MATERIALS

All the data generated or analyzed during this study are included in this published article and its supplementary files.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

COMPETING INTERESTS

A declaration was required that the panelists in this study had experience of antibiotics usage and had no significant financial conflicts of interest.

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Molecular characterization of *Staphylococcus aureus* in Benin: what about the clones identified with patients and healthcare workers?

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IN PREPARATION

Whole-genome sequencing-based screening of MRSA in patients and healthcare workers in public hospitals in Benin

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) constitutes a serious public health problem that imposes a considerable impact on patients' health and brings substantial healthcare costs. Because asymptomatic healthcare workers and patients can also serve as MRSA reservoirs in hospitals, we investigated the molecular characteristics and genomic diversity of MRSA strains circulating in public hospitals in Benin.

Material and methods

Between January 2019 and January 2021, we collected samples from patients and healthcare workers (HCWs) in six public hospitals in Benin. Using standard microbiological procedures, we processed pus specimens collected from obstetrics and gastrointestinal surgery wards, and nasal swabs collected from healthcare workers. In Belgium, Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass-Spectrometry was used for second confirmation. Whole-genome sequencing was used on confirmed MRSA isolates to characterize their genomic content and to study their relatedness.

Results

In total, 304 samples were collected from patients and 61 from HCWs. In wound samples we found 229 isolates with 49 strains of *S. aureus*. Thirty-two *S. aureus* samples(n=32/49, 65.3%) were resistant to methicillin. MRSA was found in 15 of the 61 nasal samples collected from HCWs. All these isolates were resistant to all beta-lactam antibiotics, and susceptible to vancomycin, teicoplanin, cefazoline, linezolid, and fusidic acid. Seventy-four percent were resistant to trimethoprim/sulfamethoxazole; 66.7% to ciprofloxacin, gentamicin, and amikacin; and 29.7% to erythromycin. The 27 isolates with high-quality WGS datasets were assigned to five sequence types (STs), the most common being ST8 (55.56%, n=15); ST152 (18.52%, n=5); and ST121 (18.52%, n=5). All isolates carried multiple virulence genes, including genes encoding the Panton-Valentine leukocidin toxin (48.15%, n=13) and the *tst* gene (29.63%, n=8), which is associated with toxic shock syndrome. No signs of direct transmission were found between isolates collected from patients and HCWs.

Conclusion

The genomic characterization of this collection provides valuable insight into MRSA circulating in public hospitals in Benin. The presence of strains carrying numerous genomic features associated with antibiotic resistance and virulence in HCWs is a major public health concern. Our study highlights the need to implement a multimodal strategy for reducing the risk of cross-transmission of MRSA in hospitals.

Introduction

Staphylococcus aureus, an important pathogen in healthcare and community settings, can cause a broad spectrum of diseases, ranging from skin and soft-tissue infections to invasive diseases such as pneumonia, septicemia, and endocarditis[1]. Methicillin-resistant *S. aureus* (MRSA) was first reported in the United Kingdom in the 1960s [2] , soon after the introduction of methicillin into the healthcare system. It has since been reported in many countries [3,4], and is known to be an important opportunistic pathogen with the ability to cause severe infections.

Worldwide, the prevalence of healthcare-associated infections (HAIs) in hospitalized patients is approximately 4.5-15.5% [5]. While surgical site infections (SSIs) are the third most common cause of HAIs in high-income countries, they are the prime source in income-poor ones [6,7]. *S. aureus* is one of the most-isolated bacteria on SSIs [8,9]. A subset of these *S. aureus* strains carries the *mecA* gene, which confers resistance to most beta-lactam antibiotics, including methicillin [10]. It is well documented that MRSA is associated not only with considerable therapeutic challenges but also with complications, deaths, and higher costs related to extended hospital stays. More importantly, with their intrinsic resistance to beta-lactam antibiotics, strains of multidrug-resistant hospital-acquired MRSA (HA-MRSA) greatly reduce treatment options, especially in developing countries [1,11,12].

The molecular characteristics of MRSA are generally investigated using methods such as pulsed-field gel electrophoresis (PGFE); *spa* typing; multilocus sequence typing (MLST); whole genome sequencing (WGS); and DNA microarrays [13–15]. The use of these techniques to study the clonal diversity of MRSA strains has improved researchers' understanding of their origin and transmission into hospitals and communities [16], showing that community-acquired MRSA (CA-MRSA) clones have spread rapidly in communities and are now increasingly present in healthcare settings worldwide. As there are no barriers between HA-MRSA and CA-MRSA, MRSA infections are a major threat to public health [16]. For example, although the production of Panton-Valentine leucocidin (PVL), a phage-borne toxin that kills cells and causes tissue necrosis, is historically associated with CA-MRSA strains, PVL-positive HA-MRSA was recently observed in hospitals in Egypt and communities in Tunisia and Algeria [17–19].

Although nasal colonization by MRSA is known to be a risk factor for subsequent infection in hospitals [20], infection control and surveillance in Benin are greatly limited by a lack of research on the *S. aureus* genotypes circulating among patients with SSIs and HCWs.

This explains the present study, which, to the best of our knowledge, is the first to report on MRSA screening of HCWs in country. As part of the MUSTPIC (Multidisciplinary Strategy for Prevention and Infection control) project, our overall aim is to improve hand hygiene and the rational use of antibiotics in surgical services. Direct observation of HCWs in an earlier phase of MUSTPIC showed that hand-hygiene compliance was relatively low [21]. Because patients and HCWs are at the interface between hospitals and communities, and because poor hand-hygiene practices can result in cross-transmission, we therefore investigated the molecular and genomic characteristics of the MRSA collected from patients in surgery wards. We also screened the HCWs who attended to patients in these same wards to determine whether they served as carriers or reservoirs for pathogenic MRSA .

Material and Methods

Study design and ethics consent

This study is a part of the larger Multidisciplinary Strategy for Prevention and infection control (MUSTPIC) project, covering the gastrointestinal and obstetrics departments of six public hospitals in Benin. The wound sampling from SSI in patients was performed from January 2019 to January 2020, and the nasal screening of HCWs was done later in 2020. The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences (FSS, Benin) under reference number: 012-19/UAC/FSS/CER-SS. Written informed consent was obtained from both patients and HCWs before enrolment into the study. Confidentiality and personal privacy were respected at all stages of the study

Sample collection

Wound swabs were taken from patients who presented clinical signs of SSI according to the Center for Diseases Control (CDC) definitions [22]. Overall 229 patients and 61 healthcare workers were sampled. The surfaces of the infected surgical sites were cleaned with normal saline before swabbing. Nasal swab samples were collected using sterile swabs, from both anterior nares of each HCW. Each swab was rubbed against the anterior first 1cm of the nasal vestibular wall of both nares. Metadata was collected at the time of swab sampling through a

standardized questionnaire containing demographic variables, antibiotic use, and hospitalization in the last six months.

Bacterial identification

The bacteriological analyses of samples were performed using conventional methods, as described previously [23] in the laboratory of microbiology (Cotonou, Benin). All the samples were delivered to the laboratory with optimal transport conditions.

- Wound swabs were inoculated on blood agar, Chapman, and chocolate agar media after incubation at 37°C for 24-48 h and was identified by standard microbiological procedures [24].
- Nasal swab samples were plated onto oxoid brilliance MRSA medium (Oxoid, Ltd., Basingstoke, United Kingdom) aerobically at 37°C and examined for growth after 24 and 48h of incubation following the manufacturer's recommendations. Colonies with typical shapes were selected for presumptive conventional phenotypic.
- A cefoxitin screen using a 30ug disc (Oxoid, United Kingdom) was performed to distinguish MSSA from MRSA. Isolates with a diameter $\leqslant$ 21 mm were classified as MRSA. Colonies were stored at -80°C in Trypticase Soya agar prior to molecular analysis.

All identifications were confirmed in Belgium by using the Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) according to the procedure described by Bizzini and Greub [25].

Quality control

Standard operating procedures (SOPs) were strictly followed during all analytical procedures, starting from sample collection, isolation, and identification. All culture media were prepared according to the manufacturer's instructions and were checked for their sterility and performance. As reference strains for quality control we used two international control bacteria strains: *E.coli* ATCC 25922 and *S.aureus* ATCC 25923.

Whole genome sequencing

The DNA of the MRSA isolates were extracted following a protocol adapted from Unal S. and collaborators[26] One colony was suspended into 50 ül of lysostaphin (0.1mg/ml) (Sigma-Aldrich, Overijse, Belgium) and incubated at 37°C for 10 min. Then, a mix of 45 ul of sterile water, 5 ul of proteinase K (2mg/ml) (Sugma-Aldrich, Overijse, Belgium) and 150 ul of TRIS-HCL (0.1 M, PH 8) was added to the suspension and incubated at 56°C for 10 min then at 95°C for 5 min and centrifuged at 13,000 *g for 5 min. Supernatants were recovered and stored at -20°C until further use. DNA purity and concentration were assessed with the Nanodrop 1000 (Isogen LifeScience, Utrecht, Netherlands). Isolate sequencing libraries were created using Nextera XT DNA library preparation (Illumina, San Diego, CA, USA) according to the manufacturer's instructions and subsequently sequenced using MiSeq V3 chemistry (Illumina) to produce 2 × 250 bp paired-end reads.

WGS data analysis

Quality control and preprocessing

Raw reads were trimmed using Trimmomatic 0.38 [27]with the following options: 'LEADING' set to 10, 'TRAILING' set to 10, 'SLIDINGWINDOW' set to '4:20', 'MINLEN' set to 40, and 'ILLUMINACLIP' set to 'NexteraPE-PE.fa:2:30:10'. Processed reads were then *de novo* assembled using SPAdes 3.13.0 [28]with the '--careful' option enabled and '--cov-cutoff' set to 10. Contigs smaller than 1,000 bp were removed using the seq function of Seqtk 1.3 (available at https://github.com/lh3/seqtk). The quality of the assemblies was evaluated using QUAST 4.4[29], providing the filtered assemblies as input. The median depth was evaluated by mapping the processed reads against the filtered assembly using Bowtie2 2.4.1 [30]with the '--end-to-end' and '--sensitive' options enabled. Afterwards, the 'depth' function of SAMtools 1.9 [31]was used with the '-a' option enabled to calculate the median depth. Kraken2 2.0.7 [32]was used to screen for contamination of the datasets with other microbial species. Reads were classified using an in-house constructed database containing all NCBI RefSeq 'Complete genome' entries (database accessed on the 11th of February 2021) with accession prefixes NC, NW, AC, NG, NT, NS, and NZ of the following taxonomic groups: archaea, bacteria, fungi, human, protozoa, and viruses. This database also contained a selection of birds, mammals, and arthropods reference genomes, which are listed in Supplementary Table S1. Datasets with >1%

of reads assigned to a species outside of the *Staphylococcus* genus were contaminated. Lastly, datasets were screened for intra-species contamination using ConFindr 0.7.4 with default settings[33]. Datasets were screened for intra-species contamination using ConFindr 0.7.4 with default settings. Only datasets that were not contaminated (i.e. not flagged by Kraken2 or ConFindr), with a median depth over 25x, and an N50 over 10,000 bp were retained for the analysis.

Isolate characterization

The online SCCmecFinder 1.2 tool [34]was used to check for the presence of the *mecA* gene and to determine SCC*mec* types. Blastn 2.6.0 [35]was used with default options to determine *spa* types by aligning the assembled contigs against the *spa* repeat sequences collected from the Ridom *spa* server (available at https://spaserver.ridom.de/). Sequence typing was performed as described previously[36], using the *S. aureus* MLST and core-genome MLST schemes retrieved from PubMLST.org (accessed 1st of May 2022). The assembled contigs were screened for antimicrobial resistance (AMR) genes using NCBI Antimicrobial Resistance Gene Finder (AMRFinder) 3.10.18 [37]with the '--coverage_min' option set to 0.9, the '--organism' option set to '*Staphylococcus aureus*' and database version '2021-12-21.1'. The presence of genes encoding virulence factors was evaluated using the blastn-based gene detection workflow described previously using the sequences from the Virulence Finder database (accessed 5th of June 2022)[36,38]. Only hits with >90% target coverage and >90% sequence identity were retained. The integrity of the open-reading frames (ORFs) of the detected genes was evaluated using GAMMA 1.4 with default settings. This additional analysis was limited to the AMR genes since the databases used for the virulence gene detection contained numerous invalid ORFs (i.e., sequences that did not start with a start codon and ended in a stop codon).

Phylogenomic investigation

A core-genome MLST allele matrix was constructed by combing the typing outputs, only considering perfect hits (i.e., 100% covered and 100% nucleotide identity). First, datasets with <90% of alleles identified were removed from the allele matrix. Afterwards, loci detected in <90% of datasets were removed. GrapeTree 2.2 [39]was then used to construct a minimum spanning tree from the filtered allele matrix with the method parameter set to 'MSTreeV2'. Separate SNP-based phylogenies were constructed for all detected sequence types (STs) with

multiple isolates from this study. SnapperDB [40] 1.0.6 and PHEnix 1-4 (available at https://github.com/phe-bioinformatics/PHEnix) were used to call Single Nucleotide Polymorphisms (SNPs) and calculate SNP addresses. The SNP address is a strain level 7-digit nomenclature based on the number of pairwise SNP differences. Each digit represents the cluster membership for the corresponding number of SNP differences, starting (right to left) with 0 (i.e., no SNP differences) to 5, 10, 25, 50, 100, and 250. Isolates sharing the same cluster digit differ by fewer than the corresponding number of SNPs to at least one other isolate in the corresponding cluster[40]. Reference genomes with matching ST were selected from the set of complete *S. aureus* genomes available in the NCBI assembly database. The sequences with accession numbers GCF_000013425.1, GCF_001444345.1, and GCF_008630695.1 were selected for ST8, ST121, and ST152, respectively. SNPs located in regions marked as phages by the online version of PHASTER [41]were removed before inserting the VCF files into SnapperDB. SNP matrices were extracted from SnapperDB using the 'get_the_snps' function. Maximum likelihood phylogenies were then constructed using MEGA 10.0.4[42], which was also used to determine the most suitable nucleotide substitution model. The 'Gaps/Missing data treatment' option was set to 'partial deletion', the 'Site coverage cutoff' to 50%, the 'Branch swap filter' to 'Very weak', the 'ML heuristic method' to 'SPR-3', and the number of bootstrap replicates to 100.

Results

Sample collection and species identification

A total of 304 wound swabs were collected from 174 patients with clinical signs of surgical site infections. We isolated 229 strains among which 49 were *S. aureus* and 65.3% (32/49) were resistant to methicillin[43].

Among 61 nasal screened samples of HCWs, 15 (24.6%) were MRSA. All of identification was confirmed in Belgium.

WGS data analysis

Preprocessing and data quality evaluation

In total, 53 WGS datasets were generated across six sequencing runs, as indicated in Supplementary Table S2. Obtaining high-quality DNA was challenging, and for six out of the 33 isolates, no high-quality WGS datasets could be generated, as indicated in Supplementary Table S3. When multiple datasets from one isolate passed all QC checks, the least fragmented (i.e., highest N50) was retained for the analysis. Raw and trimmed read counts for the 27 selected high-quality datasets are listed in Supplementary Table S4. The median number of read pairs before and after trimming were 457,715 and 423,759, respectively. An overview of the assembly statistics is provided in Supplementary Table S5, with a median N50 of 257,715 bp and a median total assembly length of 2,850,650 bp. The sequencing depth ranged from 32x to 115x, with a median of 62x.

Isolate typing

The STs, *spa* types, and SCC*mec* types for each isolate are shown in Supplementary Table S6. The 27 isolates were assigned to five STs. ST8 was the most prevalent (n=15) and contained isolates collected from HCWs (n=9) as well as patients (n=6). ST121 and ST152 were both observed in five isolates, including one HCW for ST121. ST772 and ST789 were only observed in a single isolate, collected from a patient and HCW, respectively. Multiple *spa* types were observed among the isolates assigned to ST8 and ST152. For ST8, the isolates were assigned to t4176 (n=13) and t121 (n=2). For ST152, three different *spa* types were observed: t1096 (n=2), t4690 (n=2), and t355 (n=1). The remaining *spa* types corresponded perfectly to STs: t657 (ST772), t091 (ST789), and t314 (ST121). The following SCC*mec* types were observed: IV (n=13), IVa (n=7), V (n=2), Va (n=2), Vc (n=2), and XII (n=1). For the thirteen isolates assigned to SCC*mec* type IV, the *ccrA2*, *dmecR1*, and *CCB2* genes were missing, and the prediction was based solely on homology to the complete SCC*mec* cassette, which was covered for ~55%. For isolate 2656, no hits were found based on the homology of the whole cassette, and the prediction was based solely on the detected genes.

AMR prediction

An overview of the detected genes and mutations associated with AMR is provided in Table 1. Resistance to aminoglycosides was predicted for 16 isolates due to the presence of the *aac*(6')-

Ie/*aph*(2'')-Ia (n=13), *aph*(3')-IIIa (n=1), or both (n=2) genes. Resistance to beta-lactam antibiotics was predicted for all isolates due to the presence of the *mecA* gene. Other detected genes associated with resistance to beta-lactam antibiotics were: *blaI* (n=23), *blaR1* (n=5), *blaZ* (n=23), *mecI* (n=1), and *mecR1* (n=1). Except for isolate 2022_017_1002 (ST789), all isolates were also predicted to be resistant to fosfomycin. All ST8, ST121, and ST772 isolates carried the *fosB* gene. Additionally, point mutations associated with resistance to fosfomycin were found, including: *glpT* A100V (n=10), *glpT* L27F (n=5), *murA* E291D (n=10), *murA G257D* (n=2), and *murA* T396N (n=10). Resistance to macrolides was predicted for four isolates which carried the *erm(C)* (n=2), *mph(C)* (n=2), or *msr(A)* (n=2) genes. Resistance to quaternary ammonium was predicted for eight isolates (all ST8) that carried the *qacC* gene. Point mutations associated with resistance to quionolones, including *gyrA* S84L (n=17), *parC* S80F (n=1), *parC* S80Y (n=16), were detected in 17 isolates, The *sat4* gene associated with resistance to streptothricin was detected in two isolates. All isolates carried a least one gene associated with resistance to tetracycline, including *tet(38)* (n=26), and *tet(K)* (n=16). The 2022_017_1401 isolate was the sole isolate not carrying a functional *tet(38)* gene since it contained an 1 bp deletion at position 215 resulting in an inactive gene product. Lastly, resistance to trimethoprim was predicted for 24 isolates, due to the presence of the *dfrG* (n=24) and *dfrS1* (n=12) genes.

Virulence gene detection

The detected virulence genes are shown in Table 2. All isolates carried at least one virulence gene associated with the production of exo-enzymes, indicated in green in Table 2. The *aur* gene, encoding aureolysin, was detected in all isolates. The majority of isolates carried additional serine protease genes: *splA* (n=16), *splB* (n=18), and *splE* (n=15). The five ST152 and the single ST772 isolate were the only isolates that, except for *aur*, lacked genes associated with exo-enzyme production. Except for isolate 2020_040_2090 (ST8), all isolates also carried one or more of the following genes associated with host immunity (indicated in red): Arginine Catabolic Mobile Element (ACME) (n=2), *sak* (n=25), and *scn* (n=26). The third category, indicated in blue in Table 2, contained genes associated with the production of toxins. Four of the five ST152 isolates carried the *edinB* gene. All isolates carried the *hlgA* and *hlgB* genes associated with the production of γ-hemolysin, and a subset of these isolates (n=22) additionally carried the *hlgC* gene. The *lukD* and *lukE* genes were observed in all isolates except for the five ST152 and the single ST772 isolate. The *lukF-PV* and *lukS-PV* genes were detected in 13 isolates: the two ST8 isolates with *spa* type t121, the five ST121 isolates, the five ST152

isolates, and the one ST772 isolate. Fifteen isolates carried at least one staphylococcal enterotoxin gene (e.g., *sea*, *seb*, …). Lastly, the *tst* gene was observed in eight isolates, all assigned to ST8.

Phylogenomic investigation

The core genome MLST allele matrix contained 1,654 loci after filtering. The resulting minimum spanning tree is shown in Figure 2. The clades in the phylogeny correspond to the classic MLST classification. Within the same ST, isolates with different *spa* types were clearly separated. For ST152, the number of allelic differences between isolates with different *spa* types was in the 20-40 range, indicating that the core genome of these isolates was relatively similar. In contrast, within ST32, the two isolates with *spa* type t121 differed over 258 alleles from the other ST8 isolates, which is a considerable difference. The clusters corresponding to ST8 and ST121 were the only ones that contained caretaker as well as patient isolates. The SNP-based phylogenies and corresponding SNP addresses for ST8, ST121, and ST152 are shown in Figure 4. For ST8, the SNP distances were relatively large. The two isolates with *spa* type t212 differed over 250 SNPs to the thirteen t1476 isolates. A clade of four healthcare workers from different hospitals shared the same three digits of the SNP address (i.e., 2.2.7; the 25-50 SNP level), which was a subclade of the *spa* type t1476 clade which shared the first two digits (50-100 SNP level). This larger clade contained isolates collected from nine HCWs and four patients, originating from the six hospitals that participated in this study. For ST121, relatively large distances existed between the isolates, since the SNP addresses only shared the first two digits (50-100 SNP level). Despite the high genomic similarity (e.g., virulence genes, *spa* type), no signs of a close phylogenetic relationship were observed between these isolates. For ST152, the topology of the SNP phylogeny corresponded to the *spa* type classification (i.e., isolates with the same *spa* type clustering together). The two-spa type t1096 isolates (2020_040_907 and 2020_040_907), both collected from patients, differed by two SNPs, indicating that they are highly likely to be related infections, especially since they were collected in the same hospital less than a year from each other.

Discussion

Characterization of the MRSA strains in circulation is essential to the implementation of efficient measures for their control and prevention. To the best of our knowledge, this is the

first study to describe the genomic characterization of MRSA strains collected from hospitals in Benin. We found that most isolates were resistant to oral antibiotics such as penicillin, tetracycline, fluoroquinolones, and cotrimoxazole (trimethoprim/sulfamethoxazole), all of which could be purchased without a prescription, potentially contributing to this high rate of resistance. Our findings are similar to those of a study conducted in Ghana in 2020, which reported similar rates of resistance to these antibiotics in a collection of 28 MRSA strains isolated from chronically infected wounds [44].

Antimicrobial resistance genes and rate of MRSA

Our finding of a high rate of MRSA in pus samples (32/49; 65.3%) and 24.59% in healthcare workers is much higher than the rate previously reported in hospitalized patients in Burkina Faso in 2016 (3.9%) [45]. It suggests that the rate is now accelerating much more rapidly than the acceleration found over two decades by a study conducted in Algeria, which showed a nearly tenfold rate of increase from less than 5% in 1996-1997 [46] to 50% in 2011 [47], and 62% in 2014 [48].

Many isolates in our study also showed resistance to cotrimoxazole, as in an earlier cases [49]. In Benin, cotrimoxazole is inexpensive, orally administered, and available over the counter without a doctor's prescription. For society, and particularly for pharmacists and physicians, awareness, education, and training are essential to responsible antibiotic use.

Distribution of sequence type, PVL and toxic shock syndrome toxin

PVL-encoding genes were detected in a substantial number of the isolates collected from patients and HCWs. These genes encode a bi-component cytotoxin that binds to the complement receptor C5a of neutrophils, whose cytotoxic effect on granulocytes leads to severe tissue necrosis [50,51]. The high dissemination of PVL+ *S. aureus* in Sub-Saharan Africa has previously been linked to three major clones. The first was ST15. The second and third, ST121 and ST152 [52,53], accounted for ten of the thirteen PVL+ strains in our dataset. Two studies published in 2012 and 2008 reported that ST152 was the most prevalent clone circulating in West Africa, accounting for 40-60% of infections, both in the community and in hospital settings [54,55]. Cases of this clone have also been reported in Europe [56,57], Turkey [58], and Haiti [59].

Another important virulence factor of *S. aureus* is toxic shock syndrome toxin (TSST) [41]. The *tst* gene encoding TSST was detected in eight isolates in this study, all assigned to ST8, and collected from an equal number of patients (n=4) and HCWs (n=4). These isolates all carried many other genes associated with virulence. As isolates co-harboring the *tst* gene and other toxin genes have been described as highly pathogenic [60], the spread of these highly pathogenic strains poses a public health risk. Implementing measures for their prevention should be a public health priority.

Relatedness between isolates, including patients and HCWs

Our phylogenomic investigation identified several groups of potentially related isolates with very similar core genomes, indicating the possibility of circulation amongst HCWs and patients. For example, the five isolates assigned to ST121 differed by only two core-genome alleles on a total of 1,654 loci. This potential cluster contained four isolates collected from patients and a single isolate collected from an HCW. Within those groups of related isolates, Our SNP-based approach found no evidence of direct transmission. However, potentially linked isolates may have been missed, due to the relatively low number of samples included in the WGS-based analysis (i.e., only a few isolates per hospital) and the sampling that did not occur in the same period. Despite this lack of evidence, two factors suggest that the strains colonizing HCWs may be involved in the clinical infection of patients: the great genomic similarity in terms of STs, AMR genes and mutations; and the similarity between the strains of the virulence genes collected from patients and those collected from HCWs. Two strains from patients visiting the same hospital showed a very great genomic similarity (only 2 SNPs difference), indicating that these infections were very likely related to each other, and possibly transmitted within the community.

Importance of screening in prevention of infection

Because nasal screening and selective decolonization may significantly mitigate the risk of surgical site infections[61], insight into the risk of subsequent *S. aureus* infection can be provided by characterizing the colonizing isolates. As described elsewhere, screening of HCWs is recommended if PVL+ producing MRSA infections was acquired in hospitals[62] or if they are unusual epidemiological aspects of outbreak or if they are persistent MRSA carriage by staff [45]. Given the sub-optimal hand-hygiene compliance we observed in hospitals in Benin

[63], it is possible that HCWs contributed to the spread of MRSA in this setting. Even though various studies have highlighted the fact that screening and decolonization before certain types of surgery play important roles in reducing rates of surgical site infection, proper hand hygiene remains a relatively easy preventive measure for reducing MRSA in LMICs.

Strengths and limitations

Our study has strengths and limitations. It was a multi-center study and may reflect the local epidemiology situation in the south of country and molecular data have indicated PVL-positive MRSA clones. However, the limited number of isolates and the large observed phylogenomic diversity indicate that our study did not fully capture the diversity of the strains circulating in the hospitals.

Conclusion

The severe complications than can result from MRSA SSIs pose a substantial burden on the healthcare systems worldwide. Our study shows that drug-resistant pathogenic MRSA strains are circulating in hospitals in Benin. Although WGS and other surveillance methods can provide valuable information that will help combat these infections and reduce the spread of AMR, the wide phylogenomic diversity found in this study makes more research and screening essential.

Figures

Figure 1: Overview of the sample collection approach for samples to be used for WGS

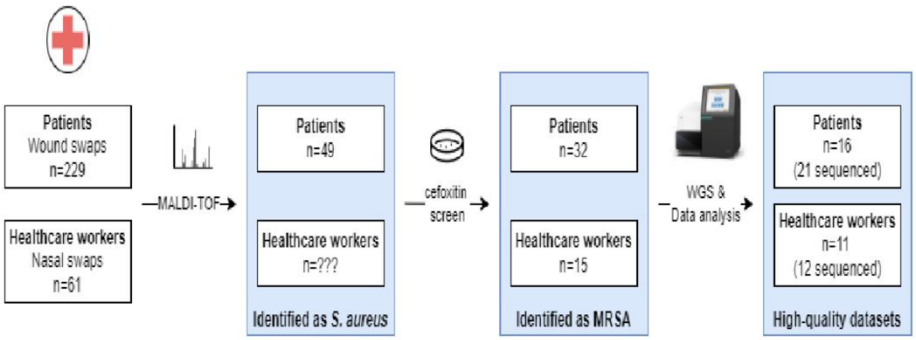
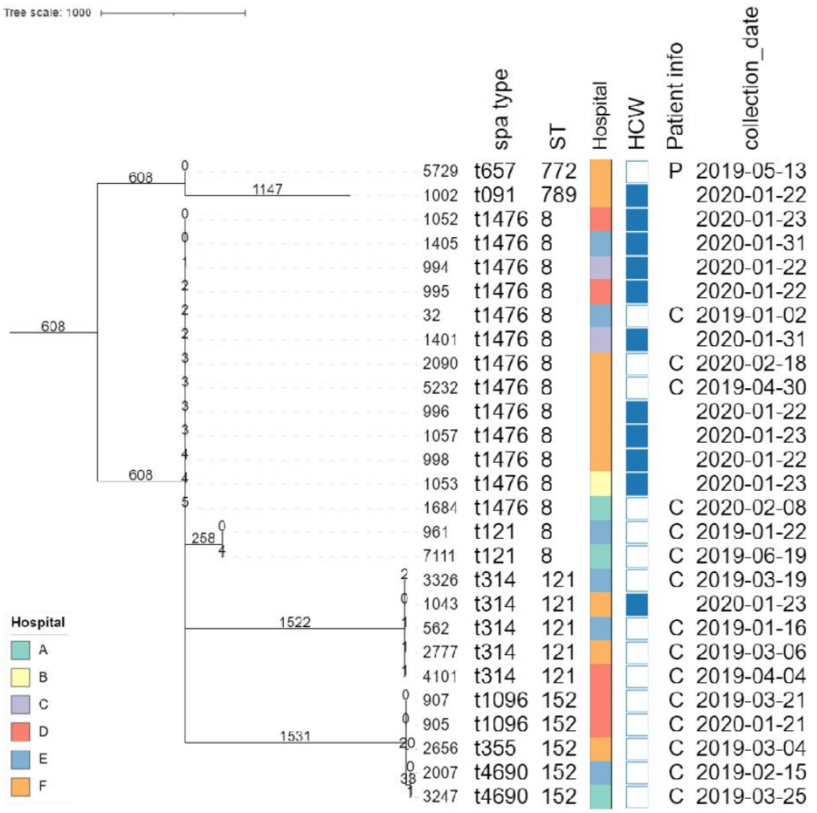


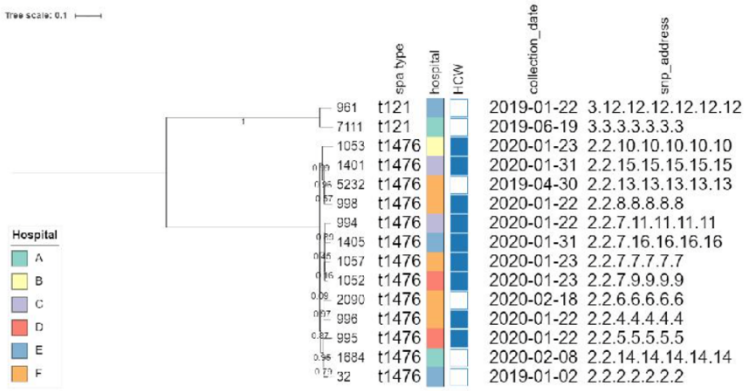
Figure 3: Core-genome MLST phylogeny



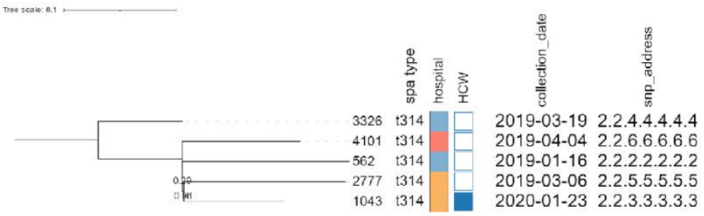
Branch lengths and the scale bar are expressed as the number of allele differences. The annotations are from left to right: *spa* type, sequence type (ST), hospital code (see legend in the bottom-left), HCW (a filled rectangle indicates that the corresponding isolate was collected from an HCW), patient information (P: peritonitis, C: cesarean), and collection date. The six hospitals participating in the study were randomly assigned an identifier from A to F.

Figure 4: SNP-based phylogenies for ST8, ST121, and ST152

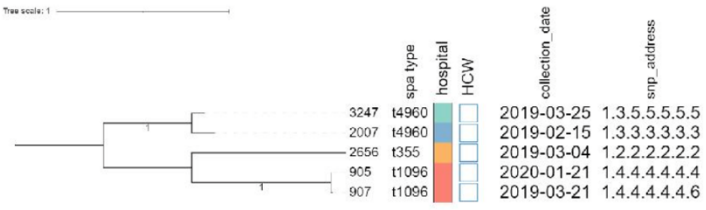
A - ST8



B – ST121



C – ST152



This figure shows the SNP-based phylogeny for ST8 (A), ST121 (B), and ST152 (C). Branch lengths and the scale bar are expressed as average substitutions per site. The bootstrap values are shown above the corresponding branches. The annotations are from left to right: isolate name, *spa* type, hospital, HCW (a filled rectangle indicates that the corresponding sample was collected from a HCW), collection date, and SNP-address. The six hospitals participating in the study were randomly assigned an identifier from A to F and are colored according to the legend.

Tables

Table 1: Detected genes and mutations associated with AMR

ST	spa type	isolate	AMG		BLA				FOF					ML			QA	QN		ST	TET		TMP	
			aac(6')-Ie aph(2'')-Ia	aph(3')-IIIa	blaI	blaR1	blaZ	mecA	mecI	mecR1	fosB	glpT A100V	glpT L27F	murA E291D	murA G257D	murA T396N	erm(C)	mph(C)	msr(A)	qacC	gyrA S84L	parC S80F	parC S80Y	sat4	tet(38)	tet(K)	dfrG	dfrS1
8	t121	2020_040_7111	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0
8	t121	2020_040_961	0	1	1	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	1	0	1	1	1	0	0	0
8	t1476	2020_040_1684	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
8	t1476	2020_040_2090	1	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0	1	1	1	1
8	t1476	2020_040_32	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	1
8	t1476	2020_041_5232	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1
8	t1476	2020_045_998	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1
8	t1476	2022_017_1052	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
8	t1476	2022_017_1053	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
8	t1476	2022_017_1057	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1
8	t1476	2022_017_1401	1	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1	1	1
8	t1476	2022_017_1405	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
8	t1476	2022_017_994	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	0
8	t1476	2022_017_995	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	1
8	t1476	2022_017_996	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1	1
121	t314	2019_071_3326	0	0	1	0	1	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0
121	t314	2020_040_2777	0	0	1	0	1	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0
121	t314	2020_040_4101	0	0	1	0	1	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0
121	t314	2020_040_562	0	0	1	0	1	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0
121	t314	2020_041_1043	0	0	1	0	1	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0
152	t1096	2020_040_905	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0
152	t1096	2020_040_907	0	0	1	1	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0
152	t355	2020_040_2656	1	0	0	0	0	1	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0
152	t4690	2020_040_2007	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0

152	t4690	2020_040_3247	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0
772	t657	2020_040_5729	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0
789	t091	2022_017_1002	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0

Overview of detected genes and mutations associated with AMR. The first, second, and third columns list the STs, *spa* types, and isolate names, respectively. The subsequent columns indicate the presence of the corresponding gene or mutations. A '1' indicates that the feature was detected, and '0' indicates that it was absent. Features associated with AMR were grouped based on the classes defined in the AMRFinder database as indicated by the top row. Abbreviations: sequence type (ST), aminoglycosides (AMG), ß-lactamases (BLA), bleomycin (BLE), fosfomycin (FOF), macrolides (ML), quaternary ammonium (QA), quinolones (QN), streptothricin (ST), tetracycline (TET), trimethoprim (TMP).

Table 2: Detected virulence genes

| ST | spa type | isolate | Exo-enzymes | | | | Host immunity | | | Toxins | | | |

Overview of detected virulence genes. The first, second, and third columns list the STs, *spa* types, and isolate names, respectively. The subsequent columns indicate the presence of the corresponding gene. A '1' indicates that the gene was detected, and '0' indicates that the gene was absent. Colors correspond to the three categories of virulence genes as defined in the Virulence Finder[38] database, shown in the top row of the table. Abbreviations: sequence type (ST).

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4 GENERAL DISCUSSION

4.1 WHAT WE DID AND WHAT WE FOUND

The Multidisciplinary Strategy for Prevention and Infection control (MUSTPIC) project aimed to improve hand hygiene practices, antimicrobial prophylaxis and globally contribute to the prevention of hospital acquired infections in Benin. There are three different theses associated to this project, each with specific objectives. The objectives of this thesis were 1) to describe hand hygiene attitude among healthcare workers and understanding enablers and barriers of adherence to good HH practices; 2) to determine the causative bacteria involved in surgical site infections (SSIs) as well as their molecular characterization; 3) to implement some actions that will help in the prevention of SSIs in the country.

Surgical site infections (SSIs) constitute a nightmare for surgeons. The first and only national point-prevalence study on them in Benin was organized in the context of the first International Conference of Health Ministers of Africa (CIMSEF 2012) held in Cotonou, Benin in 2012. For this study, all hospitals were invited to participate between 10-26 October 2012 using the protocol developed by the "Hospital in Europe Link for Infection Control through surveillance" (HELICS) project. The rate of SSIs in the 39 included hospitals was 24.7%[1]

In developing countries, SSIs are an example of healthcare-associated infections (HAIs) that can be prevented by established techniques. In our country, a further limitative factor is the lack of national infection control policies that would help to reduce the rates of SSIs. Nothing is known about hand-hygiene practices or about molecular description

of the multidrug-resistant bacteria isolated in SSIs. It is within this context that we carried out MUSTPIC project. Our choice of surgery departments in six hospitals was due to the fact that gastrointestinal interventions were performed there. The caesarean section was an important choice, as caesareans have been free of charge since 2009, along with the use of a kit containing antibiotics [2].

In **chapter 1.1**, we conducted an observational study in hospitals. All HCWs were observed unobtrusively during their daily tasks. The WHO hand-hygiene observation tool was used to collect opportunities. We found that the HCWs missed two out of three opportunities to perform HH correctly, and that when they respected these opportunities, the duration and technique were not respected. We observed a self-protective attitude amongst the HCWs, who were more compliant after touching patients and after body-fluid exposure but were less compliant before patient contact. Only two-thirds of these HCWs respected the prerequisites of HH. They also lacked infrastructures, regarding not only their number but also their location. Hand-washing remained the most practiced technique.

In **chapter 1.2,** to a better understanding of the HCWs' behavior, interviews were conducted in one of the six hospitals. This qualitative study constitutes to our knowledge the first in its field. The theoretical domain frameworks were used to identify enablers and barriers. Although HH is recognized to be an important cornerstone for the prevention of infections, almost all participants highlighted the unavailability of good infrastructures and resources and reported the

lack of frequent training about hand hygiene in hospitals. This qualitative study is the first step that allow description and prioritization of barriers that provoke a non-adherence to HH hygiene best practices. Based on chapter1, targeted training and feedback to the hospital of the MUSTPIC was provided. With the WHO implementation strategy, the project gives to these hospitals' posters and reminders, that could help to improve hand hygiene daily practice.

In **chapter 2.1**, we first realized the identification of bacteria by using the classical microbiology identification (biochemical tests, API 20 E) in Benin and then all strains were confirmed in Belgium by MALDI-TOF mass spectrometry. This double identification in Belgium showed almost 100% concordance between the first identification performed in our laboratory in Benin and the MALDI-TOF. For antibiogram two methods were used: The Kirby-Bauer disc diffusion and automated antibiogram system of phoenix. In gastrointestinal surgery, the top three bacteria isolated were *Escherichia.coli, Klebsiella. peumoniae* and *Enterobacter cloacae*. In maternity, the three most common were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. These bacteria were less susceptible to antibiotics such as beta-lactams, the third and fourth generation of cephalosporins. Almost 91% of strains were resistant to more than three classes of antibiotics. Due to their cost and frequent unavailability, the most susceptible of antibiotics remain carbapenem and amikacin.

The **chapter 2.2** with the whole genome sequencing we reported a case of *Acinetobacter baumannii* that was resistant to almost all antibiotics, isolated in a patient who previously received as antimicrobial prophylaxis ceftriaxone. We detected the first New Delhi metallo-beta lactamase-producing *Acinetobacter baumannnii* for which it was impossible to find correct therapeutic options.

In the **chapter 2.3,** the whole-genome sequencing performed on extended spectrum beta-lactamases producing *Escherichia. coli* showed that CTX-M_{15} was the dominant beta-lactamases and a diversity of clones that were not linked to hospital transmission

In the **Chapter 2.**4 we described all strains that were resistant to at least one carbapenem and highlighted the role of good antibiotic prophylaxis in surgery. In our laboratory the detection of carbapenem resistant organisms (CRO) is performed by the Modified Hodge Test. This test is obsolete and less specific than chromatographic and molecular tests.

Based on the antibiogram of isolated bacteria, researcher team and experts of 2 countries (Benin-Belgium) elaborated and validated the antimicrobial surgery guidelines. These guidelines will be used in hospitals as reference for antimicrobial prophylaxis and for treatment of surgical site infections. This study was described in the **chapter 3.1.**

To explain more about the frequency of methicillin-resistant *Staphylococcus aureus* and the presence of Panton Valentine leucocidin toxins in the wound's swabs, we analyzed in **Chapter 3.2.,** the screening samples of HCWs. The clones identified and the antibiogram profiles were found to be the same. Nonetheless, no link of transmission was found between strains.

In our setting, characterized as it was by such a lack of resources and infrastructures for hand-hygiene practices, our first action should have been not to screen HCWs in order to prevent the transmission of MRSA, but to change the system altogether by promoting good adherence to HH.

As described in the last chapter, based on antibiogram results and the results of two other researchers (Angele and Ariane) (such as the audits of surgical antimicrobial prophylaxis (SAP) in maternity and gastrointestinal surgeries), we propose a set of interventions such as feedback on audits by hospitals, a series of trainings on hand hygiene in all hospitals that targeted HCWs who were members of CLIN (Comité de lutte contre les Infections Nosocomiales). These feedback sessions were much appreciated by the partner hospitals, and discussions provided a rich series of contributions.

4.2 CRITICAL APPRAISAL

In this chapter we discuss the added value, strengths and limitations of the research conducted in this thesis.

4.2.1 Research relevance

Except for the first and single national point-prevalence study of 2012, we started MUSTPIC project on a blank page. The rate of SSIs described then was 24.7% [3]. As several studies described, SSIs constitute the hate of surgeons due to the increasing mortality, morbidity, and antibiotic resistance they cause [4]. Hand hygiene – one of the most important measures for reducing the transmission of bacteria in routine care – was found to be a considerable challenge.

❖ **Hand hygiene**

During this thesis we observed and analyzed the behavior of HCWs. As described in other studies, their non-optimal compliance was due mainly to the lack of environmental resources and infrastructures. In certain hospitals, the resource problems included the lack of a continuous supply of safe water, soap, and towels. Almost two-thirds of the HCWs we observed washed their hands but failed to perform alcohol-based hand rubbing (ABHR). This was due not only to the ignorance of the efficacity of ABHR, but also to the non-availability of gel dispensers.

HH compliance was often better among nurses than among medical doctors, possibly because, unlike many nurses during their training, doctors in Benin

have never received hygiene training during their university course. But we also encountered an unstated feeling about the negative consequences of ABHR use. Some HCWs preferred washing their hands with soap to using alcohol, as the latter caused dryness.

Additionally, as some interviewees stated, “HCWs in at least one of the hospitals doubted the quality of the water that was used for washing.” Many of these HCWs washed their hands more than they practice ABHR. More information and raise awareness will be necessary to educate people. For example, during the feedback, different steps of ABHR were described and their reminders and posters were out on place in these hospitals. Regarding the interviews analysis, the role of a model was also highlight and will be promote in hospitals. This model will help colleagues to correctly perform hand hygiene.

Finally, even if gel dispensers were available, they were often empty. During the Covid-19 pandemic in the country, many packets of hydroalcoholic gel were therefore distributed in hospitals, and the project also made some available to partner hospitals.

The literature testifies to a growing interest in producing ABHR locally according to the WHO formulation [5,6], which was found to be less expensive than commercially available ABHR. In Benin, however, there was no site for local production; a reliable supply of ingredients and dispensers was one problem, and quality control of ABHR solution was another. For example, Benin is a traditional sugar producer. This sugarcane can be used as feedstock for production of ethanol on a consequent scale to be used for industries to prepare alcohol-based hand rub like other countries such as

Brazil [7,8]. We must promote the add value of use of the sugarcane to product alcohol and consequently promote ABHR in the hospitals.
In the intervention phase, the MUSTPIC project (Researcher 3) proposed that alcohol solution should be produced and members of CLIN should be trained. All steps were planned up to quality control by the analytical chemistry laboratory of the health faculty (FSS, UAC). Implementation of production according to the WHO multimodal strategy was planned to start after an audit in the third year of project [4,9]. However, these plans were deflected during the Covid 19 pandemic, when hospitals received frequent donations of ABHR solution. Simultaneously, temporary local production sites have appeared. While these factors probably have helped alcohol solutions to reduce the rate of MDR bacteria, the research team was unable to properly implement the training phase of ABHR preparation in partner hospitals. It will therefore be important to estimate the daily consumption of alcohol solution per hospital and per HCW, and to promote the sustainable distribution of solutions in healthcare settings [10–12]. As recommended by the WHO, the system change including the availability of ABHR at the point of care is a core element of the "Clean Care Is Safer Care" campaign [13].

❖ Etiological bacteria involving in SSIs

As described by others, *Escherichia coli* and *Klebsiella pneumoniae* were predominant in gastrointestinal surgery [14–17]. In maternity, however, *Staphylococcus aureus* and *pseudomonas aeruginosa* were the most common. Despite the importance of antibiotic prophylaxis (i.e. a very brief course of antibiotics initiated closely before the start of operative procedures to reduce

post operative suppuration) [18], the unavailability of first-generation cephalosporins in Benin makes the choice of antibiotic a continuing challenge. According to the type of surgical intervention, patients and women who underwent caesarean section took antibiotic prophylaxis no more than 30 minutes before operation. In two thirds of the cesarean cases, ceftriaxone was the most used for the SAP, and half of the women took more than one antibiotic, either for a long time or in acute treatment [19]. Ceftriaxone is known to cause extended-spectrum beta-lactamases [20], which may explain the higher number of ESBL-producing *E.coli* and *K. pneumonia*e as well as the alarming rate of multidrug-resistant bacteria. As *Pseudomonas aeruginosa* is also intrinsically resistant to ceftriaxone, it explains why, despite SAP, we frequently isolated it in maternity wards.

In addition, the high rate of multidrug-resistant bacteria could also be explained by self-medication practices in Benin. As observed by Dohou et al, before caesarean, 56.7% of the 141 women included in the second phase of this project used antibiotics prior to hospitalization, over half of whom took more than one antibiotic, either for a long time or in acute treatment [21]. Therefore the government and health ministry enacted a law in 2020 prohibiting all sales of antibiotics without prescription.

Another important reason for the misuse of antibiotics and the ensuing increase in the rate of MDR in hospitals is that physicians are often forced to use antibiotics without having done an antibiogram. Unfortunately, few laboratories are able to carry out all process of bacteriology for specimens. For example, when implementation of the resistance-control “WHO GLASS” project started in Benin, the criteria were met by only six of the 60 public hospitals. These criteria were set according to the realities on the ground and

not according to international standards. Combating AMR is a permanent battle, and unfortunately Benin does not belong to internationals such as the Fleming fund project, which supports LMICs in generating, sharing, and using data to improve antimicrobials use and to encourage investment in AMR [22].

The frequency of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in SSIs is frequently associated with increased mortality and morbidity. These bacteria are found mostly in moist environments, including hospital water systems [23]. Colonization of hospital water plumbing systems with *P. aeruginosa* has been shown to be an important source of the bacteria's facilitation of transmission to patients. In Tanzanian public hospitals, *P. aeruginosa* was found to contribute significantly to wound infections [24]. As the resistant bacteria are transmitted between humans, animal, and environment, understanding the frequency of certain bacteria in SSIs requires a common "one world one Health" approach. The studies on the bacteria isolated in hospital environments and specifically in the included hospitals previously conducted in Benin are scarce. In the literature, the role of a clean environment in reducing rates of HAIs has been encapsulated in the core components of an effective infection control program [25]. Nevertheless, "*the lack of effective implementation strategies and most particularly funding leaves issues of environmental sanitation and hygiene low on the priority list*"[25]. Further studies must establish the profiles of bacteria isolated in hospital environments, and particularly in surgery services. The only study conducted in two public hospitals in 2019 in Benin showed that strains that were isolated most in their environments were respectively *Escherichia coli*

and *Klebsiella pneumoniae* [26]. Moreover, the role of ventilation systems in operating room was highlighted in many studies [27,28]. Biological particles in the operating room air environment can cause surgical site infections render airborne a significant source of exogenous source of wound contamination as well as the hand hygiene contribute to the transmission of bacteria from patient to patient, from healthcare workers to patient or vice versa [29]. Thus in LMICs they used some strategies that helped for reducing airborne contaminants. We can recommend the use of :

-laminar air flow ventilation

-limiting personnel in the operating room

-complete hair and body coverage for all the staff in the operating room

-UV lights to eliminate airborne contaminants [30–32]. Nonetheless most of these measures are not yet applicable in our hospitals where the buildings are old. We will have promoted the creation of modern operating rooms with a frequent air exchanges.

❖ **Molecular characterization of multidrug-resistant bacteria**

This project first describes the molecular aspects of ESBL-producing *Escherichia coli* in a clinical setting. The most described beta-lactamases were $bla_{CTX\text{-}M15}$, which in recent years has represented the most cited beta-lactamases worldwide [33,34]. As CTX-M-15 are associated with plasmids, they are also resistance genes in aminoglycosides, quinolones, and trimethoprim [35–37]. The plasmid-mediated quinolone-determining region (PMQR) and Qnr genes are described in half of sequenced *E. coli.*

The phylogeny of strains shows a high diversity of sequence types without a clear link of transmission between hospitals. In fact, the low number of *E. coli* isolates and the MLST technique were not enough to find this link. To detect the link of transmission of strains such as the Pulsed-field Gel Electrophoresis (PGFE) [38], further molecular techniques would be appropriate [39].

In our laboratories, carbapenemases were detected by the Modified Hodge Test (MHT), an obsolete phenotypic test that does not detect all enzymes [40]. However, it is essential to define the corresponding enzymes, not only to enable physicians to provide the correct treatment, but also to complete any additional precautions the situation requires (such as isolation). Due to the lack of dedicated equipment in Benin, molecular analysis was performed in Belgium. Very few laboratories in Benin are able to perform bacteriology antibiogram with respect of all process (quality of control, respect of guidelines and correct microbiological process). The lack of microbiological laboratories in Benin explains the limited number of hospitals included in this study – to which we should add that we chose hospitals closest to the research centers and one of the best equipped laboratories in the country. Although it is difficult to imagine analysis of MDR by whole-genome sequencing in Benin, there are cheaper chromatographic tests that are more adaptable in the context of developing countries [41,42]. As well as the whole genome sequencing is a high and expensive tool, the MHT test is obsolete and should be abandoned in our laboratories.

❖ First interventions for prevention

The guidelines proposed by this project will be considered for validation by the national authorities by the end of 2022. It is important to specify that some aspects must also be considered, such as the cost of the proposed antibiotics, and, above all, the political will to improve the practice of SAP in the country. The guidelines will be share between HCWs of included hospitals. The research team is committed to explain the importance of these guidelines and their correct use to healthcare professionals in the surgery services. Then, we will promote its use in other hospitals that were not involved in the initial project and finally we will organize audit of use of guidelines in hospitals and if necessary, improve the first edition later.

In relative terms, MRSA screening rates in Benin were higher than those reported in Burkina Faso and Taiwan in 2018 [43,44]. The two thirds of methicillin-resistant *Staphylococcus aureus* isolated on the pus samples were positive for the detection of Panton Valentine Leukocidin, a toxin associated with severe necrotizing pneumonia and other soft tissue infections that destroy white cells [45,46]. Most bacteria found in the SSIs in maternity were *S.aureus.* Even if nasal screening was not done in the same period, MRSA strains in patients were genotypically similar to those described in HCWs. The dominant clones in patients were the same as those in HCWs. MRSA carriage occurred in healthcare settings, probably representing hospital acquisition. Although screening HCWs, and particularly patients, before intervention is helpful for controlling MRSA dissemination in hospitals, it is applicable specially to major clean surgery, such as cardiothoracic and orthopedic surgery, and involves the insertion of implant devices [47].

In LMICs, the preoperative identification of carriers of MRSA followed by decolonization before surgery should be considered with caution due to the risk that resistance to mupirocin may emerge. Nevertheless, in our settings, decolonization remains the responsibility of the patients or HCWs, who will not benefit from any support in this sense. After decolonization, the positive cases have not received any treatment. Among strategies to prevent or minimize the rate of SSIs we can cite the decolonization patients with *S.aureus* especially before elective surgery [48]. This method is subject of controverses in literature and for authors another approach recommends that all patients due for surgery should be decolonized without prescreening for *S. aureus* [49,50]. Many studies involved elective cardiothoracic and prosthetic joint surgery highlighted the positive role of a combination of MRSA screening, contact precautions and decolonization which resulted in decreased MRSA rates [51,52]. But in our country without a national insurance for health all the costs for screening and decolonization would be in charge of patients and remains impossible for all.

Once again, the molecular characterization of MRSA strains represents the "visible tip of the iceberg," and further studies will be needed, especially in the hospital environment, to describe in greater detail the clones that circulate there. Cross-transmission in hospitals will also be improved by promoting proper adherence to HH. We don't need molecular methods to control antimicrobial resistance in the country as the first step remains a correct identification and early detection of resistance genes that could be screen by phenotypic methods and chromatographic screening tests[53].

4.2.2 Strengths

To answer research questions regarding MUSTPIC project, we explored a wide range of research methods and conducted a cross sectional study and mixed methods (quantitative and qualitative studies). This study is the first in the field of antimicrobial resistance and infection control that explore data collected on six public hospitals in Benin.

❖ **Mixed method approach used for hand-hygiene investigation**

- Hand-hygiene practices were investigated by direct observation. Although this method suffers from the "Hawthorne effect" [54], interviews and discussions with healthcare workers are appropriate methods for better understanding their behavior. Qualitative study thus provides the backbone for implementing interventions to promote HH
- Another strength of our study was the healthcare settings, i.e., the six different hospitals and surgery wards. This is to our knowledge the first study that include two wards on surgery through six public hospitals. The choice of maternity because of the availability of caesarean kit free of charge and gastrointestinal surgery because they are frequently gastrointestinal intervention cases in all selected hospitals.
- To our knowledge, this study is the first in its field in Benin.
- We started with the components of WHO multimodal strategy: feedback was provided per hospital in the form of audits focusing on weaknesses and the perspectives for further improvements. A series

of trainings was also organized, reminders of the need for HH were distributed and posted in all six hospitals.

❖ **The prospective study and use of next-generation analytical methods**

- In all hospitals, pus samples were collected for one year in real time. As all initial analyses were performed in the same laboratory. Reproducibility was guaranteed by full adherence to the EUCAST procedures and guidelines for antibiograms.
- Before the start of the project and again after six months, all nurses were trained in correct wound sampling. We implemented in some hospitals firstly the procedure regarding the wound sampling. As well as the detailed descriptions of these procedures, each hospital received swabs and consent forms. A special network, "MUSTPIC SSIs," was created to facilitate discussion and debate on the availability of results, on restarting contaminated samples and on other technical questions.
- The results of classical identification and disk-diffusion antibiograms performed previously in Benin were confirmed by mass spectrometry (MALDI-TOF) and Phoenix antibiograms.
- The use of WGS and multi locus sequence typing not only provided complete characterization of antimicrobial resistance genes at hospital level, but also served as a basis for the establishment of an antimicrobial stewardship program and of infection prevention and control initiatives in country. However, failing to have WGS and other molecular technics, we have work for improving availability of phenotypic tests for detection of MDR.

- All of isolates received in our laboratory analyzed by conventional methods were confirmed in Belgium (Clinique universitaire Saint Luc) with automated methods. This performance is linked to the correct organization or sampling process during the study and the high performance of the laboratory of bacteriology of Cotonou.

The prospective nature of the study remains very positive because we collected data in real time and limit errors and biases. This kind of study is to our knowledge the first in this field with inclusion of six hospitals.

❖ **All samples free of charge**

For the whole duration of the project with the support and funding received by ARES (Académie de la Recherche pour l'Enseignement Supérieur), all pus samples were taken and freely analyzed.

The results were also brought back immediately to the wards concerned. The great difficulty is that there is no health insurance for everyone in our setting. This fact makes impossible for patients to afford the cost of additional checkups. Finally, the acknowledge goes on the ARES for their valuable support which allow to impact both the population and healthcare system with strong manuscript published in international journal.

❖ **Intervention phases and concrete actions based on results**

Personalized feedback in hospitals, posters, and reminders (HH poster), and training on HH.

The principal challenge for this project was to develop a guideline on the use of antibiotics in surgery. As a follow-up of this research, and in combination

with the findings of the two other researchers, global surgery guidelines on the rational use of antibiotics were developed and validated by experts based on the Delphi method.

❖ **The number of specialties involved in the study:**

They are various specialties involving in the MUSTPIC project allowing rich discussions with partners in hospitals. Three doctors are trained and made available to the university of Abomey-Calavi (UAC). I was trained to perform qualitative research with anthropologists and globally the project allows improvement of knowledge in the field of bacteriology in Benin and Belgium through expertise of microbiologists involved in.

- All results (articles, support, and the WHO link) were made available on the project site via https://uclouvain.be/en/research-institutes/ldri/must-pic.html

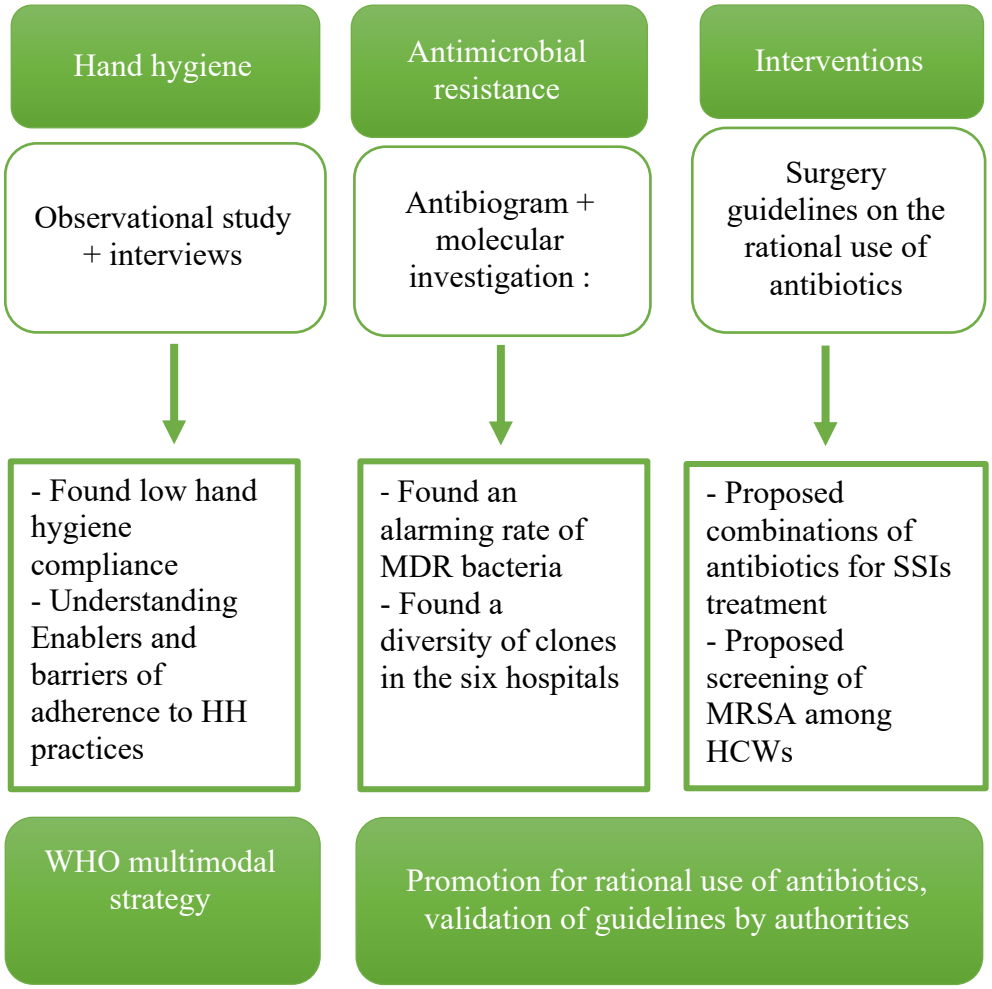


Figure 7: Steps, results and interventions observed in this thesis

4.2.3 Limitations and validity

Although the following limitations and difficulties of this research work need to be addressed, we do not believe that they discredit our results.

❖ *1. For the hand-hygiene study:*

During direct observation, the wearing of gloves was not audited.

Initially, all hospitals had planned to support focus-group discussion and interviews that were intended to provide greater understanding of HCWs behavior and the perspectives for improving HH compliance. For organizational and practical reasons, however, interviews took place at only one hospital without a focus group. This constitutes an important limitation of qualitative study as we have interviewed only nine healthcare workers in one hospital. As well as we acknowledge the relatively low number of interviewees, this is a first insight and prerequisite for implementation of WHO multimodal strategy. The direct observation of HCWs will be restart frequently in hospital after the implementation phasis.

❖ *2. For the sampling process and antibiogram results*

- Slow start-up of pus sampling

Due to unexpected delays and inactivity of the local ethics committee, the sampling steps started six months late. Without this delay, we believe that we would have had more isolates and possibly more interesting ones. The positive point is that this project made it possible to reactivate the ethics committee of health faculty of university of Abomey-Calavi. .

- Organizational challenges within hospitals

As, research are relatively rarely introduced in hospitals in Benin we noticed some difficulties. In two hospitals, all sampling was performed by the head nurse. When he or she was not present, no samples were taken – this fact explains why we did not have the expected number of samples. Moreover, for financial reasons, some nurses failed to perform sampling properly. Some of them later admitted that they had hoped they would earn more during the project. For other, they described the fearless or criticism of colleagues if they sent a lot of suppuration samples. It was very apparent from the flow of samples that, in some hospitals, there were more samples in the weeks when we checked the sampling team. In the beginning, the project has paid off for the hospital's management the equivalent of 1000 samples but for one year, we only received 304 samples through the 6 hospitals. The following table summarize the number of samples per hospital. Even if we understand well that the hospitals which have more affluence send more samples, the number is globally less than what we expected. We could have taken advantage of the fact that the results were free to boost the samples.

Table 8: Summary of the number of isolates received per hospital during MUSTPIC project

Hospitals	Number of isolates n (%)
A	102 (33.6)
F	98 (32.2)
D	40 (13.2)
E	35 (11.5)
C	21 (6.9)
B	08 (2.6)
Total	304 (100)

- Strict anaerobes were not detected amongst specimens

Surgical infections are largely polymicrobial, and the pathogenesis of the infections encountered during this project involved both aerobic and anaerobic bacteria. Due to limited facilities and expertise, strict anaerobes were not isolated during our study. The number of bacteria isolated that were found to be negative may therefore have been underestimated.

Although anaerobic bacteria are recognized as important pathogens in SSIs, the tedious culture techniques and the longer turn-around times meant that they were the microorganisms that were most overlooked by the laboratory. Strain anaerobic culture refers to the culture of microorganisms in an oxygen-free environment. As well as the modified candle-jar system, which is common in our setting, techniques include the anaerobic bag method, the

biological consumption method, the pyrogallic acid method, and the blister medium method [55]. Although the modified candle-jar system was found to be a sensitive and cost-effective alternative that might be used in LMICs, our laboratory does not systematically perform cultures; neither, for technical nor financial reasons, does it routinely perform susceptibility testing for anaerobic bacteria. Despite the latter, it is also important to educate technicians to reject unacceptable specimens. For example, rather than collecting pus or superficial specimens, specimens for anaerobic culture should collect at the infection site at the advancing wound edge [56].

- The rate of surgical site infections was not determined

The main limitation of this study was the unavailability in hospitals to the total number of surgical interventions per wards during the period of sampling. Moreover some hospitals sent a few number of specimen and it is possible that they missed some cases of SSIs because of the fear of being judged or criticized about surgical procedures.

For the correct estimation rate it would be better if we include all post discharge cases of SSIs while we have just included all positive cases (based on the CDC definitions) discovered during a wound dressing or in hospitalization room. The most important inclusion criteria were that the surgical procedure must have been done in six included hospitals. But we did not have to call the patients to ask about the presence or not of suppuration signs.

Moreover, in hospitals they are a lack of informatic system with unique code for hospitalized patients. In fact, unfortunately it was impossible to follow patients from first entrance day to the end of hospitalization.

- The follow-up of the HCWs positive for MRSA screening.

No treatment was planned for the cases of MRSA. We just gave the results to the HCWs concerned. Many have come closer to better understand the results, but this remains a limitation to diagnose HCWs without treat them. Traditionally in our setting the "word screening" is not correctly accepted and this fact would be explained by the limited number of HCWs who gave their consent to participate to this screening. We need to change this traditional culture and promote the advantages of screening in hospital settings. For direct observations of hand hygiene, we found in six hospitals 432 HCWs but just 61 have accepted to be tested. As well as its not during the same period, the number of HCWs for screening is relatively low for 6 hospitals. We will for further research promote and educated HCWs about advantages of screening particularly in surgery services where it's constitutes a key element for prevention.

❖ *The challenges of molecular analysis in MUSTPIC project*

- The low number of sequenced isolates and the link of transmission

The low number of MDR included in the whole genome sequencing analysis and limits of multi locus sequence type (MLST) that not always allowed to find a link of transmission between hospitals. Among *Enterobacterales, E. coli* remains the most common isolates followed by *Klebsiella pneumoniae* in our study. This fact explains why these strains are the first choice for molecular analysis. Regarding the whole genome sequencing, the ability to

identify abnormal variants is depending on the presence of certain sequence variants in the sequencing data.
Concerning the method, although pulsed-field gel electrophoresis (PGFE) is the "gold standard" for bacterial molecular typing, multilocus sequence typing (MLST) has clear utility in studies of extended spectrum beta-lactamases-producing *E. coli,* based on a greater discriminatory ability and reproducibility than PGFE and the ability to a priori define genetically related bacterial strains [57].

- The scarcity of molecular studies in clinical area

Discussion about molecular study realized in SSIs in other African countries was difficult because of scarcity of studies performed with the same methods. Moreover, we need to compare data with the global one in environment and in the livestock, but nothing was described in the country. Even if it is possible that partial studies are carried out, they are not published.

4.2.4 Generalizability of findings

This prospective study was performed in the south of country and even if they are a globally resources and infrastructure problems, the six hospitals are located near to the university of Abomey Calavi, health faculty and other research centers and it contains the minimum in terms of hand-hygiene necessity. Finding in six hospitals located in south a compliance of 33.3% probably represents more than what we can imagine in the north of country where we find some hospitals without running water, without qualified health

personnel and important lack of infrastructures compared to the hospitals in the south. This HH compliance is probably not representative to the global compliance when we considered all departments in country. However, it's an evidence that to change the system we need to ensure the availability of ABHR in all hospitals and to promote "WHO five moments". We will base on the first results of HH to raise awareness among HCWs and authorities. It is true that the availability of solutions improved during the pandemic of COVID-19, but it is important to continue training and awareness. Therefore, this project will propose another set of activities among which there will be diploma courses on hand hygiene with association of many authors and specialists of hygiene (Belgium-Benin-research team).

The small number of *E.coli* may limit generalization of the findings and do not allow finding a link of transmission between strains. However, the antimicrobial resistance genes identified in this study are the same than those described worldwide and in African countries such as Congo and Tanzania amongst ESBL producing *E. coli*.[33,58]. Through 19 ESBL producing *E. coli,* we described diverse sequence types and particularly those who are known to belonged to the most virulent with carbapenemases genes. Even if we admit that further analysis with a lot of strains in national level is required, the molecular characteristics of *E. coli* isolated on pus samples represents an important piece of puzzle in the molecular epidemiology of MDR bacteria in the country.

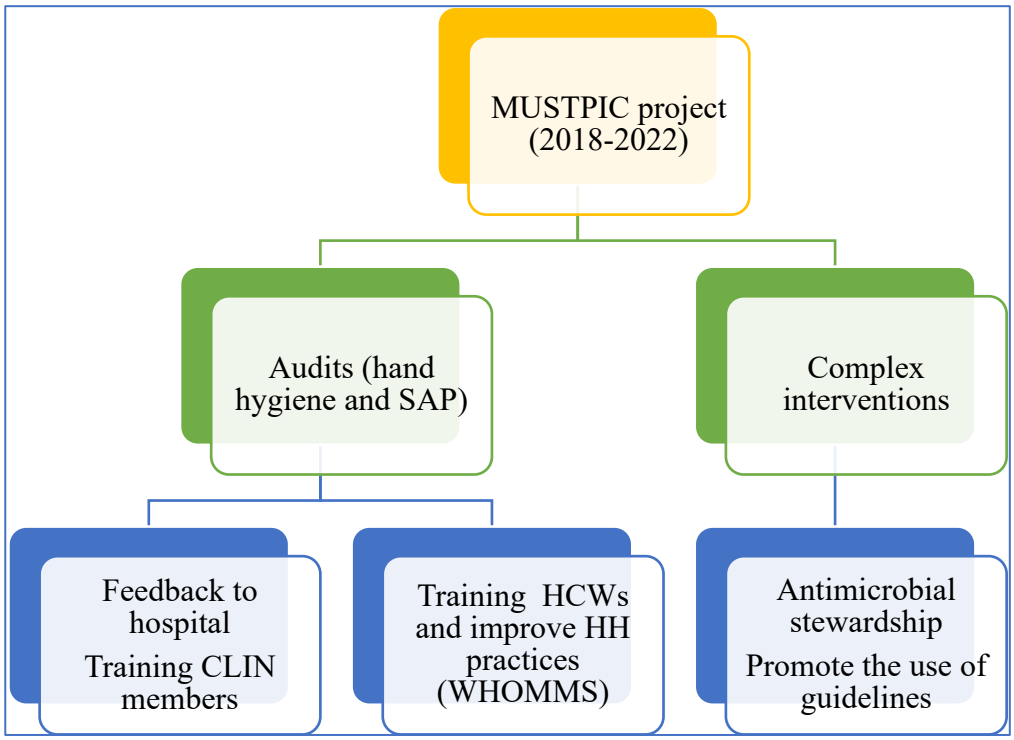


Figure 8: Interventions proposed for generalizability of the project

SAP: surgical antimicrobial prophylaxis, HH: hand hygiene, MDR: multidrug-resistant bacteria, WHOMMS: World health organization multimodal strategy. .

4.3 PERSPECTIVES

4.3.1 Clinical perspectives

- **For hospital healthcare workers**

We provide for each hospital feedback of direct observation of hand hygiene and organize a series of training with a set of posters and reminders for actors. HCWs were able to reproduce audit in their hospitals. Many actors highlighted importance of official and graduate education in hygiene or

prevention and control of infection in university. From these exchanges were born other ideas with in particular a project of continuation and we will have to organize trainings for HCWs and those who are interest. During the training HCWs discuss lot with other colleagues from neighboring hospitals and create a solid network. For the future the members of CLIN as they were trained on MUSTPIC project will frequently reorganize training about hand hygiene practices and work to maintain this solid network.

- **For nurses**

During the past years we organized a session of training about the methodology of swab sampling with the nurse's team in included hospitals. They are a series of reminders and technical procedures support in each hospital. Before starting sampling, practical aspects and demonstration were detailed through hospitals.

- **For general practitioners and members of "CLINs"**

Practicians are now unaware of resistance of some antibiotics and how they can prescribe in a setting according to the results of local epidemiology. We organized a series of restitutions specifically with member of CLINs (Comité de Lutte contre les Infections Nosocomiales) and presented the cartography of bacteria isolated per hospital. This constitutes a kind of sensibilization and training about the problematic of antimicrobial resistance in Benin. The guide for SSIs is also available and benefit for practicians, this guide will serve as basis for the SAP and for the treatment of SSIs in hospitals. This project and training sessions allowed to members of "CLINs" to re-organize the plan of

their activities and to accept their primary role in reducing infections. In fact these structures exist in most hospitals but are not always efficient. These committees do not have an annual action plan or budget for activities. The audit of “CLINs” and feedback were detailed by another researcher of the MUSTPIC project. The CLIN members will help and continue improvement of hand hygiene practices in hospitals through tools and support of MUSTPIC project.

- **For healthcare policy makers**

We presented the results. During this project, they are specific note of health ministry that recommend to all pharmacy the requirement of a prescription before selling antibiotics.
The guide exists and would be disclose in all sections of health ministry department. This project represents a kind of motivation for the authorities for implementation of the GLASS WHO. The first steps of GLASS are supported by the WHO and would be continued by the health ministry department. The researchers are frequently soliciting as experts to support activities of implementation.

- **For community pharmacists**

With the support of promoter and coordinator who is the National Order of Physicians in Benin, the government and authorities were promoted a series of legislations and initiatives designed to limit unnecessary antibiotic use (Benin, Ministry of health, 2020). Pharmacists should no longer sell antibiotics without medical prescription. The pharmacists should also be helped for the preparation of SHA.

- **Sincere collaboration between general practitioners and laboratory members**

All results were sent in real time to practicians and all MDR bacteria results were communicate before sending of the physical results. Practicians were also curious and called or approached the laboratory for a better

understanding of ESBL, CPE or MRSA codes and to discuss regarding the therapeutic options. We will help to promote in hospitals direct feedback for practicians and collaboration laboratory-clinicians.

4.3.2 Research perspectives

It is necessary in our setting to encourage involvement in the research and specifically in the antimicrobial resistance and infection control. We will promote and organize the production of ABHR as the pivot of the WHO multimodal strategy. For this purpose, we will designate in hospitals hand role models and facilitators of change of practices. While hand hygiene compliance remains sub optimal after the implementation phasis, they observed an increasing rate between compliance before and after implementation [59]. In 2019, the hand hygiene self-assessment framework (HHSAF) score indicated an intermediate hand hygiene implementation level positively associated with country income level with presence of ABHR in many healthcare facilities [60]. The implementation of WHO multimodal strategy in our country will be follow by the HHSAF survey that is a useful tool that allow evaluate and improve the hand hygiene promotion strategies [61].

It is also important to establish and promote health insurance for all to facilitate access to laboratory analyses.

The table 9 present the research perspectives and expected results.

Table 9: Point of perspectives and expected results

CHAPTER	CLINICAL PERSPECTIVES	RESEARCH PERSPECTIVES	EXPECTED RESULTS
HAND HYGIENE	***Importance of system change (core component of WHO multimodal strategy)*** • Promote and train in hospitals the preparation of alcohol-based hand rub (ABHR). • estimation of daily consummation by each hospital by using the self-assessment grid (WHO) • Discuss with authorities to support or implement local production of ABHR (in health faculty with the analytical chemistry laboratory for example) • Empowering a "Champion" for the respect of hygiene in hospitals	- Assessment of patient involvement in promotion of hand hygiene in Benin (observational before after study, qualitative study) - Assessment of hand hygiene compliance after ABHR preparation in hospitals (observational study)	*Improvement of HH by change of system With ABHR solution available in all settings* *The patients and HCWs all together help and improve the hand hygiene practices*

CHAPTER	CLINICAL PERSPECTIVES	RESEARCH PERSPECTIVES	EXPECTED RESULTS
	• Develop and discuss with HCWs the "empowerment of patient" in the daily practice of HH ***Promotion of a patient-centered approach*:** the objective is to allow patients to help HCWs in their daily practices *"Please doctor don't forget to wash your hands" "Sorry doctors, take care of my health, wash your hands*"		

CHAPTER	CLINICAL PERSPECTIVES	RESEARCH PERSPECTIVES	EXPECTED RESULTS
ANTIMICROBIAL RESISTANCE	***Importance to support laboratories to correctly perform antibiogram with respect of guidelines in country*** • Promote with the support of authorities and health ministry, the necessity to complete laboratory infrastructures by chromatographic tests for detection of carbapenemases producing organisms • Involvement of myself as microbiologist to all activities and particularly the new project of WHO "GLASS"" for the surveillance of resistance in Benin	• Restart the etiological study in hospitals in center and north of BENIN on Pus samples (for all types of surgery): Sample size proposed : 5000 swabs • Improve the detection of anaerobic bacteria with adequate media and culture methods. • Continue the molecular analyses with high number of samples to correctly discuss the epidemiological link between samples. • Promote the use of PGFE analysis	*Improvement of surveillance and control of AMR in Benin*

CHAPTER	CLINICAL PERSPECTIVES	RESEARCH PERSPECTIVES	EXPECTED RESULTS
	• Organize and continue collaboration with practicians to discuss about antibiogram • Promote at least for central laboratory the implementation of "WGS" dedicated to the research. • Promote with authorities the support of country with international funding for AMR such as the "Funding fund" that support LMICs countries.		
PREVENTION OF SSIs	***Promotion and awareness of guidelines proposed by the project*** • Audit will be organized regarding the use of this MUSTPIC guidelines" in 2024		*Implementation of guidelines in all*

CHAPTER	CLINICAL PERSPECTIVES	RESEARCH PERSPECTIVES	EXPECTED RESULTS
	• Implementation of antimicrobial stewardship with the support of partners and authorities.		*hospitals and control AMR*

For the chronological activities the following scheme show the different steps for the improvement of infection control in chronological order:

1. **System change** : ABHR in all hospitals or at least in central hospitals (training of HCWs previously)

↓

2. **Support laboratories** to correctly perform antibiogram with respect of guidelines (training of HCWs regarding antibiogram lecture, EUCAST guidelines). Few laboratories are able to perform correctly antibiogram

↓

3. **Promote national use of guidelines** proposed by the project (with the support of authorities and based on feedback of results) in hospitals and in the community

Figure 8: Chronological activities for improving infection control in Benin

5 CONCLUSION

Conclusion

Surgical site infections are not only a strictly medical but also a social problem. Their prevention remains a priority and a challenge. Hand hygiene remains the cornerstone of prevention of surgical site infections. The effectiveness of implementation of WHO multimodal strategy in Benin depends on the availability in healthcare settings of the alcohol-based hand rub all the time. It is also capital to train all of actors in hospitals and to promote WHO my five moments. The determination of the profile of bacteria Involved in SSIs constituted the first step in establishment of guidelines. The MUSTPIC project have the challenge to implement in the physician curriculum the hand-hygiene courses. Further analysis and discussion have been done with authorities concerning the possibility to manage locally the preparation of ABHR solutions for all hospitals. With this project, they are a new rules regarding the selling of antibiotics on the counter. Before promoting the rational use of antibiotics in the hospitals and in community, the practice of surgical antimicrobial prophylaxis would be promoted. A particular focus will be made on the training of HCWs and medical students about antibiotics prophylaxis and treatment.

The time has now come to promote antimicrobial stewardship in Benin. Interventions focusing on respect of hand hygiene, rational use of antibiotics in hospitals and in community will help to decrease the rate of surgical site infections. In Benin, we suffer from a lack of resources and infrastructures, good equipment's, and phenotypic tests for detection of carbapenemases producing organisms. However, this thesis shows that it is feasible to audit, to do good quality analysis and to establish with partners the guidelines for practice of SAP. To reduce surgical site infections, priority must be given to

hand hygiene. "*Moreover, it only takes as long to wash your hands well as it takes twice to sing Happy birthday*".

Table 10: Point of activities performed by year in Benin and Belgium

Year	BENIN (9 months)	BELGIUM (3 months)
2018	-Training of the students who help for the observation of HCWs	**-i**nternship in the microbiology laboratory (Cliniques universitaires Saint Luc)
2019	-Training of nurses in hospitals -Implementation of procedures (pus samples) in laboratory	-First step of microbiology analysis by mass spectrometry and Phoenix automated antibiogram -PCR on MDR samples -First round of WGS (sciensano)

2020	-follow-up of the samples (January 2020)	-Antibiogram, PCR and second round of WGS (Sciensano)
2021	**-**Feedback to hospitals with results on HH observation and antibiogram -Training with researcher team on HH and antibioprophylaxis	-End of microbiological analysis
2022	**-**Presentation of the surgery guideline proposed by the project to the hospitals	

PATIENT EMPOWERMENT TOOL FOR PREVENTION AND INFECTION CONTROL.

Recommandations pour faire d'un hôpital un réel champion du partenariat patient

- Les patients peuvent utiliser un carnet des points (carte de score). Le score permettra de stimuler le soignant à se désinfecter les mains. Il permettra aux CLINs d'évaluer la compliance à l'HDM des soignants
- Les patients peuvent porter une blouse ayant la mention « Avez-vous nettoyé vos mains ? ». Le reflexe du soignant sera de nettoyer ses mains.
- Les soignants peuvent porter un tablier portant la mention « Ok de demander l'HDM ». Ils encouragent ainsi les patients à s'engager dans l'HDM.
- Les patients peuvent utiliser les outils de responsabilisation du patient (PET: Patient Empowerment Tool) pour rappeler les soignants l'HDM.



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APPENDIX

APPENDIX 1:

INFECTIONS DU SITE OPERATOIRE AU BENIN : DESCRIPTION DES PRATIQUES D'HYGIENE ET MECANISMES DE RESISTANCE DES GERMES ASSOCIES

Formulaire de collecte de données

N°	Libellés des questions	Modalités	Codes
Identification de la fiche			
1	Numéro de la fiche	/____/___/____/	
2	Date du remplissage de la fiche	/____/___/_____/	
3	Nom de l'auditeur	----------	
NB : Pas d'observation pendant les situations d'extrême urgence du patient			
Données relatives au centre			
4	Nom de l'hôpital	1- CNHU-HKM ☐ 2- Bethesda ☐ 3- Suru-léré ☐ 4- HZ-Abomey-Calavi ☐ 5- CHU-MEL ☐ 6- HZ Porto-Novo ☐	
5	Nom du service	.....	
6	Quel est le lieu où se font les soins ?	1- Salle de pansement ☐ 2- Couloir ☐ 3- Salle d'hospitalisation ☐ 4- Bloc opératoire ☐ 5- Autre : ☐	
7	Quel est le nombre de patients présents	/___/___/	

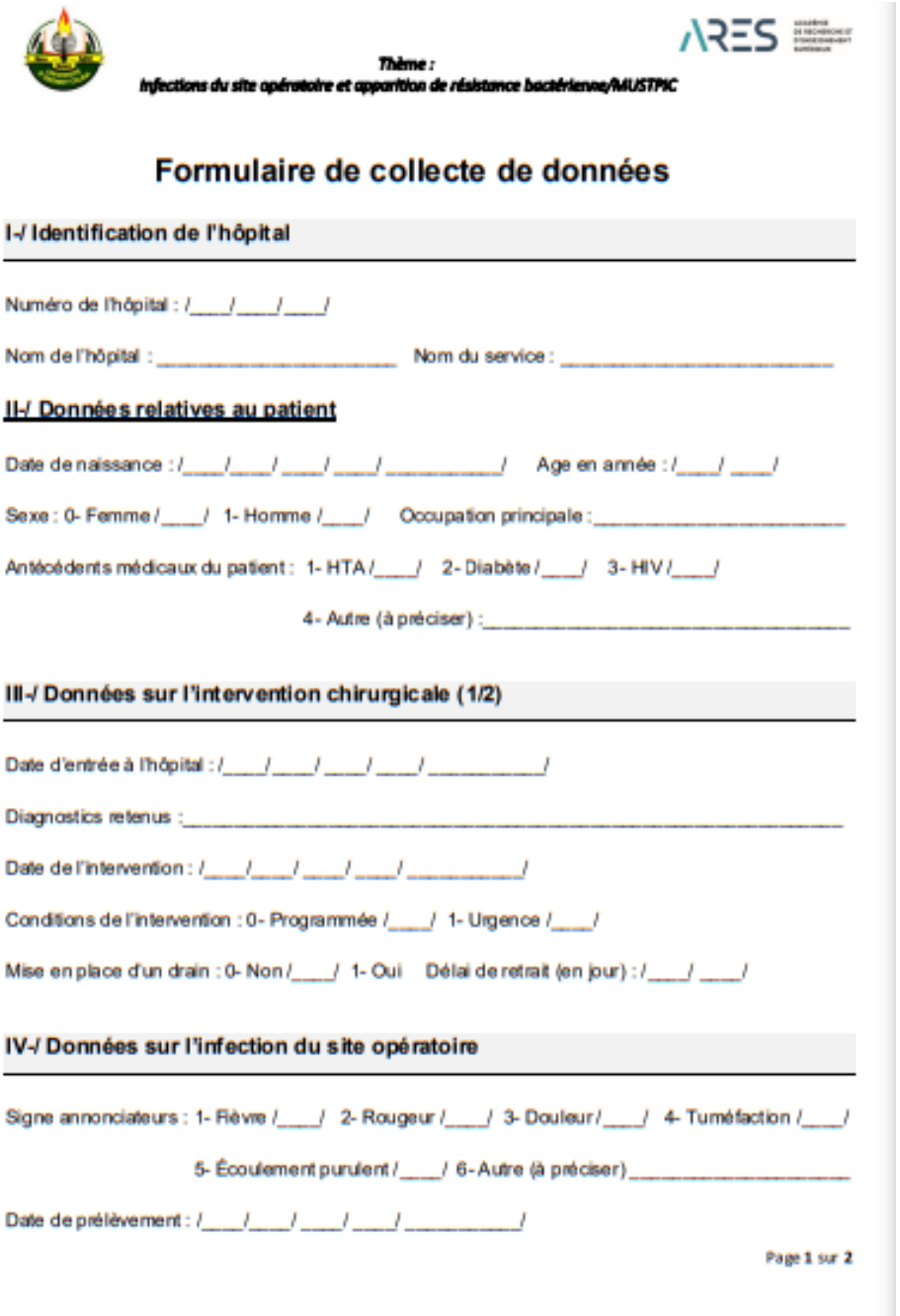
N°	Libellés des questions	Modalités	Codes
Données sur l'agent audité			
8	Quelle est la catégorie professionnelle de l'agent audité ?	1- Médecin ☐ 2- IDE ☐ 3- Aide-Soignant ☐ 4-Autre : ☐	
9	Quel est son sexe ?	1- Homme ☐ 2- Femme ☐	
10	Quelle est la période d'observation	1- 08h00-12h59 ☐ 2- 13h00-17h59 ☐ 3- 18h00-07h59 ☐	
Observation des préalables à l'hygiène des mains			
11	Tenue réglementaire de bloc opératoire ?	1- Oui ☐ 2- Non ☐	
12	Quelle est la tenue portée par l'agent audité ?	1- Manches courtes ☐ 2- Manches longues ☐ 3- Pantalon + Tunique ☐ 4- Autre : ☐	
13	Présence de bijoux aux mains et avant-bras	1- Oui ☐ 2- Non ☐	
14	Quelle est la caractéristique des ongles du personnel observé	1- Ongles courts sans vernis ☐ 2- Ongles courts avec vernis ☐ 3- Faux ongles ☐ 4- Sans vernis ☐	
Observation de la technique de lavage simple			
1er temps : Mouillage			
15	L'opérateur a-t-il mouillé les mains	1- Oui ☐ 2- Non ☐	

N°	Libellés des questions	Modalités	Codes
2ème temps : Savonnage			
16	L'opérateur a-t-il pris du savon doux liquide ?	1- Oui ☐ 2-Non ☐	
17	Si non, quel est le produit utilisé ?	---------- ---------- ----------	
18	L'opérateur a-t-il savonné les mains	1- Oui ☐ 2- Non ☐	
19	Quelle est la durée du savonnage ?	/___/ ___/ seconde	
3ème temps : Rinçage			
20	L'opérateur a-t-il réalisé un rinçage complet ?	1- Oui ☐ 2- Non ☐	
4ème temps : Séchage			
21	L'opérateur a-t-il utilisé des essuie-mains à usage unique ?	1- Oui ☐ 2- Non ☐	
22	L'opérateur a-t-il fermé le point d'eau et éliminé les essuie-mains sans contact ?	1- Oui ☐ 2- Non ☐	
Observation de la Friction hydro-alcoolique			
23	A-t-il de la friction hydro-alcoolique ? *(si non, aller à la question 28)*	1- Oui ☐ 2- Non ☐	
24	Le produit est-il réparti sur la totalité des mains ?	1- Oui ☐ 2- Non ☐	
25	Si non, quelles sont les zones frictionnées ?	1- Poignets ☐ 2- Paumes ☐ 3- Dos des mains ☐ 4- Espaces interdigitaux ☐ 5- Doigts ☐ 6- Pouces ☐ 7- Ongles ☐ 8- Autre : ---------- ☐	

N°	Libellés des questions	Modalités	Codes
26	Quelle est la durée de la friction (en seconde) ?	/____/_____/ seconde	
27	Les mains sont-elles visuellement sèches à l'arrêt de la friction ?	1- Oui ☐ 2- Non ☐	
Observation de la Friction Chirurgicale			
Lavage			
28	Quel est le type de lavage observé ?	1- Initial ☐ 2- Entre deux interventions ☐ 3- Lavage non observé ☐	
Observation de la technique de lavage			
29	A-t-il utilisé du savon liquide antiseptique ?	1- Oui ☐ 2- Non ☐	
Désinfection			
1er temps de friction			
30	Le produit est-il réparti sur la totalité des mains et avant-bras, coudes inclus ?	1- Oui ☐ 2- Non ☐	
31	Quelles sont les zones frictionnées ?	1- Paumes ☐ 2- Poignets ☐ 3- Dos ☐ 4- Avant-bras ☐ 5- Doigts ☐ 6- Ongles ☐ 7- Coudes ☐ 8- Autre : ---------- ☐	
2ème temps de friction			
32	Le produit est-il réparti sur la totalité des mains et avant-bras (manchettes) coudes Exclus ?	1- Oui ☐ 2- Non ☐	

N°	Libellés des questions	Modalités	Codes
33	Quelles sont les zones frictionnées ?	1- Paumes ☐ 2- Poignets ☐ 3- Dos ☐ 4- Avant-bras ☐ 5- Doigts ☐ 6- Ongles ☐ 7- Coudes ☐ 8- Autre : ---------- ☐	
34	La friction des bras est-elle faite de la main vers le coude ?	1- Oui ☐ 2- Non ☐	
35	Les mains sont-elles visuellement sèches à l'arrêt de la friction ?	1- Oui ☐ 2- Non ☐	

APPENDIX 2:



Thème :
Infections du site opératoire et apparition de résistance bactérienne/MUSTPIC

Formulaire de collecte de données

I-/ Identification de l'hôpital

Numéro de l'hôpital : /____/____/____/

Nom de l'hôpital : _____ Nom du service : _____

II-/ Données relatives au patient

Date de naissance : /____/____/ ____/ ____/ _____/ Age en année : /____/ ____/

Sexe : 0- Femme /____/ 1- Homme /____/ Occupation principale : _____

Antécédents médicaux du patient : 1- HTA /____/ 2- Diabète /____/ 3- HIV /____/

4- Autre (à préciser) : _____

III-/ Données sur l'intervention chirurgicale (1/2)

Date d'entrée à l'hôpital : /____/____/ ____/ ____/ _____/

Diagnostics retenus : _____

Date de l'intervention : /____/____/ ____/ ____/ _____/

Conditions de l'intervention : 0- Programmée /____/ 1- Urgence /____/

Mise en place d'un drain : 0- Non /____/ 1- Oui Délai de retrait (en jour) : /____/ ____/

IV-/ Données sur l'infection du site opératoire

Signe annonciateurs : 1- Fièvre /____/ 2- Rougeur /____/ 3- Douleur /____/ 4- Tuméfaction /____/

5- Écoulement purulent /____/ 6- Autre (à préciser) _____

Date de prélèvement : /____/____/ ____/ ____/ _____/

Page 1 sur 2





Thème :
Infections du site opératoire et apparition de résistance bactérienne/MUSTPHC

Type de prélèvement : 0- Seringue /____/ 1- Écouvillons

V-/ Données sur l'intervention chirurgicale (2/2)

Type d'intervention :_____ Durée de l'intervention : /____/ ____/

Classe de contamination :_____

Score ASA : _____

VI-/ Données sur les antibiotiques

1. Antibiotiques reçus avant l'intervention :_____
2. Antibiotiques reçus pendant l'intervention.....

	Nom de l'antibiotique	Dose administrée	Heure d'administration
1			
2			

APPENDIX 3:



Abomey-Calavi, le _____ __ ___

DEPISTAGE DU *STAPHYLOCOCCUS AUREUS* RESISTANT A LA METICILLINE (SARM)

Formulaire de collecte de données

1.Nom. /----------/

2.Prénom /----------/

3.Sexe : M /….. /. F /. /

4.Age (en années) /---------/

5.Profession: Médecin /---/ , infirmier /----/, aide-soignant /-----/, autres (Préciser) /---------- /

6.Hôpital : /----------/

7.Service : Maternité /---/ ; Chirurgie /-----/ , Bloc opératoire /----/

8.Expérience professionnelle : moins d'un an /----/, 1-5 ans /-----/, 5-10 ans /------/, > à 10 ans /-------/

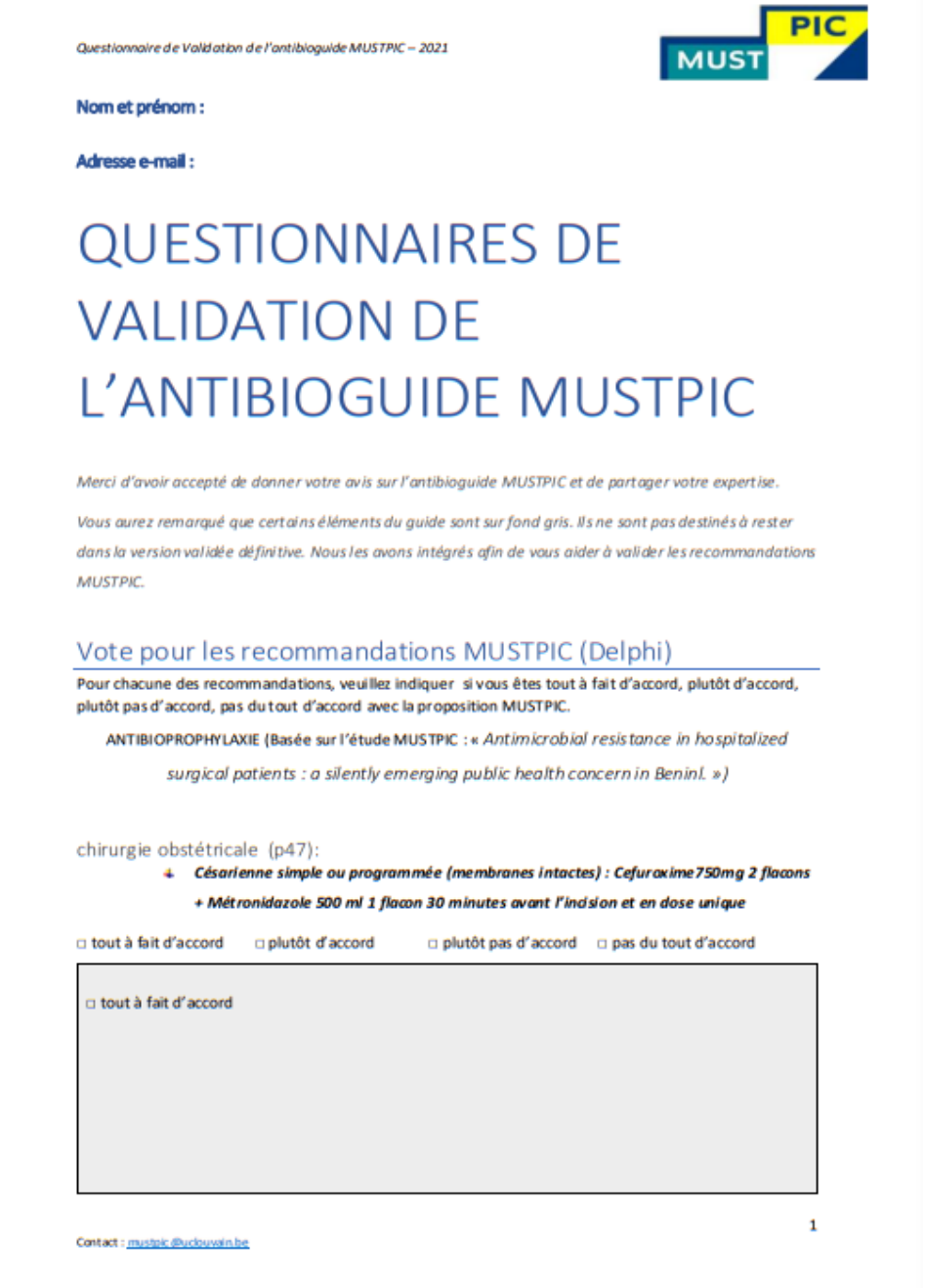
9.Notion de prise d'antibiotiques pendant ces 12 derniers mois ? oui /---/-Non /----/

10.Affection chronique ou antécédents particuliers? Non /-----/

11.Préciser si oui /----------/

12.Avez-vous été hospitalisé pendant ces six derniers mois ? oui /---/ ; Non /---/

APPENDIX 4:



Questionnaire de Validation de l'antibioguide MUSTPIC – 2021

Nom et prénom :

Adresse e-mail :

QUESTIONNAIRES DE VALIDATION DE L'ANTIBIOGUIDE MUSTPIC

Merci d'avoir accepté de donner votre avis sur l'antibioguide MUSTPIC et de partager votre expertise.

Vous aurez remarqué que certains éléments du guide sont sur fond gris. Ils ne sont pas destinés à rester dans la version validée définitive. Nous les avons intégrés afin de vous aider à valider les recommandations MUSTPIC.

Vote pour les recommandations MUSTPIC (Delphi)

Pour chacune des recommandations, veuillez indiquer si vous êtes tout à fait d'accord, plutôt d'accord, plutôt pas d'accord, pas du tout d'accord avec la proposition MUSTPIC.

ANTIBIOPROPHYLAXIE (Basée sur l'étude MUSTPIC : « *Antimicrobial resistance in hospitalized surgical patients : a silently emerging public health concern in Benin.* »)

chirurgie obstétricale (p47):

- ***Césarienne simple ou programmée (membranes intactes) : Cefuroxime 750mg 2 flacons + Métronidazole 500 ml 1 flacon 30 minutes avant l'incision et en dose unique***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

□ tout à fait d'accord

1

Contact : mustpic@uclouvain.be

- ***Césarienne compliquée : Cefuroxime 750 mg 2 flacons + Métronidazole 500 mg 1 flacon + Gentamicine 80 mg 2 flacons 30 minutes avant l'incision si la fonction rénale est bonne***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

□ tout à fait d'accord

chirurgie digestive (p47)

- ***Chirurgie non-intestinale, éventration, Hernie avec mise en place d'une plaque prothétique :***
  - ***1er choix Cefuroxime 1,5g en IV lente + Métronidazole (500 mg)***
    - ***Si la durée de l'intervention est supérieure à 2h, 0,75g de cefuroxime.***
  - ***2ème choix si allergie : Gentamicine (5mg/kg/j/IV) + clindamycine (900 mg IV lente)***
    - ***Si la durée de l'intervention est supérieure à 4h, réinjecter 600 mg de clindamycine***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

□ tout à fait d'accord

- ***Pour les hernies simples, chirurgie biliaires et les chirurgies vésiculaires par voie laparoscopique sans facteur de risque : Pas d'antibioprophylaxie***

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□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

Tout à fait d'accord

- ***Pour les chirurgies intestinales (chirurgie colorectale, appendiculaire et de l'intestin grêle) + plaies de l'abdomen :***
  - ***1er choix : Cefuroxime 1,5 g IV lente +Métronidazole 1g en perfusion en dose unique.***
  - ***En cas d'allergie mettre la Gentamicine 5mg/kg/J/IV avec un contrôle de la fonction rénale.***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

tout à fait d'accord

- ***chirurgie proctologique : mettre des imidazoles (1g en perfusion) 30 minutes avant l'incision.***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

□ Plutôt d'accord

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ANTIBIOTHERAPIE (BASEE SUR LA LITTÉRATURE)

Infection du site opératoire : CHIRURGIE OBSTETRICALE:

- ***1ere intention : Clindamycine : Par voie injectable 1800 à 2700 mg en 2 à 4 doses égales pendant 10 jours. Le relais par voie orale se fera toutes les 6 heures par du Clindamycine 150 à 450 mg.***
- ***2ème intention : Lincomycine par voie injectable 600 mg à 1g toutes les 8 à 12 heures pendant 3 à 8 jours selon la gravité et relais per os par du Lincomycine 500 mg 3 fois par jour pendant 8 jours.***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

Tout à fait d'accord

Infection du site opératoire : CHIRURGIE DIGESTIVE

- ***première intention par : Ceftriaxone + sulbactam***

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

Tout à fait d'accord

Recommandations internationales dans d'autres infections.

Pour les autres types d'infection, en ambulatoire et en milieu hospitalier, nous proposons la référence BAPCOC, en accès libre.

□ tout à fait d'accord □ plutôt d'accord □ plutôt pas d'accord □ pas du tout d'accord

Plutôt d'accord

Contact : mustpic@uclouvain.be

Autres sections du guide :

Avez-vous des commentaires ou des corrections à apporter

- sur la liste des abréviations, la liste des tableaux, la liste des figures et la liste des symboles (6 à 7)
- sur la section Généralistés sur les antibiotiques (9 à 14)
- sur la section Usage rationnel et réévaluation d'un traitement antibiotique (14 à 19)
- sur la section interactions médicamenteuses (19 à 20)
- sur la section allergies (p22 à 23)
- sur la section terrains particuliers (p23 à 24)
- sur la section arsenal thérapeutique au Bénin (p25 à 29)
- sur la section la résistance bactérienne (p29 à 33)

- **Les corrections sont faites déjà dans le texte**
- **Il manque globalement des références**
- **Suggestion d'une revue de littérature associée pour mieux étoffer les analyses**
- **Pour les autres types d'infection, en ambulatoire et en milieu hospitalier, il serait bien d'y pencher aussi pour sortir des propositions dans notre contexte**

Contact : mustpic@uclouvain.be

Autres commentaires

Honneur et plaisir de participer à ce travail

Merci pour votre participation,

L'équipe MUST-PIC

Contact : mustpic@uclouvain.be

APPENDIX 5:

info • vol. XXIV n°2, 2020

CELA S'EST PASSÉ LOIN DE CHEZ NOUS

Simple et complexe : le défi de l'hygiène des mains dans les hôpitaux du Bénin.

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4 *Pharmacy,Cliniques universitaires Saint-Luc, Université catholique de Louvain, UCLouvain, Brussels, Belgium.*
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Introduction

Les infections associées aux soins (IAS) constituent un problème majeur de santé publique dans le monde et particulièrement dans les pays en voie de développement où les infrastructures sont souvent limitées. Ces IAS entrainent entre autres, un allongement du séjour hospitalier, une augmentation de la mortalité et la morbidité et évidemment des coûts financiers additionnels aux patients et à l'hôpital [1].

Au cours des soins, les mains des praticiens constituent le vecteur principal de transmission de ces IAS. Les micro-organismes sont véhiculés facilement d'un patient vers un autre lorsque l'hygiène des mains n'est pas correctement réalisée. Celle-ci inclut le lavage à l'eau et au savon et la friction hydroalcoolique qui est même plus efficace, plus simple et ne nécessite pas, surtout dans notre contexte, de grandes infrastructures pour sa réalisation. Selon les données de l'Organisation Mondiale de la Santé, la compliance à l'hygiène des mains varie entre services, entre catégories socio-professionnelles et même selon les conditions de travail d'un hôpital à un autre et dépasse rarement 50%.[2] De nombreux facteurs peuvent influencer l'adhésion à une bonne pratique de l'hygiène des mains. Il s'agit par exemple, du manque de ressources et d'infrastructures appropriées, certaines situations comme le port de gants et des catégories professionnelles telles que les médecins décrits comme étant les mains compliants.

L'hygiène des mains constitue la pierre angulaire des stratégies de lutte contre les IAS [1,3]. C'est fort de cette évidence, que nous avons réalisé « mes cinq moments de l'hygiène des mains » à l'aide de la grille d'évaluation de l'OMS, dans six hôpitaux du pays. Cet article fait un état des lieux et décrit les diverses stratégies mises en oeuvre pour améliorer l'observance des soignants.

Compliance, déterminants et points des infrastructures

Malgré les biais et l'effet « Hawthorne », l'observation directe est le gold standard pour le monitoring de l'hygiène des mains. Dans six hôpitaux publics et dans les services de chirurgie et de gynécologie nous avons observé les soignants pendant les soins durant huit semaines. Qu'il s'agisse des salles de pansement, d'hospitalisation, de consultation ou au bloc opératoire, tout soignant était observé. La grille de L'OMS a permis de calculer la compliance. Nous avons recueilli aussi des informations concernant les prérequis à une bonne hygiène des mains (Ongles, Cheveux, bracelets...) et les infrastructures.

Pendant 60 jours, 432 acteurs de soins ont été observés. Les infirmiers et aides- infirmiers représentaient la catégorie la plus représentée [4]. La compliance était de 33,3% et elle variait selon le service. Comme dans beaucoup d'études l'attitude d'autoprotection des soignants était assez marquée. et se traduisait par une compliance plus élevée pour toutes les opportunités intervenant après contact avec des patients (37,5% après avoir touché les patients et 54,5% après exposition à un liquide biologique) comparativement à celles se situant avant un contact avec un patient (25.2%) ou avant un geste aseptique (33%) (cf. Tableau 1).

Tableau 1 : Tableau présentant la compliance des acteurs de soins au Benin, audit 2018.

Caractéristiques	Opportunités	Actions	Compliance (%)	P value
Total	1315	438	33,3	
Service				
Maternité	841	204	24,3	P=0,001
Viro/chir	471	234	49,4	
Cinq Moments de l'OMS				
Avant contact patient	393	99	25,2	
Avant geste aseptique	209	69	33	
Après exposition à un liquide biologique	77	42	54,5	P=0,001
Après contact patient	508	191	37,6	
Après contact avec l'environnement du patient	127	57	29,1	
Sexe				
Homme	671	177	26,4	P=0,001
Femme	644	261	40,5	

La friction des mains au moyen d'une solution hydro-alcoolique (SHA) est aujourd'hui le standard de qualité des pratiques d'hygiène des mains. Elle doit être privilégiée dans toutes les situations, si les mains ne sont pas souillées par des sécrétions, du sang ou tout autre liquide biologique. L'utilisation de solutions hydro-alcooliques n'est pas encore dans les habitudes de soins. Parmi les 438 actions d'hygiène réalisées, seulement 27,9% étaient consacrées à la friction. Dans la très grande majorité des cas où l'observance était respectée, la durée et la technique n'étaient pas correctes. En effet, seulement 6% des acteurs de soins respectaient les trente secondes de la friction hydroalcoolique tandis qu'aucun d'entre eux ne respectait la durée des 60 secondes du lavage au savon. Il est cependant bien connu que le temps de contact constitue un paramètre d'importance majeure pour assurer une efficacité optimale. En effet, compte tenu de la dynamique de colonisation bactérienne des mains, seule l'application d'un agent antiseptique rapidement disponible, constitue une alternative compatible avec l'enchainement des processus de soins [3].

Beaucoup reste à faire également en ce qui concerne les prérequis de l'hygiène des mains. Environ un quart des soignants portaient des bijoux 77% des soignants n'avaient pas de bijoux. >90% des soignants avaient des cheveux courts et/ ou attachés pendant les soins. Concernant les ongles, 91% avaient des angles courts sans vernis, 7.2% des ongles courts avec du vernis et 1.8% portaient de faux angles.

Grace à la grille d'infrastructures de l'OMS, nous avons fait le tour des hôpitaux à la fin de chaque mois pour collecter les différentes infrastructures disponibles. Cette grille est constituée de 28 questions et nous présentons dans le tableau 2 le récapitulatif des items clé.

Les infrastructures existantes dans les hôpitaux sont souvent mal situées dans les lieux de soins. Un lave-main bien loin des salles de consultation. L'alcool existant une semaine sur trois et une absence dans certains hôpitaux de « reminders ».

Tableau 2 : Récapitulatif des infrastructures et ressources disponibles dans les hôpitaux du Bénin, 2018.

CARACTERISTIQUES	HOP A	HOP B	HOP C	HOP D	HOP E	HOP F
Eau courante ?						
Type de robinet	Manuel	Manuel	Manuel	Manuel	Manuel	Manuel
Papier essuie-tout ?						
Savon toujours disponible ?						
Alcool toujours disponible ?						
Présence de poster ?						
Audit précédemment réalisé ?						
Autres types de rappels ?						

Légende : Les trois couleurs utilisées rappelant les feux tricolores signifient :
Rouge = absence totale d'infrastructure
Orange = Intermittence disponibilité par moments (Nécessité d'amélioration)
Vert = infrastructure disponible chaque fois.

Les défis des prochaines années

- Primo, il s'agira de préparer et d'apprendre à préparer la solution hydro-alcoolique dans les hôpitaux pour au moins faciliter l'usage fréquent pendant des soins.

- Secundo, nous formerons les infirmiers et les membres des CLIN (Comité de Lutte contre les Infections Nosocomiales) sur la technique et l'importance de l'hygiène dans la réduction des IAS. En effet les CLIN existent dans tous ces hôpitaux mais ils n'ont pas toujours un plan d'action annuel validé et exécuté.

- Tertio, nous allons réaliser et implémenter différents supports (affiches, poster, flyers). Les acteurs de soins des hôpitaux aideront à vulgariser les affiches dans les salles de pansement et d'hospitalisation.

- Quarto, il nous faut mettre le patient et les visiteurs au cœur de l'hygiène des mains. Certains l'ont fait avec succès[5]. De petites pancartes avec des émoticônes placées dans les couloirs de l'hôpital par des patients avec des phrases

incitatives telles que : « N'oubliez pas de vous laver les mains», « Êtes-vous sûrs que vos gants sont propres ? », « Lavez-vos mains, protégez-nous ». Enfin les divers services et le ministère de la santé seront totalement impliqués dans ces divers processus pour nous aider à relever le défi « Clean your hands save lives » au Bénin.

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CURRICULUM VITAE

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PUBLICATIONS IN RELATIONSHIP WITH THIS THESIS

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SUMMARY

SUMMARY

Surgical site infections (SSI) are one of the most common healthcare associated infections and contribute significantly to increase the patient's morbidity, healthcare costs and antibiotic resistance. The problem is more dramatical in low-and middle-income countries where healthcare systems are often less developed with important lack of resources and infrastructures. In Benin, due mainly the irrational prescription of antibiotics, and poor infection prevention and control programs, we observed a scarcity of local data regarding the rates of SSIs and the frequency of multidrug bacteria isolated.

Hand hygiene, one of the most important measures for reducing the transmission of bacteria in routine care was found to be a considerable challenge in our hospitals. Almost two-thirds of the healthcare workers we observed washed their hands but failed to perform alcohol-based hand rubbing (ABHR). Almost all Gram-negative bacteria isolated on pus samples in Benin were resistant to at least three classes of antibiotics. *Staphylococcus aureus* isolates were mostly harbored the Panton Valentine Leucocidin toxin and their molecular analysis showed different clones without clear link of hospital transmission.

These findings pave the way for designing and implementing guidelines and strategies in order to prevent SSIs in the country. Interventions focusing on implementation of WHO multimodal strategy with high availability of alcohol solution in hospitals. The project proposed a guideline for SAP and SSIs, that need to be promoted. However, future work is needed to largely describe the molecular epidemiology of MDR and to measure the impact of implementation in healthcare system.

RESUME

Les infections du site opératoire constituent l'une des plus fréquentes après les pneumonies et les infections urinaires et elles entrainent une augmentation de la mortalité, de la morbidité un allongement du séjour hospitalier et par conséquent une résistance aux antibiotiques. Dans notre pays, vu la prescription irrationnelle des antibiotiques et l'absence de programme de prévention des infections, nous avons objectivé au cours de ces quatre dernières années une rareté de données locales concernant la fréquence des infections du site opératoire et le taux de bactéries multi-résistantes.

L'hygiène des mains, l'une des mesures les plus importantes pour réduire la transmission manu portée des bactéries était très constitue un challenge dans nos hôpitaux .Presque 2/3des acteurs de soins qui ont été observé ont pratiqué le lavage à l'eau et au savon mais très peu s'étaient frictionné les mains avec la solution hydroalcoolique. La plupart des bactéries isolées dans les suppurations étaient résistantes à plus de trois classes d'antibiotiques. *Staphylococcus aureus,* l'une des bactéries prédominantes après *Escherichia coli* et *Pseudomonas aeruginosa* étaient productrices de la toxine de Panton Valentine une fois sur deux.

Sur la base de nos résultats en particulier et de ceux du projet en général, des interventions ont été conduites telles que le feedback personnalisé des résultats d'audits aux hôpitaux suivi de formations sur l'hygiène des mains et l'antibiothérapie. De même les résultats bactériologiques ont aidé à proposer de nouvelles associations d'antibiotiques tant pour la pratique de l'antibioprophylaxie que pour le traitement des infections du site opératoire. Cependant cette étude nécessite d'être complétée à plus grande échelle dans d'autres régions du Bénin et surtout pour apprécier l'impact du projet sur notre système sanitaire.