High prevalence of Antimicrobial Resistance in rural Burkina Faso. Assessment of risk factors for prevention and control

Thesis submitted in fulfilment of the requirement for the degree of

Doctor in Medical Sciences

JURY

President — **Prof. Anne-Catherine Pouleur**
Head of the Cardiology Department
Cliniques Universitaires Saint Luc
Insuffisance Cardiaque &Transplantation Cardiaque
Université catholique de Louvain, Bruxelles, Belgique

Secretary — **Prof Jean Cyr Yombi**
Head of the Infectious Diseases Department
Cliniques Universitaires Saint Luc
Institut de Recherche Expérimentale et Clinique
Faculté de Médecine et Médecine Dentaire
Université catholique de Louvain, Bruxelles, Belgique

Promotor — **Prof Annie Robert**
Head of the Epidemiology and Biostatistics Department
Institut de Recherche Expérimentale et Clinique
Faculté de Santé Publique
Université catholique de Louvain, Bruxelles, Belgique

Co-promotor — **Prof. Hector Rodriguez Villalobos**
Head of the microbiology department
Cliniques Universitaires Saint Luc
Institut de Recherche Expérimentale et Clinique
Université catholique de Louvain, Bruxelles, Belgique

Member — **Prof Andrea Penaloza Baeza**
Head of the Emergency Department
Cliniques Universitaires Saint Luc
Institut de Recherche Expérimentale et Clinique
Faculté de Médecine et Médecine Dentaire
Université catholique de Louvain, Bruxelles, Belgique

External members

South promotor — **Prof Halidou Tinto**
Head of the Clinical Research Unit of Nanoro
Institut de Recherche en Sciences de la Santé
Direction Régionale du Centre-Ouest, Nanoro, Burkina Faso
Associate Professor
Institut des Sciences de la Santé (INSSA)
Université Nazi Boni, Bobo- Dioulasso, Burkina Faso

Members

Prof. Marianne A.B. van der Sande
Head Department of Public Health
Full Professor Public Health Epidemiology
Institute of Tropical Medicine, Antwerp, Belgium
Associate Professor
Julius Center for Global Health, Julius Center for Health Sciences and Primary Care
University Medical Center Utrecht, the Netherlands

Prof. Olivier Vandenberg
Head of the Technological Research and Innovation Unit
Laboratoire Hospitalier Universitaire de Bruxelles (LHUB-ULB)
Université Libre de Bruxelles

SCIENTIFIC CURRICULUM VITAE

Education

- Ph.D. in Medical Sciences, Université catholique de Louvain, IREC, EPID, Belgium, March 2025.
- Master in Sciences of Public Health – Diseases Control, Institute of Tropical Medicine, Antwerp, Belgium, July 2019
- University Diploma, Methods and Practice in Epidemiology, University of Bordeaux, France, July 2017
- Doctorate in Medicine, University of Ouagadougou, Burkina Faso, July 2014

Training

- Clinical research: from project drafting to study completion, Université catholique de Louvain, May 2024
- How to write a protocol for systematic reviews and how to conduct systematic reviews in the clinical context, Cochrane Belgium, March 2023
- Qualitative and Mixed Methods in International Health Research, Institute of Tropical Medicine, Antwerp, March 2022
- Participatory Photography/ Photovoice, Institut Pasteur, Paris, France, March 2022
- Coding and certification of cause of death, Institute of Disease Modelling (IDM)/ Clinical Research Unit of Nanoro (CRUN), January 2020
- Clinical research on vaccines, Centre MURAZ Bobo Dioulasso, Burkina Faso, January 2018
- Introduction to the principle and practice of clinical research, National Institutes of Health (NIH), March 2015

Publications as first author

- **Valia D**, Ingelbeen B, Nassa GJW, Kaboré B, Kiemdé F, Rouamba T, Compaoré A, Kouanda JS, Robert A, Rodriguez-Villalobos H, Van Der Sande MAB, Tinto H. Antibiotic use by clinical presentation across all healthcare providers in rural Burkina Faso: a healthcare visit exit survey. J Antimicrob Chemother. 2024 Jul 25:dkae252. doi: 10.1093/jac/dkae252.

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Scientific communication

Oral presentation

- **Daniel Valia**, Brecht Ingelbeen, Bijou Mbangi et al. Effect of a community-based behavioural intervention bundle to improve antibiotic use, quality of primary care, and household infection prevention/control in rural Burkina Faso and DR Congo: preliminary analysis of a cluster controlled trial. *Presented at the "3èmes Journées Scientifiques de l'Institut de Recherche en Sciences de la Santé, in Koudougou, Burkina Faso, from 22 to 24 October 2024 (JSI_2024)"*

- Esther van Kleef, **on behalf of the CABU-EICO study group**: The effect of a community-based intervention bundle on household transmission of ESBL-producing E. coli in rural Burkina Faso. *Presented at the 34th Congress of the European Society of Clinical Microbiology and Infectious Diseases in Barcelona, Spain, from 27 to 30 April 2024*

- Tiendrebeogo L Alix, Yougbaré Sibidou, **Valia Daniel** et al. Prévalence des infections à *Klebsiella*, *Enterobacter*, *Serratia* et *Citrobacter* productrices de beta-lactamase à spectre élargi en milieu rural au Burkina Faso. *Presented at the "3èmes Journées Scientifiques de l'Institut de Recherche en Sciences de la Santé, in Koudougou, Burkina Faso, from 22 to 24 October 2024 (JSI_2024)"*

Poster Presentation

- **Daniel Valia**, Brecht Ingelbeen, Guétawendé JW, Nassa et al. Antibiotic dispensing per type of healthcare provider in rural Burkina Faso: A healthcare visit exit survey. *Presented at the 34th Congress of the European Society of Clinical Microbiology and Infectious Diseases in Barcelona, Spain, from 27 to 30 April 2024*

- Juste Stéphane Kouanda, Linda Campbell, Edwin Wounters, Marie Meudec, Welgo Aminata, Papa Mamadou Diagne, Tamara Giles Vernick, **Daniel Valia**, Brecht Ingelbeen et al. Perceptions des facteurs de risques d'exposition et de prolifération des infections dans le District Sanitaire de Nanoro, Burkina Faso : une expérience de l'application de l'approche photovoice pour un changement de comportement. *Presented at the "3èmes Journées Scientifiques de l'Institut de Recherche en Sciences de la Santé, in Koudougou, Burkina Faso, from 22 to 24 October 2024 (JSI_2024)"*

- Tiendrebeogo L Alix, Yougbaré Sibidou, **Valia Daniel** et al. Portage communautaire de *Klebsiella*, *Enterobacter*, *Serratia* et *Citrobacter* productrices de beta-lactamase à spectre élargi en milieu rural au Burkina Faso. *Presented at the "3èmes Journées Scientifiques de l'Institut de Recherche en Sciences de la Santé, in Koudougou, Burkina Faso, from 22 to 24 October 2024 (JSI_2024)"*

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SUMMARY

In Burkina Faso, from 2012 to 2018, extended-spectrum β-lactamase-Producing *Escherichia coli* (ESBL-EC) and *Klebsiella pneumoniae* (ESBL-KP) have been increasingly isolated from community-acquired invasive infections. This increase could be reflecting a high and increasing prevalence of faecal colonisation with these resistant bacteria in the community, as far as gut colonisation was identified as precursor to bloodstream infections. The overall objective of this thesis was therefore to assess the extent of these resistant bacteria in the community in rural Burkina Faso and to understand associated factors, in order to inform tailored infection prevention and control (IPC) interventions.

Our data showed an estimated prevalence of faecal colonisation with ESBL-EC and ESBL-KP at 61.3% in rural Burkina Faso. This prevalence was higher during the rainy season compared to the dry season (70.2% vs 53.6%, p<0.001) and higher among study participants reporting not washing hands with soap before meals compared to those who did (62.5 vs 49.0%, p<0.001). In both bacteria, *bla*$_{\text{CTX-M-15}}$ was the most prevalent (47.3% in *E. coli* and 19.9%; in *K. pneumoniae*) β-Lactamase genes. Plasmid-mediated quinolone resistance (PMQR) genes as *qnr* (48.1% in *E. coli* and 81.1% in *K. pneumoniae*), *aac(6')-ib-cr* (21.2% in *E. coli* and 18.9% in *K. pneumoniae*) as well as *OqxAB* (5.8% in *E. coli*, and 78.4% in *K. pneumoniae*) were found along with β-Lactamase genes.

In patients with severe acute febrile illness attending the Nanoro district hospital, 39.5% reported pre-hospital antibiotic use. This pre-hospital antibiotic use was significantly higher among patients referred from primary healthcare centers than among those who self-referred (54.0% vs 26.7%, p<0.001). Among all pre-hospital antibiotic use reported (424), Watch antibiotics were more frequently reported by referrals compared to self-referred patients (42.2% vs 28.1%, p=0.004).

The investigations to understand the role of different healthcare providers and knowledge of antibiotics, showed that 33.5% healthcare were seeking outside

healthcare facilities, including informal medicine vendors (47.7%), self-medication with left-over medicines kept at home (26.5%), medicine vendors in formal pharmacies (16.4%), traditional healers (9.4%) and only the latters (traditional healers) were not antibiotic dispensers. Reported reasons for seeking healthcare outside healthcare facilities included financial limitation, proximity to informal drug vendors, long waiting times at healthcare facilities and health professionals' non-empathetic attitudes towards their patients. Antibiotics knowledge (only for illnesses of bacterial origin) was limited among healthcare professionals, very limited among medicines vendors in formal pharmacies, and non-existent among informal medicine vendors and the general community.

While investigating antibiotic use by clinical presentation across all healthcare providers, we found that outpatient antibiotic use was more frequent after health center visits (54.8%, of which 16.5% Watch, n = 1249) than after visits to pharmacies (26.2%, 16.3% Watch, n = 328) and informal medicine vendors (26.9%, 50.0% Watch, n = 349). Across all healthcare providers, patients presenting with clinical presentations for which antibiotics were not recommended such as malaria, rhinopharyngitis, bronchitis, gastroenteritis, pain and wound were dispensed (Watch) antibiotics. Compliance with WHO's AWaRe Antibiotic Book could have averted at least 68.4% of all Watch antibiotic use in outpatients at health centers. Community-wide, 2.9 DDD (95% CI 1.9–3.9) were used per 1000 adult inhabitants per day, health centers representing 89.7% of it.

We concluded that the challenge of controlling antimicrobial resistance in such a setting should be multifaceted and combine both tailored antimicrobial stewardship (AMS) across all healthcare providers and the community to reduce antibiotic selective pressure and community-based hygiene interventions to break the cycle of transmission in order to mitigate and/or prevent spread. Antimicrobial stewardship program should be particularly intensified in health centers and should include dedicated education and awareness on AMR for healthcare workers, improved diagnostic tools to differentiate bacterial from non-bacterial infections, patient management algorithms based on the latest WHO recommendations for antibiotic prescription. At formal pharmacies, regulation on antibiotic sales should

be strengthened, in combination with regular AMR awareness activities and monitoring to mitigate over-the-counter dispensing. At informal medicine vendors, AMR awareness programs should help self- restriction of Watch antibiotic sales. At community level, awareness activities should include risk behaviours leading to emergence and spread of resistant microorganisms in the community. Regarding community-based hygiene interventions, improving hand hygiene practices and enhancing sanitation can be effective steps toward mitigating the burden of antimicrobial resistance.

Our studies were based on rigorous methodology. However, we are aware of some limitations that we have tried to overcome whenever possible.

Regarding all questions on antibiotic use the last three months before the day of survey in the community, community members surveyed might have used medicine, not knowing whether it was an antibiotic or not. To mitigate response biases, we provided each fieldworker with any type of antibiotic available from the range of healthcare providers in the study area (Pharmacies in healthcare facilities, private pharmacies, informal medicine vendors). Once in the household, in case medicine use the last three months was reported, fieldworkers should ask the interviewed person to identify which one(s) among the batch of antibiotics they acknowledge having used. As well, selection of isolates for molecular characterization might have led to the absence of less phenotypically expressed genes. To mitigate this, we first divided all isolates into their different expressed phenotypic group (seven groups) and performed random selection among each group.

In pre-hospital antibiotic use evaluation through patients attending the Nanoro district hospital, data was collected via a survey capturing use during the two weeks before consultation at the hospital, potentially underestimating actual use. Whenever possible, reported antibiotic use was verified from referral forms, patient medical files (healthcare booklet), and antibiotic packaging or blisters.

During investigations on places of healthcare seeking the last three months, Participants may struggle to accurately recall where they sought healthcare, especially if multiple visits occurred. Minor illnesses visits may have been forgotten or misreported. Respondents may have also reported seeking care at formal healthcare facilities rather than informal or traditional healers, even if they used both, due to perceived judgment. Likewise, a severe illness or a visit closer to the time of the survey may be more easily recalled than earlier visits, which may lead to over reporting of recent experiences. To mitigate responses biases, we used a more or less short recall period (e.g. three months instead of six months). For each participant, field workers had to ask about symptoms or illnesses presented and for each of these illnesses, ask thereafter all the providers visited. Field workers also have to provide memory aid by recalling all healthcare providers in the surrounding. Visits were also crosschecked with medical records whenever possible.

In the investigation of antibiotic use by clinical presentation across all healthcare providers, reported prevalence, means and proportions were corrected for clustering by healthcare provider, in two strata (Nanoro and Nazoanga), except for informal medicine stores, for which neither store nor health area were recorded to ensure confidentiality.

Despite these limitations that we have tried to overcome as much as possible, this thesis reports robust findings that provide a full picture of population-level antimicrobial resistance in a rural setting of low-and Middle-income countries and the interconnected factors that may be underlying it, in order to set up tailored infection prevention and control interventions.

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LIST OF ABBREVIATIONS

AMR	:	Antimicrobial resistance
AMS	:	Antimicrobial stewardship
AWaRe	:	Access, Watch and Reserve
CM	:	Centre médical
CMA	:	Centre medical avec Antenne chirurgicale
CHN	:	Centre hospitalier national
CHR	:	Centre hospitalier régional
CHU	:	Centre hospitalier universitaire
CRUN	:	Clinical Research Unit of Nanoro
CSPS	:	Centre de santé et de promotion social
DNA	:	Deoxyribonucleic Acid
EML	:	Essential medicines list
ESBL	:	Extended-spectrum β-lactamase
GDP	:	Gross Domestic Product
GLASS	:	Global Antimicrobial Resistance Surveillance System
HDSS	:	Health and Demographic Surveillance System
HIV/AIDS	:	Human immunodeficiency virus/Acquired immunodeficiency syndrome
IPC	:	Infection prevention and control
PHC	:	Primary healthcare center
WASH	:	Water, sanitation and hygiene
WHO	:	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Antimicrobial resistance

1.1.1 Definition and mechanism

Antimicrobial Resistance (AMR) is the phenomenon when microorganisms such as bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines to which they were previously susceptible, making infections difficult or impossible to treat and leading subsequently to a higher disease spread, severity, disability, and death (1,2). When AMR refers to the resistance of bacteria to antibiotics, it is called antibiotic resistance (3).

Acquisition of resistance can occur through two main processes: a) mutations in the cell's DNA during replication or b) DNA transfer. In the case of mutations, mutant strains can pass the mutation to their offspring through vertical transmission. The second method by which bacteria gain resistance is through horizontal gene transfer (transformation, transposition, and conjugation), transferring antibiotic-resistant genetic material from the antibiotic-resistant bacteria to the non-resistant-bacteria that become resistant to antibiotic (4,5).

1.1.2 Burden

World Health Organisation (WHO) has declared AMR as one of the top global public health and development threats facing humanity (1). Indeed, in 2019, bacterial AMR cost about 900 billions USD globally and related deaths were between 1.27 to 4.95 millions (Figure 1) (6).

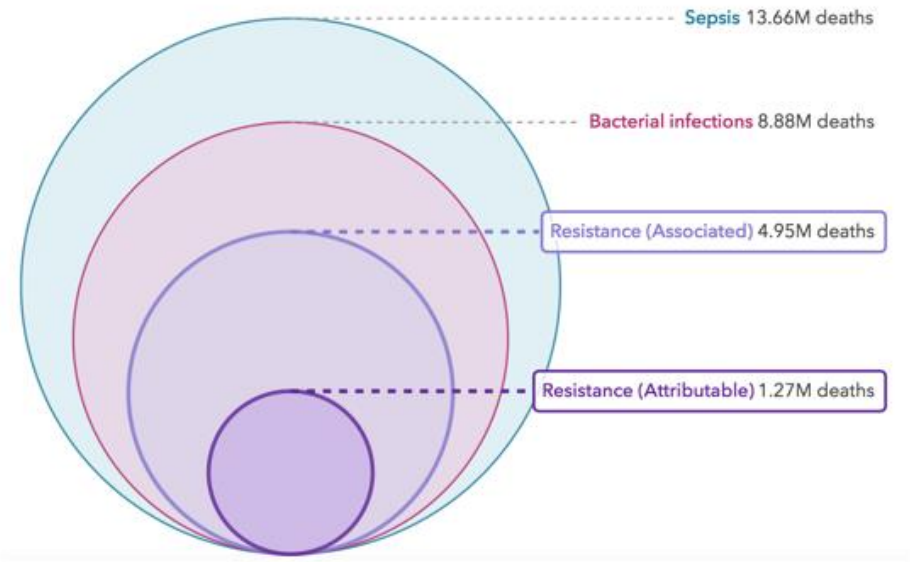

Figure 1: Composition of global infection-related deaths

Associated deaths measures people with a drug-resistant infection that contributed to their death. The infection was implicated in their death, but resistance may or may not have been a factor. Attributable deaths measures people who would not have died of infection if it was treatable (i.e., if there was no AMR) for whom resistance can be said to have caused their death. (Institute for Health Metrics and Evaluation (IHME), University of Oxford. MICROBE. Seattle, WA: IHME, University of Washington, 2022. Available from https://vizhub.healthdata.org/microbe) (6)

This burden was estimated to be highest in western sub-Saharan Africa with 27·3 deaths per 100 000 (Figure 2) (7).

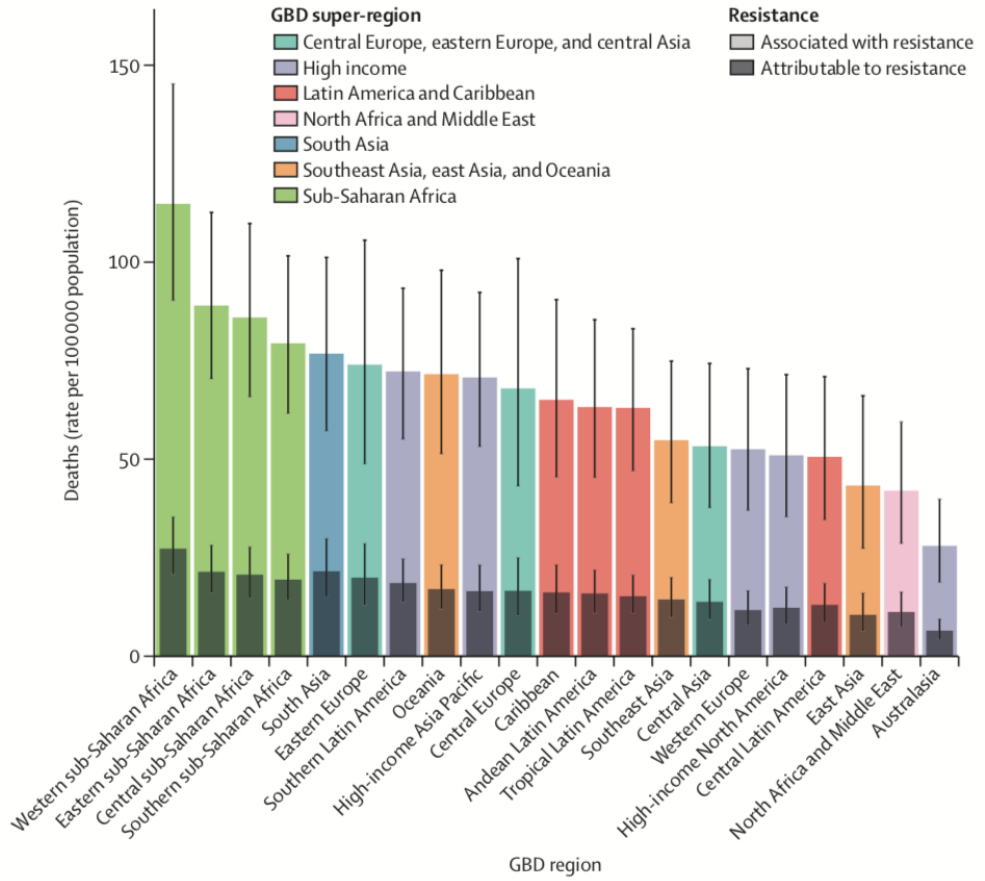

Figure 2: All-age rate of deaths attributable and associated with bacterial AMR by Global Burden of Diseases region in 2019

(Muray C et al. Lancet 2022 https://linkinghub.elsevier.com/retrieve/pii/S0140673621027240) (7)

A recent analysis of the global burden of bacterial antimicrobial resistance, showed that six pathogens, ranked by number of deaths, *E. coli*, *Staphylococcus aureus*, *K. pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, were responsible of more than 70% of all bacterial AMR-related deaths (Figure 3) (7).

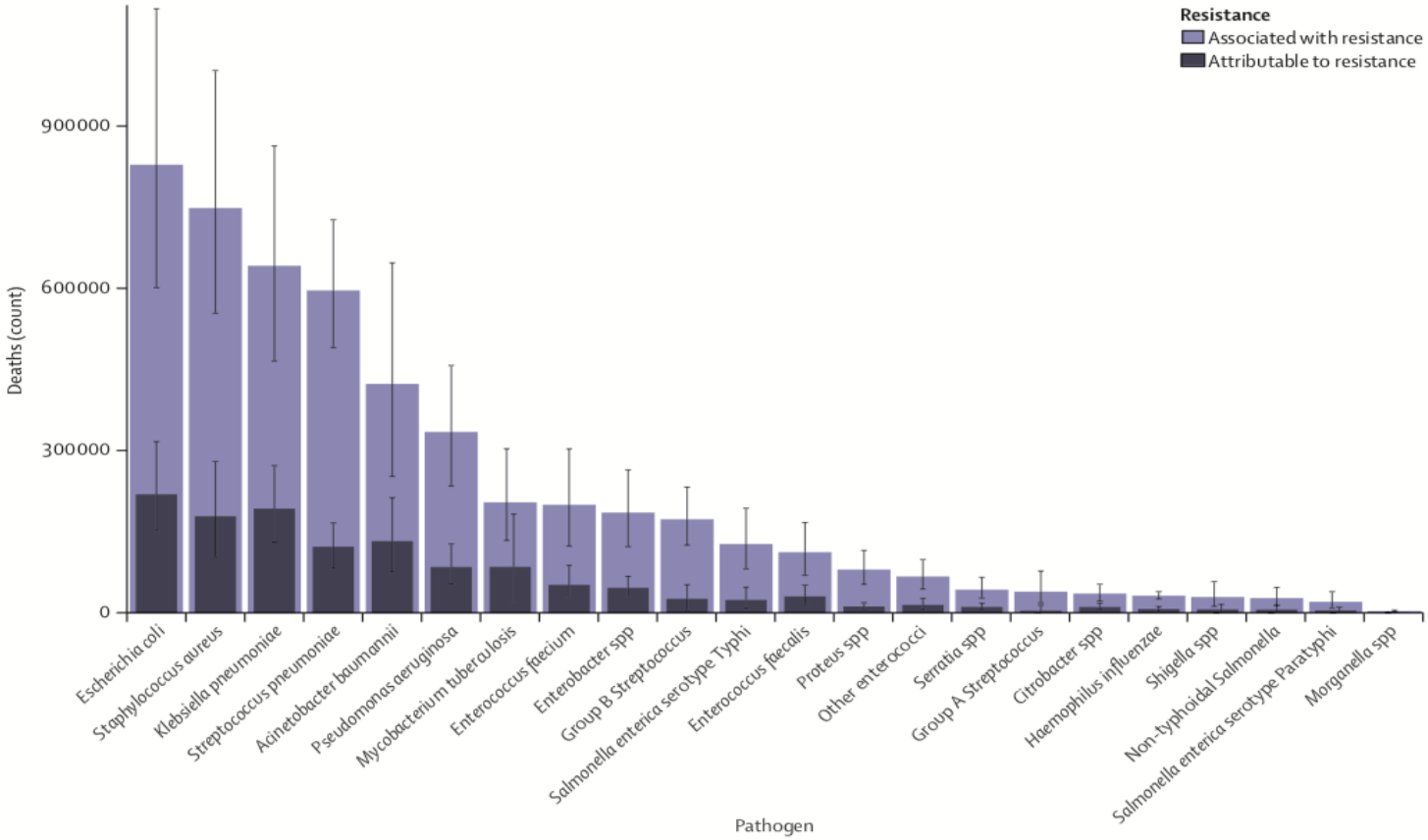

Figure 3: Global deaths (counts) attributable and associated with bacterial AMR by pathogen in 2019

(Muray C et al. Lancet 2022 https://linkinghub.elsevier.com/retrieve/pii/S0140673621027240) (7)

1.1.3 Risk factors

Acquisition of AMR, which occurs naturally over time through various mechanisms, is accelerated by incorrect antibiotic use (Figure 4) both in human and animal (8,9).

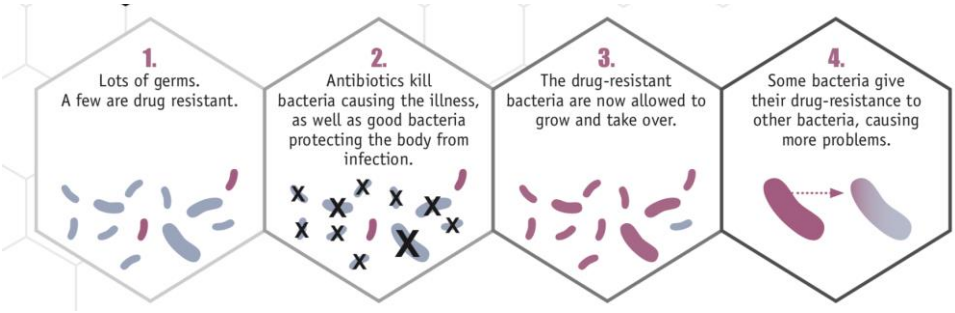

Figure 4: How resistant bacteria are selected

(Center for Diseases Control. Antibiotic Resistance Threats in the United States, 2013 https://www.cdc.gov/antimicrobial-resistance/media/pdfs/ar-threats-2013-508.pdf) (10)

As shown in Figure 5, the use of antibiotics in both humans (considering their quantity, quality and appropriateness) and animals (as feed additives) can lead to selective pressure, promoting antibiotic resistance in non-pathogenic bacteria or allowing resistant pathogens to proliferate, potentially displacing non-pathogenic strains. These resistant bacteria and their genetic material can enter the environment, which serves as a reservoir where resistance genes are exchanged among environmental bacteria through horizontal gene transfer. This facilitates the spread of resistance to other humans and animals via multiple pathways, including soil, water, and air. Additionally, resistant bacteria can be transmitted from animals to humans through the food chain (10,11).

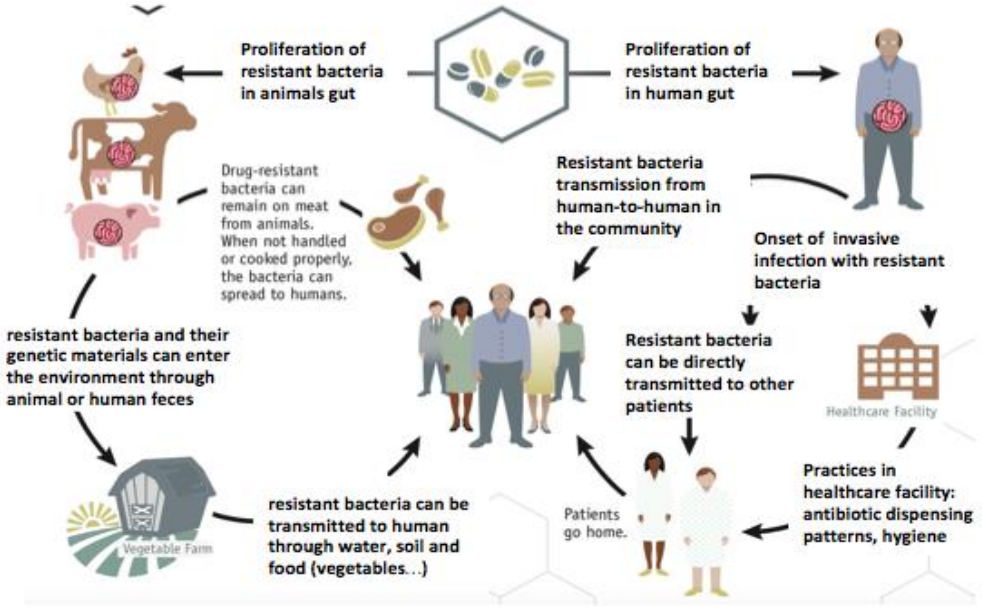

Figure 5: Transmission pathways for resistant bacteria between food animals, human and the environment

(Adapted from Antibiotic Resistance Threats in the United States, 2013. Center for Diseases Control https://www.cdc.gov/antimicrobial-resistance/media/pdfs/ar-threats-2013-508.pdf) (10)

1.1.4 Fight against antimicrobial resistance

In response to this global AMR crisis, the WHO Assembly in May 2015 adopted a global action plan with five key objectives as follow (3):

1. Improve awareness and understanding of antimicrobial resistance through effective communication, education and training.
2. Strengthen the knowledge and evidence base through surveillance and research.
3. Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures
4. Optimize the use of antimicrobial medicines in human and animal health.
5. Build the economic case for sustainable investment that considers the needs of all countries, while increasing investment for the development of new medicines, diagnostic tools, vaccines, and other interventions.

To facilitate and support a standardized global approach to AMR surveillance in alignment with the second objective of the Global Action Plan on Antimicrobial

Resistance, the Global Antimicrobial Resistance Surveillance System (GLASS) has been established in October 2015 with regular updates (12). The current AMR-related activities conducted by the GLASS are grouped into technical modules, comprising surveillance activities based on routinely available data (i.e. patient samples gathered for clinical purposes or national antimicrobial sales data), targeted activities designed to generate information for specific purposes, based on the needs of individual countries and regions as well as surveys and studies to help countries to improve the quality and representativeness of their data (Figure 6) (13).

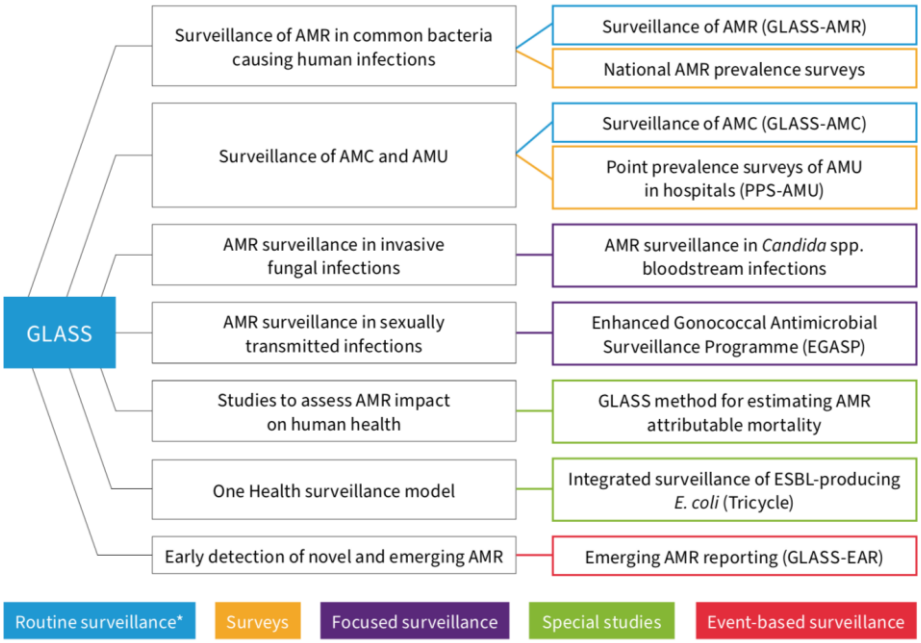

Figure 6: GLASS technical modules

(World Health Organization. Global Antimicrobial Resistance Surveillance System, 2022 https://www.who.int/publications/i/item/9789240062702) (13)

Likewise, to optimise antibiotic use, the WHO, in his model list of essential medicines (EML), has also put forward a classification of antibiotics into three large groups "Access", "Watch", "Reserve" (AWaRe) (Figure 7) according to their clinical importance and resistance potential, with specific recommendations for their appropriate use (14). To support these recommendations, the WHO AWaRe antibiotic book has been subsequently developed to provide evidence-based guidance on the choice of antibiotic, dose, route of administration, and duration of

treatment for more than 30 of the most common clinical infections in children and adults in both primary health care and hospital settings(15).

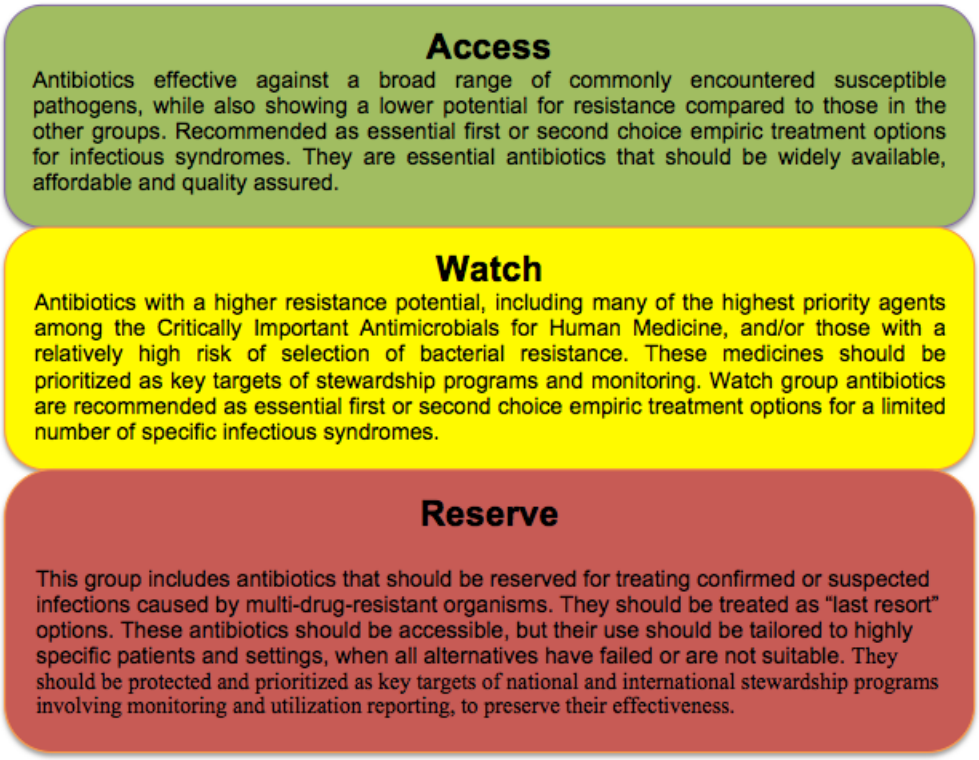

Figure 7: The WHO Access, Watch and Reserve (AWaRe) classification of antibiotics

(World Health Organization. Model list of essential medicines-23rd list, 2023 https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2023.01) (14)

A list of antibiotics for each AWaRe group has also been provided to facilitate evaluation and monitoring of use (Table 1) (16)

Table 1: List of antibiotics by AWaRe classification (1 of 2)

ACCESS		WATCH			RESERVE
Aminocystols	**Penicillins**	**Aminoglycosides**	**Fluoroquinolones**	**Carbapenems**	**Aminoglycosides**
Spectinomycin	Amoxicillin	Arbekacin	Ciprofloxacin	Biapenem	Plazomicin
	Ampicillin	Bekanamycin	Delafloxacin	Doripenem	
Aminoglycosides	Azidocililin	Dibekacin	Enoxacin	Ertapenem	**Carbapenems**
Amikacin	Bacampicillin	Isepamicin	Fleroxacin	Imipenem/cilastatin	Imipenem/cilastatin/relebactam
Gentamicin	Benzathine-benzylpenicillin	Kanamycin_IV	Garenoxacin	Meropenem	Meropenem/vaborbactam
	Benzylpenicillin	Kanamycin_oral	Gatifloxacin	Panipenem	
Amphenicols	Clometocillin	Micronomicin	Gemifloxacin	Tebipenem	**Fith-generation cephalosporins**
Chloramphenicol	Cloxacillin	Neomycin_IV	Grepafloxacin		Ceftaroline-fosamil
Thiamphenicol	Dicloxacillin	Neomycin_oral	Lascufloxacin	**Fourth-generation-cephalosporins**	Ceftobiprole-medocaril
	Epicillin	Netilmicin	Levofloxacin	Cefepime	Ceftolozane/tazobactam
Beta-lactam/beta-lactamase-inhibitor	Flucloxacillin	Ribostamycin	Levonadifloxacin	Cefoselis	
Amoxicillin/clavulanic-acid	Hetacillin	Sisomicin	Lomefloxacin	Cefozopran	**Glycopeptides**
Ampicillin/Sulbactam	Mecillinam	Streptoduocin	Moxifloxacin	Cefpirome	Dalbavancin
Sultamicillin	Metampicillin	Streptomycin_IV	Norfloxacin		Oritavancin
	Meticillin	Streptomycin_oral	Ofloxacin	**Glycopeptides**	Telavancin
Beta-lactamase-inhibitors	Nafcillin	Tobramycin	Pazufloxacin	Teicoplanin	
Sulbactam	Oxacillin		Pefloxacin	Vancomycin_IV	**Glycylcyclines**
	Penamecillin	**Beta-lactam/beta-lactamase-inhibitor_anti-pseudomonal**	Prulifloxacin	Vancomycin_oral	Tigecycline
Imidazoles	Phenoxymethylpenicillin	Piperacillin/tazobactam	Rufloxacin		
Metronidazole_IV	Pivampicillin		Sitafloxacin	**Lincosamides**	**Lipopeptides**
Metronidazole_oral	Pivmecillinam	**Beta-lactamase-inhibitors**	Sparfloxacin	Lincomycin	Daptomycin
Ornidazole_IV	Procaine-benzylpenicillin	Tazobactam	Temafloxacin		
Ornidazole_oral	Propicillin		Tosufloxacin	**Phenol derivatives**	**Monobactams**
Secnidazole	Talampicillin	**Streptogramins**	Trovafloxacin	Clofoctol	Aztreonam
Tinidazole_IV		Pristinamycin			Carumonam
Tinidazole_oral			**Steroid antibacterials**	**Phosphonics**	
			Fusidic-acid	Fosfomycin_oral	**Oxazolidinones**
					Linezolid
					Tedizolid

Table 2: List of antibiotics by AWaRe classification (2 of2)

ACCESS		WATCH			RESERVE
First-generation-cephalosporins	**Sufonamides**	**Third-generation-cephalosporins**	**Macrolides**	**Second-generation-cephalosporins**	**Other-cephalosporins**
Cefacetrile	Sulfadiazine	Cefcapene-pivoxil	Azithromycin	Cefaclor	Cefiderocol
Cefadroxil	Sulfadimethoxine	Cefdinir	Clarithromycin	Cefamandole	
Cefalexin	Sulfadimidine	Cefditoren-pivoxil	Dirithromycin	Cefbuperazone	**Penems**
Cefaloridine	Sulfafurazole	Ceftamet-pivoxil	Erythromycin	Cefmetazole	Faropenem
Cefalotin	Sulfaisodimidine	Cefixime	Fidaxomicin	Cefminox	
Cefapirin	Sulfalene	Cefmenoxime	Flurithromycin	Cefonicid	**Phosphonics**
Cefatrizine	Sulfamazone	Cefodizime	Josamycin	Ceforanide	Fosfomycin_IV
Cafazedone	Sulfamerazine	Cefoperazone	Midecamycin	Cefotetan	
Cefazolin	Sulfamethizole	Cefotaxime	Miocamycin	Cefotiam	**Pleuromutilin**
Cefradine	Sulfamethoxazole	Cefpiramide	Oleandomycin	Cefoxitin	Lefamulin
Cefroxadine	Sulfamethoxypyridazine	Cefpodoxime-proxetil	Rokitamycin	Cefprozil	
Ceftezole	Sulfametomidine	Cefsulodin	Roxithromycin	Cefuroxime	**Polymyxins**
	Sulfametoxydiazine	Ceftazidime	Solithromycin	Flomoxef	Colistin_IV
Sulfonamide-trimethoprim-combinations	Sulfamoxole	Cefteram-pivoxil	Spiramycin	Loracarbef	Colistin_oral
Sulfadiazine/tetroxoprim	Sulfanilamide	Ceftibuten	Telithromycin		Polymyxin-B_IV
Sulfadiazine/trimethoprim	Sulfaperin	Ceftizoxime	Troleandomycin	**Tetracyclines**	Polymyxin-B_oral
Sulfadimidine/trimethoprim	Sulfaphenazole	Ceftriaxone		Chlortetracycline	
Sulfamerazine/trimethoprim	Sulfapyridine	Latamoxef	**Penicillins**	Clomocycline	**Streptogramins**
Sulfamethoxazole/trimethoprim	Sulfathiazole		Aspoxicillin	Demeclocycline	Dalfopristin/quinupristin
Sulfametrole/trimethoprim	Sulfathiourea	**Quinolones**	Azlocillin	Lymecycline	
Sulfamoxole/trimethoprim		Cinoxacin	Carbenicillin	Metacycline	**Tetracyclines**
	Tetracyclines	Flumequine	Mezlocillin	Minocycline_oral	Eravacycline
Nitrofuran derivatives	Doxycycline	Nemonoxacin	Pheneticillin	Oxytetracycline	Minocycline_IV
Furazidin	Tetracycline	Oxolinic-acid	Piperacillin	Penimepicycline	Omadacycline
Nifurtoinol		Pipemidic-acid	Sulbenicillin	Rolitetracycline	
	Trimethoprim-derivatives	Piromidic-acid	Temocillin	Sarecycline	**Third-generation-cephalosporins**
Lincosamides	Brodimoprim	Rosoxacin	Ticarcillin		Ceftazidime/avibactam
Clindamycin	Trimethoprim			**Rifamycins**	
				Rifabutin	**Trimethoprim-derivatives**
Nitrofuran derivatives	**Nitrofuran-derivatives**			Rifampicin	Iclaprim
Furazidin	Nitrofurantoin			Rifamycin_IV	
Nifurtoinol				Rifamycin_oral	
				Rifaximin	

1.1.5 Antimicrobial stewardship challenges in sub-Sahara Africa

To fight against AMR, many sub-Sahara African countries have developed their national action plans (NAPs) based on the WHO global action plan, but fully implementing country-specific action plans remain challenging. Some of these challenges include lack of political commitment, inadequate funding, poor coordination and collaboration between the different sectors, inadequate surveillance data, poor infrastructure and health systems, lack of human resources and weak medicines regulatory systems (17–20). Indeed, political commitment is a key factor in successfully implementing NAPs and the lack of commitment by policymakers could be attributed to the lack of awareness and appreciation of the impact of AMR on the quality of human life (20,21). Likewise, the limited resources (characterized by a lack of continuous funding) combined with the fact that NAPs are not prioritized in health spending, results in a paucity of surveillance data, staffing inadequacy, and poor infection prevention and control (20,22–24). Similarly, multisectoral collaboration for a comprehensive one health approach in fighting against AMR face the absence of coordinating agencies, making the coordination among the different sectors problematic (25). Finally, data on antibiotic consumption/use, environmental exposure pathways, access to healthcare, morbidity and mortality rates which are critical in informing policy and actions to be undertaken at country level are lacking, constituting barriers in the successful implementation of NAPs (20).

1.2 Burkina Faso Country profile

1.2.1 Geographic location and demographic aspects

Burkina Faso is a West African country, covering an area of 274,200 km². It shares borders with Mali in the northwest, Niger in the northeast, Benin in the southeast, Togo and Ghana in the south, and Côte d'Ivoire in the southwest (Figure 8). Its population was estimated at 20 505 155 inhabitants in 2019 with 73.9% living in rural areas. Women were most represented with 51.7% and the overall population growth was 2.94% per year. The birth rate was 39.4 per 1,000, with a fairly young population, those under 15 representing 45.3%. The death rate stood at 9.2‰, and

the average life expectancy at birth was 62 years. The population density was 75.1 inhabitants/km^2 with a strong variation between regions, ranging from 31 in the Sahel to 1014 inhabitants/km^2 in the central region (26).

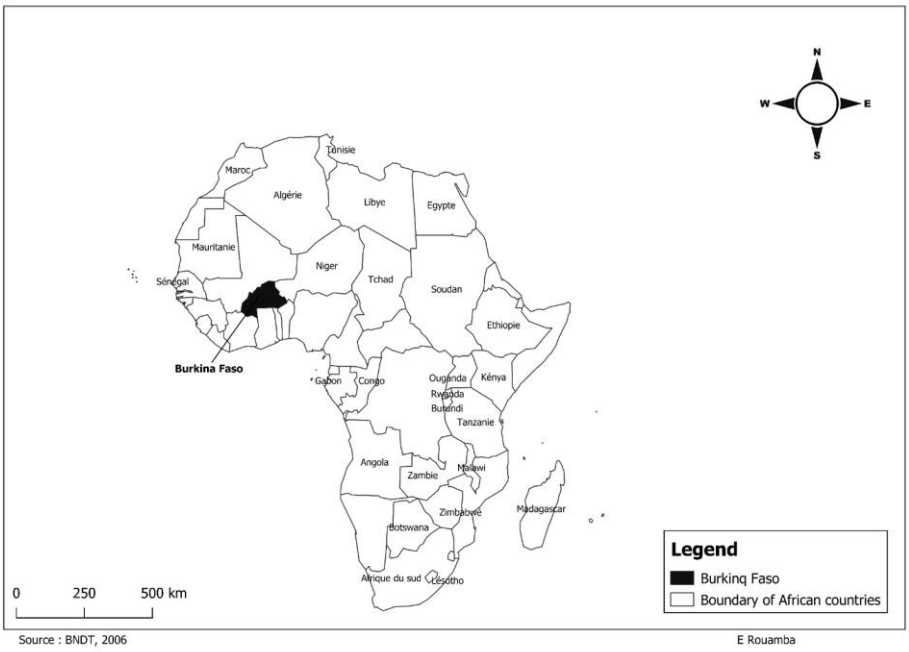

Figure 8: Burkina Faso geographic location

1.2.2 Economic situation

Burkina Faso is a low-income country, with a gross domestic product (GDP) per capita of 874.1 USD in 2023 and with more than 40% of its population living below the national poverty threshold. Its economy largely depends on agriculture, which employs around 80% of the population, although this sector remains vulnerable to climate variability (droughts, desertification) and frequently impact yields. The country is also a significant gold producer. Although agriculture continues to provide the majority of jobs in the country, it now represents less than 20% of GDP, with the gold mining sector having experienced rapid growth (77% of exports and 22% of government revenue), representing 16% of GDP, becoming the primary driver of economic growth in recent years.

In 2023, while the economy was expected to grow by 3.2%, compared to 1.5% in 2022, growth in the agricultural sector was hampered by the security context, which limited access to rural areas as well as the fall of gold production (closure of

many sites), despite its high prices on the international market. The services sector, which represents 48% of GDP, therefore remained the main driver of growth. So the country has started to consolidate its public finances. Consolidation was driven by a reduction in investments and subsidies, while military and humanitarian spending remained high. Efforts were made to support domestic revenue mobilization and even though donations from bilateral donors have declined, the risk of external over-indebtedness remains moderate (26–29).

1.2.3 Water Sanitation and Hygiene situation

Water, sanitation and hygiene (WASH) in Burkina Faso present significant challenges, particularly in rural areas.

Household access to safe drinking water was estimated at 77.2% across the whole country, ranging from 71.2% in rural areas to 91.3% in urban areas in 2021 (30). Many rural communities rely on boreholes, wells, or surface water sources like rivers and lakes (Table 2), which can be contaminated (31).

Table 3: Distribution in percentage of households' drinking water sources according to locality

Main source of drinking water supply	Type of locality					
	Ouagadougou	Bobo-Dioulasso	Other cities	Urban areas together	Rural areas together	Urban and rural together
Dam/river/watercourse/lake	0.2	0.2	0.3	0.3	2.3	1.6
Ordinary well	1.2	6.4	14.6	7.1	28.7	22.0
Ordinary lined well	0.3	1.1	3.2	1.5	5.5	4.3
Ordinary lined well equipped with a pumping system	0.4	0.4	1.4	0.8	2.8	2.2
Borehole	4.9	1.8	17.3	9.1	40.7	30.8
Public fountain	35.8	40.9	32.1	35.2	18.2	23.5
Personal water faucet with interior meter	37.2	27.4	17.9	28.3	0.7	9.3
Shared water faucet with interior meter	17.9	20.3	11.2	15.7	0.4	5.2
Water faucet in another yard	1.1	0.6	1.0	1.0	0.2	0.4
Others	1.0	0.9	1.0	1.0	0.5	0.7
Total	100.0	100.0	100.0	100.0	100.0	100.0

Even though access is better in urban settings, especially in the capital Ouagadougou, the rapidly growing population and lack of proper maintenance of water supply systems lead to frequent disruptions exacerbated by climate change (droughts and unpredictable rainfall) (30,31).

Sanitation infrastructure is generally poor across the country. In 2021, households' access to improved sanitation facilities was estimated at 15.2%, ranging from 9.2% in rural areas to 29.3% in urban areas. Many people practice open defecation in rural areas, due to the absence of latrines. The situation is somewhat better in urban areas, but a significant portion of the population still lacks access to improved sanitation facilities like flush toilets or pit latrines (30).

1.2.4 Health system organization

The health system in Burkina Faso has a pyramidal structure, organized into three main levels, taking into account administrative as well as healthcare services organization, ranging from peripheral, intermediate to central (Figure 9).

The administrative organization includes at the central level, the office of the health minister, the general secretary, the technical directorates, and affiliated programs. The intermediate level corresponds to the 13 health regions, the so-called regional

health directorates. At the peripheral level there are 70 health districts, which are the operational entity of the national health system.

The three levels of healthcare services provide primary, secondary and tertiary care. The first level includes two rungs, the first rung comprising medical centers (called CM) as well as health and social promotion centers (called CSPS). The second rung of this first level is represented by medical centers with surgery capability, which are the district hospitals (called CMA). In 2023, the country had 2233 CSPS, 116 CM and 46 CMA. CSPS and CM are supposedly patients' first point of contact with the healthcare system. There are no medical doctors in a CSPS; nurses are in charge of diagnosing and prescribing treatment. In CM there is a medical doctor, but most consultations are done by nurses. In CM and CSPS, malaria rapid diagnostic tests are available to support febrile disease diagnoses. Patients with severe cases can be monitored for maximum of 2 days, while more complicated cases should be referred to the CMA, the reference center for CM and CSPS. The CMA has hospitalization wards, medical doctors and a laboratory. CM and CSPS will be further referred to as primary healthcare centers (PHCs). The second level of care is represented by the regional hospital (called CHR), which serves as a reference for the CMA. In 2023, the country had 9 CHR. The third level of care is the university hospital (called CHU), which is the highest level of reference. There were 6 CHU in the country in 2023. At each level of care, health centers have medicine stores, where medicines should only be dispensed following prescription. Healthcare costs are paid out of patients' pocket, without any reimbursement plan, except for children under five year olds and pregnant women who receive free healthcare. Alongside these public healthcare centres organized in a pyramidal way, there are also private healthcare centers participating in the healthcare provision in Burkina Faso as well as private community pharmacies (private pharmacies outside public health centers). In 2023, the country had 1102 private healthcare facilities and 1143 private community pharmacies. In addition to these facilities, we also have pharmacopoeia and traditional medicine. The doctor-to-patient ratio across the country in 2023 was 1:9872 (32,33).

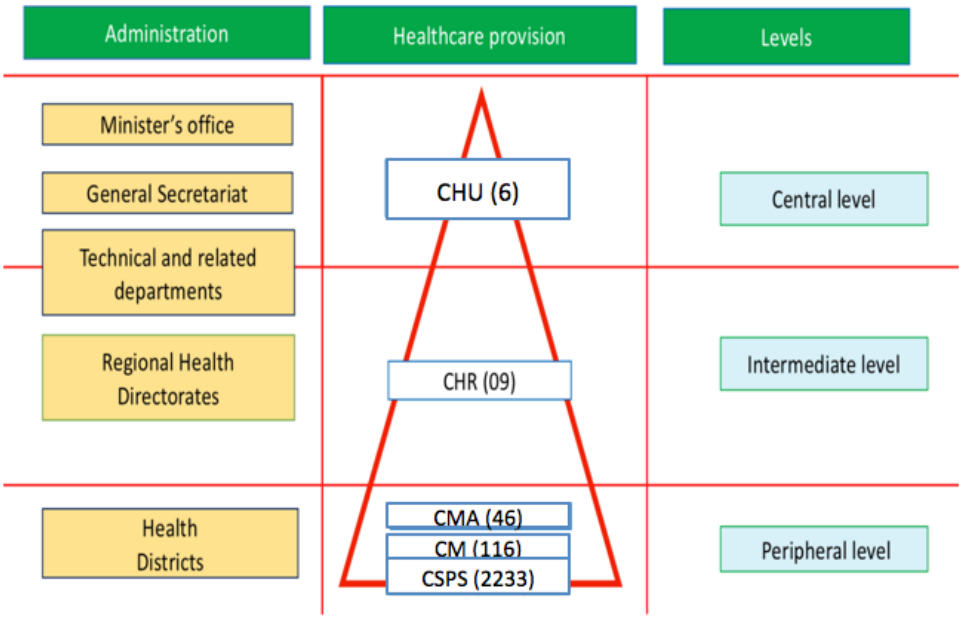

Figure 9: Administrative and technical organization of the health system in Burkina Faso

(Adapted from Ministère de la Santé et de l'Hygiène Publique Burkina Faso. Etat de santé de la population du Burkina Faso.Rapport2019.https://dicames.online/jspui/bitstream/20.500.12177/3982/1/Rapport_Etat_Sante_Pop_BF%202019_Final.pdf) (34)

Note: CHU: Centre Hospitalier Universitaire; CHR: Centre Hospitalier Régional; CMA: Centre Médical avec Antenne chirurgicale; CM: Centre Médical; CSPS: Centre de Santé et de Promotion Sociale

1.2.5 Antimicrobial stewardship in Burkina Faso and knowledge gaps

In alignment with the WHO global action plan, Burkina Faso has developed and validated a multisectoral national strategic plan in a One Health approach in 2017, integrating human, animal, and environmental health sectors (35). This plan emphasizes the importance of multisectoral collaboration in preventing and containing AMR, supported by the National One Health Coordination Platform (PNOH) which guide various health security actions, including AMR containment, across the ministries of human health, animal health, and the environment (36). The laboratory-based surveillance of antimicrobial resistance, through sentinel laboratories across the country, is a key component of this plan (35,37) and data on antimicrobial resistance and on antimicrobial consumption are also reported in GLASS-AMR and GLASS-AMC in 2021 (13).

Despite these efforts, fully implementing the multisectoral national strategic plan remain challenging as observed in other sub-Sahara African countries. Indeed, financial and human resources for implementation are yet to be dedicated (38). A clear and functioning governance structure for the NAP activities still need to be put in place (38). Although strengthening laboratories capacity is necessary to improve AMR surveillance for evidence-based decision-making, sample collection on patients admitted in regional or university hospitals (35,37) certainly reflect both hospital-and community-acquired infections, not allowing to assess the extent of each of them. Moreover, factors underlying the level of bacterial resistance observed in collected samples need deep understanding both at hospital and community level for targeted antimicrobial stewardship interventions. Likewise, antimicrobial use reporting, based on aggregated data (13,35), may not reflect actual use and do not allow an assessment of dispensing practices across different healthcare providers in the country, yet necessary to inform targeted stewardship activities. Similarly, lack of implementation of awareness and knowledge activities targeting healthcare workers was found as a gap to be filled in the review of progress in the human health sector of the NAP (38) but prior assessment of AMR knowledge across all antimicrobial dispensers (supply) and the general community (demand) may help developing targeted awareness activities which may have greater impact in the fight against AMR.

CHAPTER 2: RESEARCH RATIONALE AND OBJECTIVES

2.1 Research rationale

Resistant bacteria such as Extended-spectrum β-lactamase-Producing *Escherichia coli* (ESBL-EC) and *Klebsiella pneumoniae* (ESBL-KP) are major contributors of AMR global burden. Their increasing prevalence in bloodstream infections (BSI) (39) necessitates more frequent treatment with carbapenems, which significantly increases healthcare costs and contributes to high mortality rates. Western sub-Saharan Africa is the most affected (7,40–46).

In Burkina Faso, these resistant bacteria have been increasingly isolated from community-acquired invasive infections over the last ten years (43,47,48). This increase could be reflecting a high and increasing prevalence of ESBL-EC and ESBL-KP carriage in the community, as far as gut colonisation was found to be highly associated with BSI onset (49,50). Due to the ease of spread of ESBL-producing genes through mobile genetic material transfer (51), it is crucial to know the extent of the problem in the community, and the context-specific factors that may be underlying it to inform targeted antimicrobial stewardship (AMS) interventions.

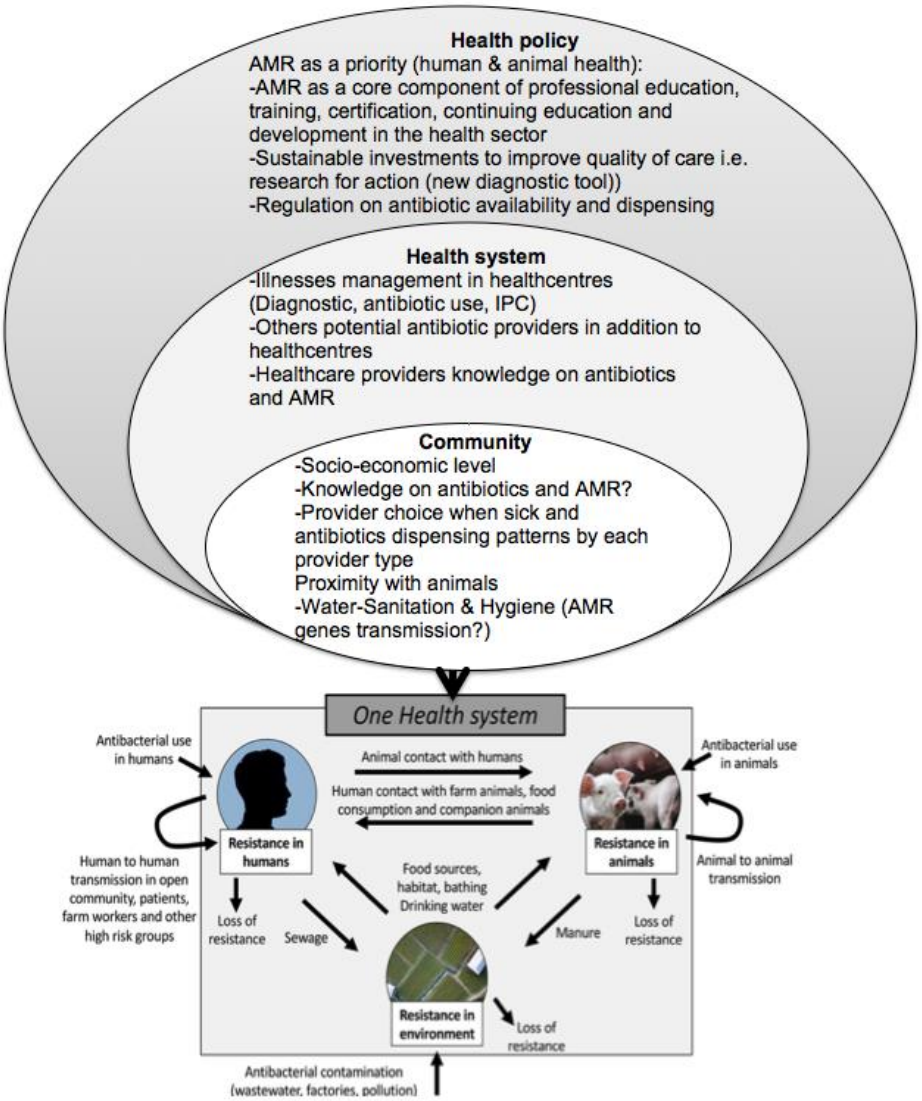

Figure 10: Analytical model of the problematic of antimicrobial resistance emergence and spread in the community in rural Burkina Faso

Source: Adapted from Coque et al. 2023 and Booston et al. 2021 (52,53)

2.2 Hypotheses

Based on our analytical model of the AMR emergence and spread in the community in rural Burkina Faso (Figure 10), we hypothesized that:

1. ESBL-EC and ESBL-KP faecal colonisation is widespread in the community and might be fuelling the burden of community-acquired invasive infections.

2. Watch antibiotics are widely used in the community

3. Community members' knowledge of antibiotics is promoting incorrect use of these antibiotics with high resistance potential

4. Community members' behaviour's regarding water, sanitation and hygiene (WASH) are contributing to the spread of resistant bacteria

5. Community members' socio-economic level is guiding their provider choice when seeking for healthcare and provider-specific dispensing of high resistance potential antibiotics might be differently fuelling the local burden of AMR

2.3 Objectives

2.3.1 General objective

The overall objective of this thesis was to determine the extent of resistant strains *E. coli* and *K. pneumoniae* (major contributors of AMR attributable deaths in Western sub-Saharan Africa) in the community in rural Burkina Faso and factors underlying it, in order to inform targeted infection prevention and control (IPC) interventions.

2.3.2 Specific objectives

1. To determine the extent of antibiotic resistant bacteria (ESBL-EC and ESBL-KP) as well as molecular characteristics of these ESBL-*Enterobacterales* in healthy community members

2. To investigate community antibiotic use before attending the Nanoro district hospital

3. To understand the role of different healthcare providers and other antibiotic dispensers in a rural community

4. To explore antibiotic dispensing practices by type of healthcare provider

CHAPTER 3: METHODS

3.1 Study settings

Investigations were conducted in two health areas of the Nanoro health district (Figure 11), which include 30 PHCs (of which only 4 have medical doctors) and the district hospital. The two health areas, Nanoro and Nazoanga, are located in central-west Burkina Faso, about 90 km from Ouagadougou. Nanoro health area includes five villages (Nanoro, Poessi, Gouroumbila, Basziri and Goulouré) with 17 300 inhabitants. Nazoanga health area includes three villages (Nazoanga, Zimidin et Sitaon) with 8800 inhabitants. Nanoro has a district hospital, a PHC (with a medical doctor), two private community pharmacies (one run by a pharmacist, although actual medicine dispensing is ensured in both pharmacies mainly by non-pharmacists), three informal medicine vendors (non-licensed vendors) and two traditional healers. Nazoanga has a PHC (without a medical doctor), two informal medicine vendors and one traditional healer. Both health areas are embedded in the Clinical Research Unit of Nanoro (CRUN) Health and Demographic Surveillance System (HDSS). The HDSS covers 24 of 57 villages in the area. Four-monthly household visits are planned by the HDSS to update individual participants' and households' data (54)

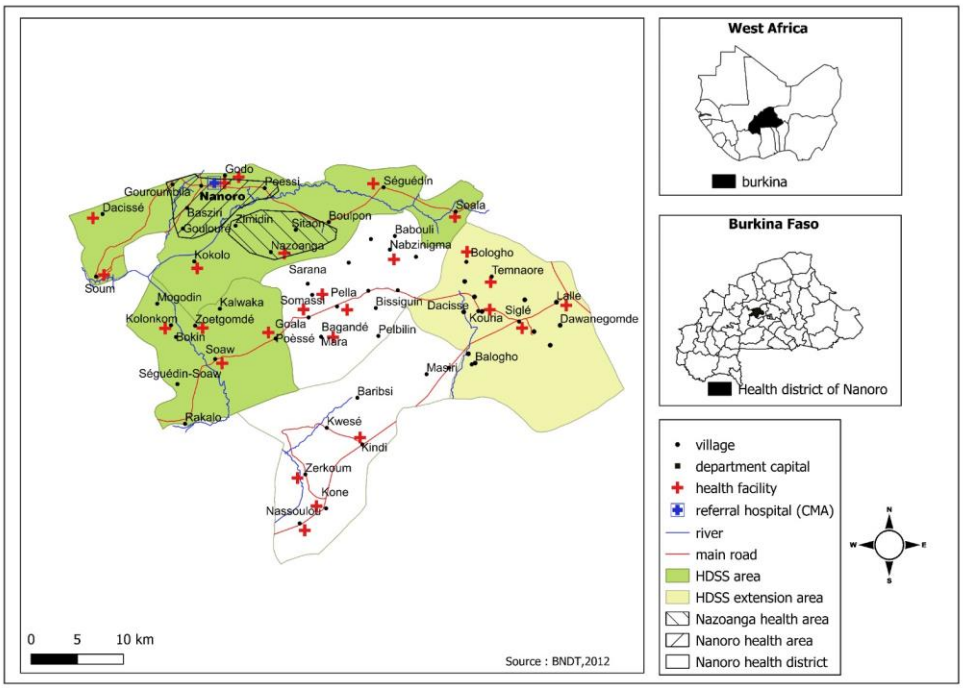

Figure 11: Nanoro health district showing the Nanoro and the Nazoanga health areas embedded in the Health and Demographic Surveillance System area

In Nanoro health district, the most frequently reported diagnosis is malaria (55),which in children is sometimes (< 3%) associated with a community-acquired invasive bacterial co-infection (47,56).Malaria is endemic with a high malaria transmission season from July to November. This period overlaps with the rainy season, which extends from June to October. The low malaria transmission season is from December to June.

3.2 Study design

Methods are extensively detailed in published manuscripts in chapter 4.

Table 4: Study specific objective, design, sample and publication reference

Specific objective	Study design	Sample and Year of data collection	Publication title, journal, year, vol, pages
To determine the extent of antibiotic resistant *Enterobacterales* (ESBL- Producing *Escherichia coli* & ESBL- *Klebsiella pneumoniae*) in the community	Community-based cross sectional study	In two health areas of the Nanoro health district, 1482 healthy community members were randomly selected and surveyed from May 2021 to May 2022. Stool samples were collected and isolated *E. coli* and *K. pneumoniae* went through antibiotic susceptibility testing (AST). Based on AST results bacteria were subsequently randomly selected for WGS	Faecal colonisation with extended-Spectrum β-Lactamase – Producing *Escherichia coli* and *Klebsiella pneumoniae* in rural Burkina Faso (to be submitted)
To investigate community antibiotic use before attending the Nanoro district hospital	In a clinical series of patients attending the Nanoro district hospital with severe acute fever, a cross sectional survey was conducted to assess pre-hospital antibiotic use	In the Nanoro district hospital, a total of 920 febrile patients were surveyed from March 2016 to June 2017	Use of WATCH antibiotics prior to presentation to the hospital in rural Burkina Faso. Antimicrobial Resistance & Infection Control. 2022 Apr 13;11(1):59.
To understand the role of different healthcare providers and other antibiotic dispensers in a rural community	Mixed-methods study: a) Qualitative study with purposive sampling b) Quantitative study: community-based cross-sectional household survey	Both types of data collected in the same two health areas of the Nanoro health district from October 2020 to December 2021 a) Qualitative study: 4 FGDs with community members, 1 FGD with community healthcare workers, 6 IDIs (1 nurse, 2 midwives 2 public pharmacy vendors and 1 private pharmacy vendor), 4 ICs combined with direct observations towards 4 Informal vendors. b) Quantitative study: 1582 households' randomly selected and 10,693 household members surveyed.	Healthcare seeking outside healthcare facilities and antibiotic dispensing patterns in rural Burkina Faso: A mixed-methods study. Tropical Medicine & International Health. 2023 May;28(5):391-400.
To explore antibiotic dispensing practices by type of healthcare provider	Healthcare visit exit survey	In patients completing healthcare visit for acute illnesses, 2108 were surveyed at exit of medicine stores, after buying their medications from October 2021 to February 2022	Antibiotic dispensing across formal and informal healthcare providers in rural Burkina Faso: A healthcare visit exit survey Journal of Antimicrobial Chemotherapy. 2024 July 25;79(10):2534-2542.

Note: FGD: Focus Group Discussion; IDI: In-Depth Interview, IC: Informal Conversation

Study 1: A community-based cross-sectional study to determine the extent of antibiotic resistant bacteria

We conducted a community-based cross-sectional survey from May 2021 to May 2022. Sample size per study area was calculated assuming a 50% prevalence of colonisation, 95% confidence interval limits of +/- 4%, 10% of non-respondents. In each health catchment area, we calculated the number of participants to be included per village, proportional to villages' population size. This resulted in a targeted sample size of 651 and 621 respectively in the Nanoro and the Nazoanga health catchment area. Using the HDSS household members as sample frame, we randomly selected the number of individuals allocated to each village. We included 798 participants in the Nanoro health catchment area and 684 in the Nazoanga health catchment area. Isolated bacteria were subsequently selected based on their phenotypic characteristics for whole genome sequencing to characterize ESBL-encoding genes, virulence factors, plasmids, and clonal relationships.

Study 2: A cross sectional survey among patients with severe acute fever attending the Nanoro district hospital to investigate their pre-hospital antibiotic use

From March 23, 2016 to June 30, 2017, the PaluBac study recruited patients aged >3 months presenting to the Nanoro district hospital with acute fever (tympanic temperature ≥ 38.0 ° C) or history of fever in the last 48 hours and ≥1 symptom(s) of respiratory distress, generalized weakness (prostration), impaired consciousness, seizures (one or more episodes), clinical jaundice, signs of shock, or with suspected severe malaria, invasive bacterial infection, or severe viral infection. A total of 920 patients of whom 344 (37.4%) inpatients and 576 (62.6%) outpatients were included. From March 23 to November 8, 2016, only inpatients were recruited; from November 9, 2016 to June 30, 2017 also outpatients were included. PaluBac evaluated the performance of an automated cell counter to quantify malaria parasitaemia. Our embedded cross-sectional study recorded patients' pre-hospital antibiotic use.

Study 3: A mixed-method study to understand the role of different healthcare providers and other antibiotic dispensers in a rural community

We conducted an exploratory mixed-method design from October 2020 to December 2021. Data were collected in three distinct stages, the results of each

stage fed into the design and development of subsequent stages. With a first qualitative design, we explored illness perceptions, the range of healthcare providers in the community, antibiotics knowledge and reasons to seek healthcare outside healthcare facilities. Based on the results of the initial qualitative study, we refined a quantitative questionnaire to survey household members in the same community on episodes of illness and healthcare utilisation within the last three months. Subsequently, with a final qualitative design we captured how private drug store vendors and informal drug vendors generally manage symptoms that households reported during the survey, with a special focus on antibiotic dispensing.

For illness perception, range of healthcare providers, and reasons for the choice of provider, five FGDs were conducted, four with community members and one with community healthcare workers. All community members were farmers. In the Nanoro health area, three FGDs were conducted, two with community members (a group of twelve women aged 30 to 40 years old and a group of twelve men aged 35 to 55) and one with community healthcare workers (a group of eight community healthcare workers aged 25 to 35 years old). In the Nazoanga health area, two FGDs were conducted with community members (a group of twelve women aged 20 to 35 years old and a group of twelve men aged 20 to 35 years old). Participants were selected purposively on their ability to provide in-depth and pertinent information regarding our research question, based on the principles of purposive sampling for qualitative data collection. All community informants from each village from both health areas were involved in this selection. Data was analyzed as the fieldwork progressed: in an iterative cyclic process, the topic guide for subsequent FGDs was adjusted based on what had emerged from the previous sessions. Subsequently, using snowball sampling, interview participants were selected. Six IDIs were conducted, four in Nanoro health area (with a nurse, a midwife, and both the private drug store vendor and the public drug store vendor located in the Nanoro's medical center) and two in Nazoanga health area (with a midwife and the public drug store vendor in Nazoanga's PHC). Four informal conversations were conducted, with two informal drug vendors in the Nanoro health area and two others in the Nazoanga health area. Interviews with informal drug vendors were an opportunity for direct observations of available medicines at their drug outlets. The findings from the different methods permitted data

triangulation. The process of participant selection was ended after theoretical saturation was reached.

For the cross-sectional household survey recording healthcare utilization, we selected a random sample of 802 and 780 households respectively in Nanoro and Nazoanga health areas, using each health area households' list (available in the HDSS database) as sample frame. The sample size (number of households) per health area was calculated assuming a proportion of the population having sought healthcare of 0.2, a design effect (adjusting for intra household clustering) of 2, an average household size of 7 (based on the Nanoro HDSS data), 95% confidence intervals, confidence limits of +/- 4%. In an electronic questionnaire, all members of each selected household were asked about symptoms during the three months prior the survey, the frequency and the type of healthcare provider visited. Data collection in both health areas was combined with the most recent HDSS routine data during which ownership of assets (television/video/radio/telephone/mobile devices, computers, laptops, cars, motorcycles, bicycles, refrigerator) was recorded and used to rank the socio-economic level of each household.

For symptoms management at informal drug outlets and private drug stores, five informal conversations were conducted, two with informal drug vendors in both health areas and one with a vendor in the Nanoro private drug store to know how they usually manage patient symptoms as detailed in the household healthcare utilization survey. We specifically explored how they dispense Watch group antibiotics if seen at their outlets during direct observations. Participants were purposively selected on their ability to provide detailed information based on data collected during first stage.

Study 4: A healthcare visit exit survey to explore antibiotic dispensing practices by type of healthcare provider

We conducted a healthcare visit exit survey from October 2021 to February 2022. We surveyed patients exiting each type of provider (identified in the previous study) after consultation for an acute illness. A consecutive sample of patients presenting with acute illness was taken by selecting the next patient exiting when the interview with the previous patient was completed. We planned to interview at least 50 outpatients who had received an antibiotic per type of healthcare provider to obtain 95% CI limits with a margin of error of 5%, assuming 15% of Watch antibiotic use. In addition to outpatients, all inpatients admitted to the paediatric and medicine

wards of the Nanoro district hospital and patients under observation in Nazoanga and Nanoro PHCs were interviewed once.

3.3 Data collection

Data collection process is extensively detailed in published manuscripts and variables collected were aligned with the objectives of each study.

For the community-based cross-sectional study, the household survey in the mixed-method study and the visit exit survey, data were electronically captured on Redcap by trained field workers. For the cross-sectional study to evaluate patients' pre-hospital antibiotic use, data were collected on paper case report forms. Qualitative data (Focus group discussions, in-depth interviews, informal conversations) were audio recorded. Results of laboratory analyses were paper-based reported.

3.4 Data management and statistical analysis

Data collected on paper case report forms were double entered either on OpenClinica version 3.1 or Redcap version 13.8.1 database. Data (electronically collected and double entered) were subsequently exported on Stata version 18.2 (StataCorp LLC, Texas, USA) for cleaning and analysis. Reported frequencies were corrected for clustering and were summarized either on frequency tables or in bar charts. Chi-square or F-tests were used to compare frequencies, variables from the univariate analysis were included in a multivariate analysis to control for potential confounding factors if $p \leq 0.2$ in univariate analysis. Likelihood-ratio test (LRT) or Wald test p-values were reported and significance level was set at 5%.

For qualitative data, the audio recordings were transcribed into English. Transcripts were reviewed to match audio records before being imported into NVivo software, version 12. Codes were then deductively and inductively generated according to the research question being addressed as well as new codes emerging from collected data. Codes were grouped into broader categories to generate themes.

3.5 Ethical considerations

Studies protocols were approved by the Ethics Committee for Health Research of Burkina Faso, reference numbers 2017-01-001, 2020-8-171 and 2021-03-052. Regarding quantitative surveys throughout each study, written informed consent was obtained from all studies participants aged at least 18 years. For participants aged between 14 and less than 18 years, oral assent was obtained in addition to parents or caretakers' written informed consent. For study participants under 14 years, written informed consent was obtained from parents or caretakers.

For the qualitative study, verbal informed consent was obtained from all participants. This verbal consent was documented in a form signed by the researchers, certifying that verbal inform consent was obtained from each participant. Both qualitative and quantitative data were anonymised to preserve study participants' confidentiality.

CHAPTER 4: RESULTS

4.1 Faecal colonisation with extended-spectrum β-lactamase – producing *Escherichia coli* and *Klebsiella pneumoniae* in rural Burkina Faso

Authors

Daniel Valia[1, 2, 3*], Brecht Ingelbeen[2,4], Ibrahima Karama[1], Zakaria Garba[1], Palpouguini Lompo[1], Bérenger Kaboré[1], François Kemdé[1], Alix L. Tiendrebeogo [1], Eric W. Tiendrebeogo [1], Esther van Kleef[5], Sandra van Puyvelde[6] , Anais Prizzon[7], Anne-Thérèse Vandenbroucke[7], Maria A. Argudín[7], Benoît M. Kabamba[7], Jan Jacobs[8] , Halidou Tinto[1,2], Marianne A.B. van der Sande[2, 9], Annie Robert[3] and Hector Rodriguez-Villalobos[7]

Author affiliations

1. Institut de Recherche en Sciences de la Santé, Direction Régionale du Centre-Ouest/Clinical Research Unit of Nanoro, Nanoro, Burkina Faso
2. Department of Public Health, Institute of Tropical Medicine (ITM), Antwerp, Belgium
3. Epidemiology and Biostatistics Unit, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium
4. International Centre for Antimicrobial Resistance solutions (ICARS), Copenhagen, Denmark
5. Oxford Nuffield department of medicine, university of Oxford, Oxford, England
6. university of Antwerp, Antwerp, Belgium
7. Microbiology unit, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium
8. KU Leuven, Department of Microbiology, Immunology and Transplantation, Leuven, Belgium
9. Julius Center, University Medical Center Utrecht, Utrecht, The Netherlands

Corresponding author

*Daniel Valia E-mail: valiadaniel@yahoo.com, ORCID ID: https://orcid.org/0000-0003-0851-9352

Abstract

Background

Faecal carriage of extended-spectrum β-lactamase- producing *Enterobacterales* (ESBL-E) is a growing public health concern, given its role as a reservoir for the transmission of antimicrobial resistance. In rural Burkina Faso, we explored its extent, associated factors and molecular characteristics.

Methods

In a community-based cross-sectional survey we collected fresh stool samples of randomly selected community members in two rural health catchment areas and recorded water, sanitation and hygiene conditions, presence of animals, household composition and healthcare utilisation, between May 2021-May 2022. We estimated the prevalence of faecal colonisation of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* and evaluated factors associated with colonisation. Using disk diffusion, we screened all isolates for ESBL production and tested for antibiotic susceptibility. Based on the antibiotypes and the real time PCR screening, isolates were selected for molecular characterization by whole genome sequencing.

Results

The estimated overall prevalence of faecal colonisation with ESBL-E was 61.3% (58.8%-63.7%), prevalence of ESBL-*E. coli* being at 53.0% (50.5%-55.5%) and that of ESBL-*K. pneumoniae* at 22.3% (20.3%-24.5%). The prevalence of faecal colonisation with ESBL-E was higher during the rainy season compared to the dry season (70.2% vs 53.6%, p<0.001). Participants reporting not washing hands with soap before meals were more frequently colonised with ESBL-E compared to those who did (62.5% vs 49.0%, p<0.001). High resistance to ciprofloxacin were reported for both ESBL-*E. coli* (66.1%) and ESBL-*K. pneumoniae* (68.1%) isolates. In both bacteria, $bla_{\text{CTX-M-15}}$ was the most prevalent ESBL gene (47.3% in *E. coli* and 19.9% in *K. pneumoniae*). A carbapenemase $bla_{\text{NDM-5}}$ was found in one *E. coli* isolate. The most common plasmids in *E. coli* isolates were IncFII (25/52), IncFIA (23/52), IncFIB(AP001918) (23/52) while IncFIB(K) (27/37), IncFII (18/37), IncFII(K) (12/37), and IncR (10/37) were predominant in *K. pneumoniae* isolates.

Conclusion

Frequent ESBL-*Enterobacterales* colonisation underlines a critical need for action to address the spread of multidrug resistant-bacteria in the community. Improving hand hygiene practices, enhancing sanitation and establishing AMR surveillance, can be effective steps toward mitigating the burden of AMR in rural Burkina Faso.

Keywords

Antimicrobial resistance, Extended-spectrum β-lactamase-producing *Enterobacterales*, Water-Sanitation and Hygiene, Burkina Faso

Introduction

Extended-spectrum β-lactamase-Producing *Escherichia coli* (ESBL-EC) and *Klebsiella pneumoniae* (ESBL-KP) are major contributors of the global burden of antimicrobial resistance (AMR) (1). Since 2000, a rapid global emergence of CTX-M-producing ESBL-EC has been widely documented. Initially, this was primarily linked to the pandemic and virulent ST131 clone of extra intestinal *E. coli*, harbouring multidrug-resistant IncFII plasmids, related to community-associated urinary tract infections (2). Since then, the reported rates of ESBL-E community carriage have continued to rise everywhere, with different dynamics according to geographical area (3). The increasing occurrence of these resistant pathogens as causative agents of bloodstream infections (BSI) requires more frequent treatment with carbapenems. Access and affordability of carbapenem is not self-evident in many low-resource settings including sub Saharan Africa, and their use associated with exacerbation of carbapenem-resistance (1,4).

In low-resource settings, including rural Burkina Faso (BF) and Kenya, most infections, including those managed in healthcare facilities, are community-associated, with the most common causative pathogens isolated being *Salmonella* spp., *Streptococcus pneumoniae*, and *E. coli* (5,6). With gut colonisation identified as a precursor to BSI, understanding the extend of human colonisation at community-level, can aid the targeting of intervention strategies to mitigate their spread (7). What is more, over the last ten years, in Burkina Faso, ESBL-EC and ESBL-KP are increasingly isolated from community-acquired invasive infections (5,8,9). Community-level ESBL-EC acquisition has previously been largely attributed to human-to-human transmission (10). While such evidence on source-attribution of AMR is largely confined to well-resourced settings, it is possible that the surge observed in Burkina Faso and elsewhere, may be driven by high and increasing prevalence of colonisation with ESBL-E in the community. ESBL-producing genes are known to spread through mobile genetic material transfer, maintained and facilitated by inappropriate use of wide spectrum antibiotic and poor water sanitation and hygiene (WASH) conditions (10–15).

The latest study on ESBL-E faecal colonisation was carried out over ten-years ago, in 2014, among a small sample of 101 healthy volunteers in Bobo Dioulasso an urban area of Burkina Faso, reporting a prevalence of 22% (95% CI: 15%-31%) of ESBL-EC and ESBL-KP which was associated with previous hospitalization and

previous antibiotic use (16). We conducted a comprehensive cross-sectional study, which aimed to estimate the prevalence of ESBL-EC and ESBL-KP faecal colonisation, identified factors associated with such colonisation; as well, we analyzed the circulating strains at the molecular level, their virulence, and antibiotic susceptibility. Hence we aim to improve our understanding of the drivers of AMR in similar sub-Saharan African settings, and help guide community-based AMR infection prevention and control interventions.

Methods

Study area

This study was conducted in the Nanoro health district, which is located in central-west Burkina Faso, at about 90 kilometres from Ouagadougou, the capital city. The Nanoro health district covers 30 primary healthcare centers (PHCs). Each PHC covers a health catchment area, which includes a number of villages. Stool samples were collected in two health catchment areas of the district. The first, Nanoro, includes five villages and covers a population of 17300 inhabitants, and Nazoanga which includes three villages and covers a population of 8800 inhabitants (11). Both health catchment areas are embedded in the Clinical Research Unit of Nanoro (CRUN) Health and Demographic Surveillance System (HDSS). Six-monthly household visits are regularly conducted by the HDSS to update individual participants' and households' data (17).

In the Nanoro health district, the most frequently reported diagnosis in healthcare facilities is malaria (18), which for children is sometimes associated with a community-acquired invasive bacterial co-infection (19,20). Malaria is endemic with a high malaria transmission season occurring from July to November. This period overlaps with the rainy season, which lasts from June to October.

Study design

We conducted a cross-sectional survey from May 2021 to May 2022. Sample size per study area was calculated assuming a 50% prevalence of colonisation, 95% confidence interval limits of +/- 4%, 10% of non-respondents. In each health catchment area, we calculated the number of participants to be included per village, proportional to villages' population size. This resulted in a targeted sample size of 651 and 621 respectively in the Nanoro and the Nazoanga health

catchment area. Using the HDSS household members as sample frame, we randomly selected the number of individuals allocated to each village. Using an electronic questionnaire, each eligible household member in a household participating in the HDSS was approached by field workers, using households' geographical coordinates from the HDSS. Questionnaires recorded the following information: (i) antibiotic use in the last three months, (ii) healthcare utilization in the last three months, (iii) hospital stay in the last three months, (iv) source of drinking water, (v) use of toilets, (vi) hand washing with soap before eating and (vii) proximity to livestock. A labelled container was provided thereafter to provide a fresh stool sample the next morning. The date and time of sample collection was recorded as well as participant ID. Collected samples were transported to the laboratory in a cool box and within eight hours of production, for screening of ESBL-EC and ESBL-KP. Data collection in both health catchment areas was combined with the most recent socio-economic level of HDSS households based on repeatedly recorded household income and ownership of assets (television/video/radio/telephone/mobile devices, computers, laptops, cars, motorcycles, bicycles, refrigerator) (17,21).

Laboratory procedures

Bacterial isolation and species identification

Ten microliters of stool samples were plated onto ESBL-selective agar plates (CHROMagar™ ESBL, Paris, France) and incubated at 35 ± 2°C for 24 hours. Following incubation, colonies exhibiting red or pink coloration were identified as *E. coli*, while green colonies were categorized as members of the KESC group (*Klebsiella, Enterobacter, Serratia*, and *Citrobacter*), in accordance with the manufacturer's instructions. *E. coli* and KESC group colonies were then selected for purification on eosin methylene blue agar. Identification of *Klebsiella* colonies was performed using the API20E system (Biomérieux, Marcy-l'Étoile, France), according to the manufacturer's recommendations.

Antimicrobial susceptibility testing and ESBL production test

A total of 903 presumptive ESBL-EC and 274 ESBL-KP were tested against 25 antibiotic agents (Table S1) using the disc diffusion method on Mueller-Hinton agar (Manufactured by Becton Dickinson, USA), and interpreted according to the American Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines (22).

The presence of ESBL was tested in all isolates by double disk synergy test with ceftriaxone, cefepime, and amoxicillin-clavulanic acid, in accordance with the CLSI 2023 guidelines. Among the presumptive ESBL-EC and ESBL-KP, 900 *E. coli* and all *K. pneumoniae* isolates were confirmed as ESBL producers. Based on the antimicrobial susceptibility test results, the isolates were categorized into seven phenotypic groups: resistant to temocillin and piperacillin-tazobactam (TEMO-R+PTAZ-R); resistant to carbapenems (CARBA-R); resistant to cephalosporins and aminoglycosides without co-resistance to cotrimoxazole (CAZ/CTX/CPME-R+SXT-S+GM/AK-S); resistant to cephalosporins and aminoglycosides with co-resistance to cotrimoxazole (CAZ/CTX/CPME-R+SXT-R+GM/AK-S); resistant to cephalosporins, aminoglycosides, gentamicin and intermediate or susceptible to amikacin (CAZ/CTX/CPME-R+GM-R+AK (I/S)); susceptible to ceftazidime (CAZ-S) and exhibiting co-resistance to cephalosporins and cefoxitin (AMPC/FOX-R). Random selection within each group yielded 124 isolates, which were stored in sterile tubes with glycerin at -80°C for further analysis in Belgium, including microdilution and molecular characterization.

Upon arrival at the Microbiology Laboratory of Cliniques Universitaires Saint-Luc, identification of isolates was confirmed using mass spectrometry (MALDI-TOF, MALDI Biotyper, MBT Smart, Bruker). The determination of minimum inhibitory concentration (MIC) values for 27 antibiotics was conducted by microdilution (Table S2), using the automated Phoenix M50 system (Becton Dickinson, USA) with the N-MIC 474 panel, in accordance with the manufacturer's recommendations for the 124 selected isolates (87 ESBL-EC and 37 ESBL-KP).

Molecular characterization of ESBL genes

Real time PCR screening of ESBL genes

Real-time PCR screening for CTX, TEM, SHV genes and 16S RNA was performed using primers and probes sequences previously described (23,24). The PCR mix was adapted for use with the FastStart Essential DNA Probes Master (Roche), and 1 µL of DNA was used per reaction. PCR amplification was carried out in a LightCycler® 96 instrument (Roche) under the conditions described by Roschanski et al.(23). CTX-positive samples were further analyzed for the characterization of CTX-M group by CTX-M-1, CTX-M-2, and CTX-M-9 real-time PCR using previously described primers and probes (25). The PCR mix was again adapted for the

FastStart Essential DNA Probes Master (Roche), and 1 µL of DNA was used per reaction, with amplification conditions as described (25).

Whole genome sequencing, De Novo Assembly, and analysis

Based on the seven phenotypic groups and the PCR screening, random selection was performed to sequence 52 ESBL-EC. All ESBL-KP screened by PCR (n=37) were also sequenced.

Bacterial DNA was extracted from strains incubated for 24 hours on Columbia blood agar under a laminar flow hood. The DNA extract was prepared by resuspending bacterial in nuclease-free water within PowerBead tubes and vortexed before centrifugation. The supernatant was mixed with pre-vortexed SPB (AMPure XP Beckman Coulter) in sterile tubes and placed on a magnetic rack to isolate the double-stranded DNA (dsDNA). After washing with 80% ethanol, the beads were resuspended in RSB (Kit Truseq RNA access Library Prep Guide Illumina). The purified dsDNA was stored at -20 °C until further use. DNA concentration was measured using a Qubit.

For the library preparation phase, extracted DNA was combined with tagmentation and amplification reagents following Illumina's protocol. The procedure employed a thermocycler for tagmentation and amplification steps and involved magnetic bead-based purification to ensure clean libraries. These libraries were analyzed using a Tapestation to verify fragment size distribution, ensuring readiness for sequencing. The final sequencing was conducted on the NextSeq 1000 system.

E. coli STs were assigned in silico by uploading assembled genomes to the multilocus sequence typing (MLST) 2.0 tool of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/) using the scheme of Wirth et al. (26) for *K. pneumoniae* STs were assigned silico by uploading assembled genomes to the Institut Pasteur MLST database (https://bigsdb.pasteur.fr/klebsiella/).

Acquired antimicrobial-resistant genes were identified by uploading assembled genomes to the ResFinder 4.6.0 tool of the Center for Genomic Epidemiology. *E. coli* virulence genes were identified by uploading assembled genomes to the VirulenceFinder 2.0 tool of the Center for Genomic Epidemiology. *K. pneumoniae* virulence genes were identified by uploading assembled genomes to the Institut

Pasteur MLST database. The incompatibility (Inc) and colicinogenic (Col) plasmid types were identifies by uploading assembled genomes to the PlasmidFinder 2.1 tool of the Center for Genomic Epidemiology.

Data analysis

We estimated the prevalence of ESBL-EC and ESBL-KP, overall and by subgroup with 95% CI correcting for the finite population (fraction of residents sampled from the total population within each village from each health catchment area) and sampling weight (n population_of_village/n study_population_of_village). Both, ESBL-EC and ESBL-KP are further referred to as ESBL-*Enterobacterales* (ESBL-E)

Ownership of assets recorded from households during routine HDSS rounds was used to rank each household in quintiles by socio-economic level, according to the Demographic and Health Surveys (DHS) wealth index (21).

We used F-test to compare the frequency of ESBL–E between sex (female vs male), age groups, occupation categories, socio-economic level, hand-washing practices (washing hands before meals vs not washing), categories of latrine, sources of drinking water, proximity with livestock categories (inside vs outside household), antibiotic use the last three months (any use vs no use), hospitalization status the last three months (hospitalized vs not hospitalized), season of survey (rainy vs dry) and finally between health catchment area (Nanoro vs Nazoanga). Variables were included in a multivariate logistic regression analysis to control for potential confounding factors if the p-value from univariable Wald tests ≤ 0.2.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee for Health Research of Burkina Faso (CERS ref n° 2021-03-052). Written informed consent was obtained from all study participants aged at least 18 years old. For study participants aged between 14 and less than 18 years, oral assent was obtained in addition to parents or caretakers' written informed consent. For participant less than 14 years old, written informed consent was obtained directly from their parents or caretakers.

Results

Characteristic of the study population

Out of 1500 participants surveyed, 1482 provided stool samples (798 in Nanoro health area and 684 in the Nazoanga health area). Of these, 693 (47.0%) were men, 337 (22.2%) were aged 0-4 years old, 220 (16.2%) were from the lowest socio-economic quintile. Open-air defecation was reported by 1144 (75.1%) participants, 350 (21.3%) reported drinking water from unprotected well and 1339 (90.8%) reported not washing hands with soap before meals (Table 1).

Table 1: Characteristic of study participants who provided stool samples

Participant characteristics	Health area			
	Nanoro (n = 798)		Nazoanga (n = 684)	
	n	%	n	%
Sex				
Female	417	52.3	372	54.4
Male	381	47.7	312	45.6
Age (years)				
0-4	162	20.3	175	25.4
5-17	251	31.5	274	40.0
18-49	182	22.8	145	21.3
≥50	203	25.4	90	13.3
Occupation				
No occupation	309	38.7	288	42.11
Housewife	116	14.5	132	19.3
Farmer	217	27.2	106	15.5
Student	110	13.8	157	23.0
Trader-Artisan-Civil servant	46	5.8	1	0.2
Socio-economic level[a]				
Highest quintile	147	18.9	98	14.5
Fourth	102	13.1	68	10.2
Third	155	19.9	175	26.1
Second	219	28.1	269	39.5
Lowest	155	19.9	65	9.6
Hand-washing with soap before meals[b]				
No	721	90.7	618	91.0
Yes	74	9.3	61	9.0
Latrine[c]				
Open air	503	64.4	641	93.9
Unimproved	110	14.0	25	3.6
Improved	169	21.6	17	2.5
Drinking Water source[d]				
Tap water	88	11.1	17	2.5
Borehole	429	53.9	196	28.9
Protected well	181	22.8	213	31.4
Unprotected well	97	12.2	253	37.2
Place of livestock keeping[e]				
Outside house	318	53.4	345	60.3
Inside house	275	46.6	230	39.7
Antibiotic use the last three months[f]				
No	609	76.5	625	92.0
Yes	187	23.5	54	8.0
Hospitalization the last three months[g]				
No	773	97.2	668	98.5
Yes	22	2.8	11	1.5
Visit to health centres the last three months[h]				
No	693	87.4	592	87.5
Yes	100	12.6	87	12.5
Visit to informal medicine vendors the last three months[i]				
No	673	84.7	597	88.0
Yes	122	15.3	82	12.0
Season				
Dry season	303	37.9	558	82.4
Rainy season	495	62.1	126	17.6

[a]: 29 missing values for Socio-economic level of which 20 in the Nanoro health catchment area; [b]: 8 missing values for hand-washing with soap of which 3 in the Nanoro health catchment area; [C]: ownership of latrine data were missing for 17 participants of which 16 in the Nanoro health catchment area; [d]: drinking water source data were missing for 8 study participants of which 3 in the Nanoro health catchment area; [e]: proximity with animals was evaluated on 1168 study participants who have livestock; [f]: data on antibiotic use the last three months before the survey were missing for 7 study participants of which 2 in the Nanoro health catchment area; [g]: data on hospitalization the last three months were missing for 8 study participants of which 3 in the Nanoro health catchment area; [h]: data on visit to health centers the last three months were missing for 10 study participants of which 5 in the Nanoro health catchment area; i: data on visit to informal vendors the last three months were missing for 8 study participants of which 3 in the Nanoro health catchment area

Prevalence and risk factors of fecal colonisation with extended-Spectrum β-Lactamase – Producing *E. coli* and *K. pneumoniae*

Estimated overall prevalence of fecal colonisation with ESBL-E was 61.3% (58.8%-63.7%), ranging from 53.0% (49.4%-56.6%) in Nazoanga to 65.9% (62.6%-69.0%) in Nanoro. ESBL-EC prevalence was at 53.0% (50.5%-55.5%), ranging from 45.3% (41.8%-48.9%) in Nazoanga to 57.4% (54.0%-60.7) in Nanoro and the one of ESBL-KP was estimated at 22.3% (20.3%-24.5%), going from 17.9% (15.3%-20.8%) in Nazoanga to 24.8% (22.0%-27.9%) in Nanoro. ESBL-E Prevalence was higher among participants who reported not washing hands with soap before meals (818, 62.5%) compared to those who reported washing hands with soap before meals (65, 49.0%, p<0.001). The prevalence of faecal colonisation was higher in the rainy season (433, 70.2%) compared to the dry season (455, 53.6%, p<0.001) (Table 2)

Table 2: Univariate and multivariate analysis of factors associated with faecal colonisation with ESBL-E

	Presence of extended-Spectrum β-Lactamase – Producing *E. coli* and/or *K. pneumoniae*					
Participant characteristics	n	% (95% CI)	OR (95% CI)	Wald test p-value	aOR (95% CI)	Wald test p-value
Sex				0.50		-
Female	480	62.0 (58.7-65.3)	1		-	
Male	408	60.4(56.8-63.9)	0.9 (0.8-1.1)		-	
Age (years)				0.003		0.22
0-4	181	54.7 (49.4-59.9)	1		1	
5-17	306	59.7 (55.5-63.7)	1.2 (0.9-1.6)		1.1 (0.8-1.5)	
18-49	218	68.0 (62.9-72.7)	1.8 (1.3-2.4)		1.6 (0.8-3.3)	
≥50	183	63.6 (58.0-68.9)	1.4 (1.1-2.0)		1.2 (0.6-2.6)	
Occupation				0.001		0.25
No occupation	321	55.1 (51.1-59.0)	1		1	
Housewife	150	61.5 (55.5-67.1)	1.3 (1.0-1.8)		0.9 (0.4-1.7)	
Farmer	212	67.1 (61.9-71.9)	1.7 (1.3-2.2)		1.0 (0.5-2.0)	
Student	172	65.5 (59.8-70.9)	1.5 (1.2-2.1)		1.4 (1.0-2.1)	
Trader-Artisan-Civil servant	33	70.8 (- - -)	2.0 (1.0-3.7)		1.0 (0.4-2.6)	
Socio-economic level				0.01		0.04
Highest quintile	145	61.0 (54.9-66.8)	1		1	
Fourth	94	55.7 (48.1-63.1)	0.8 (0.5-1.2)		0.8 (0.5-1.2)	
Third	190	58.3 (53.0-63.4)	0.9 (0.6-1.2)		1.0 (0.7-1.4)	
Second	287	60.7 (56.5-64.8)	1.0 (0.7-1.3)		1.1 (0.8-1.5)	
Lowest	155	71.1 (64.8-76.6)	1.6 (1.1-2.3)		**1.6 (1.1-2.3)**	
Washing hands with soap before meals				0.002		<0.001
No	818	62.5 (59.9-65.0)	1		1	
Yes	65	49.0 (40.5-57.5)	0.58 (0.4-0.8)		**0.4 (0.3-0.6)**	
Latrines				0.007		0.59
Open air	659	59.0 (56.2-61.7)	1		1	
Unimproved	93	69.0 (60.8-76.1)	1.5 (1.1-2.3)		1.2 (0.8-1.8)	
Improved	127	68.3 (61.4-74.6)	1.5 (1.1-2.1)		1.2 (0.8-1.7)	
Drinking Water source				0.96		-
Tap water & Borehole	430	60.9 (57.4-64.2)	1		-	
Protected well	242	61.7 (56.8-66.3)	1.0 (0.8-1.3)		-	
Unprotected well	211	61.5 (56.5-66.3)	1.0 (0.8-1.3)		-	
Place of livestock keeping				0.30		-
Outside household	384	59.0 (55.3-62.6)	1		-	
Inside household	305	61.9 (57.7-66.0)	1.1 (0.9-1.4)		-	
Antibiotic use the last three months				0.60		-
No	742	60.9 (58.3-63.6)	1		-	
Yes	142	62.8 (56.7-68.5)	1.1 (0.8-1.4)		-	
Hospitalization the last three months				0.65		-
No	862	61.1 (58.6-63.6)	1		-	
Yes	21	64.9	1.2 (0.6-2.4)		-	
Visit to health centers the last three months				0.54		-
No	767	60.9 (58.2-63.4)	1		-	
Yes	114	63.2	1.1 (0.8-1.5)		-	
Visit to informal vendors the last three months				0.7		-
No	762	61.4 (58.8-64.0)	1		-	
Yes	121	60.0 (53.1-66.6)	0.9 (0.7-1.3)		-	
Health area				<0.001		0.04
Nazoanga	362	53.0 (49.4-56.6)	1		1	
Nanoro	526	65.9 (62.6-69.0)	1.7 (1.4-2.1)		**1.3 (1.0-1.7)**	
Season				<0.001		<0.001
Dry season	455	53.6 (50.3-56.9)	1		1	
Rainy season	433	70.2 (66.5-73.6)	2.0 (1.6-2.5)		**1.7 (1.3-2.1)**	

Antimicrobial susceptibility of extended-Spectrum β-Lactamase – Producing *E. coli* and *K. pneumoniae*

A total of 898 (76.4%) *E. coli* and 277 (23.6%) *K. pneumoniae* were isolated. The percentage of resistance and the cumulative percentage of both *E. coli* and *K. pneumoniae* strains inhibited by MIC of each antibiotic are presented in figure1, figure 2 and table S2 respectively. Susceptibility testing by disc diffusion (Figure 1&2) revealed high resistance rates to ciprofloxacin (66.0% *E. coli* and 68.2% *K. pneumoniae*), sulfamethoxazole/trimethoprim (69.4% *E. coli* and 86.6% *K. pneumoniae*), nitrofurantoin (21.8% *E. coli* and 26.0% *K. pneumoniae*, and gentamicin (16.6% *E. coli* and 26.4% *K. pneumoniae*). Overall, carbapenems showed the best activity with <1% of resistance isolates and 98% of isolates with MIC values lower than 0,5 mg/L (Table S2). Despite this activity, eight strains (six *E. coli* and two *K. pneumoniae*) showed resistance to meropenem by disc diffusion technique, of which one *E. coli* was confirmed resistant to meropenem and imipenem with MIC values > 16 mg/L. Fosfomycin was the second most active antibiotic, with 99.2% of ESBL-EC isolates and 93.5% of ESBL-KP isolates being susceptible; of these, 90% exhibited a minimum inhibitory concentration (MIC90) of ≤ 16 mg/L. ESBL-EC demonstrated higher MIC values for recently marketed antibiotics, with 11% of isolates having MIC values for ceftolozane-tazobactam ≥ 8 mg/L, and 2% showing MIC values for ceftazidime-avibactam ≥ 16 mg/L. In contrast, no resistance was observed in ESBL-KP for ceftazidime-avibactam (MIC100 values ≤ 1 mg/L), and lower MIC values were recorded for ceftolozane-tazobactam, with only 3% exhibiting values ≥ 8 mg/L.

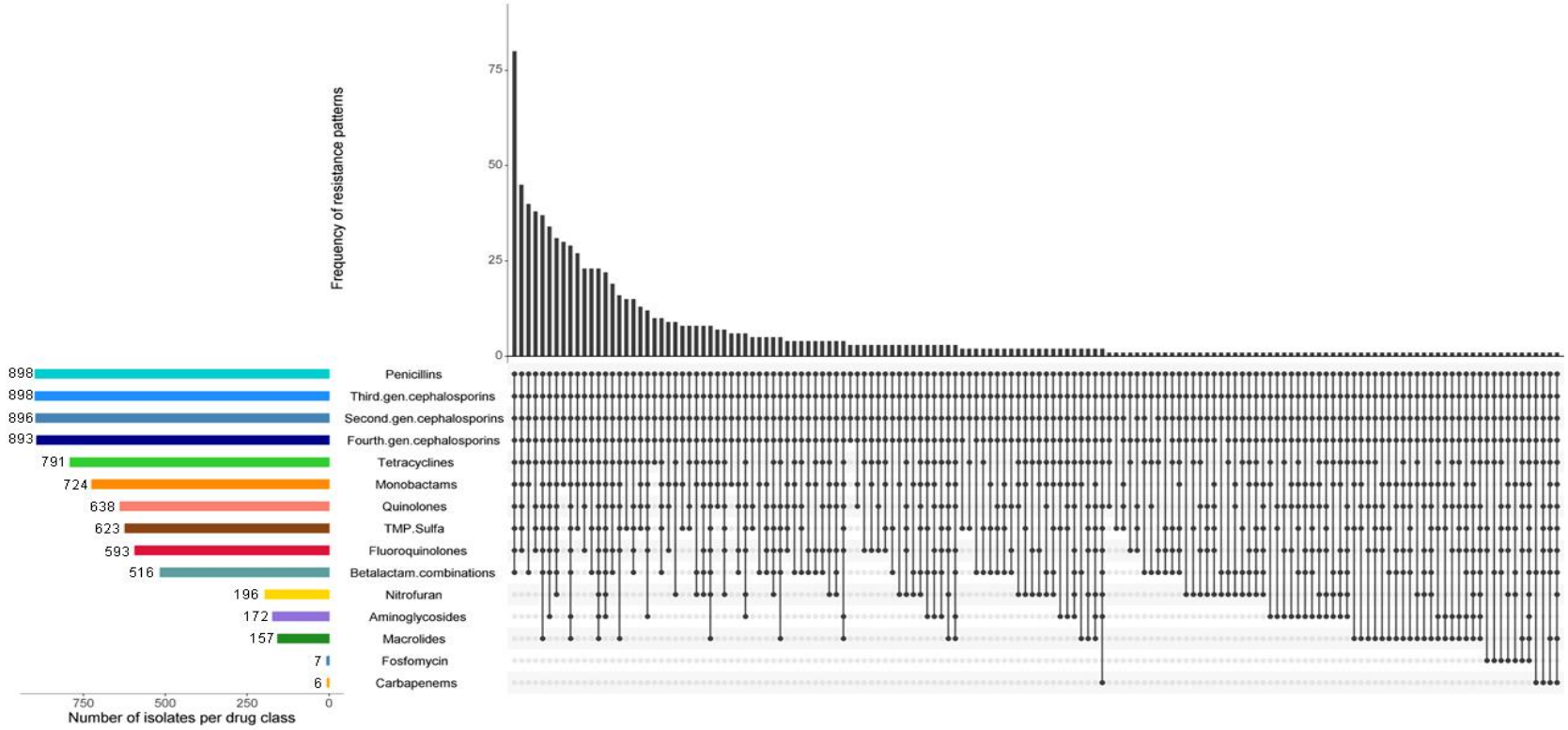

Figure 1: ESBL-EC antibiotic susceptibility testing by disc diffusion

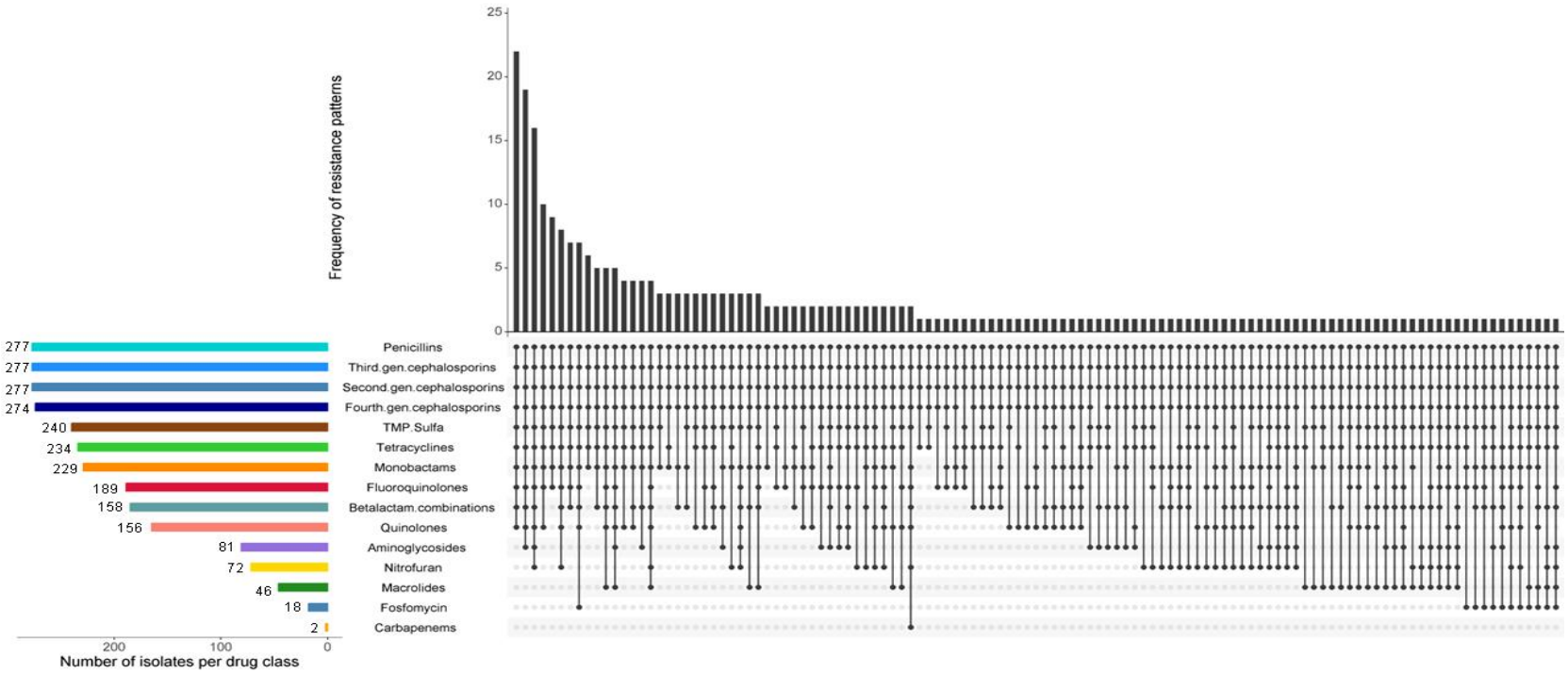

Figure 2: ESBL-KP antibiotic susceptibility testing by disc diffusion

Genotypic aspects of extended-Spectrum β-Lactamase – Producing *E. coli* and *K. pneumoniae*

In total 52 *E. coli* and 37 *K. pneumoniae* ESBL isolates were subject to whole genome sequencing.

Resistance genes

β-Lactamase genes for both *E. coli* and *K. pneumoniae* are presented in figure 3 and figure 4 respectively. In total, 93 β-Lactamase genes were found in *E. coli* (28 *E. coli* harboring two or more β-Lactamase genes) and 171 in *K. pneumoniae* (all of them carrying at least two β-Lactamases). CTX-M enzymes were the most prevalent, including CTX-M-1, CTX-M-14, CTX-M-15, and CTX-M-27, collectively representing 33.3% of all β-lactamases identified. The *bla*$_{\text{CTX-M-15}}$ gene was the most frequently detected in both species, present in 84.6% of *E. coli* (Figure 3) and 91.9% of *K. pneumoniae* isolates (Figure 4).

In *K. pneumoniae*, *bla*$_{\text{CTX-M-15}}$ was frequently associated with a diverse array of SHV-type β-lactamases, including ESBL *bla*$_{\text{SHV}}$ genes (SHV-13, SHV-27, SHV-40, SHV-70, and SHV-98), inhibitory resistance *bla*$_{\text{SHV-26}}$, and other *bla*$_{\text{SHV}}$ variants (SHV-11, SHV-28, SHV-56, SHV-78, SHV-79, SHV-81, SHV-85, SHV-89, SHV-94, SHV-96, SHV-106, SHV-110, SHV-145, SHV-172, SHV-179, SHV-194, and SHV-199). In *E. coli*, ESBL genes such as *bla*$_{\text{SHV-28}}$ (3.9%), *bla*$_{\text{SHV-81}}$ (1.9%), *bla*$_{\text{SHV-106}}$ (3.9%), and *bla*$_{\text{SHV-110}}$ (1.9%) were also identified.

Co-occurrence of CTX-M with TEM-1B and OXA-1 was observed in 38.5% and 19.2% of *E. coli* isolates and in 75.7% and 18.9% of *K. pneumoniae* isolates, respectively. Notably, no TEM ESBL genes were detected. The plasmid-acquired cephalosporinase *bla*$_{\text{CMY-2}}$ was identified in two *E. coli* isolates, both of which co-harboured *bla*$_{\text{CTX-M-15}}$.

Plasmid-mediated quinolone resistance (PMQR) genes were frequently detected alongside β-lactamase genes (Supplementary Table 2). The *qnr* genes were present in 48.1% (n=25) of *E. coli* and 81.1% (n=30) of *K. pneumoniae* isolates, while *aac(6')-Ib-cr* was detected in 21.2% (n=11) and 18.9% (n=7) of *E. coli* and *K. pneumoniae* isolates, respectively. Additionally, *OqxAB* was identified in 5.8% (n=3) of *E. coli* isolates and 78.4% (n=29) of *K. pneumoniae* isolates.

Finally, one *E. coli* isolate was found to carry the carbapenemase gene *bla*$_{\text{NDM-5}}$, co-harbouring *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{OXA-1}}$.

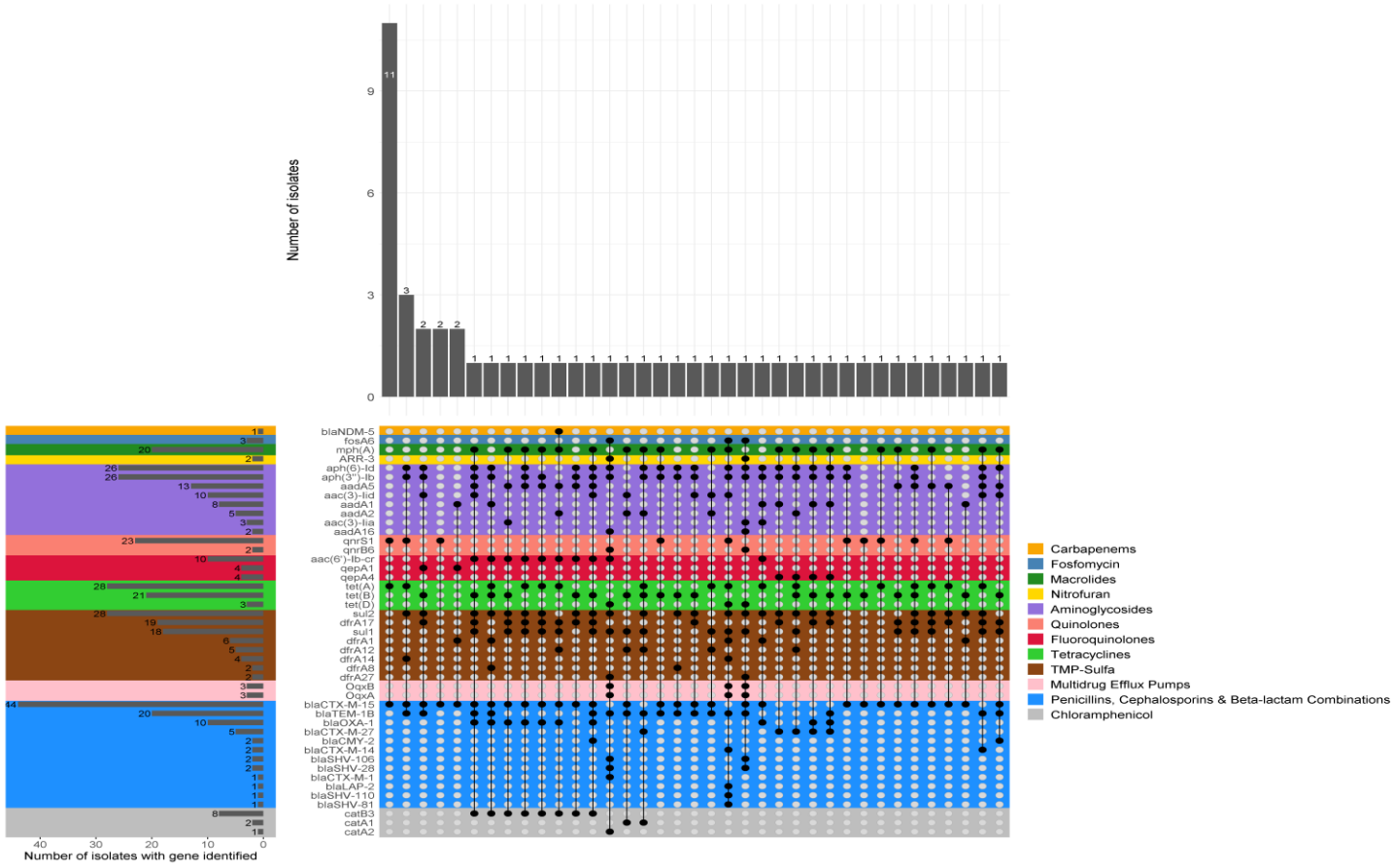

Figure 3: ESBL-EC resistant genes

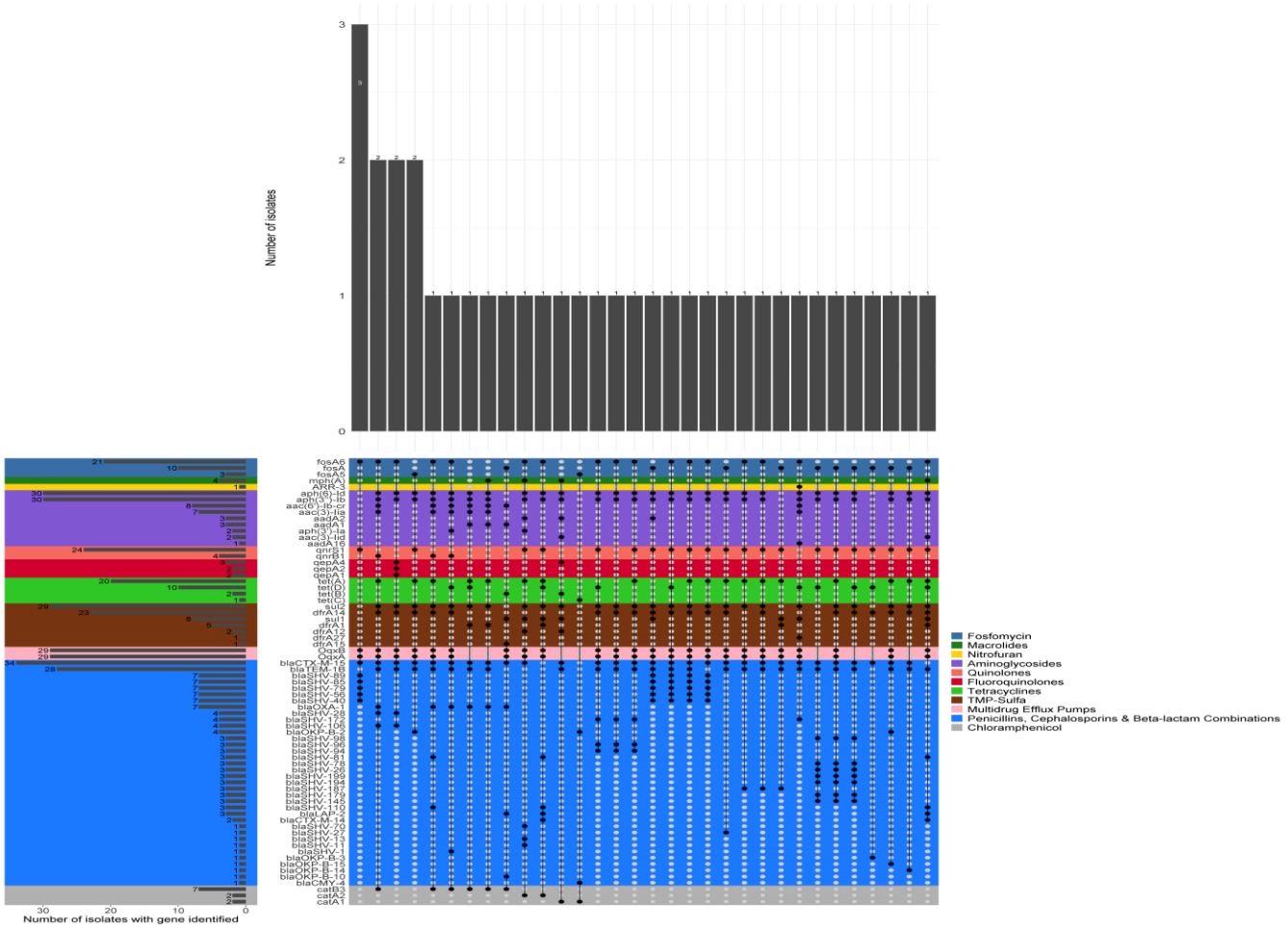

Figure 4: ESBL-KP resistant genes

Virulence genes

A diversity of virulence genes were detected among *E. coli* and *K. pneumoniae* isolates sequenced. The most common virulence genes in *E. coli* strains were *ompT* (51/56), *nlpl* (51/56), *csgA* (48/52), *hlyE* (46/52), *TerC* (46/52), *fdeC* (39/52), *fimH* (36/52), *Alsa* (25/52). Capsular genes *kpsE* (11), *kpsMll_K5* (6), *kpsMll_K4* (1) were detected in *E. coli* strains. *aggR*, *agg3A*, *agg3C*, *agg3D* were detected only in two *E. coli* isolates. As for *K. pneumoniae* isolates, fimbrial genes (*mrkABCDHJ*) and aerobactin siderophore ferric receptor protein encoded gene (*ituA*, 36/37) were widely carried. Cluster genes [(*ybtAEPQSTUX*) and (*irp1*, *irp2*, *fyuA*)] were detected in 14 and 15 *K. pneumoniae* isolates respectively. Aerobactin (*iucABCD*) encoded genes were detected in only two isolates of *K. pneumoniae*.

Plasmid

Plasmid analysis identified the presence of several incompatibility (Inc) and colicinogenic (Col) plasmid types both in *E. coli* and *K. pneumoniae* strains (Table S3). A total of 141 and 115 different plasmids were detected in *E. coli* and *K. pneumoniae* isolates respectively. The IncF group plasmids were most common in both *E. coli* and *Klebsiella pneumoniae* strains with 110 and 87 different plasmids respectively, followed by Col group with 14 and 10 different plasmids harboured respectively. The most common plasmids in *E. coli* isolates were IncFII (25/52), IncFIA (23/52), IncFIB(AP001918) (23/52) while IncFIB(K) (27/37), IncFII (18/37), IncFII(K) (12/37), and IncR (10/37) were predominant in *K. pneumoniae* isolates.

Sequence type

Sequence types (STs) for both *E. coli* and *K. pneumoniae* are presented in table S4. A total of 27 distinct STs were detected among *E. coli* isolates. The most frequently identified STs were ST10, ST710, ST3268, and ST12780, each found in four isolates, while ST354, ST410, and ST6359 were present in three isolates. Notably, the globally disseminated ST131 was identified in a single *E. coli* strain.

For *Klebsiella pneumoniae*, 23 different STs were identified. The most common was ST1741, found in four isolates, followed by ST17 and ST307, each detected in three isolates. Additionally, ST25, ST708, and ST1224 were present in two isolates each. The multidrug-resistance ST37 was detected in one *Klebsiella* isolate.

Discussion

The prevalence of ESBL-E faecal colonisation among healthy community members in rural Burkina Faso in 2021-22 was 61.3% (58.8%-63.7%), almost three times higher than what was recorded ten years earlier in healthy volunteers in Bobo Dioulasso, an urban area of Burkina Faso. This prevalence is also higher than those reported in high income countries as in Europe where community prevalence was ranging from 3.7% in Spain in 2003 to 7.3% in England in 2014 (27–30) and in the United States with a community prevalence estimated at 3.5% in healthy children in 2015 (31). It is even higher than the 18% pooled prevalence reported in healthy community members in other sub-Saharan African countries (32), which ranged from 5% in Gambia in 2015 to 59% in Central African Republic in 2013 (33,34), yet comparable to those reported in Asia, with a pooled prevalence of 46% (35), ranging from 6.4% in Japan in 2010 to 73.9% in China in 2014 (15,36). Almost all identified β-Lactamase genes were plasmid mediated and were highly associated with PMQR genes suggesting location on the same plasmid (37,38) leading to the co-occurrence of this dual resistance widely reported (39–43). CTXM-15 was the most prevalent β-Lactamase gene, which was also the most reported β-Lactamase gene isolated from clinical samples and from healthy volunteers in Burkina Faso in 2014 and 2016 (16,44,45). It was also the most reported β-Lactamase gene in many other sub-Saharan African countries (46–49) and the most frequently identified worldwide (50).

This widespread faecal colonisation of ESBL-EC and ESBL-KP among healthy individuals in the general population represents a significant reservoir of resistant bacteria. While the role of human-to-human transmission risk at household-level has not been extensively quantified, and is particularly unclear for sub Saharan African settings, gut carriage of resistant *E. coli* has been shown to cluster at household- as well as host-level (51). Therefore, our identified high ESBL-E faecal colonisation may be a results of community-level drivers, promoting household-level transmission of these commensals (10,52). Indeed, community-wide antibiotic use in the study area was lower or at most comparable to those reported in other sub-Saharan African countries (53–56), the frequency of healthcare seeking is equally lower (11), suggesting that frequent transmission of resistant bacteria as reflected in the elevated prevalence of faecal colonisation is most likely resulting from community transmission, human to human or involving environment or animals, promoted by the poor WASH conditions or practices. Although specific

STs from environmental samples in Burkina Faso and in sub-Saharan African countries are lacking to further reinforced this assumption, the association we observed between colonisation and hygiene practices supports this (57). Moreover, the increased colonisation rate observed during the rainy season, consistent with findings in other sub-Saharan African settings (58–61), may be due to the high proportion of open-air defecation, leading to wider environmental contamination of water sources, soil, food supplies from faecal material moved by flowing rainwater.

High colonisation rates could also explain observed frequent community-onset invasive infections (5,8,9), evidenced by the presence of some virulence genes like *hlyE*, *fimH*, *ituA* and *fyuA*, conferring high pathogenicity to *Enterobacterales* involved in urinary tract infections and BSI (62–65). Furthermore, the high resistance rate to ciprofloxacin observed, likely reflecting the high prevalence of PMQR genes, suggests a multi-drug resistance pattern which further reduces treatment options (1,66–68).

As no use of carbapenems was reported in this area, the NDM-5 gene found in one resident must have been acquired following a stay in urban areas where are located the highest reference level hospitals and where carbapenemase-producing *Enterobacterales* were found both in clinical and environmental samples (45,69,70). However, the detection of some clones as ST307 and ST410 (71–77), along with plasmids like IncFIB(K) and IncFIA (78,79) associated with carbapenem-resistant and MDR strains transmission, increases the risk of widespread dissemination in case carbapenems are introduced in this setting as a first- or second-line treatment options for infections caused by ESBL-E, without prior caution. Moreover, the 11% of ESBL-EC showing resistance to ceftolozane/tazobactam (MIC≥ 8 mg/L), a last resort antibiotic, is concerning.

Findings hence stress the need for focused interventions to reduce transmission. Such interventions should include community-based hygiene interventions, yet evidence on which specific intervention should be prioritized is scarce (80,81). Interventions might include promoting or facilitating handwashing with soap before meals (82), as our findings suggest plays a role; improving drinking water safety, including collection, transport and storage in households. These community-based hygiene measures to be implemented should be particularly reinforced during the rainy season.

As prevalence of ESBL-E faecal colonisation in human is a good indicator of the burden of antibacterial resistance in a given community (83), efforts should focus on monitoring antibiotic resistance patterns to guide empirical treatment choices and inform public health strategies. Setting up regular surveillance to track AMR trends (including ESBL/carbapenemase) within the community and healthcare facilities could also be helpful on guiding timely interventions to contain spread (84,85).

Further research to identify key sources of AMR transmission and human behavior influencing transmission could provide a more complete picture of the factors contributing to the high ESBL prevalence, necessary for targeted, context-adapted, effective interventions.

Conclusion

We observed a high prevalence of faecal colonisation with ESBL-EC and ESBL-EC with significant associations to hygiene practices and seasonal variation. The increased prevalence among individuals not washing hands with soap and overall, during the rainy season emphasizes the importance of targeted public health interventions focused on improving hygiene and sanitation. The high levels of ciprofloxacin resistance and the presence of *bla*$_{\text{CTX-M-15}}$ and PMQR genes highlight the potential for these bacteria to act as reservoirs for multidrug resistance. The frequent detection of IncF plasmids in association with resistance genes, underscores the role of plasmid-mediated dissemination in the spread of antimicrobial resistance in the community. These findings emphasize the need for population-based surveillance, improved hygiene practices, which should be combined to stringent antibiotic stewardship to, on the one hand, mitigate the spread of ESBL-producing *Enterobacterales* and, on the other hand, prevent expansion of carbapenemase-producing ones in rural communities in low- and middle-income countries.

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4.2 Use of WATCH antibiotics prior to presentation to the hospital in rural Burkina Faso

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Use of WATCH antibiotics prior to presentation to the hospital in rural Burkina Faso

Daniel Valia[1,2,3*], Brecht Ingelbeen[2], Bérenger Kaboré[1], Ibrahima Karama[1], Marjan Peeters[2], Palpouguini Lompo[1], Erika Vlieghe[4], Annelies Post[5], Janneke Cox[6], Quirijn de Mast[5], Annie Robert[3], Marianne A. B. van der Sande[2,7], Hector Rodriguez Villalobos[8], Andre van der Ven[5], Halidou Tinto[1] and Jan Jacobs[2,9]

Abstract

Background: In low- and middle-income countries, the prevalence of antimicrobial resistance (AMR) is increasing. To control AMR, WHO recommends monitoring antibiotic use, in particular Watch antibiotics. These are critically important antibiotics, with restricted use because at risk of becoming ineffective due to increasing AMR. We investigated pre-hospital antibiotic use in rural Burkina Faso.

Methods: During 2016–2017, we collected data from patients aged > 3 months presenting with severe acute fever to the rural hospital of Nanoro Health District, Burkina Faso, including antibiotic use in the two weeks prior to consultation or hospitalization. We analysed reported antibiotic use by applying the WHO Access, Watch, Reserve classification.

Results: Of 920 febrile participants (63.0% ≤ 14 years), pre-hospital antibiotic use was reported by 363 (39.5%). Among these 363, microbiological diagnoses were available for 275 (75.8%) patients, of whom 162 (58.9%) were non-bacterial infections. Use of more than one antibiotic was reported by 58/363 (16.0%) participants. Of 491 self-referred patients who did not previously visit a primary health care center, 131 (26.7%) reported antibiotic use. Of 424 antibiotics reported, 265 (62.5%) were Access and 159 (37.5%) Watch antibiotics. Watch antibiotic use was more frequent among patients > 14 year olds (51.1%) compared to those 0–14 year old (30.7%, $p < 0.001$) and among referrals from the primary health care centers (42.2%) compared to self-referred patients (28.1%, $p = 0.004$). Most frequently reported Watch antibiotics were ceftriaxone (114, 71.7%) and ciprofloxacin (32, 20.1%).

Conclusion: The reported frequent use of Watch group antibiotics among febrile patients prior to presentation to the hospital in rural Burkina Faso highlights the need to develop targeted interventions to improve antibiotic use in community settings as part of strengthening antibiotic stewardship in low- and middle-income countries. This should include facilitating referral, access to qualified prescribers and diagnostic tools in rural primary health care centers.

Trial registration ClinicalTrials.gov identifier: NCT02669823. Registration date was February 1, 2016.

Keywords: Community antibiotic use, Antimicrobial resistance, Burkina Faso, AWaRe

Introduction

The emergence of antimicrobial resistance (AMR) is driven by appropriate and inappropriate use of antimicrobials. Increasing microorganisms' exposure to antibiotics results in selection pressure, while suboptimal

*Correspondence: valiadaniel@yahoo.com
[1] Institut de Recherche en Sciences de La Santé, Direction Régional du Centre-Ouest/Clinical Research Unit of Nanoro, Nanoro, Burkina Faso
Full list of author information is available at the end of the article

Introduction

The emergence of antimicrobial resistance (AMR) is driven by appropriate and inappropriate use of antimicrobials. Increasing microorganisms' exposure to antibiotics results in selection pressure, while suboptimal regimen allows selective survival of resistant microorganisms(1,2). Globally, mortality attributable to AMR infections has been estimated to increase from 700,000 annual deaths in 2014 to 10 million in 2050 (3). The World Health Organization's 2015 Global Action Plan on AMR urges a.o. to strengthen surveillance of AMR and antibiotic use, and to optimize the use of antibiotics (4). A global surveillance network for monitoring concurrently AMR and antibiotic use, the Global Antimicrobial Resistance Surveillance System (GLASS), has since been set up, reporting country-wide AMR prevalence of key human pathogens and antibiotics (5). To monitor antibiotic use, WHO proposed a classification of antibiotics for human use in three groups, "Access", "Watch" and "Reserve", according to their clinical importance, specific recommendations for their appropriate use, and resistance potential (6). Antibiotic sales data during 2000-2015 showed that the human use of so-called Watch antibiotics, critically important antibiotics particularly at risk of AMR emergence, was declining in high-income countries, while importantly increasing in middle income countries for which data were available (7,8)

In sub-Saharan Africa, self-medication with over-the-counter antibiotics from private pharmacies or informal drug stores, without prior prescription by a qualified health worker, is frequent and facilitates uncontrolled antibiotic use (8–11). To optimize antibiotic use, it is crucial to understand the epidemiology of antibiotic use, in the community as well as in the hospital. Furthermore, also in local primary health care centers, antibiotic prescriptions are not always rational (9,12). Peripheral health workers' poor understanding of the correct use of antibiotics and risks of AMR, limited availability and use of diagnostic tools and difficult access to qualified referral healthcare are likely to be fuelling irrational prescribing of antibiotics (13,14). National level antibiotic consumption estimated from wholesale data, as in Burkina Faso, is limited to the official healthcare sector and usually aggregates in- and outpatient use (15). To our knowledge, no studies so far have investigated community-level antibiotic use. The purpose of this study is to understand community-level antibiotic use in febrile patients prior to presentation to the hospital in a rural district of Burkina Faso.

Methods

Study population

From March 23, 2016 to June 30, 2017, the PaluBac study recruited patients aged >3 months presenting to the Nanoro district hospital with acute fever (tympanic temperature ≥ 38.0 ° C) or history of fever in the last 48 hours and ≥1 symptom(s) of respiratory distress, generalized weakness (prostration), impaired consciousness, seizures (one or more episodes), clinical jaundice, signs of shock, or with suspected severe malaria, invasive bacterial infection, or severe viral infection. A total of 920 patients of whom 344 (37.4%) inpatients and 576 (62.6%) outpatients were included. From March 23 to November 8, 2016, only inpatients were recruited; from November 9, 2016 to June 30, 2017 also outpatients were included. PaluBac evaluated the performance of an automated cell counter to quantify malaria parasitaemia (16). Our embedded cross-sectional study recorded patients' pre-hospital antibiotic use.

The study was conducted in the Nanoro Health District in the West-Central region of Burkina Faso, about 90 km from Ouagadougou, the capital city of Burkina Faso. The district has 24 primary health care centers and one district hospital, "Centre Médical avec Antenne Chirurgicale" (CMA). Primary health care centers are meant to be the patients' first point of contact with the healthcare system, during outpatient consultations. There are no medical doctors attached. More complicated cases are referred from the primary health care centers to the district hospital (17). The district hospital has hospitalization units, medical doctors and a clinical microbiology laboratory. In the health district, the most frequently reported diagnosis is malaria (18), in children often (< 3%) associated with a community-acquired invasive bacterial co-infection (19,20). Malaria is endemic with a *high malaria transmission season* from July to November. This period overlaps with the rainy season, which extends from June to October. The *low malaria transmission season* is from December to June.

Antibiotics dispensing in Burkina Faso

In Burkina Faso health districts, antibiotics can be obtained from formal public or private drug stores after prescription by a Medical Doctor or a nurse. Importation and quality of antibiotics (and medicines in general) is under the control of the national pharmaceutical regulatory agency (21). However, some medicines escape

from this control and are available over-the-counter in public places such as markets (22). Also in formal drug stores, antibiotics can be purchased without prior consultation or prescription. In primary health care centers, the only diagnostic tools available to support febrile diseases management are malaria rapid diagnostic tests (RDT). If such tests show a negative result, this often leads to systematic antibiotic prescription (23).

Data collection

At inclusion, study clinicians completed case report forms with medical history, including the use of antibiotics and antimalarials in the two weeks prior to consultation, which we refer to as *pre-hospital antibiotic use*, the date of symptom onset, and whether the patient was referred or not from a primary health care center. *Referral patients* first attended a primary health care center where a nurse decided about the need for higher-level care and referral to the district hospital, based on clinical symptoms. Proper inpatient care is not possible at a primary health care center, the patient can only be observed for a maximum of 48 hours. *Self-referred patients* were those presenting directly at the district hospital without first attending a primary health care center.

Data analysis

Pre-hospital antibiotics were classified (i) according to WHO's Access, Watch, Reserve (AWaRe) classification and (ii) according to products name as stated in the WHO essential medicines list (58). For the latter, we combined ampicillin and amoxicillin use into one category (“ampicillin or amoxicillin”), and zoomed in on the use of the most prevalently prescribed antibiotic in the Watch group in particular. Anti-tuberculosis drugs were excluded from analysis. We report frequencies of antibiotics used by product name and by AWaRe group, of the use of more than one antibiotic, and compared by age (0-14 years vs >14 years), by malaria transmission season, by hospitalisation status after arrival to the hospital (in- vs outpatients) and by referral vs self-referred, using chi-square tests. We stratified the frequencies of pre-hospital antibiotic use by malaria transmission season. Data was analysed with Stata version 14.2 (StataCorp LP, Texas, USA).

Results

Characteristics of the study population

Of 1212 screened febrile patients, 920 (75.9%) patients were included in the study and had complete data available about pre-hospital antibiotic use. Of these 920, 344 (37.4%)were hospitalized upon arrival at the hospital and 576 (62.6%) were treated as outpatients. Children aged 0-14 years represented 580 (63.0%) of patients and 533 (57.9%) patients were male. A total of 428 (46.5%) patients were referrals from primary health care centers, the remaining were self-referred (Table 1).

Table1: characteristics of the study population according to age, gender, pre-hospital antibiotic use, malaria season and care status from March 23, 2016 to June 30, 2017 at the district hospital of Nanoro, Burkina Faso (n=920)

		Number (n)	Percentage (%)
Age (years)	0-14	580	63.0
	>14	340	37.0
Gender	Male	533	57.9
	Female	387	42.1
Pre-hospital antibiotic use	Yes	363	39.5
	No	557	60.5
Referrals from primary health care centers*	Yes	428	46.6
	No	491	53.4
Patients in high malaria season	Yes	377	41.0
	No	543	59.0
Hospitalized upon arrival to the hospital	Yes	344	37.4
	No	576	62.6

*Missing data in 1 patient

Pre-hospital antibiotic use

Pre-hospital antibiotic use was reported by 363/920 (39.5%) patients, of whom 58 (16.0%) used more than one antibiotic. Proportion of use tended to be more prevalent among 0-14 year olds (240, 41.4%) compared to those >14 (123, 36.2%) (p=0.12). Referral patients significantly more frequently reported pre-hospital antibiotic use (231, 54.0%) than self-referred patients (131, 26.7%, p<0.001). Fewer patients (131, 34.7%) reported antibiotic use during the high malaria transmission season than outside (232, 42.7%, p<0.001).

Microbiological diagnoses (malaria microscopy, blood culture, PCR of nasopharyngeal swabs) were available for 275/363 (75.8%) patients who reported pre-hospital antibiotic use. Of these, 113 (41.1%) were bacterial infections; the other diagnoses were malaria (70, 25.5%), viral infections excluding HIV (85, 30.9%), and HIV (7, 2.6%) HIV.

AWaRe distribution of antibiotics

Overall, 424 antibiotics were reported by 363 patients: 265 (62.5%) antibiotics belonged to the Access group, 159 (37.5%) to the Watch group and none to the Reserve group. Watch antibiotics were more frequently reported by >14 year olds (72, 51.1%) than by 0-14 year olds (87, 30.7%, p<0.001) and by referrals (117, 42.2%) compared to self-referred patients (41, 28.1%, p=0.004). There was no

difference in the proportion of Watch antibiotics used between the high and low malaria-transmission season (Table 2).

Table 2: Bivariate risk factors associated with pre-hospital Watch group antibiotic use among patients reporting pre-hospital antibiotic use from March 23, 2016 to June 30, 2017 at the district hospital of Nanoro, Burkina Faso (424 antibiotics used by 363 patients reporting antibiotic use)

		Number of antibiotics reported (n=424)	**Watch group antibiotic use (n=159)**		**Odds Ratio (95%CI)**
			n	%	
Age (Years)	0-14	283	87	30.7	1
	>14	141	72	51.1	**2.35** (1.55-3.56)
Malaria season	High	146	56	38.4	1
	Low	278	103	37.1	0.95 (0.63-1.43)
Care status	Not hospitalized	286	113	39.5	1
	Hospitalized	138	46	33.3	0.77 (0.50-1.17)
Referral status*	Self-referred	146	41	28.1	1
	Referred from primary health care centers	277	117	42.2	**1.87** (1.22-2.89)

* Missing data in 1 patient, n is the number either of all antibiotics reported or Watch group antibiotics reported

Antibiotics reported

Ampicillin or amoxicillin use (Access) was the most reported antibiotic, accounting for 137/424 (32.3%) of all antibiotics reported. The most frequently reported Watch antibiotics were ceftriaxone (114, 26.9% of all antibiotics reported) and ciprofloxacin (32, 7.5% of all antibiotics reported). Among antibiotics reported by referral patients (n = 277 antibiotics), ceftriaxone was recorded 100 times (36.1%) and ciprofloxacin 12 times (4.3%). Among antibiotics reported by self-referred patients (n = 146), ciprofloxacin was recorded 20 times (13.7%) (Table 3).

Table 3: Distribution of pre-hospital antibiotics used for severe febrile illness by WHO AWaRe classification and by referral status from March 23, 2016 to June 30, 2017 at the district hospital of Nanoro, Burkina Faso (424 antibiotics used by 363 patients reporting antibiotic use)

Antibiotics reported	Number of antibiotics	WHO AWaRe classification	Referral status*			
			Referrals		Self-referred	
			n	%	n	%
phenoxymethylpenicillin	3	Access	3	1.1	0	0.0
ampicillin or amoxicillin	137	Access	95	34.3	42	28.8
amoxicillin + clavulanic acid	10	Access	1	0.4	9	6.2
cefadroxil	2	Access	1	0.4	1	0.7
trimethoprim + sulfamethoxazole	45	Access	18	6.5	27	18.5
metronidazole	45	Access	21	7.6	24	16.4
gentamicin	21	Access	21	7.6	0	0.0
thiamphenicol	2	Access	0	0.0	2	1.4
Total Access antibiotics	**265**		**160**	**57.8**	**105**	**71.9**
ceftriaxone	114	Watch	100	36.1	13	8.9
ciprofloxacin	32	Watch	12	4.3	20	13.7
erythromycin	12	Watch	5	1.8	7	4.8
cefixime	1	Watch	0	0.0	1	0.7
Total Watch antibiotics	**159**		**117**	**42.2**	**41**	**28.1**
Total number of antibiotics	**424**		**277**	**100**	**146**	**100**

*For one ceftriaxone course reported, data was missing on referral status. Numbers refer to total numbers of products reported by patients; percentage relates to the total number of antibiotics (n = 277 and n = 146 for referrals and self-referred patients respectively).

Discussion

Nearly 40% of patients presenting with acute fever to a referral hospital in rural Burkina, reported pre-hospital antibiotic use; more than half of patients who were referred by a primary health care centre, and a quarter of self-referred patients. Nearly 60% of febrile patients with pre-hospital antibiotic use for whom a microbiological diagnosis was available did not have a bacterial infection confirmed.

While empirical first- or second-choice (Access) antibiotics were most frequently used, Watch antibiotics were used by 42% of patients referred from a primary health care center. The high use of Watch antibiotics in referred patients is worrisome particular since ceftriaxone (Watch) is not on the Burkina Faso medicine list for use in primary health care centers and is recommended at this level only in

case of a meningitis outbreak (which did not occur during our study period) (24). Moreover, Watch antibiotics accounted for 28% of antibiotics used by self-referred patients, who presumably self-medicated with antibiotics obtained without prescription at private pharmacies or from informal drug sellers. Use of Watch antibiotics was not associated with severity or seasonality as there was no increased risk for patients admitted compared to those treated as outpatients nor during low-malaria transmission season compared to high-malaria transmission season.

High and increasing use of Watch antibiotics has been observed in other low- and middle-income settings (7,25,26). To optimize antibiotic use, it is important to better understand the origins of and reasons why Watch antibiotics are used, both at primary health care center level and without prescription at the community-level. Our study illustrates the need to monitor antibiotic use among official and informal healthcare providers. Nationwide antibiotic use estimated from official sales data found that, in 2015, 75% of antibiotics used in Burkina Faso were Access and 24% Watch group, achieving the WHO target of at least 60% of antibiotics used to be Access antibiotics (15). This use of Watch antibiotics is lower than the proportion of Watch antibiotics reported in the present study. In comparison, the frequency of pre-hospital antibiotic use by febrile patients admitted to the Nanoro district hospital in 2012-2013 was lower (28.2%), and the proportion of Access antibiotics used was higher (45.9% amoxicillin or ampicillin vs 32.3% now and 35.1% trimethoprim + sulfamethoxazole vs 10.6% now) (19), further confirming the need to halt and reverse this high prevalence of Watch antibiotic use.

Despite ampicillin being the treatment of choice for patients with severe infectious diseases at primary health care center level pending referral, it is increasingly ineffective against *Enterobacterales*. Indeed, most (90.5%) of non-Typhi *Salmonella* and 87.5% of *Escherichia coli* were resistant to ampicillin in Nanoro during 2012-2013 (19). This could explain (systematic) use of ceftriaxone when - in the absence of a microbiological diagnosis - bloodstream infection is suspected. Rapid referral to the district hospital, where laboratory testing should be available as recommended in the WHO model list of essential in vitro diagnostics (27) is then indicated. Because cephalosporin use has been associated to the emergence of beta-lactamase-producing pathogens, particular caution must be taken to optimize its use (28). Integrating point-of-care CRP or procalcitonin tests at primary health

care center level, to differentiate between bacterial and non-bacterial causes of fever, could be explored (29).

Some caution interpreting these findings is needed. First, the inclusion of outpatients during the second half of the study period might have resulted in some changes in the study population and pre-hospital antibiotic use. However, we observed no difference in the prevalence of antibiotic use, when comparing the two populations from both recruitment periods. Further, data about pre-hospital antibiotic use were missing for a quarter of screened patients. Also, antibiotic use was collected via a survey capturing use during the two weeks before consultation at the hospital, potentially underestimating actual use. Whenever possible, reported antibiotic use was verified from referral forms, patient medical files (healthcare booklet), and antibiotic packaging or blisters. Dosage and duration of antibiotic used were not available and the final diagnoses at the district hospital may not correspond to those that had triggered prescription or self-medication within the two preceding weeks.

Conclusion

The frequent use of Watch antibiotics in primary health care centers and at community-level in rural Burkina Faso is concerning in a context of increasingly ineffective first-line antibiotics. It points to the need to set up antibiotic use monitoring and antibiotic stewardship in rural communities in low- and middle-income countries (LMIC. As no alternatives for the currently available Watch antibiotics are available nor affordable in many LMIC settings, it is imperative to act to avoid even more alarming levels of resistance in years to come.

List of abbreviation

AMR: Antimicrobial resistance

AWaRe: Access, Watch, Reserve

CMA: Centre Médical avec Antenne Chirurgicale

CRP: C-Reactive Protein

GLASS: Global Antimicrobial Resistance Surveillance System

HIV: Human Immunodeficiency Virus

RDT: Rapid Diagnostic Test

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

The PaluBac study protocol and an amendment to include pre-hospital antibiotic use were approved by the Ethics Committee for Health Research of Burkina Faso (ref 1029/15 and 2017-01-001). Written informed consent was obtained from all included patients or their caretakers.

Consent for publication

All the patients included in the study or their caretakers gave their consents for publication.

Availability of data and materials

All data generated or analysed during this study are included in this published article and datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The Authors declare that they have no competing interests

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Authors' contributions

Conceptualization: DV, BI, MABvdS ,JJ

Data curation: DV

Formal analysis: DV

Investigation: BK, IK, MP, PL, AP, JC

Methodology: DV, BI, MABvdS, JJ

Software: DV

Supervision: HT, MABvdS, JJ

Validation: HT, MABvdS, JJ

Writing – Original Draft Preparation: DV

Writing – Review & Editing: BI, BK, MP, PL, EV, AP, JC, QdM, AR, MABvdS, HRV, AvdV, HT, JJ.

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4.3 Healthcare seeking outside healthcare facilities and antibiotic dispensing patterns in rural Burkina Faso: A mixed methods study

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RESEARCH ARTICLE

Healthcare seeking outside healthcare facilities and antibiotic dispensing patterns in rural Burkina Faso: A mixed methods study

Daniel Valia[1,2,3] | Juste Stéphane Kouanda[1] | Brecht Ingelbeen[2,4] | Karim Derra[1] | Bérenger Kaboré[1] | François Kiemdé[1] | Toussaint Rouamba[1] | Eli Rouamba[1] | Franck Sovi Hien[1] | Linda Campbell[5,6] | Marie Meudec[2] | Annie Robert[3] | Halidou Tinto[1,2] | Marianne A. B. van der Sande[2,4] | Hector Rodriguez Villalobos[7]

[1]Institut de Recherche en Sciences de la Santé, Direction Régionale du Centre-Ouest/Clinical Research Unit of Nanoro, Nanoro, Burkina Faso
[2]Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium
[3]Epidemiology and Biostatistics Unit, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium
[4]Julius Center, University Medical Center Utrecht, Utrecht, The Netherlands
[5]Centre for Population, Family and Health, University of Antwerp, Antwerp, Belgium
[6]Department for Public Health and Primary Care, University of Ghent, Ghent, Belgium
[7]Microbiology Unit, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium

Correspondence
Daniel Valia, Institut de Recherche en Sciences de la Santé, Direction Régionale du Centre-Ouest/Clinical Research Unit of Nanoro, Nanoro, Burkina Faso.
Email: valiadaniel@yahoo.com

Funding information
Belgian Directorate of Development Cooperation; Conseil de l'Action International de l'Université Catholique de Louvain

Abstract
Objective: Optimising antibiotic use is important to limit increasing antibiotic resistance. In rural Burkina Faso, over-the-counter dispensing of antibiotics in community pharmacies and non-licensed medicine retail outlets facilitates self-medication. We investigated its extent, reasons and dispensing patterns.
Methods: In an exploratory mixed-method design conducted between October 2020 and December 2021, this study first explored illness perceptions, the range of healthcare providers in communities, antibiotics knowledge and reasons for seeking healthcare outside healthcare facilities. Second, frequencies of illness and healthcare utilisation in the last 3 months were quantitatively measured.
Results: Participants distinguished between natural and magico-religious illnesses, according to origins. For illnesses considered to be 'natural', healthcare was mainly sought at healthcare facilities, private pharmacies and informal drug outlets. For illnesses considered as magico-religious, traditional healers were mainly visited. Antibiotics were perceived in the community as medicines similar to painkillers. Healthcare-seeking outside healthcare facilities was reported by 660/1973 (33.5%) participants reporting symptoms, including 315 (47.7%) to informal vendors. Healthcare seeking outside facilities was less common for 0–4-year-olds (58/534, 10.9% vs. 379/850, 44.1% for ≥5-year-olds) and decreased with improving socio-economic status (108/237, 45.6% in the lowest quintile; 96/418, 23.0% in the highest). Reported reasons included financial limitation, and also proximity to informal drug vendors, long waiting times at healthcare facilities, and health professionals' non-empathetic attitudes towards their patients.
Conclusion: This study highlights the need to facilitate and promote access to healthcare facilities through universal health insurance and patient-centred care including reducing patients' waiting time. Furthermore, community-level antibiotic stewardship programmes should include community pharmacies and informal vendors.

KEYWORDS
antibiotic use, antimicrobial resistance, Burkina Faso, community setting, cross-sectional studies, healthcare utilisation, informal drug outlets, mixed methods, sub-Saharan Africa

Sustainable Development Goal: Good Health and Wellbeing

Introduction

Inappropriate use of antimicrobials speeds up the pace of antimicrobial resistance (AMR) (1). Unlike in high-income countries where AMR burden is largely attributed to healthcare facilities, in low-and middle-income countries (LMICs), AMR is mainly associated with community-acquired infections (2–4). While prescriptions in healthcare facilities are not always appropriate, inappropriate use is also facilitated by unrestricted access to a wide range of antibiotics in community pharmacies where a prior prescription is not needed, and the plethora of non-licensed drug retail outlets in communities, promoting uncontrolled antibiotic dispensing (5–8). Point-of-care tests, which differentiate bacterial from non-bacterial infections in healthcare facilities that do not have microbiology laboratories, has been valuable in reducing unnecessary antibiotic prescriptions: nevertheless over-the counter dispensing escapes such interventions (9). Therefore, it is crucial to understand the reasons for seeking healthcare outside healthcare facilities as well as identify symptoms that trigger antibiotic dispensing in such settings: the already high AMR burden will continue to grow without comprehensive action to optimise antibiotic use (10).

Despite legislation to prohibit medicine dispensing from non-licensed drug retail outlets (11–13) in Burkina Faso, they continue to coexist next to healthcare facilities and community pharmacies. Over-the-counter dispensing of antibiotics in community pharmacies, without prior prescription, also largely escapes any official control (13–15).

Previous studies evaluating antibiotic access and use outside facilities in LMICs concluded that long waiting times in healthcare facilities, easy and unrestricted accessibility of drug retail outlets in communities, and a lower cost per treatment course dispensed were the main reasons for accessing antibiotics without prior formal consultation (16–18). While largely overlooked in antimicrobial stewardship, antimicrobial dispensing and use outside healthcare facilities may contribute towards maintaining a high AMR burden (19–21). Studying the reasons underlying healthcare seeking outside healthcare facilities is crucial to develop context-specific interventions and to inform policy change. The purpose of this study was to assess the prevalence and the factors influencing healthcare seeking outside healthcare facilities as well as understand symptoms that trigger antibiotics dispensing without prescription in a rural district of Burkina Faso.

Methods

Study area

This study was conducted in the Nanoro health district (Figure 1), which is located in central-west Burkina Faso, about 90 kilometres from Ouagadougou. The Nanoro health district includes 24 primary healthcare centers and 4 medical centers. Each primary healthcare center or medical center covers a health area, which is made up of villages .The data were collected in two health areas of the district; Nanoro health area which includes five villages (Nanoro, Poessi, Gouroumbila, Basziri and Goulouré) and covers a population of 17300 inhabitants and Nazoanga health area which includes three villages (Nazoanga, Zimidin and Sitaon) and covers a population of 8800 inhabitants. In Nanoro health area, a medical center is the patients' first point of contact with the healthcare system; in Nazoanga health area, it is a primary healthcare center (PHC). PHCs have no medical doctors. Malaria rapid diagnostic tests are available to support febrile disease diagnoses. More complicated cases should be referred from the PHC or the medical center to the district hospital. Both health areas are embedded in the Clinical Research Unit of Nanoro (CRUN) Health and Demographic Surveillance System (HDSS). The HDSS covers 24 villages out of 57 in the area in total. Four-monthly household visits are planned by the HDSS to update individual participants' and households' data (22).

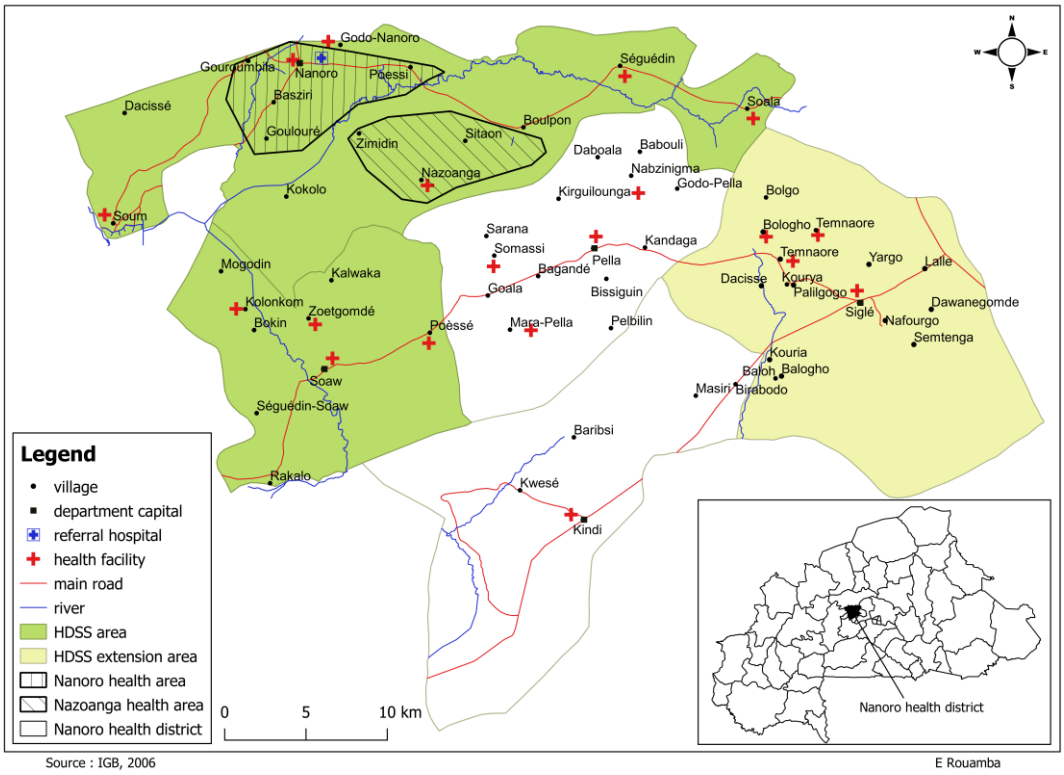

Figure 1: Nanoro health district showing the Nanoro and the Nazoanga health areas embedded in the HDSS area

In the Nanoro health district, the most frequently reported diagnosis is malaria (23), which in children is sometimes (< 3%) associated with a community-acquired invasive bacterial co-infection (24,25). Malaria is endemic with a *high malaria transmission season* from July to November. This period overlaps with the rainy season, which extends from June to October. The *low malaria transmission season* is from December to June.

Study design

We conducted an exploratory mixed-method design from October 2020 to December 2021. Data was collected in three distinct stages. The results of each stage fed into the design and development of subsequent stages. First, using focus group discussions (FGD), in-depth interviews (IDIs) and informal conversations (ICs), we explored illnesses perceptions, range of healthcare providers in the community, antibiotics knowledge and reasons to seek for healthcare outside healthcare facilities. Based on the results of the initial qualitative study, we refined a quantitative questionnaire to survey household members in the same community

on episodes of illness and healthcare utilization within the last three months. Subsequently, with the private drug store vendors and informal drug vendors, we conducted informal interviews to capture how they generally manage symptoms that households reported during the survey, with a special focus on antibiotic dispensing.

Data collection

Illness perception, range of healthcare providers, and reasons for the choice of provider

Five FGDs were conducted, four with community members and one with community healthcare workers. All community members were farmers. In the Nanoro health area, three FGDs were conducted, two with community members (a group of twelve women aged 30 to 40 years old and a group of twelve men aged 35 to 55) and one with community healthcare workers (a group of eight community healthcare workers aged 25 to 35 years old). In the Nazoanga health area, two FGDs were conducted with community members (a group of twelve women aged 20 to 35 years old and a group of twelve men aged 20 to 35 years old). Participants were selected purposively on their ability to provide in-depth and pertinent information regarding our research question, based on the principles of purposive sampling for qualitative data collection (26). All community informants from each village from both health areas were involved in this selection. Data was analyzed as the fieldwork progressed: in an iterative cyclic process, the topic guide for subsequent FGDs was adjusted based on what had emerged from the previous sessions. Subsequently, using snowball sampling, interview participants were selected. Six IDIs were conducted, four in Nanoro health area (with a nurse, a midwife, and both the private drug store vendor and the public drug store vendor located in the Nanoro's medical center) and two in Nazoanga health area (with a midwife and the public drug store vendor in Nazoanga's PHC). Four informal conversations were conducted, with two informal drug vendors in the Nanoro health area and two others in the Nazoanga health area. Interviews with informal drug vendors were an opportunity for direct observations of available medicines at their drug outlets. The findings from the different methods permitted data triangulation. The process of participant selection was ended after theoretical saturation was reached. FGDs, IDIs and informal conversation sessions were conducted in French, the official language in Burkina Faso and in Mooré, the most

spoken local language in the research area. Recording and facilitation were undertaken for all sessions by the CRUN anthropologists after verbal informed consent was obtained from the participants and recorded by the interviewers.

Household healthcare utilization

For the cross-sectional household survey recording healthcare utilization, we selected a random sample of 802 and 780 households respectively in Nanoro and Nazoanga health areas, using each health area households' list (available in the HDSS database) as sample frame. The sample size (number of households) per health area was calculated assuming a proportion of the population having sought healthcare of 0.2, a design effect (adjusting for intra household clustering) of 2, an average household size of 7 (based on the Nanoro HDSS data), 95% confidence intervals, confidence limits of +/- 4%. In an electronic questionnaire, all members of each selected household were asked about symptoms during the three months prior the survey, the frequency and the type of healthcare provider visited. Data collection in both health areas was combined with the most recent HDSS routine data during which ownership of assets (television/video/radio/telephone/mobile devices, computers, laptops, cars, motorcycles, bicycles, refrigerator) was recorded and used to rank the socio-economic level of each household (22,27).

Symptoms management at informal drug outlets and private drug stores

Five informal conversations were conducted, two with informal drug vendors in both health areas and one with a vendor in the Nanoro private drug store to know how they usually manage patient symptoms as detailed in the household healthcare utilization survey. We specifically explored how they dispense Watch group antibiotics if seen at their outlets during direct observations. Participants were purposively selected on their ability to provide a detailed information based on data collected during first stage

Data analysis

Qualitative data

Data were analysed using a thematic analytical approach. The audio recordings of the interviews were transcribed into English. Transcripts were reviewed to match audio records before being imported into NVivo software, version 12. After familiarisation with the data, codes were deductively and inductively generated

according to the research question being addressed as well as new codes emerging from collected data. Codes were grouped into broader categories and themes were named; first, on the perceptions of illness and the range of healthcare providers in the community for their management; second, the reasons for seeking healthcare outside healthcare facilities; third, on perceptions and knowledge regarding antibiotics; and fourth, on the management of acute illnesses at informal drug outlets and private drug stores (after the household utilization survey). Triangulation between quantitative and qualitative data allowed for the examination of complementing and contrasting data.

Quantitative data

We reported frequencies of the type of healthcare provider visited in the last three months prior to the survey. Combinations of symptoms during the three months preceding the survey were grouped into five combinations of symptoms, (i) fever only, (ii) gastroenteritis, indicated by diarrhoea and/or vomiting with or without fever, (iii) respiratory tract symptoms, indicated by cough and/or rhinorrhoea with or without fever, (iv) urinary tract symptoms with or without fever and (v) sore throat and ear pain and/or discharge with or without fever. We then reported frequencies for each combination of symptoms.

Ownership of assets recorded during the routine HDSS round of which the survey was combined, were used to rank households in quintiles at the corresponding socio-economic level according to the steps outlined to construct the new Demographic and Health Surveys (DHS) wealth index (27).

We used chi square tests to compare the frequency of healthcare utilisation between age groups, health area (Nanoro vs Nazoanga), gender (female vs male), symptom category, socioeconomic level quintiles. Variables from the Univariate analysis were included in a multivariate analysis to control for potential confounding factors if $p<=0.2$. Likelihood-ratio test (LRT) p-values were reported and significance was set at 5%.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee for Health Research of Burkina Faso (ref n° 2020-8-171). For the quantitative study, written informed consent was obtained from households heads and each household member aged

at least 18 years old. For household members aged between 14 and less than 18 years, oral assent was obtained in addition to household heads' written informed consent. For household members less than 14 years old, written informed consent was obtained from parents or caretakers. For the qualitative study, verbal informed consent was obtained from all participants. This verbal consent was documented in a form signed by the researchers, certifying that verbal inform consent was obtained from each participant.

Both qualitative and quantitative data were anonymized to preserve study participants' confidentiality

Results

Household healthcare utilization

In 1582 households included in the survey (802 in Nanoro and 780 in Nazoanga) with median household size 6 (IQI 4-9), symptoms and healthcare visits of 10693 participants were recorded. Acute symptoms were reported by 1973 (18.5%) participants. Of these, 907 (46.0%) were men, 535 (27.1%) were 0-4 years old and 237 (12.0%) were from the lowest socio-economic quintile. Main symptoms reported were fever only (1063, 53.9%), followed by respiratory tract symptoms (669, 33.9%). Healthcare seeking outside healthcare facilities was reported by 660 (33.5%) participants **(table 1)**.

Table1: Characteristics of household members who reported acute symptoms

	Health area			
	Nanoro (n=1132)		**Nazoanga** (n=841)	
	n	**%**	**n**	**%**
Gender				
Female	616	54.4	450	53.5
Male	516	45.6	391	46.5
Age (years)				
0-4	256	22.6	279	33.2
5-17	399	35.3	273	32.5
18-49	293	25.9	150	17.8
≥50	184	16.3	139	16.5
Socioeconomic level*				
Highest quintile	339	30.0	79	9.4
Fourth	244	21.6	181	21.6
Third	254	22.5	259	30.8
Second	183	16.2	194	23.1
Lowest	110	9.7	127	15.1
Self-reported acute symptoms				
Fever only	538	47.5	525	62.4
Respiratory tract symptoms	475	42.0	194	23.1
Gastroenteritis	97	8.6	112	13.3
Urinary tract symptoms	15	1.3	4	0.5
Sore throat and ear pain	7	0.6	6	0.7
Place of healthcare sought*				
Formal HC	728	64.5	581	69.1
Outside formal HC	400	35.5	260	30.9

*Socioeconomic level data were missing for 2 study participants in Nanoro health area and for 1 study participant in Nazoanga health area. *Place of healthcare sought data were missing for 4 study participants in Nanoro health area

Among the 660/1969 (33.5%) of participants who reported visiting healthcare providers outside healthcare facilities, 315 (47.7%) reported having visited informal drug vendors, 175 (26.5%) self-medicated with left-over medicines kept at home, 89 (13.5%) visited formal private drug stores without prior consultation, 62 (9.4%) visited traditional healers and 19 (2.9%) sought healthcare in public drug stores without prior medical consultation.

Over a quarter (534/1969) of reported healthcare episodes were in 0-4 year olds, which had the lowest proportion of visits outside facilities (58/534, 10.9%), compared to 42.0% (602/1435, p<0.001) of visits among other age groups **(table 2)**. Respiratory tract symptoms led more frequently to visits outside healthcare facilities (273, 40.9%) than other combinations of symptoms (387, 29.7%, p=0.027). Participants from the lowest socioeconomic level (108, 45.6%) more

frequently sought healthcare outside healthcare facilities than those from the highest level (96, 23.0%, p<0.001).

Table 2: Univariate and multivariable analysis of factors associated with looking healthcare outside healthcare facilities

		Reported healthcare sought outside healthcare facilities			
	n	%	OR (95% CI)	LRT p-Value	aOR (95% CI)	LRT p-Value
Health area				0.035		0.12
Nanoro	1128	35.5	1		1	
Nazoanga	841	30.9	0.81 (0.67-0.99)		0.84 (0.68-1.05)	
Gender				0.63		-
Female	1065	34.0	1		-	
Male	904	33.0	0.95 (0.79-1.15)		-	
Age (years)				<0.001		<0.001
0-4	534	10.9	1		1	
≥5	1435	42.0	5.93 (4.43-7.94)		6.01 (4.46-8.10)	
Socioeconomic level				<0.001		<0.001
Highest quintile	418	23.0	1		1	
Fourth quintile	424	32.1	1.58 (1.17-2.15)		1.68 (1.22-2.31)	
Third quintile	511	36.0	1.89 (1.41-2.52)		2.28 (1.67-3.11)	
Second quintile	376	35.9	1.88 (1.38-2.56)		2.17 (1.56-3.03)	
Lowest quintile	237	45.6	2.81 (1.99-3.96)		3.34 (2.30-4.85)	
Fever only				0.13		0.16
No	908	35.2	1		1	
Yes	1061	32.0	0.87 (0.72-1.01)		1.81 (0.79-4.17)	
Respiratory tract symptoms				<0.001		0.003
No	1302	29.7	1		1	
Yes	667	40.9	1.64 (1.35-1.99)		2.57 (1.11-5.94)	
Gastroenteritis				<0.001		0.86
No	1760	35.3	1		1	
Yes	209	18.7	0.42 (0.29-0.60)		1.09 (0.44-2.67)	

aOR, adjusted odds ratio for health area, age, socioeconomic level, fever only, respiratory tract symptoms and gastroenteritis

Illness perception and the range of healthcare providers in the community for their management

It emerged that communities were facing illnesses of diverse origins: illnesses were divided into two categories by participants; both logical and magico-religious diseases depending on whether their origins were perceived to be natural or unnatural (witches, genies). Certain disorders - like mental disorders or any other illness of an origin considered to be unnatural by the community - were considered “magico-religious”. Healthcare was therefore generally sought from traditional healers or in churches:

"*Sometimes, illnesses are due to evil spirits or witches, or you can simply be sick without understanding why and according to your faith, you can seek healthcare from the traditional healers or in churches*" (FGD, women, 30-40 years, Poessi)

"*Some are doubtful about the natural cause of their disease and instead of going to the healthcare facility, they will rather go to the traditional healers because they think that their diseases are of mystical origin*" (IDI, public drug store vendor, Nazoanga)

For illnesses considered "natural" by communities such as malaria, urination burn, cough, common cold, diarrhea, stomachache, treatment was generally sought at healthcare facilities, at informal drug outlets and at private drug stores:

"*For these illnesses, we generally seek for healthcare in healthcare facilities or directly from informal drug vendors*" (FGD, women 20-35 years, Sitaon).

Illnesses might be perceived as natural at first but reconsidered to be magico-religious if symptoms were not resolved by formal healthcare:

"*Sometimes, some patients come here to seek for healthcare, we treat them but without health condition improvement. They therefore prefer to go to churches or to traditional healers, rather than coming back here*" (IDI, responsible nurse, Nanoro).

"*You need to know that there are some illnesses that cannot be treated in formal health centers such as some stomach aches,* ***persistent illnesses after many treatments***" (FGD, women 20-35 years, Sitaon)

Traditional medicine might also be regarded as complimentary to formal healthcare:

"*Most of the time, we go to consultation in formal health centers, but, between two consultations, we can also seek for healthcare to the traditional healers*" (FGD, men 20-35 years, Nazoanga).

Knowledge and perceptions regarding antibiotics

In the community antibiotics were usually perceived as medicines that are similar in nature to painkillers. Even when discussing antibiotics by name, participants continued to confuse their function with pain medication:

"*Well, I will try! Antibiotics are medicines to fight against pain when wounded*" (FGD, women 30-40 years, Poessi).

"*Amoxicillin can be used for joints pain, as well, when you get wounded you can take it orally as medication*" (FGD, men 20-35 years, Nazoanga).

For community healthcare workers, antibiotics were considered to be wound treatments and stated that they could also be used to protect the body from disease:

"*Antibiotic is used for wounds treatment and can be used to reinforce the body in order to prevent it from diseases*" (FGD, community health workers, Nanoro, 25-35 years).

Among informal vendors, knowledge on antibiotic use and function was very limited. They generally based their vague knowledge of the function of antibiotics on pictures on the packaging even though they could not tell whether medicines were antibiotics or not:

" *Do you see the picture on this package?... The manufacturer is clever, as the picture shows, you can see that medicine is for headache. It is how we choose which medicine to dispense according to our patients complaints*" (Informal conversation, informal drug vendor, Nazoanga).

For formal private drug store vendors, antibiotics could be used to kill microbes and treat infection diseases:

"*An antibiotic is a substance use to kill microbes. In other words, I can say that it is a substance used to treat infectious diseases*" (IDI, private drug store vendor, Nanoro)

Only a formal healthcare worker (nurse) was able to give an answer including antibiotics' ability to combat bacterial infections:

"*Antibiotic is used against pathogens, we can talk about bacteria, pneumococcus, meningococcus, typhoid fever germs*" (IDI, nurse, Nazoanga).

Reasons to seek healthcare outside healthcare facilities

When asking the reasons for seeking healthcare outside healthcare facilities, interviewees raised many factors of which, financial limitations were prominent:

"*It is always the lack of money that makes us look for healthcare to the informal drug vendors in markets, and all categories of people, young and old go there*" (FGD, women 20-35 years, Sitaon).

"*In the market, medicine prices can be negotiated and we can buy them for only 100 or 150 CFA (0.15 or 0.23 euro). In addition, from informal drug vendors, payment can be delayed, meaning that one can have the medicine when he needs it and come back to pay later when he gets money, which is not possible in formal drug stores*" (FGD, men 35-55 years, Nanoro).

"*Some customers take the medicines here without paying immediately, they come back later to pay when they get money, which is not possible in healthcare facilities. There, they will give you a prescription*" (informal interview with an informal drug vendor in Nanoro).

"*It must be recognized that some are afraid to go to the public drug stores at the risk of being refused to be given medicines without prior consultation which is additional cost* " (informal interview with an informal drug vendor in Nazoanga).

"*Some patients find our medicines of high cost and cannot afford them*" (IDI, formal public drug store vendor).

Geographical access limitations were also underlined. Informal drug vendors lived in the community, and were therefore closer to people and easier to reach than healthcare facilities. Furthermore, in the rainy season healthcare facilities were frequently inaccessible:

"*During the rainy season, it is difficult to reach the healthcare facility because the bridge to get there is flooded, so informal drug vendors are mostly solicited*" (FGD, women 20-35 years, Sitaon);

"S*ometimes it is the remoteness of healthcare facilities that lead people to the informal drug vendors*" (FGD, men 20-35 years, Nazoanga).

Health system factors were also raised: long waiting times and unprofessional attitudes of formal healthcare workers towards patients were remarked upon:

"*Sometimes, you get to the healthcare facility and there is no healthcare worker to listen to you and readily take care of you and that leads people to informal drug vendors*" (FGD, men 20-35 years, Nazoanga).

It also emerged that medicines from markets were used as first-line treatment before looking to formal healthcare if symptoms still persist:

"W*e treat our children having diarrhea with medicine from the market before we look for formal healthcare, because healthcare workers will not immediately take care of them*" (FGD, women 30-40 years, Poessi).

Finally, medicines sold in the market were considered to be stronger than those in formal drug stores, as illustrated in the following quotes:

"*I remember when I was sick and under observation in the primary healthcare center, the nurse was about to refer me to the Nanoro district hospital. I just ordered a child to buy me a medicine from the market; when I took it my health condition improved and I was not referred anymore*" (FGD, men 20-35 years, Nazoanga)

"*Our medicines quickly heal them*" (informal interview with an informal drug vendor in Nanoro).

Symptoms management at informal drug outlets and the private drug store

Fever without any additional symptoms was managed differently in the private drug store as compared to informal drug outlets. While these patients were referred to the healthcare facility by the private drug store vendor, all informal drug vendors reported treating them as malaria cases with paracetamol and an anti-malaria monotherapy (amodiaquine hydrochloride) or an artemisinin-based combination therapy (artemether-lumefantrine). Respiratory tract symptoms were managed with oral route amoxicillin by the private drug store vendor while informal drug vendors reported treating them with drugs only available in their outlets. When reading the components, the latter did not contain antibiotics. But both informal drug vendors and the private drug store vendor reported dispensing amoxicillin (and oral route ampicillin, only available at informal drug outlets) for treatment of wounds. Gastroenteritis was managed with metronidazole by both the private drug store vendor and the informal drugs vendors regardless of the type of diarrhoea. Urinary tract symptoms were referred to the healthcare facility by the private drug store vendor while informal drug vendors reported treating all illnesses that manifest by burns, meaning urination burns, stomach burns, with ciprofloxacin and erythromycin. For them both antibiotics played the same role and were only

different in colour. When asking the private drug store vendor how he dispensed ciprofloxacin, erythromycin and azithromycin, he reported dispensing ciprofloxacin for typhoid fever or stomach burns and erythromycin for dermatosis like pimples all other the body. He declared not selling azithromycin without medical prescription because he did not know this medicine very well.

Regarding dosage and length of treatment, informal drug vendors stated that ciprofloxacin and erythromycin dosed at 500 milligrams should be given as half a tablet twice a day for adults and a quarter of a tablet twice a day for children; they furthermore stated that the course of antibiotic should be stopped when symptoms ended. The private drug store vendor reported that he mainly sold these medicines to adults at a dosage of one tablet of 500 milligrams twice a day for seven days, regardless duration of symptom.

Discussion

This article aimed to examine factors influencing healthcare seeking outside healthcare facilities and antibiotic dispensing patterns in a rural district of Burkina Faso. Nearly 34% of people who went through acute illnesses in the last three months prior to the survey reported that they had sought healthcare outside of healthcare facilities and almost 50% among them went to the informal drug vendors. Low income and high costs related to formal healthcare seeking as well as lack of patient-centered care and difficult geographical access were mentioned as reasons for seeking healthcare outside healthcare facilities. People from the highest socio-economic quintile and with children aged 0-4 years old were more likely to attend official healthcare facilities when seeking healthcare.

Communities' high demand of care from informal vendors is worrisome. Indeed, the latter's poor antibiotics knowledge - such as questionable antibiotics indications - may lead to excessive and inappropriate dispensing. Additionally, ciprofloxacin and erythromycin dispensing even for non-infectious diseases at poor dosage and at treatment length according to symptoms duration is striking and may be fuelling the growing resistance level to these high resistance potential antibiotics (20,28,29). The high financial incentive yield might be a reason for not looking for alternative activities even though informal drug selling is prohibited by the authorities. Additionally, their proximity to the population, the ease of payment methods, meaning possibility of delaying payment and only buying the number of tablets one

can afford, make them the healthcare providers of choice in the community. Sometimes, they are even the first contact with patients before they look for formal healthcare. The availability of Access and Watch antibiotics in these informal stores, dispensed over-the-counter without any rule, suggest that interventions to optimise antibiotics use should not only target healthcare facilities, but all antibiotics providers including formal and informal drug stores. Developing and implementing a context-specific behavioural intervention may help optimise antibiotic dispensing. Such interventions should involve a component targeting community as well, in order to raise health literacy and reduce unnecessary antibiotics demand and overuse.

In Burkina Faso healthcare is free at healthcare facilities for children aged 0-4 years old but out-of-pocket payment prevails for those above this age group. As healthcare-seeking outside healthcare facilities and out-of-pocket payments have been found to be associated with uncontrolled antibiotic dispensing in other low- and middle-income countries (5,30,31), reflecting on how to set up universal health insurance could be crucial. In addition, promoting patient-centered care In healthcare facilities is vital, including reducing patients' waiting times when possible, empathic attitude of healthcare workers towards patients, taking patient's needs into account to the extent that they are rational and sensitization about the dangers of self-medication both at individual and at community-level, may help reduce healthcare seeking outside healthcare facilities (32–35). However, while promoting healthcare seeking at healthcare facilities, febrile illnesses management should be improved and unnecessary antibiotics prescription reduced at this level, e.g. by using C-reactive protein (CRP) or procalcitonin tests to differentiate bacterial from non-bacterial infection where microbiology labs are lacking (9,36,37).

Our findings largely align with findings from other studies in Sub-Saharan Africa (38–40), and will be used to tailor community-based antibiotic stewardship interventions to tackle uncontrolled and likely inappropriate dispensing and (over) use. We found a strong association between financial limitations and healthcare seeking outside healthcare facilities sustained by both qualitative and quantitative data. Of note, the few study participants who sought healthcare outside healthcare facilities by going to traditional healers were not further investigated. Indeed, based on what emerged from qualitative data, such patients when presenting likely "natural" illnesses might have unsuccessfully tried other options before going to the traditional healers, rather than being triggered by financial reasons. Likewise, due

to low levels of knowledge of antibiotics in the community reported in the first part of this study, it was difficult to measure the amount and type of antibiotics used the last three months prior to the household survey. Although the third part of this study reported how healthcare providers outside healthcare facilities managed symptoms presented in the community, exit interviews from each provider will be more suitable to measure in real time the quantity of antibiotics per provider type, prescription dosages, treatment length and symptoms or illnesses for which each type of antibiotic is dispensed. This will better inform antibiotic dispensing inappropriateness per provider type and therefore highlight where to stress interventions to reduce antibiotics (over) prescription.

Conclusion

Frequent healthcare seeking outside healthcare facilities and antibiotics dispensing with unclear indications, dosage and duration, as well as community members' low levels of knowledge around antibiotics are concerning in the context of increasing antimicrobial resistance. This suggests that while facilitating formal healthcare access by universal health insurance and patient-centered care, interventions to reduce the uncontrolled or inappropriate dispensing and (over) use of antibiotics should target all antibiotic providers (formal and informal) as well as the entire community.

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4.4 Antibiotic use by clinical presentation across all healthcare providers in rural Burkina Faso: a healthcare visit exit survey

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Antibiotic use by clinical presentation across all healthcare providers in rural Burkina Faso: a healthcare visit exit survey

Daniel Valia [1,2,3]*, Brecht Ingelbeen[2,4], Guétawendé Job Wilfried Nassa[1], Bérenger Kaboré[1], François Kiemdé[1], Toussaint Rouamba[1], Adélaïde Compaoré[1], Juste Stéphane Kouanda[1], Annie Robert[3], Hector Rodriguez-Villalobos [5], Marianne A. B. Van Der Sande[2,4] and Halidou Tinto[1,2]

[1]*Institut de Recherche en Sciences de la Santé, Direction Régionale du Centre-Ouest/Clinical Research Unit of Nanoro, Nanoro, Burkina Faso;* [2]*Department of Public Health, Institute of Tropical Medicine (ITM), Antwerp, Belgium;* [3]*Epidemiology and Biostatistics Unit, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium;* [4]*Julius Center, University Medical Center Utrecht, Utrecht, The Netherlands;* [5]*Microbiology unit, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium*

*Corresponding author. E-mail: valiadaniel@yahoo.com

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Background: To guide antibiotic stewardship interventions, understanding for what indications antibiotics are used is essential.

Methods: In rural Burkina Faso, we measured antibiotic dispensing across all healthcare providers. From October 2021 to February 2022, we surveyed patients in Nanoro district, Burkina Faso, following visits to health centres (3), pharmacies (2), informal medicine vendors (5) and inpatients in health centres. We estimated prevalence of antibiotic use and the proportion of Watch group antibiotics by provider type and by clinical presentation, assessing compliance with WHO's AWaRe Antibiotic Book. We estimated per capita antibiotic use by multiplying prevalence of antibiotic use, mean DDD per adult treatment course, and the rate of healthcare visits per 1000 inhabitants per day, estimated from a prior household survey.

Results: Outpatient antibiotic use was more frequent after health centre visits (54.8%, of which 16.5% Watch, $n=1249$) than after visits to pharmacies (26.2%, 16.3% Watch, $n=328$) and informal medicine vendors (26.9%, 50.0% Watch, $n=349$). The frequency of antibiotic use was highest for bronchitis (79.9% antibiotic use, of which 12.6% Watch), malaria (31.9%, 23.1% Watch), gastroenteritis (76.0%, 31.7% Watch), rhinopharyngitis (40.4%, 8.3% Watch) and undifferentiated fever (77.0%, 44.8% Watch). Compliance with WHO AWaRe guidance could have averted at least 68.4% of all Watch antibiotic use in outpatients at health centres. Community-wide, 2.9 DDD (95% CI 1.9–3.9) were used per 1000 adult inhabitants per day.

Conclusions: Most Watch antibiotic use at community level or primary care deviated from WHO guidance. Antibiotic stewardship should focus on key clinical presentations and include primary care and self-medication.

Introduction

In sub-Saharan Africa, increasing antimicrobial resistance (AMR) threatens effective treatment with accessible antibiotics. Over the last 10 years, Enterobacterales isolates from patients have had high and increasing resistance to fluoroquinolones and third-generation cephalosporins.[1–3] To combat AMR, optimizing antibiotic use is one of the five objectives in the Global Action Plan on Antimicrobial Resistance.[4] In many low- and middle-income countries (LMICs), the presence of informal medicine stores (non-licensed), as well as over-the-counter dispensing, i.e. without prescription, in private community pharmacies, are facilitating self-medication with antibiotics.[5–8] To effectively guide and target interventions to optimize antibiotic use, it is essential to know where, to whom and for what antibiotics are used at community level.[9]

In Burkina Faso, primary health centres should be patients' first contact when seeking healthcare. Nevertheless, in a prior

Introduction

In sub-Saharan Africa, increasing antimicrobial resistance (AMR) threatens effective treatment with accessible antibiotics. Over the last ten years, *Enterobacterales* isolates from patients had high and increasing resistance to fluoroquinolones and third generation cephalosporins[1–3]. To combat AMR, optimizing antibiotic use is one of the five objectives in the Global Action Plan on Antimicrobial Resistance[4]. In many LMICs, the presence of informal medicine stores (non-licensed) as well as over-the-counter dispensing, i.e. without prescription, in private community pharmacies, are facilitating self-medication with antibiotics[5–8]. To effectively guide and target interventions to optimize antibiotic use, it is essential to know where, to whom and what for antibiotics are used at community-level[9].

In Burkina Faso, primary health centers should be patients' first contact when seeking healthcare. Nevertheless, in a prior study, 27% of patients admitted to hospital had used antibiotics without prior healthcentre visit[11]. In health centers, malaria rapid diagnostic tests (mRDT) are the only available tool to guide febrile illness diagnoses, resulting in frequent unnecessary antibiotic use when the test is negative[10]. Interventions to improve antibiotic use should target all community-level healthcare or medicine providers substantially contributing to community-wide antibiotic use. Knowledge of indications that trigger antibiotic use, antibiotic dose, or treatment duration, could guide antibiotic stewardship intervention options.

Objectives

The objective of this study was to quantify antibiotic use by type of healthcare or medicine provider, including prevalence and quantity of antibiotic use, dose, duration, clinical presentations instigating antibiotic use, and providers' compliance with the WHO Access, Watch, Reserve (AWaRe) Antibiotic Book[12], in a rural district of Burkina Faso.

Methods

Study area

In Burkina Faso, primary health centers (PHCs) are supposedly patients' first point of contact with the healthcare system. Generally, nurses diagnose and prescribe treatment, yet in a so-called medical centre, also a medical doctor is present but

most consultations are done by nurses. In PHCs (including medical centres), patients with severe cases can be observed for maximum two days; more complicated cases should be referred to the district hospital, which has hospitalization wards, medical doctors and a laboratory. Each PHC and district hospital has a medicine store, where medicines should only be dispensed following prescription. Healthcare costs are paid out-of-pocket by patients, without any reimbursement plan, except for under 5 years olds and pregnant women for whom healthcare is free[13]. Of private community pharmacies (Private pharmacies outside PHCs and district hospitals), the health district and the national pharmaceutical regulatory agency are responsible to inspect origin and quality of medicines and whether dispensing of certain medicines follows medical prescription[14].

This study was conducted in two health areas of the Nanoro health district (which include 30 PHCs (of which only 4 have medical doctors) and the district hospital), Nanoro and Nazoanga, located in central-west Burkina Faso, about 90 kilometers from Ouagadougou. Nanoro health area includes five villages with 17300 inhabitants. Nazoanga health area includes three villages with 8800 inhabitants. Nanoro has the district hospital, a PHC (with a medical doctor), two private community pharmacies (one run by a pharmacist, although actual medicine dispensing is ensured in both pharmacies mainly by non-pharmacists), three informal medicine vendors (non-licensed vendors), and two traditional healers. Nazoanga has a PHC (without a medical doctor), two informal medicine vendors, and one traditional healer.

In Nanoro health district, the most frequently reported diagnosis is malaria[15], which in children is sometimes (< 3%) associated with community-acquired invasive bacterial co-infection, caused by non-Typhi *Salmonella* spp. in particular[1,16]. Malaria is endemic with a high malaria transmission season from July to November. This period overlaps with the rainy season, which extends from June to October. The low malaria transmission season is from December to June.

Study design

We conducted a healthcare visit exit survey from October 2021 to February 2022. In a preceding qualitative study, all healthcare providers in both health areas were identified. The district hospital and PHCs with integrated medicine stores, private community pharmacies (integrated medicine stores and private community pharmacies are further referred to as formal pharmacies) and informal medicine

vendors were included. Traditional healers were not included because a pilot study found they did not dispense antibiotics(59). A consecutive sample of patients presenting with acute illness was taken by selecting the next patient exiting when the interview with the previous patient was completed. We planned to interview at least 50 patients who had received an antibiotic per type of healthcare provider to obtain 95% confidence interval limits with a margin of error of 5%, assuming 15% of Watch antibiotic use(60). In addition, all inpatients admitted in the paediatric and medicine wards of the Nanoro district hospital and patients under observation in Nazoanga and Nanoro PHCs, were interviewed once. Using an electronic questionnaire, field workers approached patients or caretakers after buying medication to collect data on symptoms that had triggered healthcare seeking, type of healthcare provider visited, diagnoses if any received, type of medicines dispensed/bought (antibiotic or not), dose, route of administration, uptake frequency, treatment duration and whether dispensing followed a prescription during a healthcare consultation or not.

Data analysis

We analysed antibiotic use by type of healthcare provider, by clinical presentation, and by age group (0-4 years, 5-17, 18-49 and ≥50). If medicine dispensing from any medicine outlet – i.e. medicine stores of the Nanoro district hospital, the Nanoro or Nazoanga PHC or the two private pharmacies located in Nanoro – followed a formal healthcare consultation, we assigned it as healthcentre medication, or if otherwise, as self-medication in formal pharmacies. In healthcentre, all patients surveyed in observation (PHCs) and hospitalized (district hospital) were defined as inpatients. We assigned clinical presentations to presumed diagnoses reported by the healthcare provider, or – if none reported – to a combination of reported symptoms (Table S1) following the symptoms reported for each disease in the WHO AWaRe antibiotic book [12]. In case a patient reported only one symptom, the latter was reported as the clinical presentation. At healthcentre level, a malaria infection was generally diagnosed based on positive mRDT, systematically used for any fever. For self-medicated patients, malaria was reported as a clinical presentation when assumed by the healthcare provider visited or the patient himself. In case a patient presented with more than one clinical presentation, we prioritized reporting the one, which would have led to an antibiotic prescription. Patients with chronic diseases such as high blood pressure,

diabetes, asthma, epilepsy, mental illness and HIV, without any additional acute illness were excluded from this analysis.

We analysed antibiotic use frequencies, class, mode of administration, and AWaRe (Access, Watch Reserve) distribution[18], and median treatment duration with 95% confidence interval (CI), by provider type, by age group, by clinical presentation. In outpatients, frequencies of antibiotic use as well as the percentage of Watch antibiotic use were compared between types of provider using chi-square tests, p-values were reported and significance was set at 5%. Mean defined daily doses (DDD) were calculated for adults only. Combining the DDD with number of adult outpatient visits per 1000 inhabitants per day in a preceding household healthcare utilization survey conducted in the same study area [5] with the frequencies of antibiotic use per adult visits and per type of provider, we generated the DDD used per 1000 adult inhabitants per day (DID). Reported prevalence, means and proportions were corrected for clustering by healthcare provider, in two strata (Nanoro, Nazoanga), except for informal medicine stores, for which neither store nor health area were recorded to ensure confidentiality. We classified antibiotic use as 'necessary' if used for a clinical presentation for which an antibiotic should be administered according to guidance in the WHO AWaRe antibiotic book [12]. For adult outpatients, we also classified antibiotics uptake regimens as optimal versus suboptimal. An uptake regimen was considered optimal if daily dosage and treatment duration for a clinical presentation for which this antibiotic was used were in line with the treatment guide in the WHO AWaRe antibiotic book. In case of unnecessary use, we nevertheless classified uptake regimen as optimal regardless of the clinical presentation for which it was used, if daily dosage was between the lower and the higher dose recommended for such an antibiotic and duration within the recommended duration range for mild to moderate cases in which it should be used as recommended in the WHO AWaRe antibiotic book. For all antibiotics for which treatment duration recommended for mild to moderate cases was limited to a maximum of 5 days for adults, we added a margin of 2 days, thus considering a duration up to 7 days as correct.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee for Health Research of Burkina Faso (ref no. 2020-8-171). Written informed consent was obtained from all patients aged at least 18 years. For patients aged between 14 and less than 18,

oral assent was obtained in addition to parents or caretakers' written informed consent. For patients under 14 years, written informed consent was obtained from parents or caretakers.

Results

Characteristics of the study population

Out of 2196 patients interviewed, 2108 (96.0%) had an acute illness, 86 (3.9%) a chronic illness, 2 (0.1%) attended for antenatal care or a delivery. Out of the 2108 acute illness cases in this study, 1431 (67.9%) attended health centers (182 (12.7%) inpatients, 1249 (87.3%) outpatients); 328 (15.6%) self-medicated at formal pharmacies; 349 (16.6%) self-medicated at informal medicine vendors (Table 1). Children aged 0-4 years were most frequently seen in health centers (94.5%, 622/658) rather than self-medicating (either in formal pharmacies or informal medicine vendors) (5.5%, 36/658). Adults aged 18-49 years most often self-medicated (56.4%, 473/839) than consulting in health centers (43.6%, 366/839). Patients who presented with fever most often consulted in health centers (84.5%, 1027/1215) rather than self-medicated (15.5%, 188/1215).

Inpatient antibiotic use

Among 182 inpatients, 90.7% (95%CI 77.9%-96.4%, n=165) used antibiotics of which 59.4% (95%CI 16.1%-91.8%, n=98) more than one antibiotic. Among inpatients who used antibiotics 64.2% (95% CI 8.13%-97.3%, n=106) used Watch antibiotics (Table 2). Malaria, bronchitis, and gastro-enteritis were the most frequent clinical presentations, together accounting for 62.6% (114 out of 182) of inpatients, 61.2% (111 out of 165) of antibiotic use, and 54.7% (58 out of 106) of Watch antibiotic use.

Outpatient antibiotic use

Antibiotics use was more frequent after consultation in health centers (54.8%, 95%CI 32.3%-75.5%, n=685) than at formal pharmacies (26.2%, 95%CI 10.3%-52.4%, n=86, p<0.001) and informal medicine vendors (26.9%, n=94, p<0.001). Among these patients who used antibiotics, the percentage of those with Watch antibiotics use was higher at informal medicine vendors (50.0%, n=47) than in health centers (16.5%, 95%CI 10.6%-24.8%, n=113, p<0.001) or at formal pharmacies (16.3%, 95%CI 15.0%-17.8%, n=14, p<0.001) (Table 3).

AWaRe distribution of all antibiotics used

Among all antibiotics used in inpatients 60.3% (95%CI 32.2%-82.9%, n=176) were Access, 39.7% (95%CI 17.1%-67.8%, n=116) were Watch; none Reserve (Figure 1). By clinical presentations, malaria, bronchitis, undifferentiated fever, gastroenteritis and wounds accounted together for 70.7% (n=82) of all inpatient Watch antibiotics used. Ceftriaxone was the most used Watch antibiotic among inpatients.

Among all antibiotics prescribed in outpatients at health centers, 85.2% (95%CI 80.9%-88.7%, n= 675) were Access and 14.8% (95%CI 11.3%-19.1%, n= 117) were Watch. Clinical presentations as malaria, rhinopharyngitis, bronchitis, undifferentiated fever, gastroenteritis, pain, dermatosis and wound for which antibiotics were not recommended (apart from dermatosis where Access group antibiotics were adequate) accounted together for 68.4% (n=80) of all Watch antibiotics used, which could have been avoided. Ciprofloxacin was the most dispensed Watch antibiotic in health centers (35.0%, n=41), followed by erythromycin (38, 32.5%) and ceftriaxone (26, 22.2%).

Among all antibiotics received after formal pharmacies visits, 84.6% (n=77) were Access and 15.4% (n=14) were Watch. Clinical presentations as rhinopharyngitis, bronchitis, gastroenteritis, dermatosis and pain accounted for 64.3% (n=9) of all Watch antibiotics used, which could have been avoided. Ciprofloxacin was the most used Watch antibiotic by self-medication in formal pharmacies (71.4%, n=10) followed by erythromycin (28.6%, n=4).

Among all antibiotics used after informal medicine vendors' visits, 52.5% (n=53) were Access and 47.5% (n=48) were Watch. Clinical presentations as rhinopharyngitis, bronchitis, gastroenteritis, pain and wound, for which antibiotics were avoidable, accounted for 50.0% (n=24) of all Watch antibiotics used. Oxytetracycline was the most used Watch antibiotic following informal medicine vendors' visits (45.8%, n=22) followed by norfloxacin (27.1%, n=13), ciprofloxacin (25.0%, n=12) and erythromycin (2.1%, n=1).

Class of antibiotics

Third-generation-cephalosporins were the most used antibiotic class in inpatients (35.6% 95%CI 10.3%-72.8%, n=104) while penicillins were the most used class by

outpatients who consulted in health centers (40.3% 95%CI 27.7%-53.3%, n=319) or self-medicated in formal pharmacies (49.5% 95%CI 16.6%-82.8%, n=45) or at informal medicine vendors (31.7%, n=32) (Figure 2).

Antibiotics route of administration, duration and dosage

Injectable antibiotics accounted for 68.2% (95%CI 8.2%-98.1%, n= 199) of antibiotics used by inpatients, 6.9% (95%CI 2.7%-16.5%, n=54) of antibiotics used by outpatients who visited health centers. None of the antibiotics used by self-medicated was injectable.

The median duration of treatment was 5 days (IQR 4-7) among healthcentre outpatients, 5 days (IQR 5-7) in formal pharmacies and 3 days (IQR 2-3) at informal medicine vendors.

In adults outpatients, 60.7% (95%CI 28.7%-85.5%, n/N=74/122) of antibiotics prescribed in health centers and 69.1%% (n/N=67/97) of antibiotics used by self-medicated, including 59.1% (95%CI 0.2%-99.9%) in formal pharmacies and 90.3% (n/n=28/31) in informal medicine vendors were taken suboptimally.

The mean DDD per antibiotic treatment course among adult outpatients was higher in health centers (5.9 DDD, 95%CI 3.9-8.0) and formal pharmacies (4.7 DDD, 95% CI 2.6-6.8) than at informal medicine vendors (1.8 DDD).

Community-wide antibiotic use

The rate of healthcare utilization was 1.46 visits per 1000 inhabitants per day (655 healthcare visits among 4984 adult household members in the past 3 months/90 days): 0.97 visits per 1000 to health centres (n=434), 0.36 to informal medicine vendors (n=162), and 0.13 to private community pharmacies (n=59).

The community-wide rate of antibiotic use was 2.9 DID (95% CI 1.9-3.9 DID), consisting of 2.6 DID (95% CI 1.7-3.5 DID) from health centers, 0.14 (95% CI 0.08-0.2 DID) from community pharmacies, and 0.15 DID from informal medicine vendors. This gap is explained by differences in healthcare utilization as well as the prevalence of antibiotic use per type of provider visited

Discussion

Main findings and comparison with other LMICs

Community-wide antibiotic use in rural Burkina Faso in 2021-22 (2.9 DID) was lower than the median estimate of lower middle-income countries in 2015 (10.8 DID), yet comparable to estimates from DR Congo in 2019-20 (1.75 to 10.2 DID in rural and peri-urban populations, respectively)[6,19]. The 54.8% prevalence of antibiotic use during healthcentre visits was within the prediction interval of 44%–60% antibiotic use during primary care centre visits in a systematic review in 27 LMICs [17]. The percentage of outpatients with Watch antibiotic use after healthcentre visits (16.5%) was lower than that observed in most studies globally (range 7.5-90.3%) yet comparable to that in other west- or central African settings (range 10.0-23.6%) [6,17]. Nevertheless, nearly 70% of Watch antibiotic use in public health centers could have been avoided if adhering to treatment guidance in the 2022 WHO AWaRe Antibiotic Book.

Antibiotics dispensing mechanisms and informed antimicrobial stewardship in health centers

The high proportion of inpatients dispensed (Watch) antibiotics may be related to prescription being guided by perceived disease severity, as in other LMICs[20]. Likewise, the high proportion of outpatients diagnosed with conditions very unlikely to be of bacterial origin such as rhinopharyngitis, bronchitis, gastroenteritis (watery diarrhoea), who were nevertheless prescribed (Watch) antibiotics when attending health centers, confirms that also there, antibiotics are not only prescribed when recommended, as observed in other LMICs[21–23]. Similarly, inadequate use of antibiotic classes, such as imidazoles and sulfonamide-trimethoprim-combinations for pneumonia were observed. In the same way, the single dose administration of injectable ceftriaxone in some outpatients with mild to moderate clinical presentations without an antibiotic indication as well as a high proportion of antibiotics dispensed suboptimally, are worrisome. These practices by healthcare workers could be related to limited diagnostic resources in health centers, inducing concerns of missing a potential bacterial co-infection, which resulted in an (over) prescription of wide-spectrum antibiotics. Lack of knowledge on the rational choice and use of antibiotics according to patients' clinical presentations and the implications of incorrect use could have further perpetuated or exacerbated such practices. Interventions to improve quality of care and reduce AMR in health

centers should be multi-faceted, combining different strategies including dedicated education and awareness about AMR for healthcare workers, improved diagnostic tools to differentiate bacterial from non-bacterial infections, patient management algorithms which take into account age, based on the latest WHO recommendations for antibiotic prescription[12,24–28].

Informed antimicrobial stewardship targeting over-the-counter dispensing

Although most antibiotics used in the community came from health centers and a large quantity was inappropriate, implying a need for more intensified interventions there, a significant amount were also dispensed over-the-counter, suggesting more involvement needed of the national pharmaceutical regulatory agency in combating AMR. At district level, this involvement could be more responsibilities allocated to the district management team (well trained on AMR), allowing them to include all private pharmacies in the district areas into their regular monitoring program with PHCs, instead of spot inspections as currently allowed[14]. Watch antibiotics should be included into the list of medicines to be dispensed following medical prescription only and should be one of the key components of this monitoring. This could also be an opportunity for ongoing AMR awareness activities towards pharmacy workers[29]. At national level, more commitment could help limit uncontrolled access to medicines of potentially poor quality, hence reducing the availability of over-the-counter antibiotics at informal medicine vendors. For this group, AMR awareness program should stress the harm of Watch antibiotics misuse (wrong indications and suboptimal regimen) for their entire community and urge them not to sell these medicines

Informed community-targeted antimicrobial stewardship

At community level, health literacy, including awareness on risk behaviours leading to emergence and spread of resistant microorganisms in the community, could significantly reduce self-medication with antibiotics[30]. Such activities should be combined with interventions facilitating and promoting access to healthcare facilities e.g. through universal health insurance and patient-centered care including reducing patients' waiting time[31–34].

Conclusion

Interventions to optimize antibiotic dispensing should be provider-type tailored:

- At formal pharmacies, strengthening regulation on antibiotics sale associated with regular AMR awareness activities and monitoring should mitigate other-the-counter Watch antibiotics dispensing.
- At informal medicine vendors, AMR awareness program should help self-restriction of Watch antibiotics sale.
- In health centers, while access should be promoted and facilitated, interventions should include healthcare professionals' education and awareness on AMR, which is a key component for the success of a multiplex intervention including improving diagnostic tools and using appropriate algorithms to reduce (over) prescription.

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Table 1: Characteristics of patients who visited healthcentre, formal pharmacies and informal medicine vendors

	Healthcentre (n=1431)		Self-medicated at formal pharmacies (n=328)		Self-medicated at informal medicine vendors (n=349)	
	n	%	n	%	n	%
Gender						
Female	737	51.5	175	53.4	148	42.4
Male	694	48.5	153	46.7	201	57.6
Age grp						
0-4 yo	622	43.5	21	6.4	15	4.3
5-17 yo	253	17.7	43	13.1	21	6.0
18-49 yo	366	25.6	206	62.8	267	76.5
>=50 yo	190	13.3	58	17.7	46	13.2
Education level						
No education	951	66.5	200	61.0	275	78.8
Primary school level	270	18.9	49	14.9	54	15.5
Secondary school level	194	13.6	70	21.3	19	5.4
University level	16	1.1	9	2.7	1	0.3
Care status						
Inpatients	182	12.7	0	0.0	0	0.0
Outpatients	1249	87.3	328	100.0	349	100.0
Fever						
Present	1027	71.8	105	32.0	83	23.8
Absent	404	28.2	223	68.0	266	76.2
Clinical presentation						
Malaria	555	38.8	88	26.8	21	6.0
Rhinopharyngitis	116	8.1	63	19.2	61	17.5
Bronchitis	211	14.7	36	11.0	51	14.6
Pneumonia	45	3.1	0	0.0	0	0.0
Enteric fever	9	0.6	4	1.2	20	5.7
Undifferentiated fever	87	6.1	0	0.0	0	0.0
Gastroenteritis	128	8.9	7	2.1	48	13.8
Stomach ache	70	4.9	32	9.8	28	8.0
Pain	65	4.5	62	18.9	75	21.5
Dermatosis	17	1.2	3	0.9	2	0.6
Wound	37	2.6	12	3.7	18	5.2
STI-Vaginal infection	13	0.9	2	0.6	0	0.0
Urinary-tract infection	6	0.4	1	0.3	4	1.2
Sepsis	5	0.4	0	0.0	0	0.0
Anorexia/Asthenia	20	1.4	2	0.6	3	0.9
Others	47	3.3	16	4.9	18	5.2

Table 2: Number of inpatients who used antibiotics and Watch antibiotics according to clinical presentation

Clinical presentation		Patients who received antibiotics		Patients who received Watch antibiotics	
	N	n	n/N(%)(95%CI)	m	m/n(%)(95%CI)
Malaria	52	40	76.9(50.5-91.6)	22	55.0(44.7-97.0)
Bronchitis	36	35	97.2(70.8-99.8)	20	57.1(0.4-99.8)
Pneumonia	11	11	100.0	10	90.9(0.03-100)
Enteric fever	5	5	100.0	1	20.0(0.04-99.3)
Undifferentiated fever	19	18	94.7(20.7-99.9)	15	83.3(3.0-99.9)
Gastroenteritis	26	26	100.0	16	61.5(10.4-95.7)
Stomach ache	9	8	88.9(25.0-99.5)	6	75.0(4.16-99.5)
Pain	2	1	50.0	0	0.0
Wound	3	3	100.0	1	33.3(0.0-100.0)
Urinary- tract infection	3	3	100.0	3	100.0
Sepsis	5	5	100.0	5	100.0
Anorexia/Asthenia	1	0	0.0	-	-
Others	10	10	100.0	7	70.0(1.8-99.7)
Total	182	165	90.7(77.9-96.4)	106	64.2(8.1-97.3)

Table3: Number of outpatients prescribed antibiotics and Watch antibiotics by type of healthcare provider according to clinical presentation

	Patients who visited healthcentre (n=1249)					Self-medicated in formal pharmacies (n=328)					Self-medicated at informal medicine vendors (n=349)				
Clinical presentation	Number of patients per clinical presentation	Patients who received antibiotics		Patients who received Watch antibiotics		Number of patients per clinical presentation	Patients who received antibiotics		Patients who received Watch antibiotics		Number of patients per clinical presentation	Patients who received antibiotics		Patients who received Watch antibiotics	
	N	n	n/N(%)(95%CI)	m	m/n(%)(95%CI)	N	n	n/N(%)(95%CI)	m	m/n(%)(95%CI)	N	n	n/N(%)	m	m/n(%)
Malaria	503	170	33.8(15.5-58.8)	27	15.9(6.9-32.6)	88	2	2.3(0.0-82.6)	0	0.0	21	0	0.0	-	-
Rhinopharyngitis	116	74	63.8(34.2-85.6)	6	8.1(5.3-12.1)	63	13	20.6(3.6-64.7)	1	7.7(1.0-40.9)	61	10	16.4	1	10.0
Bronchitis	175	161	92.0(64.9-98.6)	6	3.7(1.3-10.4)	36	31	86.1(0.0-100.0)	1	3.2(0.0-92.6)	51	11	21.6	3	27.3
Pneumonia	34	31	91.2(7.65-99.9)	1	3.2(0.1-56.4)	0	-	-	-	-	0	-	-	-	-
Enteric fever	4	3	75.0(0.0-100.0)	3	100.0	4	4	100.0	1	25.0(0.0-99.9)	20	6	30.0	3	50.0
Undifferentiated fever	68	49	72.1(42.5-90.0)	15	30.6(10.4-62.7)	0	-	-	-	-	0	-	-	-	-
Gastroenteritis	102	87	85.3(42.8-97.8)	12	13.8(1.9-56.5)	7	6	85.7	3	50.0	48	20	41.7	13	65.0
Stomach ache	61	29	47.5(24.7-71.5)	9	31.0(15.7-52.2)	32	12	37.5(20.5-58.3)	4	33.3(0.1-99.7)	28	19	67.9	14	73.7
Pain	63	7	11.1(3.7-29.0)	2	28.6(0.8-95.4)	62	3	4.8	2	66.7	75	2	2.7	2	100.0
Dermatosis	17	15	88.2(6.0-99.9)	12	80.0(57.7-92.1)	3	2	66.7	2	100.0	2	2	100.0	2	100.0
Wound	34	29	85.3(14.9-99.5)	6	20.7(6.0-51.6)	12	11	91.7	0	0.0	18	18	100.0	4	22.2
STI-Vaginal infection	13	9	69.2(12.7-97.2)	7	77.8(17.3-98.3)	2	0	0.0	-	-	0	-	-	-	-
Urinary- tract infection	3	2	66.7(0.0-100.0)	1	50.0(0.0-100.0)	1	1	100.0	0	0.0	4	4	100.0	4	100.0
Anorexia/Asthenia	19	0	0.0	-	-	2	0	0.0	-	-	3	0	0.0	-	-
Others	37	19	51.4(36.4-66.1)	6	31.6(16.5-51.9)	16	1	6.3	0	0.0	18	2	11.1	1	50.0
Total	1249	685	54.8(32.3-75.5)	113	16.5(10.6-24.8)	328	86	26.2(10.3-52.4)	14	16.3(15.0-17.7)	349	94	26.9	47	50.0

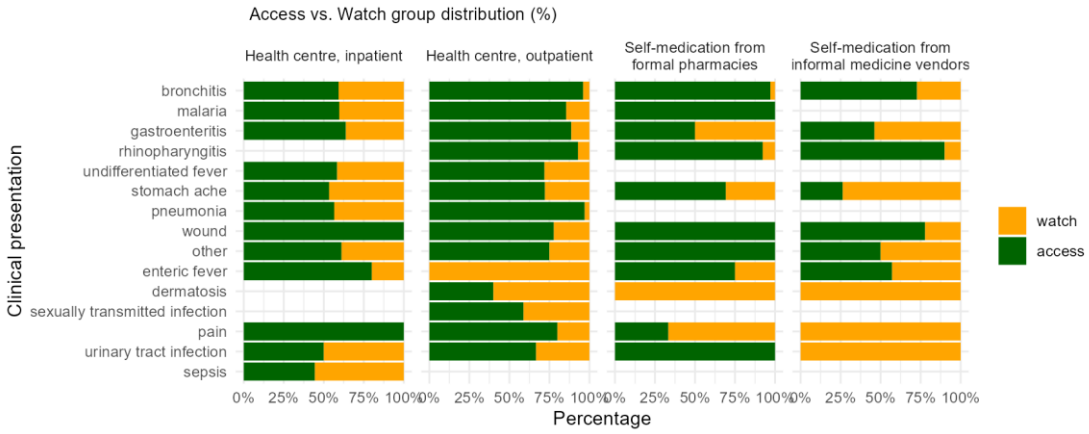

Figure 1: Distribution of AWaRe groups among all antibiotics used per type of visit

Result 4 : Antibiotic use by clinical presentation across all healthcare providers

Figure 2: Distribution of antibiotic classes among all antibiotics used, by clinical presentation and by type of visit

CHAPTER 5: SYNTHESIS OF KEY FINDINGS AND DISCUSSION

5.1 Summary of key findings

K. pneumoniae and *E. coli* were among the six leading causes of death attributable to bacterial AMR worldwide, and respectively the first and the second cause in Burkina Faso in 2021 (61). Although some studies reported the increasing prevalence of ESBL-*K. pneumoniae* and ESBL-*E. coli* in community-acquired infections, no study had investigated what may be fuelling such an increase. As ESBL-*Enterobacterales* faecal colonisation in human is a good indicator of the burden and act as a reservoir of antibacterial resistance in a given community, we explored its extent, molecular characterization and risk factors that may be underlying this carriage, in order to set up tailored strategies for infection prevention and control.

The overall objective of this thesis was to determine the extent of resistant strains *E. coli* and *K. pneumoniae* (major contributors of AMR attributable deaths in Western sub-Saharan Africa) in the community in rural Burkina Faso and factors underlying it, to inform health policies in setting up tailored strategies for infection prevention and control (IPC). To achieve this goal, our specific objectives were first to determine the extent of antibiotic resistant bacteria (ESBL- *E. coli* and ESBL- *K. pneumoniae*) as well as molecular characteristics of these ESBL-*Enterobacterales* in healthy community members, second to investigate community antibiotic use before attending the Nanoro district hospital, third to understand the role of different healthcare providers and other antibiotic dispensers in a rural community and finally to explore antibiotic dispensing practices by type of healthcare provider.

5.1.1 Synthesis of key findings

Magnitude of ESBL-*E. coli* and ESBL-*K. pneumoniae* faecal colonisation in the community, risk factors and molecular characteristics

Prevalence of faecal colonisation with ESBL-*E. coli* and ESBL-*K. pneumoniae* in the community was very high 61.3% (58.8%-63.7%). Open defecation was reported by more than 75% of community members, more than 21% reported drinking water from unprotected well and nearly 91% reported not washing hands with soap before meals. Faecal colonisation prevalence was significantly associated with hygiene practices (hand-washing with soap before meals vs not washing hand with soap before meals) and seasonal variation (rainy season vs dry season). The most prevalent extended spectrum β-Lactamase gene was *bla*$_{\text{CTX-M-}}$

15. β-Lactamase genes were highly associated with Plasmid-mediated quinolone resistance (PMQR) genes. A carbapenemase *bla*$_{NDM-5}$ was also found. A diversity of sequence types and virulence genes have also been evidenced. Resistant genes were plasmid-mediated (plasmids were found in all K. pneumoniae and in 79.9% of E. coli) mainly by the IncF group plasmids.

Extent of community antibiotic use before attending the Nanoro district hospital

Pre-hospital antibiotic use was reported by nearly 40% of patients presenting with severe acute fever at the Nanoro district hospital. Use was significantly higher in patients referred from PHCs (54%) than self-referred patients (26.7%). Among all antibiotic use reported, the percentage of Watch antibiotic use was significantly higher in referrals (42.2%) compared to self-referred patients (28.1%). Nearly 60% of febrile patients with pre-hospital antibiotic use for whom a microbiological diagnosis was available did not have a bacterial infection confirmed.

Understanding the role of different healthcare providers on antibiotic dispensing in rural communities

Frequency of healthcare seeking outside healthcare facilities was reported at 33.5%, including informal medicine vendors (47.7%), self-medication with left-over medicines kept at home (26.5%), medicine vendors in formal pharmacies (16.4%) and traditional healers (9.4%). Among these providers outside healthcare facilities, only traditional healers were found not to be antibiotic dispensers. Main reasons for seeking healthcare outside healthcare facilities were financial limitations as well as long waiting times and unprofessional attitudes of healthcare professionals towards patients. Antibiotics knowledge (only for illnesses of bacterial origin) was limited among healthcare professionals, very limited among medicine vendors in formal pharmacies, and non-existent among informal medicine vendors and the general community.

Magnitude of antibiotic dispensing across the range of healthcare provider

Outpatient antibiotic use was more frequent after healthcare facility visits (54.8%, of which 16.5% Watch) than after visits to pharmacies (26.2%, 16.3% Watch) and informal medicine vendors (26.9%, 50.0% Watch). High inappropriate (Watch) antibiotic use was recorded in patients exiting each type of provider and nearly

70% of Watch antibiotic use after healthcare facility visits could have been avoided. The community-wide rate of antibiotic use was 2.9 DID of which 2.6 DID (89.7%) recorded after healthcare facility visits.

5.2 Discussion

The prevalence of faecal colonisation with ESBL-*Enterobacterales E. coli* and/or *K. pneumoniae* in healthy community members in rural Burkina Faso in 2021-22 was 61.3% (58.8%-63.7%), almost three times higher than what was recorded ten years earlier in healthy volunteers in Bobo Dioulasso, an urban area of Burkina Faso. This prevalence is also higher than those reported in high income countries as in Europe where community prevalence was ranging from 3.7% in Spain in 2003 to 7.3% in England in 2014 (62–65) and in the United States with a community prevalence estimated at 3.5% in healthy children in 2015 (66). The prevalence is even higher than the 18% pooled prevalence reported in healthy community members in other sub-Saharan African countries (67), which ranged from 5% in Gambia in 2015 to 59% in Central African Republic in 2013 (68,69), yet comparable to those reported in Asia, with a pooled prevalence of 46% (70), ranging from 6.4%% in Japan in 2010 to 73.9% in China in 2014 (71,72). However, antibiotic use in the same study area in Burkina Faso (73,74), was lower or at most comparable to levels reported in other sub-Saharan African countries (75,76), contrasting with the high prevalence of ESBL-E faecal colonisation we observed in our findings. This suggests that the high prevalence observed in rural communities in Burkina Faso may result from the interconnection of several factors. Indeed, although the inappropriate (Watch) antibiotic use reported could have initiated the emergence of these resistant bacteria, transmission must have been facilitated by the poor WASH conditions prevailing (77), evidenced by the significant association observed between colonisation, hygiene practices and seasonal variations. These interconnected factors contribute to a “silent” reservoir, continuing to increase transmission within households and the community (78), posing significant public health risks. In addition, the frequent detection of some virulence genes like *hlyE*, *fimH*, *ituA* and *fyuA* conferring high pathogenicity to *Enterobacterales* involved in urinary tract infections and BSI, can lead to more community-onset invasive infections (79). Likewise, the high prevalence of PMQR genes associated with beta-lactamase genes reduces the treatment options (7,44,45,80). Even more concerning is the possibility of community dissemination of carbapenemase-

producing strains (*bla*$_{NDM-5}$ isolated from one *E. coli*), often the only therapeutic option for infections caused by ESBL-producing strains. Indeed, the detection of some clones as ST307 and ST410 (81–87) along with plasmids like IncFIB(K) and IncFIA (88,89) linked to the spread of carbapenem-resistant and MDR strains, makes this risk more imminent, stressing the imperative need for informed interventions to significantly reduce spread. The challenge of controlling AMR in such a setting is particularly complex and should combine both antibiotic use optimization to reduce antibiotic selective pressure and community-based hygiene interventions to break the cycle of transmission in order to mitigate and/or prevent spread.

5.2.1 Antibiotic dispensing mechanisms and informed antimicrobial stewardship

Healthcare facilities

Many patients under observation in PHCs or hospitalized in the Nanoro district hospital are dispensed (Watch) antibiotics likely guided by perceived disease severity (74,90). Likewise, a high proportion of outpatients diagnosed with conditions very unlikely to be of bacterial origin such as rhinopharyngitis, bronchitis, gastroenteritis (watery diarrhoea), who were nevertheless prescribed (Watch) antibiotics when attending healthcare facilities, confirms that there also, antibiotics are not only prescribed when recommended (74,91–93). Similarly, inadequate use of antibiotic classes - i.e. imidazoles and sulphonamide/trimethoprim combinations for pneumonia - was observed. In the same way, a single-dose administration of injectable ceftriaxone in some outpatients with mild to moderate clinical presentations without an antibiotic indication, as well as a high proportion of antibiotics dispensed suboptimally, are worrisome (74). These practices by healthcare workers could be related to limited diagnostic resources in healthcare facilities, inducing concerns of missing a potential bacterial co-infection, which resulted in an (over) prescription of wide-spectrum antibiotics (94). Lack of knowledge on the rational choice and use of antibiotics according to patients' clinical presentations (59,74) and the implications of incorrect use could have further perpetuated or exacerbated such practices. Interventions to improve quality of care and reduce AMR in healthcare facilities should be multifaceted, combining different strategies including dedicated education and awareness about AMR for healthcare workers, improved diagnostic

tools to differentiate bacterial from non-bacterial infections, patient management algorithms that take into account age, based on the latest WHO recommendations for antibiotic prescription.

Over-the-counter dispensing

A significant amount of antibiotics used in the community is also dispensed over-the-counter (74), suggesting that a greater involvement of the national pharmaceutical regulatory agency is needed in combating AMR. At the district level, this involvement could be more responsibilities allocated to the district management team (well trained on AMR), allowing them to include all private pharmacies in the district areas into their regular monitoring programme with PHCs, instead of spot inspections, as currently allowed (95). Watch antibiotics should be included in the list of medicines to be dispensed following medical prescription only and should be one of the key components of this monitoring. This could also be an opportunity to fill pharmacy workers knowledge gaps with ongoing AMR awareness activities (59,96). At national level, more commitment could help limit uncontrolled access to medicines of potentially poor quality, hence reducing the availability of over-the-counter antibiotics at informal medicine vendors. For this group, AMR awareness programmes should stress the harm of the misuse of Watch antibiotics (wrong indications and suboptimal regimen) for their entire community and urge them not to sell these medicines.

General community

At community level, health literacy should include awareness of risk behaviours leading to the emergence and spread of resistant microorganisms in the community(97). Such activities should be combined with interventions facilitating and promoting access to healthcare facilities, e.g. through universal health insurance and patient-centered care, including reducing patients' waiting time (98–101).

5.2.2 WASH conditions and informed community-based hygiene interventions

The poor WASH conditions – i.e. inadequate access to clean water, insufficient sanitation facilities, and limited handwashing infrastructure – create a favorable environment for the transmission (spread of faecal contaminants) of ESBL-*Enterobacterales* and other resistant bacteria in the community. This combination

of both faecal colonisation and poor WASH conditions observed, presents a public health threat, as it allows for continuous environmental contamination – i.e. ESBL-*Enterobacterales* can be introduced into the environment through human waste, leading to contamination of soil and water sources – which serves as an ongoing source of exposure for the community. Such environmental contamination contributes to a cycle of reinfection and spread within the community, particularly in our setting where access to clean water is limited. Additionally, environmental reservoirs of resistant bacteria complicate efforts to control AMR, as individuals are continuously re-exposed to these pathogens despite attempts to reduce antibiotic (over) use.

Informed community-based hygiene interventions should take into account several aspects. Public health campaigns promoting handwashing with soap before meals can help reduce transmission as evidenced by our findings and other studies (102). Although access to safe drinking water should be promoted, conditions of water collection, transport and storage in households should also be improved as probable ways of contamination, attested by the similar colonisation rate between people drinking from water sources considered safe (tap water, borehole) and unsafe (unprotected well). Likewise, promoting access to improved latrines alone won't be enough to reduce transmission as evidenced in our study and should be necessarily combined with these latter handling (i.e. clean, restricted to the same household members). Improving sanitation and drinking water handling should be particularly reinforced during the rainy season, given the increased risk of colonisation observed (103–106).

5.2.3 Scalability and feasibility of proposed interventions in similar low-resource settings

Antimicrobial stewardship interventions targeting healthcare facilities and over-the-counter dispensing (including greater involvement of the national pharmaceutical regulatory agency) can be applied nationwide as all health districts share the same characteristics. Regarding general community, knowledge of antibiotics, antimicrobial resistance, provider choice, and hygiene practices may differ based on rural or urban residence and education level. However, a nationwide community education campaign on risk behaviours leading to the emergence and spread of resistant microorganisms in the community (self-medication with antibiotics, no handwashing with soap before meals) could raise awareness among those

previously unaware of AMR risks, encouraging behavior change, while reinforcing good practices among those already informed.

The scalability of these AMS interventions to other LMICs depends on factors like healthcare system capacity, regulatory enforcement, infrastructure, and community engagement. Regarding intervention targeting healthcare facilities, dedicated education and awareness on AMR for healthcare workers are highly scalable, and training programs can be incorporated into existing medical curricula and continuing education programs. Likewise, in similar resource-limited settings with limited diagnostic capacity in primary healthcare facilities, introducing low-cost rapid diagnostic tests to differentiate bacterial from non-bacterial infections can also help optimize antibiotic prescription (94,107). Similarly, introducing patient management algorithms based on the latest WHO recommendations on antibiotic prescription is highly scalable to other LMICs, as far as these recommendations can be contextually adapted and integrated into clinical workflows. Awareness messages targeting community should be context-specific tailored because, even if potential contamination sources could be similar in most resource-limited settings, risk behavior promoting AMR transmission may vary and influence differently resistant bacteria spread across these settings. However, raising health literacy and promoting responsible hygiene practices including handwashing with soap before meals could be effective steps towards mitigating the burden of AMR in resource-limited settings (77,108).

5.3 Limitations and strengths

Our studies were based on rigorous methodology. However, we are aware of some limitations that we have tried to overcome whenever possible.

Regarding all questions on antibiotic use the last three months before the day of survey in the community (study 1), community members surveyed might have used medicine, not knowing whether it was an antibiotic or not. To mitigate response biases, we provided each fieldworker with any type of antibiotic available from the range of healthcare providers in the study area (Pharmacies in healthcare facilities, private pharmacies, informal medicine vendors). Once in the household, in case medicine use the last three months was reported, fieldworkers should ask the interviewed person to identify which one(s) among the batch of antibiotics they acknowledge having used. As well, selection of isolates for molecular

characterization might have led to the absence of less phenotypically expressed genes. To mitigate this, we first divided all isolates into their different expressed phenotypic group (seven groups) and performed random selection among each group.

In the study evaluating pre-hospital antibiotic use (study 2), the inclusion of outpatients during the second half of the study period might have resulted in some changes in the study population and pre-hospital antibiotic use. However, we observed no difference in the prevalence of antibiotic use, when comparing the two populations from both recruitment periods. Likewise data on pre-hospital antibiotic use was collected via a survey capturing use during the two weeks before consultation at the hospital, potentially underestimating actual use. Whenever possible, reported antibiotic use was verified from referral forms, patient medical files (healthcare booklet), and antibiotic packaging or blisters. Final diagnoses at the district hospital may also not correspond to those that had triggered prescription or self-medication within the two preceding weeks. Dosage and duration of antibiotics used were not available to assess antibiotic uptake regimens, but these were captured in the healthcare visit exit survey (study 4)

Regarding place of seeking healthcare the last three months (study 3), Participants may struggle to accurately recall where they sought healthcare, especially if multiple visits occurred. Minor illnesses visits may have been forgotten or misreported. Respondents may have also reported seeking care at formal healthcare facilities rather than informal or traditional healers, even if they used both, due to perceived judgment. Likewise, a severe illness or a visit closer to the time of the survey may be more easily recalled than earlier visits, which may lead to over reporting of recent experiences. To mitigate responses biases, we used a more or less short recall period (e.g. three months instead of six months). For each participant, field workers had to ask about symptoms or illnesses presented and for each of these illnesses, ask thereafter all the providers visited. Field workers also have to provide memory aid by recalling all healthcare providers in the surrounding. Visits were also crosschecked with medical records whenever possible.

In the healthcare visit exit survey, reported prevalence, means and proportions were corrected for clustering by healthcare provider, in two strata (Nanoro and

Nazoanga), except for informal medicine stores, for which neither store nor health area were recorded to ensure confidentiality.

Despite these limitations that we have tried to overcome as much as possible, this thesis reports robust findings that provide a full picture of population-level antimicrobial resistance in a rural setting of low-and Middle-income countries and the interconnected factors that may be underlying it, in order to set up tailored infection prevention and control interventions.

CHAPTER 6: CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

6.1 Conclusion

In settings with high ESBL-*Enterobacterales* faecal colonisation, inappropriate antibiotic use, and poor WASH conditions such as we observed in rural Burkina Faso, tackling antimicrobial resistance presents significant challenges. This thesis underscores the complex interactions between human behavior, environmental factors, and resistance dynamics that drive the persistence and spread of ESBL-*Enterobacterales* in the community. The high colonisation rate represents a silent reservoir of resistant bacteria, facilitating ongoing transmission within households and the broader community under poor WASH conditions. Inappropriate antibiotic use further exacerbates this issue, creating selective pressures that favor the survival and proliferation of these resistant strains. Lack of antibiotic stewardship and limited public awareness of antimicrobial resistance risks contribute to perpetuate a cycle of misuse, which continues to fuel resistance. The poor WASH conditions amplify this public health threat by allowing high transmission of these resistant bacteria. The seasonal variation observed – i.e. increased transmission during the rainy season - highlights the role of environmental factors in shaping antimicrobial resistance patterns in rural Burkina Faso.

Because of these challenges, interventions must be multifaceted. For dedicated education and awareness on AMR for healthcare workers, training programmes can be incorporated into existing medical curricula and, for those already working in healthcare facilities, through continuous training included in the regular supervision activities of district management teams. At community level, health literacy programmes including awareness of risk behaviours leading to the emergence and spread of resistant microorganisms in the community (poor WASH conditions and antibiotic incorrect use) can be conveyed through channels such as radio or TV broadcasts, social media and community meetings involving community health workers and community leaders. School-based programmes can also be used.

Strengthening surveillance of antimicrobial resistance and faecal colonisation will be needed to identify transmission hotspots and guide interventions accordingly. Sustainable improvements in WASH, combined with targeted public health initiatives, will be critical in mitigating the spread of ESBL- *Enterobacterales* and reducing the antimicrobial resistance burden in such a high-risk setting. Similarly, while promoting existing immunization programmes (Pneumococcal Vaccines,

Haemophilus influenzae B vaccine, etc.) due to the fact that they prevent related infections, hence antibiotic use averted, research on vaccines development targeting *E. coli* and *K. pneumoniae*, major contributors of the global AMR burden, should be encouraged.

This thesis provides valuable insights into the drivers of antimicrobial resistance in a resource-limited environment, contributing to a broader understanding, necessary for developing context-specific strategies for antimicrobial resistance containment.

6.2 Recommendations and perspectives

6.2.1 Recommendations

To the Ministry of Health of Burkina Faso

- ✓ To facilitate and to promote access to healthcare facilities trough universal health insurance.

- ✓ To promote equitable distribution of healthcare workers across all healthcare facilities in the country (urban and rural areas), which could help reducing workload and facilitate patient-centered care including reducing patients waiting time.

- ✓ To optimise antibiotic use in healthcare facilities by multifaceted interventions including:
    - To set up dedicated education and awareness on AMR for healthcare workers.
    - To improve diagnostic tools to differentiate bacterial from non-bacterial infections in PHCs.
    - To include in PHCs, patients' management algorithm based on the latest WHO recommendations for antibiotic prescription.

To the National Pharmaceutical Regulation Agency of Burkina Faso

- ✓ To allocate more responsibilities to the district management team (well trained on AMR) allowing them to include all private pharmacies in district areas into their regular monitoring programme with PHCs.

- ✓ To include Watch antibiotics in the list of medicines to be dispensed following medical prescription only and highlight it as one of the key components of the district regular monitoring programme.

- ✓ To limit uncontrolled access to medicines of potentially poor quality at national level, which will reduce availability of over-the-counter antibiotics at informal medicine vendors' outlets.

Towards formal pharmacy vendors

- ✓ To promote regular AMR awareness activities including dispensing Watch antibiotics following medical prescription only.

Towards informal medicine vendors

- ✓ Including community leaders, to promote regular AMR awareness activities, stressing the harm of the misuse of Watch antibiotics for the entire community, which would lead them to a self-restriction of the sale of these medicines.

Towards the community

- ✓ To increase general community health literacy, including awareness of risk behaviours leading to emergence and spread of resistant microorganisms in their community, which could lead to reduction of self-medication with antibiotics.

- ✓ To promote handwashing with soap before meals.

- ✓ To promote use of improved latrines, kept clean with a restriction on sharing.

- ✓ To promote drinking water from safe sources: tap water, borehole

- ✓ To improve drinking water handling: collection, transport and storage in households to avoid contamination.

6.2.2 Perspectives

Given the high prevalence of ESBL-*Enterobacterales* faecal colonisation in humans in rural Burkina Faso, which is an indicator of the burden of antibacterial resistance in the community, efforts should focus on monitoring community antibiotic resistance patterns to guide empirical treatment choices and inform public health strategies. Setting up regular surveillance to track antimicrobial resistance trends (including ESBL/carbapenemase) within the community and healthcare facilities could also be helpful on guiding timely interventions to contain spread

Further research to quantify the attributable risk of key sources of antimicrobial resistance transmission as well as to explore pathways through which human behavior influences this transmission could provide a more complete picture of the factors contributing to this high ESBL-*Enterobacterales* prevalence, necessary for more comprehensive and effective interventions.

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