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Assessment of the Quality of Injectable Antibiotics in Benin

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Abstract. Substandard and falsified medicines are an enormous threat to global health. Poor quality antibiotic preparations contribute to the development of antimicrobial resistance. In surgery, where the occurrence of healthcare-associated infections is high, healthcare teams need to rely on the quality of antibiotic prophylaxis to prevent infections. We assessed the quality of antibiotics used for surgical infection prophylaxis in Benin. Thirty-three samples were collected from six hospitals located in various departments in Benin. The antibiotics (powders for injection: amoxicillin + clavulanic acid, ampicillin, ceftriaxone; solutions for injection: ciprofloxacin, gentamicin, metronidazole) were assessed using visual inspection, pharmacotechnical tests (including uniformity of mass, pH measure, sterility test, and active pharmaceutical ingredient identification), and assay tests (including a simple analytical method thin layer chromatography) and complex analytical techniques (ultraviolet-visible spectrophotometry, high-performance liquid chromatography—diode-array detection, conductometry). Because the material needed for the methods recommended by the pharmacopeias to assess the dosage of gentamicin was not available, we developed and validated a conductometry method. Results showed that 97% ($n = 32$) of the samples passed visual inspection; 100% ($n = 33$) of the samples passed the pharmacotechnical tests, identification of active ingredients, and sterility test; 88% ($n = 29$) passed the test for percentage of active pharmaceutical ingredients. Overall, 15% of the samples did not pass the quality test (3% on visual inspection and 12% for excess active ingredients). Although most of the samples passed the quality tests, it appears important to perform routine quality control for intravenous medicines.

INTRODUCTION

Medications are important products for disease prevention and control in the healthcare system. In addition to cost, availability, and appropriate use, quality is essential for their effectiveness. Medication quality is a challenge in all countries because of substandard and falsified (SF) medicines. Ensuring equitable access to quality pharmaceuticals is thus a key development challenge and an essential component of health-system strengthening and primary healthcare reform programs worldwide.[1]

Substandard medicines, also called "out of specification," are defined as "authorized medical products that fail to meet either their quality standards or their specifications, or both."[2] Medicines may be substandard as a result of poor manufacturing practices, unsuitable packaging, or inappropriate transport or storage conditions.

Falsified medicines, also called counterfeit medicines, are defined as "medical products that deliberately or fraudulently misrepresent their identity, composition, or source."[2] SF medicines are a threat for health, and every part of the world is concerned to different degrees. The proportion of SF medicines has been estimated at less than 1% in industrialized countries, and 10% to 30% in low incomes countries.[3]

After the Bamako initiative in 1987, access to medicines in sub-Saharan African countries has improved,[4] but SF medicines have frequently been reported.[5] Since 2013, the WHO has received 1,500 reports of SF medical products, 42% of which were from the WHO African region.[2]

In low- and middle-income countries, the impact of SF medicines is high, involving 10% of all medicines, with an economic cost of US $10 billion to $200 billion.[6] SF medicines may cause prolonged illness or disease and treatment failure and can also directly harm patients through toxic effects or adverse reactions and sometimes death.[7–9]

Antibiotics are the most frequently falsified medicines, accounting for 28% of global falsified medicines and representing 5% of the global antibiotic market. Of all falsified medicines, 17% are injectable formulations.[3] Poor-quality antibiotic preparations contribute to the development of microbial resistance to antibiotics and to death.[10] Poor-quality antibiotics have been reported in Africa,[11] including cases of substandard injectable ceftriaxone in Uganda with fatal consequences[12] and cases in Kenya.[13] The evolution of antimicrobial resistance is a major risk from poor-quality drugs because of microbial selection and geographic spread.[5] The United Nations, in its N° 3.8 Sustainable Development Goal, includes access to safe, quality, and affordable medicines to reduce the threat from SF medicines.[14]

The effectiveness of antibiotics is essential in infection prevention and control, which is a key feature in all healthcare departments especially in surgery, where the occurrence of infections, notably healthcare-associated infections, is high.[15] Infection prevention and control occupies a unique position in the field of patient safety and quality universal health coverage, because it is relevant to health workers and patients at every single healthcare encounter. One of the core components of infection prevention and control is good management of antibiotic use by both oral and injectable routes.[16]

In Benin, antibiotics are the second most commonly used therapeutic class after antimalarials.[17] Numerous studies have reported poor-quality antimalarials in Benin.[18–20] Assessing the quality of antibiotics can help ensure their effectiveness

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and contribute to reducing the evolution of antimicrobial resistance.

Given the importance of the quality of antibiotics in surgical infection prophylaxis,[21] we assessed the quality of intravenous antibiotics (powders for injection of amoxicillin/clavulanic acid, ampicillin, and ceftriaxone; solutions for injection of ciprofloxacin, gentamicin, and metronidazole) frequently used for surgical infection prophylaxis in Benin.

MATERIALS AND METHODS

Materials and instrumentation. This research was conducted in the frame of a multidisciplinary project involving six hospitals in Benin. Antibiotic drugs were purchased in pharmacies located in the six hospitals in the south of Benin: Centre National Hospitalo-Universitaire Hubert Koutoukou Maga (CHNU-HKM, Cotonou), Centre Hospitalo-Universitaire de la Mère et de l'Enfant Lagune (CHU-MEL, Cotonou), Centre Hospitalo-Universitaire Départemental Ouémé-Plateau (CHUD-OP, Porto-Novo), Centre Hospitalo-Universitaire de Zone Suru Léré (CHUZ Suru-Léré), Centre Hospitalo-Universitaire de Zone d'Abomey-Calavi/Sô-Ava (CHUZ-AC/SA, Abomey Calavi), and Hôpital Bethesda (Cotonou). The hospitals are from various levels of the healthcare system and for anonymity are simply named "hospital 1" through "hospital 6" in this report.

We studied the six antibiotics frequently used in surgical infection prophylaxis as reported in a Beninese study[22] and other unpublished data: amoxicillin + clavulanic acid (J01CR02), ampicillin (J01CA01), ceftriaxone (J01DD04), ciprofloxacin (J01MA02), gentamicin (J01GB03), and metronidazole (J01XD01). The antibiotics were brand or generic forms from different manufacturers. For easy handling, the antibiotics were coded using alphabetical letters and the number of each hospital (e.g., A1 for amoxicillin + clavulanic acid from hospital 1).

We planned to collect 36 samples (the six antibiotics from the six hospitals), with each sample composed of 30 vials of the same batch of antibiotic to have sufficient for all the analyses. We therefore aimed to collect: six samples of amoxicillin + clavulanic acid powder for injection, six samples of ampicillin powder for injection, six samples of ceftriaxone powder for injection, six samples of ciprofloxacin injection, six samples of gentamicin injection, and six samples of metronidazole injection, giving a total of 1,080 vials for analysis.

An adapted form from the WHO was used to record the visual inspection information.[23] The form includes about 57 items divided into four categories (secondary packaging, primary packaging, medication leaflet, physical appearance of power or injection solution) (see Supplemental materials, Appendix 1).

The following reagents were used for high-performance liquid chromatography (HPLC):

- acetonitrile (HPLC grade) purchased from Fisher Scientific (Loughborough, United Kingdom),
- potassium dihydrogen phosphate, sodium dihydrogen orthophosphate monohydrate from VWR chemicals (Brussels, Belgium),
- acetic acid (99.8%) purchased from BDH AnalaR (London, United Kingdom),
- methanol (HPLC grade) from Honeywell (Brussels, Belgium),
- phosphoric acid (100%) from Certa (Braine-l'Alleud, Belgium),
- triethylamine from Surechem products Ltd (Ipswich, United Kingdom), and
- ultrapure water obtained locally using a PURELAB CHORUS water purification system from VEOLIA (Paris, France).

Chemical reference substances (U.S. Pharmacopeia [USP] grade) of amoxicillin (86.9%), ampicillin (99%), ceftriaxone (92.6%), ciprofloxacin (99.6%), gentamicin sulphate (67.1%), and metronidazole (100%) were purchased from the USP (Rockville, MD); clavulanic acid (100%) was purchased from Sigma Aldrich (Darmstadt, Germany).

Qualitative analyses were performed with HPLC equipment from Hitachi VWR (Brussels, Belgium), controlled using the Chromaster System Manager version 1.1 (VWR; Brussels, Belgium) and a separation module coupled to the Hitachi photodiode array (PDA) detector (VWR); an ultraviolet-visible spectrophotometer (UV-VIS Spectrophotometer 6300PC VWR; 634-6041 series); and thin layer chromatography plates (TLC Silica gel 60 F_{254}, Merk Darmstadt, Germany). Quantitative analyses were carried out using HPLC, the UV-VIS spectrophotometer, and a conductometer (WTW Cond 3210, No. 13071009, Weilheim in Oberbayern, Germany).

pH values were measured using a pH meter (pHenomenal, pH1000H, Ser. No. 13150774, VWR), accuracy $\pm$0.1 pH.

The well-characterized microbial strains *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus spizizenii* ATCC 6633, *Clostridium sporogenes* ATCC 19404, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404, purchased from Medimark Europe (Grenoble, France), were used for the sterility test. The thioglycollate broth was purchased from MERCK (Darmstadt, Germany), the casein and soy hydrolysate liquid medium (tryptone soya broth) from HiMEDIA (Mumbai, India), and peptone water from OXOID (Basingstoke Hampshire, United Kingdom).

Two Memmert (Schwabach, Germany) bacteriological incubators, a Touchclave-R (Oldham, United Kingdom) autoclave, and a Telstar (Terrassa, Spain) laminar flow hood were used for all the tests.

Methods. Each sample was accompanied by a sheet detailing the date and location of the collection, the quantity taken, and the conditions of storage (laboratory temperature). Visual inspection and pharmacotechnical tests were conducted at the Laboratoire de Chimie Analytique et Analyse des Médicaments (LCAM) of the Faculté des Sciences de la Santé (FSS) in Cotonou, Benin. The sterility tests were performed in the national laboratory (Agence Nationale de Contrôle de Qualité des produits de santé et de l'eau, ANCQ) at the Ministry of Health (Cotonou, Benin). Samples were collected from the hospitals from December 20 to 27, 2019, and analyses were conducted from January 3 to August 31, 2020.

Each sample was screened using the form mentioned earlier (see supplemental materials, Appendix 1). A medicine was defined as poor quality when it did not conform to at least one of the items on the list. To identify potential poor quality, we recorded information including integrity, expiration date, batch number, and identifying elements on the primary and secondary packaging; information including indications, contraindications, and side effects on the medication leaflet; and physical appearance, such as color, appreciation of clarity for injection solutions, appreciation of granulometry for powder forms, and odor.

A mass uniformity test was performed for powdered samples (amoxicillin + clavulanic acid, ampicillin, ceftriaxone),

realized with 10 vials taken at random from each sample and weighed individually.

An extractable volume test was done for liquid samples (ciprofloxacin, gentamicin, metronidazole). According to European pharmacopoeia, one bottle of ciprofloxacin, one of metronidazole (100 mL), and five vials of gentamicin (2 mL) were poured in different named test tubes and the volumes were read for each of them.

The pH was measured on reconstituted solutions from dissolved antibiotics powders and from antibiotics for injection as recommended by the USP. Acceptability criteria were those of the USP[24] (amoxicillin + clavulanic acid 5.0–9.0; ampicillin 8.0–10.0; ceftriaxone 6.0–8.0; ciprofloxacin 3.5–4.6; metronidazole 4.5–7.0; gentamicin 3.5–5.5).

The tests used for identification and quantification are summarized in Supplemental Table 1. We used the most common, generic, and validated analytical methods (i.e., UV-VIS spectrophotometry, HPLC-diode array detection [DAD]) described in the literature and pharmacopoeia for five of the six antibiotics: amoxicillin + clavulanic acid, ampicillin, ceftriaxone, ciprofloxacin, metronidazole. The identification and quantitative dosage of gentamicin as described in the pharmacopoeias and the literature consulted (microbiologic titration, molecule derivatization) appeared more complex and required expensive reagents and sophisticated equipment not available in Benin. We therefore used TLC to identify gentamicin and then used a validated conductometry method for gentamicin quantification.

UV-VIS spectrophotometry was used to identify ceftriaxone by comparing the UV spectra of the samples to those of reference standard. This test used the method described by Muhindo et al.[25] See supplemental material, "Methods."

UV spectrophotometric identification was performed by recording and comparing the absorption spectra from the reference and sample test solutions acquired in the range between 200 and 400 nm.

The remaining molecules (amoxicillin, ampicillin, ciprofloxacin, clavulanic acid, metronidazole) were identified using HPLC-DAD and UV-VIS spectrophotometry.

The gentamicin samples underwent identification using TLC. The test solutions and the reference solution of gentamicin sulphate (USP) were prepared in ultrapure water to obtain a concentration of 1 mg/mL (see supplementary material, section "Methods"). Gentamicin was identified by comparing the frontal ratios (Rf) and the spots of the samples to those of the reference.

Quantitative analyses of active pharmaceutical ingredients (API) were carried out using HPLC-DAD, UV-VIS spectrophotometry, and conductometry.

The test sample solution of ceftriaxone prepared according to the method of Muhindo et al.[25] was placed in a cuvet and submitted to a wavelength of maximal absorption of ceftriaxone (240 nm). Three assays were done on each sample. The mean values for the absorbances of the sample and for the chemical reference substance (CRS) solutions were used to calculate the percentage of active ingredient.

The test sample solutions for amoxicillin + clavulanic acid were prepared following the method of Durga et al.,[26] and the test sample solutions for ampicillin, metronidazole, and ciprofloxacin were prepared according to the USP.[24] See detail in supplemental material, "Methods."

HPLC analyses were carried out for amoxicillin and clavulanic acid. USP methods, with a few adaptations, were used for metronidazole, ampicillin, and ciprofloxacin as described in Supplemental Table 2. The chromatographic separation was done in isocratic elution mode. All the data were analyzed according to USP specifications.[24]

Conductometry methods were validated before analyzing the samples of gentamicin. A stock solution of calibration standard (CS) of gentamicin (CRS, 1,000 μg/mL) and sample solutions were prepared based on a method adapted from that described in the literature for aminosides[27] (see detail in supplemental material, "Methods"). Three assays were made for each sample by measuring the conductance of each solution with the conductometer. The mean values of the conductance for the sample solutions and the CRS solution were used to calculate the percentage of the active ingredient (% PA) as described in the supplemental materials. The acceptance limit was 90% to 125% according to the USP recommendation.[24]

We considered the validation criteria commonly used in analytical procedures set out in document Q2 (R1) of the International Conference on Harmonization[28]—namely, selectivity/specificity, trueness, precision (repeatability and intermediate precision), accuracy, linearity, limit of detection, and limit of quantitation (see details in supplemental materials). The acceptance limit was set at ±10% according to the International Pharmacopeia (3rd edition, 2003) and considering consumer risk and the intended use of the analytical procedure. The selected calibration model was linear regression because of its high accuracy index (between –5 and +5) and ease of routine use. The concentration results were back-calculated using the calibration curves. These concentrations were used to determine the relative bias, the precision (repeatability and intermediate precision), the β-expectation tolerance intervals at 95% probability level, and the linearity. Validation calculations were performed using e-noval version 3.0 (Pharmalex S.A, Mont-Saint Guibert, Belgium).

Sterility testing of an injectable medication is a decisive criterion contributing to drug safety. The method for culturing microorganisms to test for product sterility has existed since approximately 1930 [Cleanroom technology, 2015, 1] and involves either filtration of the pharmaceutical product (preferred method according to the European pharmacopoeia), or direct transfer of the product into a liquid culture medium. Because of the material available, we opted for direct transfer of the product into a liquid culture medium.[29] The sterility test was performed in a controlled environment under aseptic conditions [Cleanroom technology, 2015, 1]. To ensure that measures taken to prevent contamination of the critical area did not affect the growth of any microorganism present in samples undergoing sterility testing, we used positive and negative controls for each test.

The validation test for the method was performed before the sterility test to inhibit antimicrobial activity. For this, a typical inhibitor medium and a stock solution of each sample were prepared (see supplemental material).

Because the microorganisms (Supplemental Table 3) used can cause severe disease in humans and constitute a danger for directly exposed employees, we wore protective gloves when handling them and worked in class III biosafety cabinets (TELSTAR, Terrassa, Spain).

For noncompliant samples, similar cases would be looked for in the literature and a report made to the Benin medicines' regulatory agency (Agence Béninoise de Régulation Pharmaceutique, https://www.abrp.bj/) as per usual procedures.

RESULTS AND DISCUSSION

In our study, we collected 33 of the planned 36 samples in the formal supply chain. The sample size was about 990 vials of the 1,080 expected, giving a collection rate of 91.7%. Details of the sample are summarized in Supplemental Table 4. At least one of these three molecules (ceftriaxone, gentamicin, metronidazole) were out of stock in three hospitals, indicating incomplete availability of the antibiotics. This incomplete availability is similar to that observed in a study performed in Cameroon and the Democratic Republic of Congo.[11] This situation can cause delay in initiating antibiotic prophylaxis, resulting in reduced effectiveness.

For the following analyses, the results for each product are expressed in terms of nonconformity—that is, a specified requirement not being fulfilled.[30]

None of the samples had expired on the date of purchase, showing good storage management practices at the hospitals of our study. No irregularities were noticed in physical appearance. Visual inspection showed 97% ($N = 32$) conformity and 3% ($N = 1$) nonconformity. This nonconformity concerned the medication leaflet, which did not have the heading "Drug interactions" and was not translated into French as recommended. This finding indicates nonrespect of good manufacturing practices and lack of care by the provider during the supply process. This rate of nonconformity was higher than that reported in a Senegalese study (0%).[31] Contrary to our findings, nonconformity of several indicators of quality has often been reported,[9,11,32] which has a major impact on the quality of the medicine.

All the samples (100%, $N = 33$) met the European Pharmacopeia (8th edition) requirements for extractable volume and mass uniformity. When weighed individually, the deviation of the individual masses from the average mass did not exceed 10%, and the pH values were within USP specifications. Nonconformity for uniformity of mass results from poor mixing during manufacture but can also be due to uneven distribution of the powder in containers. Nonconformity for uniformity of mass or extractable volume affects the quantity of active ingredient contained in the drugs. The results from this analysis are consistent with the samples having been made using good manufacturing practices and also demonstrate good supply chain practices and good storage conditions in the formal supply chain in Benin.

For all the samples tested, 88% ($n = 29$) passed the tests for the API dosage and 12% ($N = 4$) did not.

Ceftriaxone samples collected in hospitals C1, C3, C4, C5, and C6 had spectra that stacked perfectly with the CSR spectrum. The antibiotics available in these hospitals thus contained ceftriaxone as indicated.

The frontal ratios (0.4), color, intensity, and size of spots of the five samples of gentamicin for injection (E1, E2, E3, E4, E6) were the same as those of the CRS. The antibiotics collected in these hospitals thus contained gentamicin as claimed.

As shown on the chromatogram in Figure 1, the retention time of chemical reference substances matched the major peak in the samples.

On the multi-chromatogram in Figure 2, the first set consisted of six samples, coded A1, A2, A3, A4, A5, A6, claiming to contain a fixed dose of amoxicillin and clavulanic acid (1 g/0.2 g); the second set consisted of six samples coded B1, B2, B3, B4, B5, and B6 and contained ampicillin 1 g; the third set consisted of six samples coded D1, D2, D3, D4, D5, and D6, and contained ciprofloxacin 0.2 g; the fourth set coded F1, F2, F3, F4, and F5 contained metronidazole 0.5 g.

Supplemental Table 5 summarizes the results of the content analyses. All the batches of ampicillin, ciprofloxacin, metronidazole, and ceftriaxone were close to the labeled amounts and within specifications. However, the API contents for amoxicillin and clavulanic acid showed mixed trends. The trends of samples A2 to A6 were all within the same range, whereas the content in A1 had a higher value (114.8% $\pm$ 0.6) for amoxicillin than specified and a lower value for clavulanic acid (95.8% $\pm$ 0.6). This sample was the only one with a different trademark among the six collected, which may explain the observed result.

For ampicillin, there was a trend to less than 100% content for all samples and for ceftriaxone a trend greater than 100%, with some standard deviations greater than 1. For ciprofloxacin and metronidazole, the API trends were also mixed but in the same range. It is important to highlight that some of the standard deviations were greater than 1 and 2, but this has no significant importance.

Validation of the method to assay gentamicin gave the following results. Supplemental Table 6 (see supplemental material online), we presented the most appropriate selected regression models, sorted according to the accuracy index. The accuracy profiles for gentamicin are given in Figure 3, and the validation criteria are summarized in Supplemental Table 7, where:

- trueness was expressed in terms of absolute bias (in micrograms per milliliter) or relative bias (%) at each concentration level of the validation standards. The trueness of the developed method was good with absolute and relative biases $< 1.8\%$.
- precision was expressed in terms of relative standard deviation values for repeatability and for intermediate precision and did not exceed 2.2%. This indicates good precision of the developed method.
- the linearity of an analytical method is the ability to obtain results directly proportional to the concentration (quantity) of the analyte in the sample within a definitive range. Good linearity was observed between the results (here back-calculated concentrations) and the introduced concentrations, with excellent determination coefficient (R^2 of 0.9995; Supplemental Table 7). The accuracy of the method was therefore demonstrated also by assessing the accuracy profile (Figure 3) for which the relative β-expectation tolerance intervals were within a range of –1.8 to 4.8%, and the relative expanded uncertainly was $< 4.8\%$.

Because the lower and upper tolerance bounds were within the acceptance limits for all the targeted concentration levels, each future result will fall within the acceptance range with a probability of at least 95%.[33] The dosing range is the interval between the lower and the upper limits where the procedure achieved adequate accuracy; dosing ranges were 30 to 90 μg/mL.

The validated method was then used to determine the content of API in the samples of gentamicin injection (E1, E2, E3, E4, and E6). The results obtained for the analyses, consisting of the mean percentage of claimed nominal content and the standard deviation computed on three independent samples (Supplemental Table 8). The gentamicin contents in the

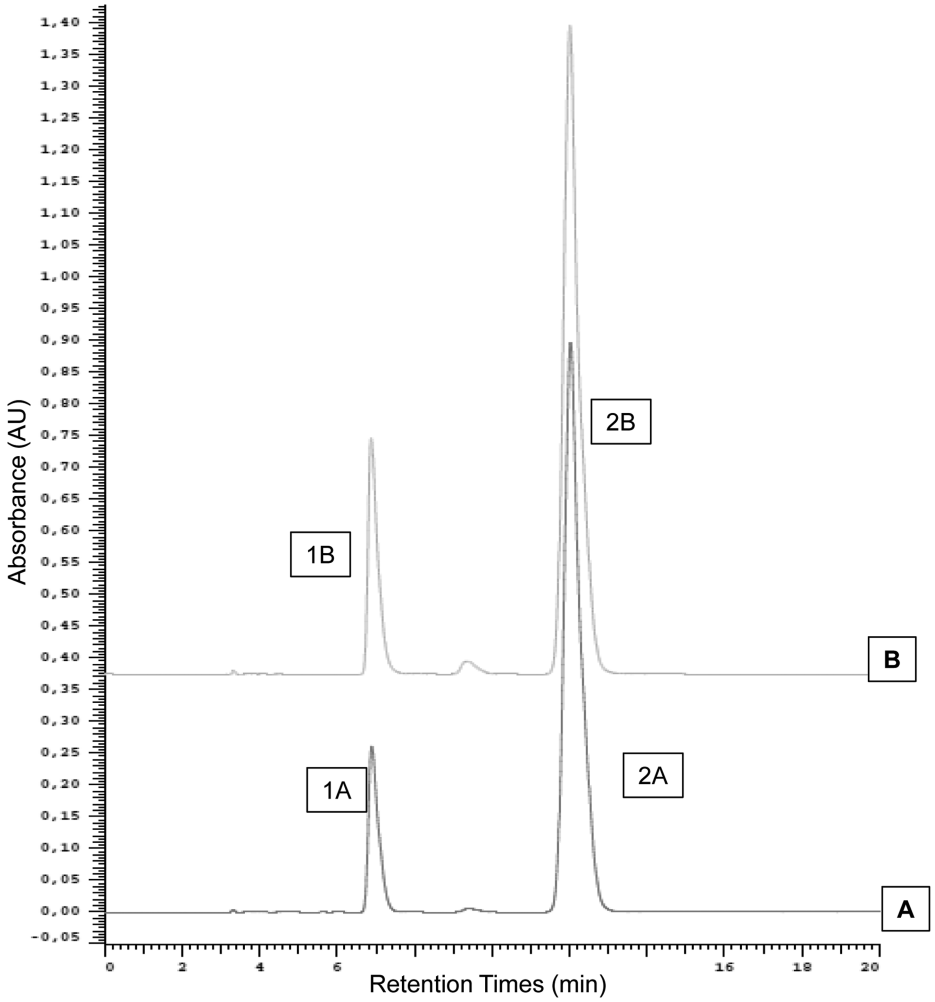


FIGURE 1. Typical chromatograms of amoxicillin and clavulanic acid chromatographic pics of clavulanic acid (**1A**) and amoxicillin (**2A**) in reference solution at 220 nm. Chromatographic pics of clavulanic acid (**1B**) and amoxicillin (**2B**) in sample solution coded E1 at 220 nm. This figure appears in color at www.ajtmh.org.

injection samples were within 124.7% to 126.5%. Four of the samples (E1, E2, E4, and E6) were out of the specification ranges, which can imply a risk of overdosage for the patient. Considering the risk of gentamicin ototoxicity and nephrotoxicity, particular attention must be paid to this product content. Especially that the hospitals do not perform the monitoring pharmaco-therapeutic in patients under gentamicin.

In view of the results, all the samples trended toward higher values. Only one sample (E3) was within the specifications. Curiously, this sample had the same manufacturer as samples E2 and E4 but a different distributor. We do not think that this difference could have an impact on the product. Moreover, falsified medicines usually have low quantities of API to make more profit.

The method applied is simple, fast, robust, easy to perform, and does not require large equipment; thus, it is well suited for routine checks. However, it is not very selective because all the ions present in the analyzed medium can interfere with the process. This factor may explain the risks of excessive doses observed during the application of the method. It could be relevant to combine a separation method (HPLC) with a conductivity detector.

After the third day for bacteria and the fifth day for fungi, no turbidity was noticed in the culture mediums, indicating the absence of growth of microorganisms. All the samples (100%, $N = 33$) met this requirement. We can conclude that the antibiotics available for prophylaxis in the hospitals did not represent a risk of contamination for patients.

In summary, most of our samples were conform (Supplemental Table 9), probably because our samples were taken from the formal supply chain. Other studies in less formal settings in developing countries reported contrasting findings.[9,34] It is likely that the good quality of the antibiotics available in the included hospitals is linked to easy access to legal providers of medicines and to the locations of the hospitals in southern Benin, where they are within range of the regulatory authorities. Furthermore, the repression operation, Operation PANGEA IX, which attempts to curtail the use of medicines from the informal supply chain, may also have had an impact.[35] Nevertheless, oral forms of antibiotics are often nonconform,[9,11,32] likely because oral forms are more widely used, their manufacture does not require many resources, and, in contrast to injectable forms, a qualified person is not needed for their use.

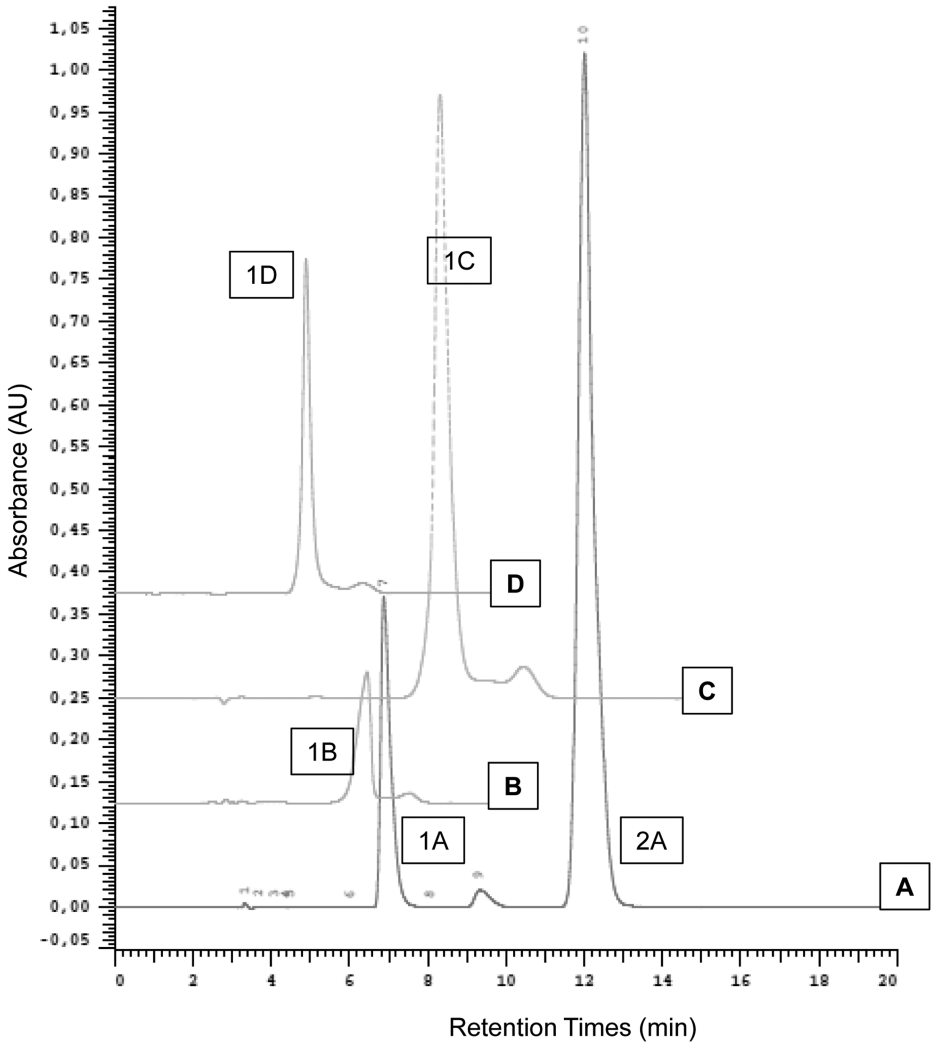


FIGURE 2. Typical chromatograms of four antibiotics chromatographic pics of clavulanic acid (**1A**) and amoxicillin (**2A**) in sample solution coded E1 monitored at 220 nm. Chromatographic pic of ampicillin (**1B**) in a sample solution coded E5 monitored at 254 nm. Chromatographic pic of ciprofloxacin (**1C**) in a sample solution coded E5 monitored at 278 nm. Chromatographic pic of metronidazole (**1D**) in a sample solution coded E5 monitored at 319 nm. This figure appears in color at www.ajtmh.org.

From our results, we can hypothesize that the occurrence of surgical site infections and antimicrobial resistance in the six Beninese hospitals[22] is not a result of poor-quality antibiotics used for prophylaxis.

Our study results are strengthened by the anonymous and in situ samplings. The antibiotics analyzed were selected on the basis of results previous studies that audited antibiotic prophylaxis practices in surgery. The hospitals where sampling was performed were from different levels of the healthcare system. Brand and generic drugs were sampled from various manufacturers, providing diversity to our sample. The samples were analyzed using low-cost and validated methods, with feasibility of routine testing in developing countries such as Benin.

Because of the lack of equipment, we did not perform the endotoxin test on the samples to guarantee their nonpyrogenic status. Because this study comprised only six hospitals located in southern Benin, our results cannot be generalized to the whole country because of unequal means and supply possibilities in hospitals far from the economic capital (Cotonou). Nevertheless, all hospitals can procure medicines from the legal, public, and central provider, Société Béninoise pour l'Approvisionnement en produits de Santé [SoBAPS]; EX-CAME), which has many offices across the country.

CONCLUSION

This study contributes to the evaluation of the quality of the antibiotics used primarily in surgical infection prophylaxis in Benin. To the best of our knowledge, this is the first study to assess the quality of intravenous antibiotics used in surgery. The Beninese national agency of quality control (Agence Nationale de Contrôle de Qualité des Produits de Santé et de l'Eau; www.ancq.bj) performs tests before all drugs are marketed, but during marketing, testing is performed mostly for oral forms. This study included 33 samples of 30 units collected from six hospitals. Results showed that 97% ($n = 32$) of the samples passed the visual inspection, 100% ($N = 33$) of the samples passed the pharmacotechnical tests and identification of active ingredients, and 88% ($n = 29$) passed the dosage of active ingredients; the remaining 12% of samples carried a risk of overdosage, with four of the five samples of

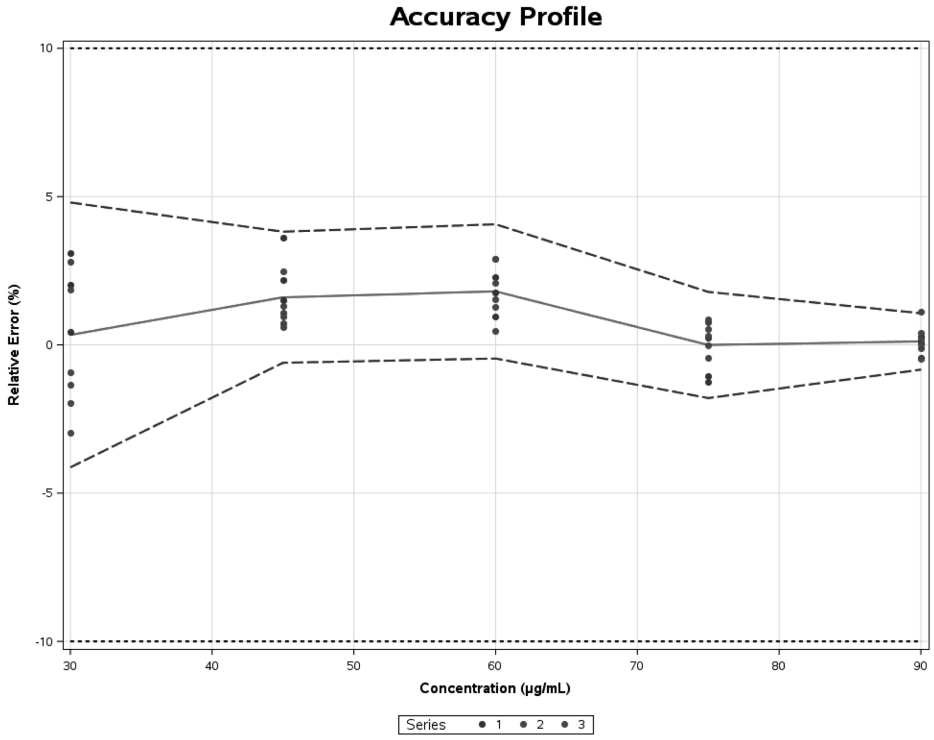


FIGURE 3. Accuracy profile obtained by using linear regression model. The plain red line is the relative bias, the dashed blue lines are the β-expectation tolerance limits, and the dashed black lines represent the acceptance limits. The dots represent the relative error of the back-calculated concentrations and are plotted with respect to their targeted concentration. This figure appears in color at www.ajtmh.org.

gentamicin sulfate not conform to the specifications of USP in terms of dosage. All the samples (100%, $N = 33$) passed the sterility test. Apart from the gentamicin, this preliminary study confirms the good quality of the antibiotics administered for surgical infection prophylaxis in Benin. Our results help reassure prescribers of the reliability of the formal drug supply chain and enable patients to be confident in the drugs provided via the formal supply route. In the context of rare routine laboratory controls, this study showed good feasibility of tests. The quality of antibiotics is a cornerstone in infection prevention and control. Thus, regular, large-scale analyses in various hospitals are important to maintain appropriate healthcare.

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Note: Supplemental materials appear at www.ajtmh.org.

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