Antimicrobial Resistance in Hospitalized Surgical Patients: a Silently Emerging Public Health Concern in Benin.

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Research

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Abstract

Background: Surgical site infections are related to high morbidity, mortality and healthcare costs. As the emergence of multidrug-resistant bacterial pathogens in hospitals is becoming a worldwide challenge for surgeons who treat healthcare-associated infections, we wished to identify the causative agents involved in surgical site infections and their susceptibility pattern in six public hospitals in Benin.

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

Results: We included 304 patients (mean age 32 ± 11 years), whose median length of stay was 9 days. A total of 259 wound swabs (85.2%) had positive aerobic bacterial growth. In obstetrics S. aureus (28.5%, n=42) was the most common isolate. In contrast, Gram-negative bacteria (GNB) were predominant in gastrointestinal surgery. The most dominant being E.coli (38.4%, n=31). Overall, 90.8% (n=208) of aerobic bacteria were multidrug resistant. Two-third of S. aureus (65.3%, n= 32) were methicillin-resistant Staphylococcus aureus (MRSA), three of which carried both MRSA and induced clindamycin resistance (ICR). GNB showed high resistance to ceftazidime, ceftriaxone and cefepime. Extended-spectrum beta-lactamases were presented by 69.4% of E.coli (n=43/62) and 83.3% of K. pneumoniae (n=25/30). Overall, twelve Gram negative bacteria (5.24%) isolates showed resistance to at least one carbapenem. No isolates showed a wild-type susceptible phenotype.

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

Background

Surgical site infection (SSI) is defined as an infection that occurs within thirty days at an operative site if no graft is left in place after surgery or within ninety days if an insert is left in place [1]. In the United States, SSIs are the third common hospital-acquired infections, accounting for 38% of all nosocomial infections according to the National Nosocomial Infection Surveillance System of the Center for Disease Control (CDC’s) [2]. In countries with limited resources, SSIs are the most common in the overall patient population, affecting up to 66% of operated patients and nine times more than in industrialized countries [3]. SSIs increase the length of post-operative hospital stay, the cost of healthcare, and the rate of hospital readmissions [4].

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

The battle between bacteria and their susceptibility to drugs remains difficult for the public, researchers, clinicians, and also for drug companies that seek effective drugs. In addition, SSI by resistant drug bacteria is now become serious problem in developing countries such as Benin, due mainly to poor infection prevention and control programs, crowded hospital environments, widespread inappropriate uses of antibiotics, and the irrational prescription of antimicrobial agents. [10]. Due to these inadequate SSI-surveillance programs healthcare centers are unable get updates on bacteria that are resistant to antimicrobial drugs [11]. The correct antibiotics are both expensive and hard to access [12]. Use of broad-spectrum antibiotics is common. In Benin, most clinical laboratories are not equipped with testing facilities that can detect multidrug-resistant bacteria [13]. Although appropriate knowledge of these pathogens, of their resistance, and of updated antimicrobial therapy is crucial to the treatment process and to infection-control measures [14], these laboratories have little data on their patterns of antimicrobial resistance. We therefore determined the bacterial pathogens from hospital-acquired SSIs and described their antimicrobial resistance patterns in six public hospitals in Benin.

Methods And Materials

Study design and setting

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

Patients who consented to participate in the study were included from January 2019 to January 2020. Patients who had initial surgery in another hospital or ward (internal medicine or emergency) and those who did not volunteer to participate were excluded from the study. We included two wards (obstetrics and gastrointestinal ) at six public hospitals: Bethesda, Centre Hospitalier Universitaire de Zone de Suru Lere, Centre National Hospitalier Université Hubert Koutoukou Maga (CNHU-HKM), Centre Hospitalier Universitaire de la Mère et L’enfant (CHUMEL), Centre Hospitalier Universitaire Départemental Ouémé/Plateau (CHUDOP) and Centre Hospitalier Universitaire de Zone d'Abomey Calavi. All participating hospitals were located in the south of Benin, thereby allowing daily transport from each hospital to the CNHU-HKM laboratory, where all collected wound swabs were analyzed. All results were confirmed in the laboratory of the Cliniques Universitaires Saint-Luc-UCLouvain (Brussels, Belgium).

Sampling and data collection
The case definitions and clinical criteria of SSIs (Superficial, incisional SSI, deep incisional SSI and organ/space SSI) were according to the guidelines on prevention of SSI by Centers for Disease Control and Prevention [2]. A preliminary step in the project consisted of training nurses in the proper sampling technique. These trained nurses collected all the samples were per hospital. One swab was collected aseptically from each patient using a sterile cotton swab on the day a clinical SSI was detected. Each sample was labeled properly with the date of sample collection, the collection method and the patient's details. The swab was immediately dipped into a sterile tube with transport medium (Amies, Beckton Dickinson) and delivered to bacteriology laboratory at CNHU-HKM in Benin.

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

**Processing of samples**

**Macroscopic and microscopic examination of samples**

All the specimens were visually examined for consistency, color, turbidity, presence or absence of blood. Gram staining of each specimen was performed [15].

**Culture of specimens and bacteria isolation**

Bacterial identification was performed according to the standards for microbiological methods of the European Committee of Antimicrobial Susceptibility Testing guidelines [16]. In Benin, cultures were incubated a total of 48h (in case of no growth at 24h) at 37°C in aerobic atmosphere, and then examined for microbial growth. Identification of Gram-positive bacteria was done by Gram staining, catalase test, coagulase test, DNase test and Pastorex staphylococci plus test (Pastorex, staph plus Biorad). Gram-negative strains were identified by different biochemical tests: oxidase, and characteristics of Analytical Profile Index (API 20E, Biomerieux, Lyon) such as Voges Proskauer (VP) test, indole test and citrate utilization.

All identifications were confirmed in Belgium by using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry (Brucker Daltonics, Bremen, Germany). Due to lack of equipment, anaerobes were not analysed.

**Antimicrobial Susceptibility Test (AST)**

Antimicrobial susceptibility testing was performed for all isolates following the modified Kirby Bauer disc diffusion technique as described in European Committee on Antimicrobial Susceptibility Testing guidelines [16]. Antibiotics were purchased from BioRad (Marnes-la- coquette, France) and included for *S. aureus*: ampicillin (10 , cefotaxime (30 g), cefoxitin (30 , gentamicin (10 ,amikacin (30 ), ciprofloxacin (5 ),
trimethoprim + sulfamethoxazole (25 g), tetracycline (30 g) and chloramphenicol (30 g). The Gram-negative organisms were tested for ampicillin 10, piperacillin 100 g, cefotaxime (30), cefoxitin (30), ceftiraxone (30 g), gentamicin (10), tobramycin (10), amikacin (30 g), ciprofloxacin (5), trimethoprim + sulfamethoxazole (25 g), imipenem (10), and meropenem (10). For non-fermentative Gram-negative bacteria, the discs used included ceftiraxone (30), ceftazidime (30), gentamicin (10), ciprofloxacin and meropenem (10 g). After 24 h of incubation at 37°C, the inhibition zones were measured and the results were analyzed.

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

MDR was defined as non-susceptible to at least one agent in three or more antimicrobial classes [17]. The presence of ESBL was detected using the double-disc synergy test (DDST) between clavulanate and third-generation cephalosporins and/or aztreonam [18].

Methicillin resistant *S. aureus* isolates were detected using the cefoxitin disk (30 g) method. The diameter of the zone of inhibition for cefoxitin was < 21mm [19]. Similarly, inducible macrolide-lincosamide streptogramin-B (iMLS\textsubscript{B}) resistance was detected in *S. aureus* with the D-test disk method using clindamycin (2ug) and erythromycin (15ug) on MHA plates. After overnight incubation, isolates with a flattened zone of inhibition adjacent to the erythromycin disk (referred to as a “D” zone) were considered to exhibit inducible clindamycin resistance [20]. The presence of resistance to at least one carbapenem was checked with the RESIST-30.K.N.ICT (Coris Bioconcept, Gembloux, Belgium), which detects OXA 48, KPC and NDM carbapenems. The final results of the ICT test were read when they became positive, at the latest after 15 min. All ESBL, MRSA and CPO strains were confirmed and characterized by whole genome sequencing.

**Quality control**

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

**Data analysis and statistical tests**

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

Socio-demographic and clinical characteristics

A total of 304 wound swabs were collected from 174 patients with clinical signs of surgical site infections. Obstetrics patients (n=148; median age 29 ± 07 years) ward represented 195 swabs (64.1%). The median length of stay in obstetrics was 9 days (6-IQR-14). In gastrointestinal surgery, the age ranged from 18-76 years with mean of 38 ± 14 years. A large majority of the patients were females (80.3%) . The length of stay in gastrointestinal surgery was nine days. Emergency surgery was the most common type of surgery (82.6%).

Surgical antimicrobial prophylaxis

Of the samples collected, 172/304 (56.6%) originated from patients who had received preoperative antimicrobial prophylaxis for more than 24h after surgery. Many patients received monotherapy with ceftriaxone 57/172 (33%) followed by ampicillin 23/172 (13%) and amoxicillin/clavulanic acid 17/172 (9.9%). The most prescribed regimen among the combination regimens was ceftriaxone + metronidazole 35/172 (20%). There was no difference between the antimicrobial classes used in obstetrics and gastrointestinal surgery.

Bacterial etiologic agents isolated per ward

Among the 304 cultures of wound swabs, 259 (85.2%) were positive for aerobic bacterial growth. Forty-five yielded negative results while 85 were excluded because they presented more than two germs. Of the 174 remaining samples, 55 yielded polymorph flora with more than one species (24%) while 119 (52%) yielded single isolates. Altogether, 229 isolates were identified while Gram-positive microorganisms represented 21.4% (n=49) of isolates 78.6% (n=180) were Gram-negative. Whereas *Staphylococcus aureus* (28.5%, n=42), *Pseudomonas aeruginosa* (21.6%, n=32) and *E. coli* (20.9%, n=31) were the most frequent in the obstetric ward. *Escherichia. coli* (38.4%, n=31), *Klebsiella pneumoniae* (21.0%, n=17) and *Enterobacter cloacae* (12.3%, n=10) were the most prevalent in gastrointestinal surgery. (Figures 1 and 2)

Drug resistance patterns of isolates to different classes of antibiotics

*Staphylococcus aureus*

All Gram-positive organisms were *S. aureus*. Almost all *S. aureus* isolates were resistant to penicillin (98%, n=48/49) and 32 isolates (65.3%) showed resistance to methicillin (MRSA phenotype). Resistance to cotrimoxazole was found in 10.2% of isolates, to gentamicin in 38.8% and to ciprofloxacin in 36.7%. Ninety-eight percent of isolates were susceptible to clindamycin and no resistance to vancomycin was observed. (Figure 3).

Gram-negative bacteria
While all GNB showed resistance to multiple antibiotics tested, amikacin and imipenem remained the most active (95.9% and 99.2% respectively). However, resistance to other aminoglycosides is important (61.5% for gentamicin). Almost all Enterobacteriales isolates (99.2%) showed resistance to ampicillin. Three quarters (75.4%) were resistant to ceftriaxone, 76.2% to cefotaxime and 73.8% to cefepime. Resistance to quinolone reached 68.9% for ciprofloxacin. There was also a high level of resistance to the cotrimoxazole (83.6%). We noted 6.6% of Enterobacteriales which were resistant to at least ertapenem (figure 4).

**Gram-negative non-fermenters**

The non-fermentative bacteria (*Acinetobacter baumannii* n= and *P. aeruginosa* n=) showed low resistance to ciprofloxacin (20.7%), ceftazidime (20.7%), gentamicin (31.0%) and Piperacillin/tazobactam (34.5%). Unfortunately, 8.6% and 10.3% of non-fermentative bacteria were resistant to imipenem and 10.3% were resistant to meropenem (Figure 5).

**Prevalence of ESBL and Carbapenem resistant isolates of gram negative rods.**

Forty-three of the 62 isolates of *E.coli* (69.4%) were ESBL producers, as were 25 of the 30 (83.3%) *K. pneumoniae* isolates, and 16 of the 21 *E. cloacae* (76.2%) isolates. Five isolates (8%) of *E. coli* and three *E. cloacae* (14%) were resistant to at least one carbapenem tested (imipenem, meropenem and ertapenem). Three isolates of *Paeruginosa* isolates (8%) and one of *A. baumannii* (5%) showed resistance to carbapenems. With the RESIST -3 O.K.N.ICT test, we detected NDM in *A.baumannii* and VIM in *Paeruginosa*. According to the PCR, the ESBL isolates harbored mainly CTX-M₃ enzymes. The molecular description of these genes will be reported soon.

**Multidrug Resistance Pattern of Bacterial Isolates**

As well as large majorities of of *E.coli* (93.5%), and *E. cloacae* (95.2 %), 69.4% of *S. aureus* strains were MDR (resistant to two or more antimicrobial classes). All *P. aeruginosa* and *A. baumannii* strains were resistant to more than five classes of antibiotic (Table 1).

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

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

### Discussion

This study provides insight into the causative bacteria and sensitivity profiles of SSIs in six hospitals in Benin. Overall, 90.8% of aerobic bacteria were multidrug resistant organisms, an MDRO rate that was higher than those described in Ethiopia and Uganda [21,22]. Three main factors may have contributed for these high rates of MDRO. The first is likely to have been associated with the country’s overall lack of antimicrobial resistance surveillance and stewardship programs. There is enough evidence to indicate that, by improving the use of antibiotics, such programs help both to understand the pattern of resistance and to prevent the development of antibiotic resistance [6]. The second reason might be associated with the lack of comprehensive national policies on antibiotics usage. Instead, it is common practice in Benin to buy antibiotics including large spectrum ones, without prescription, from private drug vendors and pharmacies. The third reason may also be due to the lack of diagnosis laboratory services before the use of antibiotics by clinicians who do not have an antibiogram or evidence of the causative agents.

The most common isolates in obstetrics were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This finding is in line with other studies that described *S. aureus* to be associated with SSIs in obstetric wards [23–25]. *S. aureus* is considered as a commensal organism of the skin and can easily contaminate a wound [26]. The high frequency of *P. aeruginosa* could be explained by the fact that this bacteria is intrinsically resistant to ceftriaxone, which was the most common used drug for prophylaxis in Benin.

According to the AST results, almost all (98%) of *S. aureus* were resistant to penicillin. While similarly high resistance of *S. aureus* to penicillin was also reported in Uganda and Nepal [21,24], resistance to ampicillin was observed in only 4% of isolates in India [27]. Such variations in the susceptibility pattern may be attributed to differences in the rational use of antibiotics. In Benin, ampicillin has also been widely used as a prophylaxis after ceftriaxone. Some two thirds of our isolates (65.3%) were resistant to cefoxitin and were reported as MRSA species. Upreti and Shrestha in Nepal found the same rate of MRSA
In a retrospective single center study conducted in 2016 by Mercy Ship during surgical outreaches in six sub-Saharan African countries (Benin, Togo, Liberia, Madagascar, Congo and Sierra-leone) Lai PS et al found the highest rates of MRSA in Benin (34.6%) and Congo (31.9%) and the lowest rate in Togo (14.3%) and Madagascar (14.5%) [30]. The difference in the rates of isolation of MRSA between studies may be due to different levels of inappropriate use of antibiotic but also to the effectiveness of hygiene programs. In a previous study [31], we found hand-hygiene compliance among Benin healthcare providers to be only 33.3%. The treatment of infections caused by MRSA may also require the use of reserve drugs such as glycopeptids or lincosamides. However, the fact that we observed no resistance to vancomycin in our study can be explained by the fact that this antibiotic was not available in Benin.

E. coli, K. pneumoniae and E. cloacae were the commonest isolates in gastrointestinal surgery in our study. The predominance of E. coli has been reported in some other recent studies [32–34]. In Morocco and Uganda, the authors showed K. pneumoniae to be the predominant Gram-negative bacteria [21,33]. This predominance could be attributed to their diverse habitat, which includes inanimate surfaces in hospitals, to their multidrug resistant pattern and to possible contamination from the intestinal tract during surgery. The bacteria most frequently involved in SSIs change from time to time and also vary with hospital settings. Our finding that GNB showed high resistance to ceftriaxone, ceftazidime, cefepime, and cotrimoxazole is in agreement with various studies worldwide [35–38]. The high rate of bacterial resistance against ceftriaxone is likely due to frequent use of this antibiotic in and outside hospitals. Our finding that almost all P. aeruginosa and A. baumannii were sensitive to amikacin and had relatively moderate resistance to cefepime (15.5%), ceftazidime (20.7%), and ciprofloxacin (20.7%) are similar to those in studies in Nepal and India [28,29], which observed moderate resistance to ciprofloxacin (6.2% to 24%). High sensitivity to imipenem and amikacin may be due to the limited exposure of these drugs to the prescription antibiotics that are relatively more expensive and are not constantly available in Benin.

The emergence of ESBL-producing Gram-negative rods have attracted increasing concern in the developing world [12]. The majority of Enterobacterales isolates in our study were ESBL producers. Upreti et al have reported that 25% of E. coli and 40% of K. pneumoniae are ESBL producers. In 2016, Benin was found to have the highest rate of third generation cephalosporin resistant enterobacterales of six sub-Saharan African countries [30]. Almost all ESBL producers isolates showed co-resistance to other class of antibiotics such as aminoglycosides, quinolones, and trimethoprim-sulfamethoxazole. Although patients in the present study received prophylactic antimicrobials such as ceftriaxone and ampicillin prior to surgery, the antibiogram results showed that the isolated organisms were resistant to these antimicrobial agents. Ceftriaxone is also known to favor the emergence of ESBL. This high antibiotic resistance implies that if immediate action is not taken, recommended antibiotics such as cefazolin might be rendered useless.

In our study, 10.3% of Paeruginosa and A. baumannii showed resistance to meropenem and constituted pan-drug resistance bacteria according to the Magiorakos classification [17]. This resistance was due to mechanisms that are expressed frequently in hospital-acquired strains of Acinetobacter and Pseudomonas such as beta-lactamases, alterations in cell-wall channel (porins) and efflux pumps. Due to
the unavailability of an effective last therapeutic option such as ceftazidime-avibactam the increasing rate of carbapenemase producing organisms in this study is a great concern [39].

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

**Limitations of study**

The strength of this study lies in its prospective nature. However, a limitation should also be noted: Our study did not isolate strict anaerobes, which may have increased the number of bacterial isolates currently reported negatives. Relevant additional information would be produced by further studies. Molecular characterization of MDRO would have generated more useful epidemiological results.

**Conclusion**

This study indicates the existence of high drug resistance bacteria in surgical site infections in Benin. As two-thirds of isolates were producers of ESBL and MRSA, this prevalence particularly concerns GNB and *S. aureus*. None of the isolates showed a wild-type susceptible phenotype. With regard to reducing the spread of multidrug-resistant bacteria in Benin, these findings represent an urgent call for the judicious use of antibiotics, for network surveillance of antimicrobial resistance, and for strict adherence to good hand-hygiene practice.

**Abbreviations**

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

**Declarations**

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**Author’s contributions**

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

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**Availability of data and materials**

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

**Ethics approval and consent to participate**

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

**Consent for publication**

Not applicable.

**Competing Interests**

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

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**Figures**
Figure 1

Flow chart of the patient enrollment and data collection. GPB: Gram-positive bacteria, GNB: Gram-negative bacteria, NFGB: Non-fermentative Gram-negative bacteria
**Figure 2**: Proportion of bacterial species per ward: Obstetrics and Gastrointestinal

**Figure 3**: Antimicrobial resistance pattern among *S. aureus* isolated from pus specimen
Antimicrobial resistance pattern among S.aureus isolated from pus specimen

Figure 4: Antimicrobial resistance pattern among Enterobacteriales isolated in pus

Antimicrobial resistance pattern among Enterobacteriales isolated in pus
Figure 5: Antimicrobial resistance pattern among non-fermentative bacteria isolated in pus

Antimicrobial resistance pattern among non-fermentative bacteria isolated in pus