*Original Research Article*

# Journal of Biological Research & Biotechnology

Bio-Research Vol. 18 No.2; pp.1164-1176(2020). ISSN (print):1596-7409; eISSN (online):9876-5432

## Molecular characterization of Enterobacteriaceae producing β-lactamase and methicillin-resistant staphylococci isolated from the hospital environment and catheters in two public hospitals in Benin, Republic of Benin

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## Abstract

Antimicrobial resistance is a real public health problem. All over the world, it has a considerable impact in hospitals. The present study was conducted to ascertain the bacterial ecology in two hospitals in Benin as well as the resistance genes present in the recovered isolates. A total of 146 environmental and catheter samples were collected at the University Hospital Center of Abomey-Calavi / So-Ava and at the Beninese Army Hospital of Cotonou. These samples were inoculated on Mannitol Salt and Eosin Methylene Blue agars. The colonies obtained were identified and their sensitivity to antibiotics were tested, using the Kirby Bauer technique. Four resistance genes encoding the production of extended-spectrum beta-lactamases (blaCTX-M1, blaCTX-M2, blaCTX-M9, blaCTX-M15) and the gene coding for methicillin resistance (mecA) were screened. The gene coding for methicillin resistance (mec*A*) was sought in staphylococci. A total of 69 (53,49%) and 60 (46,51%) strains belonging to Enterobacteriaceae family and staphylococci were identified, respectively. A predominance of *Staphylococcus aureus* (25.6%) followed by *Enterobacter cloacae* (21.0%) and coagulase negative staphylococci (21.0%) was observed. These bacterial strains showed multidrug-resistance, particularly to beta-lactams, fluoroquinolones, aminoglycosides, and macrolides. Beta-lactamases were identified in the genome of bacterial strains with a predominance of blaCTX-M15 (42.8%). The frequency of the mecA gene in staphylococci was 50%. These results show the magnitude of the antimicrobial resistance situation in the hospitals investigated. They can be used to support advocacy for urgent action at the national level, especially with regards to the management and efficient use of antimicrobials in Benin.

**Keywords**: **Antimicrobial resistance, resistance genes, hospital environment, catheters, Benin**

**https://dx.doi.org/10.4314/br.V18i2.5** 
Journal Homepage: ***http://www.bioresearch.com.ng.***
Publisher***: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.***

## INTRODUCTION

Over the past fifty years, the use of antibiotics has led to many therapeutic advances in infectious diseases. However, the massive and sometimes excessive use of antibiotics in the hospital has considerably modified microbial ecology and tends to increase the rate of resistant bacteria (World Health Organization (WHO), 2014; Stewardson et al., 2016; Koudokpon et al., 2018). Thus, the emergence and rapid dissemination of antibiotic resistance is a major problem for human health (Blair et al., 2015). The adaptive power of bacteria is manifested by their ability to appropriate new properties either by modifying their genome (mutations) or by acquiring genetic information via mobile genetic elements such as plasmids and transposable elements (Xia et al., 2012; Wang et al., 2014; Xu et al., 2019). Most bacterial species are able to integrate different determinants of resistance into their genome. Thus, the dissemination of resistance genes between bacteria has led to the emergence of bacteria resistance to several antibiotics in particular methicillin-resistant *Staphylococcus aureus* (MRSA), the enterobacteria producing extended-spectrum beta-lactamases (ESBL) and vancomycin resistant enterococci (VRE) (Naas et al., 2016; Koudokpon et al., 2017; Hagel et al., 2019). Among bacteria species resistant to antibiotics, enterobacteria producing ESBL represent a major global threat to public health (Naas et al., 2016). Often associated with urinary tract infections, they can also cause serious blood stream infections (Naas et al., 2016). *Staphylococcus aureus* is the leader in the family of staphylococci because of its involvement in suppurative, localized or severe systemic pathologies in humans (Boukhatem et al., 2015; Joshi et al., 2017). However, other species of staphylococci (coagulase negative staphylococci) can cause a lot of damage to their hosts. This is the case, for example, with *Staphylococcus saprophyticus*, which is the second most common bacterium responsible for human urinary tract infection after *Escherichia coli* (Koudokpon et al., 2017). According to WHO, carbapenem-resistant gram-negative bacteria, namely,carbapenem-resistant enterobact-eriaceae, *Actinobacter baumanni*, and *Pseudomonas aeruginosa* are critical and high priority pathogens as they are resistant to a large number of antibiotics. Six other bacteria including *S.aureus, Helicobacter pylori, Salmonella, Neisseria Gonorrhoea, Campylobacter spp*. and *Enterococcus faecium* were classified as high priority pathogens while *Streptococcus pneumonia, Haemophilus influenza*, and *Shigella spp.* are of moderate priority (WHO, 2017). Finally, for *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Shigella spp*, the urgency is moderate (WHO, 2017). More than ever, the prescription of antibiotics must consider not only the desired effect on the infection but also the effects on the bacterial ecology. Indeed, faced with the urgency of finding new therapies, preserving existing antibiotics and above all limiting the progression of resistance in the environment, the mastery and use of antibiotics particularly in hospitals is a necessity (Mangin, 2016). Therefore, antibiotic therapy must be justified in order to be prescribed. Preserving the effectiveness of antibiotics requires rapidly and appropriately reducing the use of these molecules but also the knowledge of the genes that can induce resistance (Mangin, 2016). Consequently, it is useful and urgent to have scientific data on the potential presence of resistant bacteria and associated genes in the hospital environment. This study aims to analyze the presence of resistance genes and the ecology of bacterial isolates recovered in two hospitals in Benin.

## MATERIALS AND METHODS

### Sampling

A total of 146 samples comprising environmental (n=105) and catheter (n=41) samples were sourced from patients in two different hospitals (Abomey-Calavi/So-Ava University and Hospital Center (UHC) and Beninese Army Hospital (BAH))''. The swabbed surfaces were hospital beds, internal and external door latches, benches, bedside tables, mobile phones, gallows, drums, carts, cradle, weighing table, sink, oxygen cylinder, etc. Swabs were taken according to ISO 14698-1 (ISO 14698-1, 2003) and then placed in 3 ml of Brain heart infusion broth (BHI Broth) (Lemmen et al., 2001; Cavallo et al., 2002). The samples were collected from catheter as described by Cleri et al. (1980). After disinfecting the hands and wearing disposable sterile latex gloves, the catheters were removed without antisepsis. Five centimeters from the inner end of the catheter were then cut with sterile scalpel blades and placed in a sterile tube containing 3 ml of BHI Broth. All samples were sent to the laboratory for analyzes. After 18 hours of incubation at 37 ° C, the broths were inoculated on agar plates for bacteriological analysis.

**Isolation and identification**

Isolation and identification of bacterial isolates were carried out as previously described by Dougnon et al (2016) and Afle et al. (2019). Briefly, 18-hour subcultures (broths) of bacterial isolates were seeded on agar plates (Mannitol Salt and Eosin Methylene Blue agars) followed by Gram staining of bacterial colonies isolated on each agar. Upon observation, the Gram-negative bacillus (GNB) and Gram-positive cocci (GPC) colonies were then selected. After purification, biochemical identification of GNB was carried out using API 20 E gallery, as described by Koudokpon et al. (2017). For the identification of GPC, catalase, coagulase, and DNase tests were carried out.

**Antibiotic susceptibility testing**

An overnight bacterial pre-culture of isolates was diluted to obtain a turbidity of 0.5 McFarland (in sterile distilled water). The Kirby Bauer technique was then adopted for the susceptibility testing as previously described (CA SFM, 2019). Antibiotics from different families were selected to determine the resistance profile of the recovered isolates.: aztreonam (30 µg); erythromycin (15µg); tobramycin (10µg); ceftriaxone (30µg); ciprofloxacin (5µg); ertapenem (10µg); imipenem (10µg); amoxicillin + clavulanic acid (30µg); gentamicin (10µg); kanamycin (30 µg); streptomycin (10µg) and nalidixic acid (30µg). *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as positive control.

**Molecular detection of resistance genes**

The DNA of the isolates was extracted using Miniprep Kit from Zymo Research (Irvine, United States of America) following the manufacturer's instructions. In order to assess and ensure the quality of the DNA extracts, a verification was carried out by electrophoresis on 1% agarose gel and stained with 0.1% ethidium bromide (BET). The amplified products were visualized on 1% agarose gel run in 1X concentrated tris-borate-EDTA (TBE) buffer at 100V for 15 min. The extracted DNA was stored at -20°C. In total, five specific primer pairs of resistance genes (mecA, blaCTX-M1, blaCTX-M2, blaCTX-M9, blaCTX-M15) were used to assess the resistance of the identified isolates. These resistance genes were detected by simplex PCR (Yu et al., 2007; Bittar et al., 2009). The PCR was performed at a total volume of 25 µl containing 1.5 µl of plasmid DNA; 2.5 µl of each primer (forward and reverse); 1 µl of dNTP; 2.5 µl of PCR buffer (5X); 0.25 µl of Taq polymerase; 0.5 µl of MgCl2 and 14.25 µl of sterile distilled water. Amplification products were separated by 2% agarose gel electrophoresis incorporated with 1 µl of bromide ethidium. The migration was done with TBE 1X buffer for 25 to 35 minutes at 100V. The bands were visualized using UV trans-illuminator ENDURO™ (Labnet, New York, United States of America). the electrophoretic DNA profiles were scored based on the presence or absence of bands of certain size and intensity. Table 1 shows the primers used.

**Statistical analysis**

The data were analyzed with statistical software R version 3.6.1. The difference was significant when $p < 0.05$. The GraphPad Prism 7 software was used to produce the graphs.

**RESULTS**

**Distribution of the isolated bacteria**

Out of the 146 samples collected, 129 (88.36%) bacteria were recovered, with 111(86%) of the bacteria isolated from the environmental samples and 18 (14%) isolated from indwelling catheters. Of all the bacterial strains isolated from the environment, *Enterobacter cloacae* leads with 21% (n = 27), followed by *S. aureus* and coagulase-negative Staphylococci with 17.8% respectively (n = 23). *Serratia spp*. (7.8%), *Flavimonas horyzyhabitans* (4.7%), *Enterobacter spp*. (3.9%), *Pantoae spp*. (3.9%) and *Acinetobacter baumannii* (2.3%) were also recorded. Among isolates from catheter samples, *Staphylococcus aureus* was the most isolated with 7.8% (n = 10) followed by 3.1% coagulase negative staphylococci (n = 4). The rest were *Escherichia coli*: 2 (1.6%), *Shigella spp:* 1(0.8%) and *Klebsiella pneumoniae*:1 (0.8%) (Table 2). The results remained the same in each of the two hospitals where the samples were taken. There was a high prevalence of *Enterobacter cloacae* recovered from environmental samples collected from Abomey-Calavi / So-Ava University Hospital Center (UHC) and in the Beninese Hospital of Army (BHA), with 9 (12.5%) and 18 (31.6%) respectively, followed by *S. aureus* (29.8% for BAH and 8.3% for UHC) and coagulase-negative staphylococci (11 (19.3%) for BAH and 12(16.7%) for UHC) (Table 2).

**Table 1:** Sequences of primers

| Genes | Primers | Sequences (5'-3') | Temperature in °C | References |
|---|---|---|---|---|
| blaCTX-M1 | F | GGTTAAAAAATCACTGCGTC | 51 | Bittar et al., 2009 |
| | R | TTGGTGACGATTTTAGCCGC | | |
| blaCTX-M2 | F | ATGATGACTCAGAGCATTCG | 60 | Bittar et al., 2009 |
| | R | TGGGTTACGATTTTCGCCGC | | |
| blaCTX-M9 | F | ATGGTGACAAAGAGAGTGCA | 51 | Bittar et al., 2009 |
| | R | CCCTTCGGCGATGATTCTC | | |
| blaCTX-M15 | F | CACACGTGGAATTTAGGGACT | 51 | Bittar et al., 2009 |
| | R | GCCGTCTAAGGCGATAAACA | | |
| mecA | F | TCCAGATTACAACTTCACCAGG | 51 | Yu et al., 2007 |
| | R | CCACTTCATATCTTGTAACG | | |

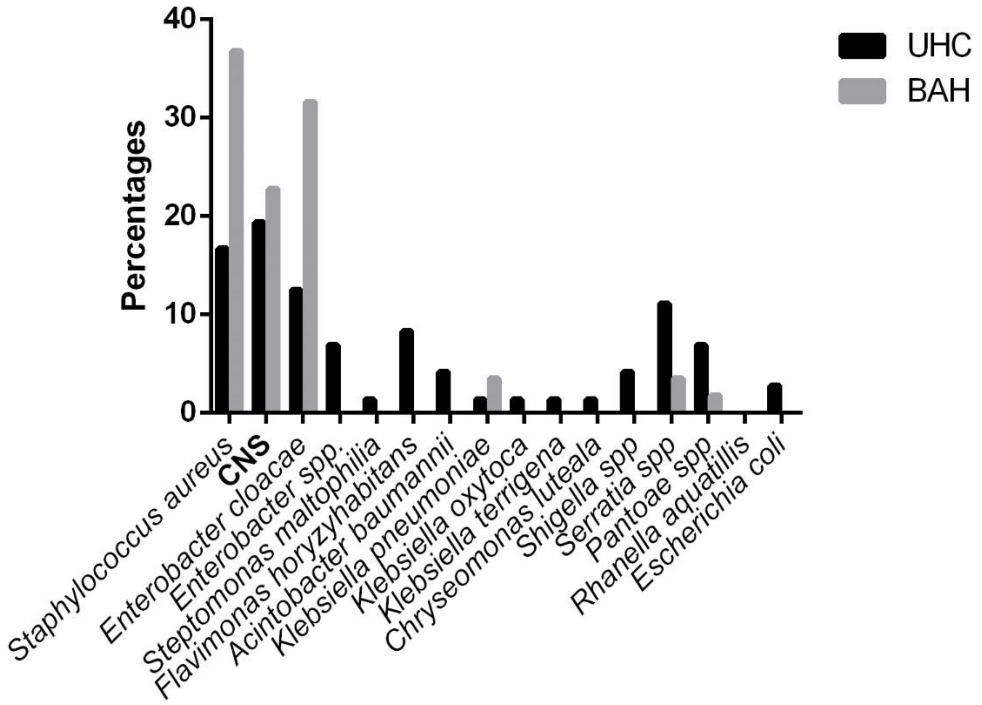



**Figure 1.** Distribution of isolates according to the sampling location

**Key**: UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital; CNS: Coagulase-negative Staphylococci

Table 2. Distribution of the isolated bacteria.

| Bacterial strains | UHC (n = 72) | | | | BAH (n = 57) | | | | Total (%) n = 129 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Environment (%) | | Catheter (%) | | Environment (%) | | Catheter (%) | | Environment (%) | | Catheter (%) | |
| *Staphylococcus aureus* | 06 | (8.3) | 06 | (8.3) | 17 | (29.8) | 04 | (7.0) | 23 | (17.8) | 10 | (7.8) |
| Coagulase-negative Staphylococci | 12 | (16.7) | 02 | (2.8) | 11 | (19.3) | 02 | (3.5) | 23 | (17.8) | 04 | (3.1) |
| *Enterobacter cloacae* | 09 | (12.5) | - | - | 18 | (31.6) | - | - | 27 | (21.0) | - | - |
| *Enterobacter spp* | 05 | (6.9) | - | - | - | - | - | - | 05 | (3.9) | - | - |
| *Steptomonas maltophilia* | 01 | (1.4) | - | - | - | - | - | - | 01 | (0.8) | - | - |
| *Flavimonas horyzyhabitans* | 06 | (8.3) | - | - | - | - | - | - | 06 | (4.7) | - | - |
| *Acintobacter baumannii* | 03 | (4.2) | - | - | - | - | - | - | 03 | (2.3) | - | - |
| *Klebsiella pneumoniae* | 01 | (1.4) | - | - | 01 | (1.8) | 01 | (1.8) | 02 | (1.6) | 01 | (0.8) |
| *Klebsiella oxytoca* | 01 | (1.4) | - | - | - | - | - | - | 01 | (0.8) | - | - |
| *Klebsiella terrigena* | 01 | (1.4) | - | - | - | - | - | - | 01 | (0.8) | - | - |
| *Chryseomonas luteala* | 01 | (1.4) | - | - | - | - | - | - | 01 | (0.8) | - | - |
| *Shigella spp* | 02 | (2.8) | 01 | (1.4) | - | - | - | - | 02 | (1.6) | 01 | (0.8) |
| *Serratia spp* | 08 | (11.1) | - | - | 02 | (3.5) | - | - | 10 | (7.8) | - | - |
| *Pantoae spp* | 05 | (6.9) | - | - | - | - | - | - | 05 | (3.9) | - | - |
| *Rhanella aquatillis* | - | - | - | - | 01 | (1.8) | - | - | 01 | (0.8) | - | - |
| *Escherichia coli* | - | - | 02 | (2.8) | - | - | - | - | - | - | 02 | (1.6) |
| Total | 61 | (84.7) | 11 | (15.3) | 50 | (87.7) | 07 | (12.3) | 111 | (86.0) | 18 | (14.0) |

Key: UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital

These percentage differences are not statistically significant ($p > 0.05$). In addition, an absence of *Enterobacter spp*, *Steptomonas maltophilia*, *Flavimonas horyzyhabitans*, *Acintobacter baumannii, Klebsiella oxytoca*, *Klebsiella terrigena*, *Chryseomonas luteala*, *Shigella spp*, *Pantoae spp* and *Escherichia coli* was noted in BAH samples (Figure 1).

**Evaluation of bacteremia linked to catheters (BLC)**

Table 3 shows the frequency of isolates responsible for catheter-related bacteremia. *S. aureus* is the main bacterial species responsible for BLC with a frequency of 6(54.54%) and 4 (54.14%) in samples collected from UHC and BAH respectively, followed incidentally by *Shigella spp*:1 (9.09 %).

Table 3. Frequency of isolates responsible for catheter-related bacteremia (BLC)

| | UHC | | BAH | |
|---|---|---|---|---|
| | Positives | % | Positives | % |
| *Staphylococcus aureus* | 6/11 | 54.54 | 4/7 | 54.14 |
| Coagulase-negative Staphylococci | 2/11 | 18.18 | 2/7 | 28.57 |
| *Escherichia coli* | 2/11 | 18.18 | - | - |
| *Klebsiella pneumoniae* | - | - | 1/7 | 14.28 |
| *Shigella spp* | 1/11 | 9.09 | - | - |

**Key:** UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital

Table 4. Resistance profile of isolates recovered from BAH and UHC hospitals

| | Enterobacteriacea | | Staphylococci | |
|---|---|---|---|---|
| | Antibiotics Type | Resistance (%) | Antibiotics Type | Resistance (%) |
| BAH | β-lactams | 13/23 (55.6%) | Fluoroquinolones | 25/36 (69.0 %) |
| | Fluoroquinolones | 10/23 (43.0%) | Aminoglycosides | 16/36 (42.8 %) |
| | Aminoglycosides | 14/23 (60.0%) | Macrolides | 17/36 (47.0%) |
| UHC | β-lactams | 36/48 (74.0%) | Fluoroquinolones | 17/27 (62.5%) |
| | Fluoroquinolones | 32/48 (69.0%) | Aminoglycosides | 17/27 (63.3%) |
| | Aminoglycosides | 32/48 (66.0%) | Macrolides | 23/27 (85.0%) |

**Evaluation of the resistance profile of the bacteria isolates**

At BAH, enterobacteria were more resistant to the aminoglycosides tested, particularly gentamicin: 14 (60%). The fluoroquinolones (ciprofloxacin, nalixidic acid) revealed a resistance of 25 (69%) of the staphylococcal isolates. High percentages of resistance were identified at the UHC. Beta-lactams (aztreonam, imipenem, ertapenem, ceftriaxone, amoxicillin + clavulanic acid) revealed resistance of 36 (74%) of the isolates of enterobacteria. As for staphylococci, macrolides (erythromycin) showed resistance level of 23 (85%) (Table 4).

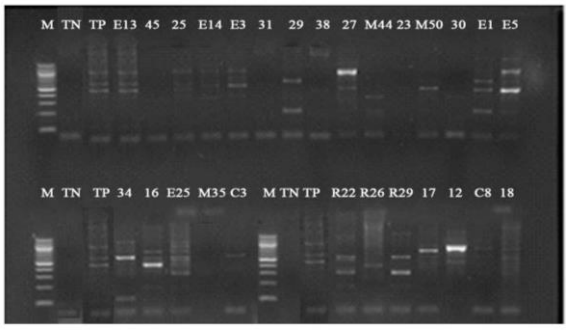
**Molecular characterization of resistance genes**

Our results further showed the predominance of resistance genes in bacteria species isolated from environmental swab samples than those from catheters. There was also a high prevalence of blaCTX-MI (35.02%) and blaCTX-M15 (42.73%) among Enterobacteriaceae family. However, among the Enterobacteriaceae isolated from catheters, the blaCTX-M2 gene was absent. Likewise, the blaCTX-M9 gene was not detected in Enterobacteriaceae isolated from BAH catheter samples. In staphylococci, the average frequency of the mecA gene was 50%. There is a higher prevalence of the presence of mecA gene in Staphylococci isolated from the environment than in those isolated from catheter samples (Table 5).

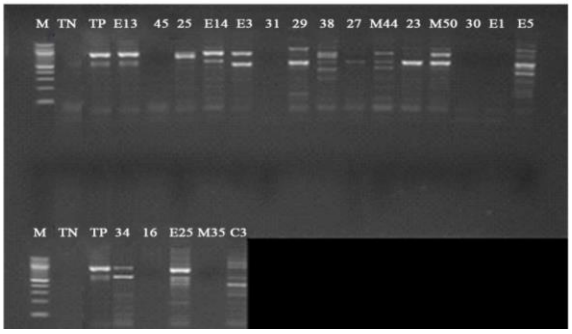
Table 5. The prevalence of resistance genes in the isolated bacteria.

| Resistance genes | BAH | | UHC | |
|---|---|---|---|---|
| | Environment (%) | Catheter (%) | Environment (%) | Catheter (%) |
| blaCTX-M1 | 8/14 (57.1%) | 1/14 (7.1%) | 20/29 (69.0%) | 2/29 (6.9%) |
| blaCTX-M2 | 2/14 (14.3%) | - | 10/29 (34.5%) | - |
| blaCTX-M9 | 4/14 (28.6%) | - | 11/29 (34.5%) | 1/29 (6.0%) |
| blaCTX-M15 | 11/14 (78.6%) | 1/14 (7.1%) | 23/29 (79.3%) | 1/29 (6.0%) |
| mecA | 16/19 (84.2) | 3/19 (15.8%) | 14/17 (82.3%) | 3/17 (17.6%) |

**Key**: UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital

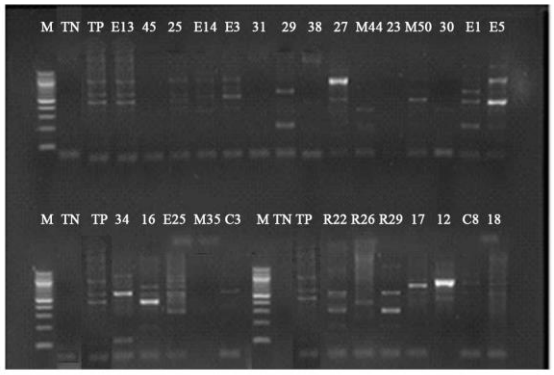



A:Gel migration of the blaCTX-M1 gene (863bp). Key: M: Size marker; TN: Negative control; TP: Positive control; E13 to C3: Samples

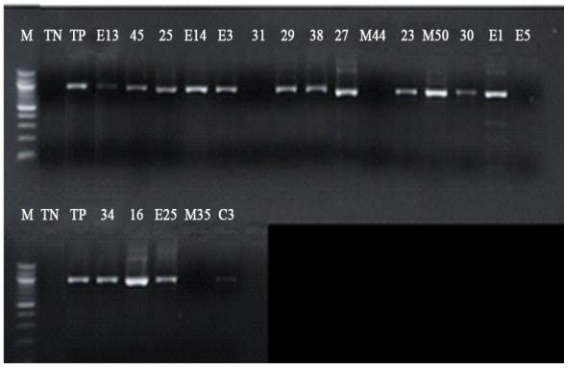



B: Gel migration of the blaCTX-M2 gene (865pb). Key: M: Size marker; TN: Negative control; TP: Positive control; E13 to C3: Samples

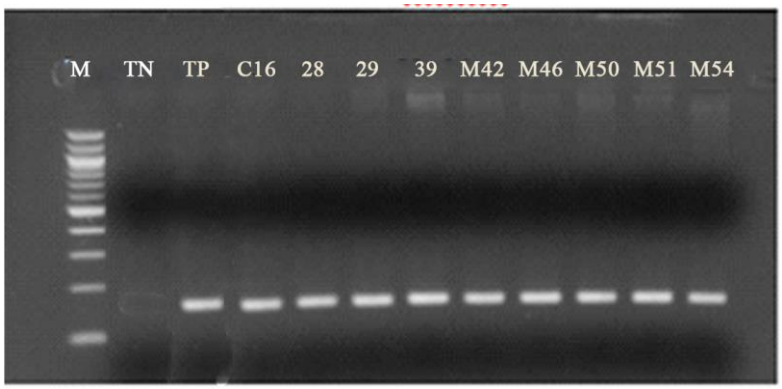
Figure 2. Pictures of the gel electrophoresis for the screening of resistance genes




C: Gel migration of the blaCTX-M9 gene (869bpb). Key: M: Size marker; TN: Negative control; TP: Positive control; E13 to 18: Samples




D: Gel migration of the blaCTX-M15 gene (995pb). Key: M: Size marker; TN: Negative control; TP: Positive control; E13 to C3: Samples




D: Gel migration of mecA gene (163pb). Key: M: Size marker; TN: Negative control; TP: Positive control; C16 to M54: Samples

Figure 2: Gel electrophoresis for the screening of the resistance genes (A: blaCTX-M1 gene (863bp) B: blaCTX-M2 gene (865pb); C: blaCTX-M9 gene (869bpb); D: blaCTX-M15 gene (995pb))

## DISCUSSION

Most bacterial isolates recovered in hospitals carry resistance genes. The study showed that 17.8% of *S. aureus* was isolated from inanimate objects/environment. This frequency of isolation of *S. aureus* is due to the opportunistic and ubiquitous nature of the germ (Nauciel and Vilde, 2009). Its ability to survive in the hospital environments makes it a common infectious agent associated with healthcare. The prevalence of contaminated catheter was 7.8%. This result is lower than the 14% found in Morocco by Oubihi (2015) and Lemsanni (2016). The most isolated bacterial species from the ends of the catheters in the two hospitals were *S. aureus* (55.6%), coagulase-negative Staphylococci (22.2%), *Escherichia coli* (11.1%), and *Klebsiella pneumoniae* (5,6 %). The occurrence of catheter-related bacteremia remains a reoccurring issue during the hospitalization period. According to Douard (1994), bacteria usually get in during the insertion of catheters. A second factor linked to the occurrence of bacteremia is the quality of disinfection (Dougnon et al., 2019). This includes hand hygiene, the quality of the antiseptic used for hand hygiene and disinfection of the operating site (SF2H, 2007). On the other hand, the inanimate environment and catheters are mostly contaminated with similar bacterial species. This may be due to transmission by hand, in the absence of hand hygiene before and/or after handling. These results are slightly different from those reported by Tajeddin et al. (2015). These authors found that most of the bacteria isolated from hospital staff were found in the hospital environment.

The sensitivity to antibiotics was tested on all the identified isolates. The antimicrobial resistance profile showed the partial resistance of the isolates to beta-lactams, aminoglycosides, fluoroquinolones, and macrolides. These results are slightly lower than those obtained by Ebongué et al. (2013) who reported higher frequencies. Other work has also reported the problem of multidrug resistance in hospitals (Vodovar et al., 2013; De Angelis et al., 2012). Microbial resistance to antibiotics is real in Benin and was reported by Ahoyo et al. (2007). This antimicrobial resistance occurs naturally over time following genetic modifications, but the excessive use of antibiotics accelerates the process (WHO, 2016).

Antibiotic resistance is therefore one of the major medical challenges of the 21st century (WHO, 2015). The major challenge today is to limit the spread of enterobacteria producing broad-spectrum beta-lactamases (ESBL) in the community and especially in hospitals. Indeed, the production of ESBL is the most widespread resistance mechanism in enterobacteria. The results obtained after the PCR confirmed the presence of the genes sought. The blaCTX-M1 and blaCTX-M15 genes are the most prevalent in enterobacteria, with an average of 35.02% and 42.73% respectively. It therefore appears that blaCTX-M15 was the most frequently found ESBL in our study. Several other studies have shown that blaCTX-M15 is the enzyme most commonly found in strains circulating in the hospital environment as well as within the community (Ogbolu et al., 2018). The emergence and spread of ESBLs in West African countries have been linked to the global expansion of the CTX-M15 type (Doi et al., 2013). Among other ESBLs of the CTX-M type, blaCTX-M9 has also been responsible for resistance at lower frequencies of 20.20% (UHC) and 14.29% (BAH). As for blaCTX-M2, it was found at 7.13% and 17.24% respectively (BAH and UHC). These results are similar to those obtained in Ghana, where half of the enterobacteriaceae (49.4%) isolated from the various infections diagnosed at Korle-Bu hospital produced ESBL (Feglo et al., 2013). Also, several isolates have produced more than one ESBL at a time, making therapy in hospitals complex. In addition to the resistance of the isolated enterobacteriaceae strains to beta-lactams, several of them have been resistant to other families of antibiotics (fluoroquinolones, aminoglycosides). This is explained by the fact that all of the ESBL-carrying plasmids also harbor other resistance genes, thus conferring resistance on the vast majority of ESBL enterobacteria to other families of antibiotics, in particular cotrimoxazole, aminoglycosides and fluoroquinolones (Reuland et al., 2013). The resistance to carbapenems (imipenem, ertapenem) observed is due to the production of ESBL class B which currently represents an emerging concern leading to ineffectiveness of carbapenems (Reuland et al., 2013). The mecA gene has been found in staphylococcal isolates. The proportion of *S. aureus* strains that carry the mecA gene at BAH was 52.62% compared to 41.17% at UHC bringing to 46.89% the average frequency of the isolates producing mecA responsible for resistance to methicillin. These results are similar to those of Akerelé et al. (2014) in Nigeria where the frequencies of MRSA varied between 20 and 47% and were much lower than those reported by Anago et al. (2015) in Benin.

The high proportion of bacteria resistant to antimicrobials observed in our study could be

explained by the practice of self-medication in patients and the use of antibiotics of questionable quality during treatment. Dougnon et al. (2016) reported that the quality of the antibiotic discs used to carry out the antibiograms could be a factor favoring the resistance of bacteria. Therefore, it is important to put an end to self-medication, the use of poor-quality antibiotics based on awareness and continuous information among all those involved in healthcare delivery. In addition, many medical treatments involve presumptive treatment based on data from the literature in developed countries which do not necessarily share the same microbial ecology and may therefore be ill-adapted (Ouedraogo et al., 2017). It is therefore important to develop antibiotic therapy protocols for the most common diseases in a multidisciplinary framework, considering the local reality of antibiotic resistance.

## CONCLUSION

This study made it possible to characterize the antibiotic resistance genes of bacterial isolates associated with hospital environments. The presence of the mecA gene reflects the resistance observed within staphylococci, particularly those of methicilin-resistant *Staphylococcus aureus* (MRSA). The presence of the blaCTX-M1, blaCTX-M2, blaCTX-M9 and blaCTX-M15 genes indicates the resistance noted within the isolates of enterobacteria. In addition, their multidrug resistance to antibiotics confirms the danger that these isolates pose on both patients and health workers. This poses the problem of non-compliance with hygiene measures in hospitals but also the urgency of judicious and responsible management of antibiotics. The two determinants of the emergence and spread of bacterial resistance to antibiotics are the exposure of the population to antibiotics and the environment to resistant strains. Knowledge of bacterial ecology and the genes involved in antimicrobial resistance are important factors in the efficient management of infections, but also a means of helping to reduce the phenomenon of antimicrobial resistance in hospitals. The present study is therefore of paramount importance as the results can be used to support advocacy for urgent action at the national level, especially with regard to the management and efficient use of antimicrobials.

### Ethical Statements

Not applicable. The manuscript contains no individual person's data.

### Competing interests

The authors declare that they have no competing interests


### Funding

No external funding was obtained to carry out this study. It was carried out with own funding from the authors and co-authors.


### Authors' contributions

EG, VD, YC, LB-M, SV, BK and CG wrote the protocol and validated it. JA and ED reviewed the protocol. JA, ED and VD supervised the microbiological analyses which were performed by KF and EG. PS, EG performed the molecular analysis.


### Acknowledgements

All the authors are grateful to Kaisa HAUKKA, University of Helsinki (Finland) for her permanent support about fighting antimicrobial resistance in Africa.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