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

Elodie GBOTCHE
Universite d'Abomey-Calavi
Victorien Tamègnon DOUGNON (victorien.dougnon@gmail.com)
Universite d'Abomey-Calavi https://orcid.org/0000-0001-9047-7299
Yossounon CHABI
Universite d'Abomey-Calavi Faculte des Sciences de la Sante
Jerrold AGBANKPE
Universite d'Abomey-Calavi
Esther DEGUENON
Universite d'Abomey-Calavi
Paulin SEDAH
Universite d'Abomey-Calavi
Kafayath FABIYI
Universite d'Abomey-Calavi
Lamine BABA-MOUSSA
Universite d'Abomey-Calavi
Clément AGBANGLA
Universite d'Abomey-Calavi
Honoré BANKOLE
Universite d'Abomey-Calavi

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Abstract

Background: Antimicrobial resistance is a real public health problem. All over the world, it has a considerable impact in hospitals. The present study aims to analyze the presence of resistance genes and bacterial ecology in two hospitals in Benin.

Methods: To do this, 146 environmental and catheter samples were collected at the University Hospital Center of Abomey-Calavi / So-Ava and at the Beninese Army Hospital. These samples were inoculated on Mannitol Salt and Eosin Methylene Blue agars. The colonies obtained were identified and their sensitivity to antibiotics was tested, using the usual bacteriological techniques. Four resistance genes encoding the production of extended spectrum beta-lactamases (blaCTX-M1, blaCTX-M2, blaCTX-M_9, blaCTX-M15) have been searched in the genome of enterobacteriaceae strains. At the level of staphylococci, the gene coding for methicillin resistance (*mecA*) was sought.

Results: At the end of this study, 69 strains of enterobacteria and 60 of staphylococci were identified. A predominance of *Staphylococcus aureus* (25.6%) followed by *Enterobacter cloacae* (21.0%) and coagulase negative staphylococci (21.0%) was noted. These bacterial strains showed to be multidrug-resistant, 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%.

Conclusions: These results show the magnitude of the antimicrobial resistance situation in hospitals. They can be used to support advocacy for urgent action at the national level, especially with regard to the management and efficient use of antimicrobials in Benin.

Background

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 at hospital has considerably modified microbial ecology and tends to increase the rate of resistant bacteria [1, 2, 3]. Thus, the emergence and rapid dissemination of antibiotic resistance is a major problem for human health [4]. 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 [5, 6, 7]. 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 appearance of bacteria resistant to several antibiotics in particular the strains of *Staphylococcus aureus* resistant to methicillin (MRSA), the enterobacteria producing beta-extended spectrum lactamases (ESBL) and vancomycin resistant enterococci (VRE) [8, 9, 10]. Among bacteria resistant to antibiotics, enterobacteria producing broad spectrum β-lactamase (ESBL) represent a major global threat in hospitals [8]. Often associated with urinary tract infections, they can also cause serious infections associated with the bloodstream [8]. *Staphylococcus aureus* is the leader in the family of staphylococci because of its involvement in suppurative, localized or severe systemic pathologies in humans [11, 12]. 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 bacteria responsible for the infection of the urinary tract after *Escherichia coli* [9].

In February 2017, WHO ranked germs based on antibiotic resistance. According to this classification, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and enterobacteria producing broad spectrum beta-lactamases represents a critical emergency as they are resistant to a large number of antibiotics. Six other bacteria including *Staphylococcus aureus*, *Helicobacter pylori*, salmonella and *Neisseria gonorrhoeae* represent a high emergency [13]. Finally, for *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Shigella spp*, the urgency is moderate [13]. More than ever, the prescription of antibiotics must take into account 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 [14]. Thus, antibiotic therapy must therefore 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 [14]. It is in this same context that this study is situated.

Methods

Sampling

It consisted of swabbing the inanimate environment of two health structures in South Benin, namely the Abomey-Calavi / So-Ava University and Hospital Center (UHC) and the 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 Heart-Brain broth [15, 16].

As for the catheter samples, they were taken according to Cleri et al. [17]. 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 4 ml of Heart-Brain broth.

In total we sampled 146 samples (Table 1). All samples were sent to the laboratory and incubated. After 16 hours of incubation at 37 ° C, the broths were inoculated on agar plates for bacteriological analysis.

Table 1
Distribution of the different types and their number according to the hospital center.

Hospital centers	Number of different types of samples		Total
	Environment	Catheter	
UHC	49	24	73
BAH	56	17	73
Total	105	41	146
UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital			

Isolation And Identification

With regard to the isolation of the bacterial strains, the 16-hour subcultures (broths) were seeded on the agar plates (Mannitol Salt and Eosin Methylene Blue agars) according to the techniques described by Dougnon et al.,

[18] and Afle et al. [19]. Gram staining was carried out directly on two to three 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 by seeding the API 20 E gallery. For the identification of GPC, catalase, coagulase and DNase tests were carried out.

Antibiotic Susceptibility Test

An overnight bacterial pre-culture of isolates was diluted to obtain a turbidity of 0.5 McFarland (in sterile distilled water). Kirby Bauer techniques were used to perform the susceptibility testing [20]. Antibiotics of different families were chosen for the resistance pattern of the isolates: aztreonam (30 µg); erythromycin (15 µg); tobramycin (10 µg); ceftriaxone (30 µg); ciprofloxacin (5 µg); ertapenem (10 µg); imipenem (10 µg); amoxicillin + clavulanic acid (30 µg); gentamicin (10 µg); kanamycin (30 µg); streptomycin (10 µg); nalixidic acid (30 µg). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 was tested for quality assurance.

Molecular Detection Of Resistance Genes

The DNA of the isolates was extracted according to the instructions of the Zyppy Plasmid Miniprep Kit from Zymo Research. In order to assess and ensure the quality of the plasmid DNA extracts, a verification was carried out by electrophoresis on a 1% agarose gel and stained with 0.1% ethidium bromide (BET). Migration was done at 100V for 15 min with 1X concentrated TBE buffer. 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 identified to assess the resistance of the identified strains. These resistance genes were detected by simplex PCR [21, 22]. The PCR was performed at a total volume of 25 µl containing 1.5 µl of plasmid DNA; 2.5 µl of each primer (Sens 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.

At the end of the reaction, the PCR products were revealed by electrophoresis on a 2% agarose gel incorporated with 1 µl of BET. 5 µl of each PCR product was deposited. The migration was done with TBE 1X buffer for 25 to 35 minutes at 100V. The bands were viewed under ultraviolet on a trans-illuminator equipped with a camera. A scoring of the electrophoretic profiles was made by the presence or absence of a band as a function of the size (in base pairs) expected from the sequence. Table 2 shows the primers used.

Table 2
Sequences of primers

Genes	Primers	Sequences (5'-3')	Temperature in °C	References
blaCTX-M1	F	GGTTAAAAAATCACTGCGTC	51	[22]
	R	TTGGTGACGATTTTAGCCGC		
blaCTX-M2	F	ATGATGACTCAGAGCATTCG	60	
	R	TGGGTTACGATTTTCGCCGC		
blaCTX-M9	F	ATGGTGACAAAGAGAGTGCA		
	R	CCCTTCGGCGATGATTCTC		
blaCTX-M15	F	CACACGTGGAATTTAGGGACT	51	
	R	GCCGTCTAAGGCGATAAACA		
mecA	F	TCCAGATTACAACTTCACCAGG	51	[21]
	R	CCACTTCATATCTTGTAACG		

Statistical Data Processing

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 isolated strains according to the nature of the sample and the place of sampling

Of the 146 samples analyzed, 129 bacterial strains were isolated with 86% (n = 111) of the strains isolated from environmental samples and 14% (n = 18) isolated from the catheter samples. Bacterial strains isolated from the environment, *Enterobacter cloacae* leads with 21% (n = 27), followed by strains of *Staphylococcus aureus* and Coagulase-negative Staphylococci with 17.8% respectively (n = 23). We also isolated, the strains of *Serratia spp*. (7.8%), *Flavimonas horyzyhabitans* (4.7%), *Enterobacter spp*. (3.9%), *Pantoae spp*. (3.9%) and *Acinetobacter baumannii* (2.3%). Regarding strains isolated from catheter samples, *Staphylococcus aureus* is the most isolated with 7.8% (n = 10) followed by 3.1% coagulase negative staphylococci (n = 4). We also isolated the strains of *Escherichia coli* (1.6%), *Shigella spp* (0.8%) and *Klebsiella pneumoniae* (0.8%) (Table 3). This difference in percentage of the different strains isolated between the two types of samples is not statistically significant ($p > 0.05$).

The results remained the same in each of the two hospitals where the samples were taken. There is a high prevalence of *Enterobacter cloacae* strains isolated from environmental samples taken both in the Abomey-Calavi / So-Ava University Hospital Center (UHC) and in the Beninese Hospital of Army (BHA), with respectively 12.5% (n = 9) and 31.6% (n = 18), followed by *Staphylococcus aureus* (29.8% for BAH and 8.3% for UHC) and coagulase-negative staphylococci (19.3% for BAH and 16.7% for UHC) (Table 3). These percentage differences are not statistically significant ($p > 0.05$). Our results showed that from a hospital point of view, we note that the prevalence of *Staphylococcus aureus* strains is higher in BAH (36.8%) than in UHC (16.7%). The same is true for

the prevalence of *Enterobacter cloacae* strains (31.6% for BAH and 12.5% for UHC) and coagulase negative staphylococci strains (22.8% for BAH and 19.4 for UHC). 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* strains was noted at BAH samples (Fig. 1.).

Table 3
Distribution of isolated strains according to the nature of sample.

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)**

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

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

Evaluation Of Bacteremia Linked To Catheters (blc)

Table 4 provides information on the evaluation of infections associated with catheter-related bacteremia type care in the two hospitals sampled. *Staphylococcus aureus* is the main bacterial species responsible for BLC with respective frequencies of 54.54% and 54.14% at the UHC and at the BAH followed incidentally by *Shigella spp* (9.09%).

Table 4
Frequency of strains responsible for catheter-related bacteremia (BLC)

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

Evaluation of the resistance profile of strains

At BAH, enterobacteria were more resistant to the aminoglycosides tested, particularly gentamicin (60%). The fluoroquinolones (ciprofloxacin, nalixidic acid) revealed a resistance of 69% of the staphylococcal strains (Table 5). High percentages of resistance were identified at the UHC. Beta-lactams (aztreonam, imipenem, ertapenem, ceftriaxone, amoxicillin + clavulanic acid) revealed resistance of 74% of the strains of enterobacteria. As for staphylococci, macrolides (erythromycin) showed resistance of 85% (Table 6).

Table 5.

Resistance profile of strains isolated at BAH

Enterobacteriacea		Staphylococci	
Families of Antibiotics	**% Resistance**	**Families of Antibiotics**	**% Resistance**
β-lactams	55.6	Fluoroquinolones	69.0
Fluoroquinolones	43.0	Aminoglycosides	42.8
Aminoglycosides	60.0	Macrolides	47.0

Table 6.

Resistance profile of strains isolated at UHC

Entérobactéries		Staphylocoques	
Families of Antibiotics	**% Resistance**	**Families of Antibiotics**	**% Resistance**
β-lactams	74.0	Fluoroquinolones	62.5
Fluoroquinolones	69.0	Aminoglycosides	63.3
Aminoglycosides	66.0	Macrolides	85.0

Molecular Characterization Of Resistance Genes

The resistance genes sought at the two hospitals concerned were found more in bacterial strains isolated from samples of the inanimate environment than in those of catheters. However, the blaCTX-M1, blaCTX-M15 genes are those with the highest prevalence in enterobacteria, with an average of 35.02% for the blaCTX-M1 gene and 42.73% respectively for the blaCTX-M15 genes Our results showed an absence of the blaCTX-M2 gene in enterobacteriaceae strains isolated from catheter samples but present in those isolated from the environment. Likewise, the blaCTX-M9 gene was not detected in enterobacteriaceae strains isolated from BAH catheter samples. In staphylococci, the average frequency of the mecA gene was 50% in the strains. There is a higher prevalence of the presence of the mecA gene in Staphylococci isolated from the environment than in those isolated in catheter samples (Table 7).

Table 7
Prevalence of resistance genes in strains isolated according to the type of sample.

Resistance gènes	**BAH**		**UHC**	
	Environment (%)	**Catheter (%)**	**Environment (%)**	**Catheter (%)**
blaCTX-M1	57.1	7.1	69.0	6.9
blaCTX-M2	14.3	-	34.5	-
blaCTX-M9	28.6	-	34.5	6.0
blaCTX-M15	78.6	7.1	79.3	6.0
mecA	84.2	15.8	82.3	17.6
UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital				

Discussion

Most bacterial strains in hospitals carry resistance genes. The study showed that *Staphylococcus aureus* was 17.8% isolated from the inanimate environment. This frequency of isolation of *S. aureus* is due to the opportunistic and ubiquitous nature of the germ [23]. Its ability to survive in the hospital environment allows it to be the source of infections associated with healthcare. We observed a 7.8% prevalence of contaminated catheter.

This result is lower than the 14% found in Morocco by Oubihi [24] and Lemsanni [25]. The most isolated bacterial species from the ends of the catheters in the two hospitals are *Staphylococcus aureus* (55.6%), Coagulase-negative Staphylococci (22.2%), *Escherichia coli* (11.1%), and *Klebsiella pneumoniae* (5,6%). The occurrence of catheter-related bacteremia remains constant during the hospitalization period. According to Douard [26], it is therefore when the catheter is placed that the bacteria are inserted. A second factor linked to the occurrence of bacteremia is the quality of disinfection [27]. This includes hand hygiene, the quality of the antiseptic used for hand hygiene and disinfection of the operating site [28]. On the other hand, the inanimate environment and the catheters were mostly all infected 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 the study by Assogba et al. [29] which showed that after a comparative study of bacteria isolated from staff and from the environment, most of the bacteria isolated from staff were found in the hospital environment.

The sensitivity to antibiotics was tested on all the strains identified. The antimicrobial resistance profile showed the partial resistance of the strains to beta-lactams, aminoglycosides, fluoroquinolones and macrolides. These results are slightly lower than those obtained by Ebongué et al. [30] who reported higher frequencies. Other work has also reported the problem of multidrug resistance in hospitals [31, 32]. Microbial resistance to antibiotics is real in Benin and was reported by Ahoyo et al. [33]. This antimicrobial resistance occurs naturally over time in general following genetic modifications, but the excessive and excessive use of antibiotics accelerates the process [34].

Antibiotic resistance is therefore one of the major medical challenges of the 21st century [35]. 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 is 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 [36]. The emergence and spread and spread of ESBLs in West African countries are therefore linked to the global expansion of the CTX-M15 type [37]. 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 186 diagnosed at Korle-Bu hospital produced ESBL [38]. 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 [39]. 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 [39]. The mecA gene has been found in staphylococcal strains. 52.62% of Staphylococcus aureus strains carried the mecA gene at BAH compared to 41.17% at UHC bringing to 46.89% the average frequency of the strains producing mecA responsible for resistance to methicillin. These results are similar to those of Akerelé et al. [40] in Nigeria where the frequencies of MRSA vary between 20 and 47% and are much lower than those obtained by Ahoyo et al. [41] 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. [18] have perceived 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 of all health actors but also to promote compliance with the measures 'hygiene. 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 can therefore be ill-adapted [42]. It is therefore important to develop antibiotic therapy protocols for the most common diseases in a multidisciplinary framework, taking into account the local reality of antibiotic resistance.

Conclusion

This study made it possible to characterize the antibiotic resistance genes of bacterial strains isolated in a hospital environment. 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 strains of enterobacteria. In addition, their multidrug resistance to antibiotics confirms the danger that these strains represent not only for patients but also for healthcare personnel. 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 by the environment of resistant strains. Knowledge of bacterial ecology and of 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.

Declarations

Ethical Statements

Not applicable

Consent to publish

The current manuscript contains no individual person’s data. Therefore consent to publish is not applicable.

Availability of data and materials

All data generated or analysed during this study is included in this published article and Additional file.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

EG, VD, YC, LB-M, 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.

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Figures

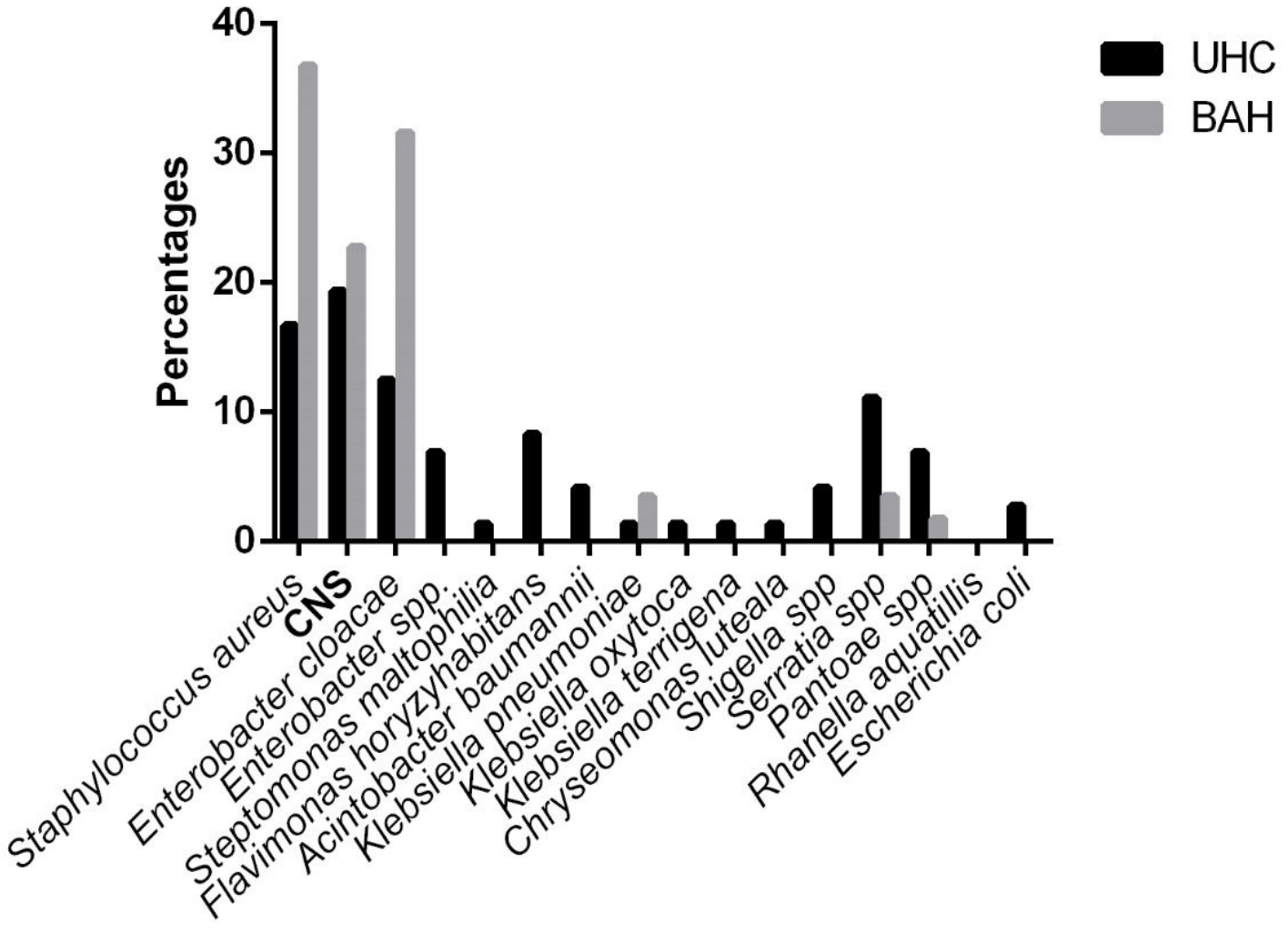

Figure 1

Distribution of isolated strains according to the sampling location. UHC: Abomey-Calavi / So-Ava University and Hospital Center; BAH: Beninese Army Hospital; CNS: Coagulase-negative Staphylococci