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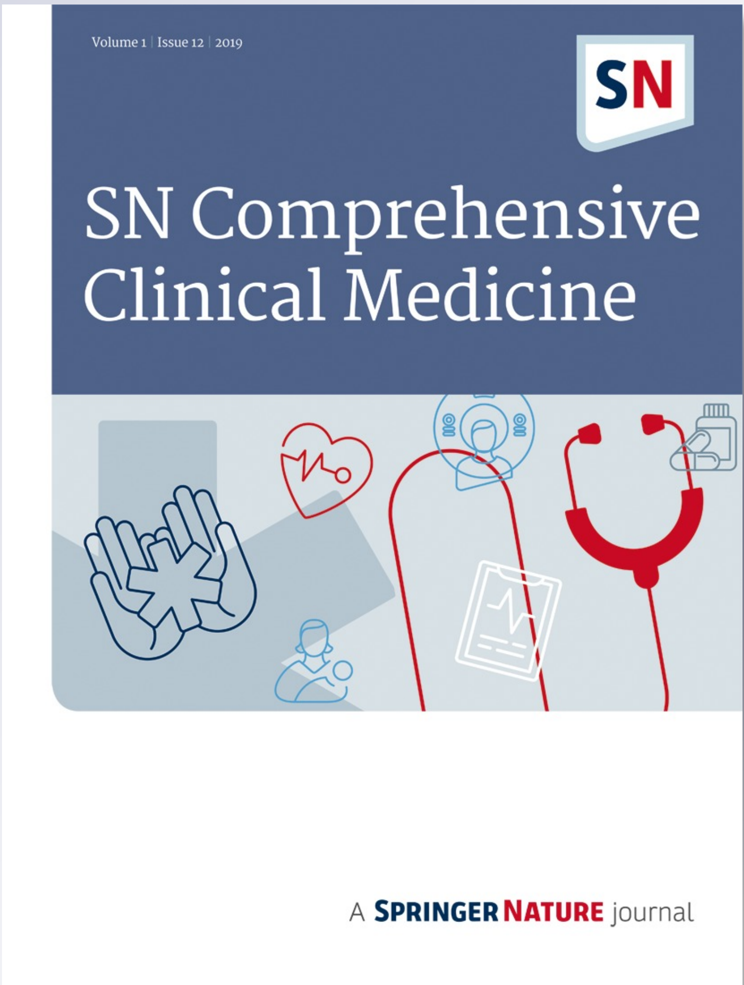

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MEDICINE

High Prevalence of Multidrug-Resistant Bacteria in the Centre Hospitalier et Universitaire de la Mère et de l'Enfant Lagune (CHU-MEL) Reveals Implications of Poor Hygiene Practices in Healthcare

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Abstract

Healthcare-associated infections represent an emerging public health issue with serious impact among hospitalized patients, including cesarized women and children with catheter. The present study determined the implications of hygiene practices and the hospital environment in wound reinfection among cesarized women and the occurrence of catheter-induced infections in children. Bacteriological analyses were performed on 100 wound swabs from cesarized women, 40 swabs from the wound dressing room, and 83 catheter tips used in children. Isolated bacteria were tested for antimicrobial susceptibility. A comparison of the resistance profile between strains isolated from wounds and those isolated from the dressing room was conducted, whereas the hygiene practices observed from the personnel during catheter removal were recorded in the case of children. The results show that 85% of wound swabs, 63% of swabs from the dressing room, and 33.7% of catheter tips were positive for bacteriological analysis. The most isolated strains in wound and environmental swabs were *Staphylococcus aureus* (56%) and coagulase-negative *Staphylococcus* (44%), followed by *Klebsiella pneumoniae* (30%) and *Enterobacter cloacae* (32%) for wounds and *Escherichia coli* (43%) and *Klebsiella pneumoniae* (28%) for the environment. The catheter tips contained mostly *Klebsiella pneumoniae* (32%), coagulase-negative *Staphylococcus* (25%), and *Enterobacter cloacae* (14%). All strains showed resistance to penicillin and cephalosporin. The comparison of the resistance profiles suggests an implication of the environmental strains in the reinfection of wounds in cesarized women. However, a significant correlation was recorded between poor hygiene practices and the contamination of the catheter tips. These findings allowed the authorities of this hospital to reinforce the knowledge and improve the hygiene management, in order to still hold the good label of the structure.

Keywords Healthcare-associated infections · Hygiene practices · Reinfection · AMR

This article is part of the Topical Collection on *Medicine*

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Table 1 List of antibiotic discs tested

Discs	Concentrations (μg)
AMP (Ampicillin)	10
AMC (Amoxicillin + Clavulanic acid)	30
CRO (Ceftriaxone)	30
ERT (Ertapenem)	10
IPM (Imipenem)	10
CT (Colistin)	50
OX (Oxacillin)	5
FOX (Fosfomycin)	50
VAN (Vancomycin)	30
DA (Clindamycin)	15
E (Erythromycin)	15
GEN (Gentamicin)	15

Introduction

Healthcare-associated infections (HAIs) are known worldwide as a public health problem, which have led to a significant increase in mortality, morbidity, and cost of patient care due to longer stays [1]. Their frequency and severity are particularly high in the intensive care settings because of the pathologies presented by the patients, the associated comorbidities, and the density of the invasive techniques used [2, 3].

Healthcare-associated infections represent one of the most common nosocomial infections of public health concern due to high mortality and longer hospital stays despite the use of antibiotics, improvement of anesthesia techniques, and progress of preventive measures [4]. They account for about 25% of all nosocomial infections (NIBs) ranking second to UTIs [5]. According to the WHO, the rate of infection of the operating site varies from 0.5 to 1.5%. It exceeds 25% in some developing countries [6]. They are also responsible for an economic problem due to the extension of the hospital stay, absenteeism, and extra care costs [7]. Among the vulnerable groups, cesarized women are at two to three times more risk of healthcare-associated infections [8].

Catheter-related infections account for approximately 18 to 25% of nosocomial infections, with increased morbidity and mortality. Mortality due to catheter bacteremia is highly variable, but it could be as high as 60%. Catheter-related bloodstream infections are therefore of a serious public health concern. They are relatively common and are among the four most studied nosocomial infections [9] with the infection rate indicated in the literature between 1.8 and 5.2 cases per 1000 day-catheter [10, 11]. The catheter-associated bacteremia cases have the highest costs and the longest hospitalizations [12].

The present study aims to assess the implication of hygiene practices and the hospital environment in the bacterial infection of wounds in cesarized women and the occurrence of catheter infections in children.

Table 2 Distribution of samples according to positivity

	Wounds	Environment	Total
Sterile	15 (15%)	15 (37%)	21
Positive	85 (85%)	25 (63%)	89
Total	100 (100%)	40 (100%)	140

Table 3 Distribution of wound samples by patient status

	Negative	Positive	Total	Chi^2 test
Non-hospitalized	9 (9%)	23 (23%)	33 (33%)	0.084
Hospitalized	9 (9%)	59 (59%)	68 (68%)	
Total	18 (18%)	82 (83%)	100 (100%)	

Materials and Methods

Framework

The present study took place at the Centre Hospitalier et Universitaire de la Mère et de l'Enfant Lagune (CHU-MEL). With an annual budget of nearly 3 billion Francs CFA, more than 30 doctors, 70 midwives, and 90 nurses, CHU-MEL is a reference institution in Benin, located in Cotonou, and entirely dedicated to the mother and the child. This hospital used to receive patients not only from Cotonou, an urban and main

Table 4 Distribution of wound samples according to the appearance of wounds

Aspect	Negative	Positive	Total	Chi^2 test
Clean	10 (10%)	34 (34%)	44 (44%)	0.759
Bloody	3 (3%)	16 (16%)	20 (20%)	
Suppurative	4 (4%)	23 (23%)	27 (27%)	
Serosity	1 (1%)	9 (9%)	10 (10%)	
Total	18 (18%)	82 (82%)	100 (100%)	

Table 5 Distribution of wound samples according to the number of wound dressings

	Negative	Positive	Total	Chi^2 test
1 Wound dressings	4 (4%)	24 (24%)	28 (33%)	0.034
2 Wound dressings	5 (5%)	34 (34%)	39 (68%)	
> 2 Wound dressings	9 (9%)	24 (24%)	33 (33%)	
Total	18 (18%)	82 (83%)	100 (100%)	

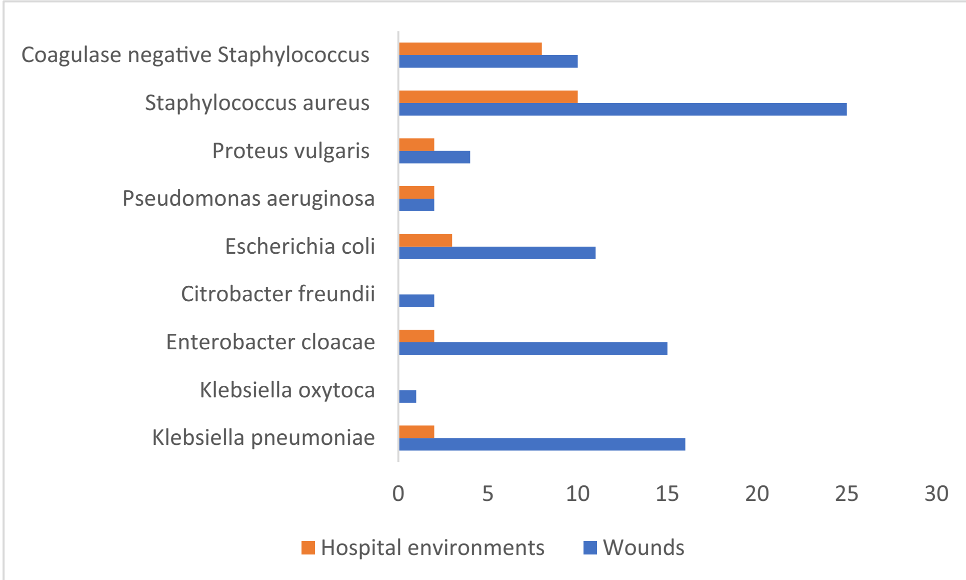

Fig. 1 Distribution of isolated bacteria

populated city, but also from areas all over the country. Approximatively, CHU-MEL got 256 beds.

Study Population

Cesarized women were enrolled in this study. Similarly, all central venous catheters (CVC) posed in the study period, from July to October 2018, in newborns and children under 10 years, hospitalized at the CHU-MEL of Cotonou specifically in the Neonatology and Pediatric departments were included regardless of the pathology, pose pattern, and duration of the catheter operation.

Criteria of healthcare-associated infections used in the study were related to the type of surgery, duration of surgery, antimicrobial prophylaxis, duration of hospitalization before surgery and after, occurrence of symptoms, and swabbing for bacteriological exams [13, 14]. For venous catheter infection, the criterion used was a positive culture of 5 cm from the inner end of the catheter [15].

Collection of Samples

Swabs were collected from cesarean section wounds before they were dressed. Swabs were also taken from the wound dressing room (bench, bed, stool) and materials (tongs, bean, tray, compress, etc.) and placed in 3 ml of Tripcase soy broth [16, 17]. After 16 h of incubation, the broth was streaked onto agar plates for bacteriological analysis.

Catheter sample was taken according to Cleri et al. [18]. After disinfecting the hands and wearing sterile latex

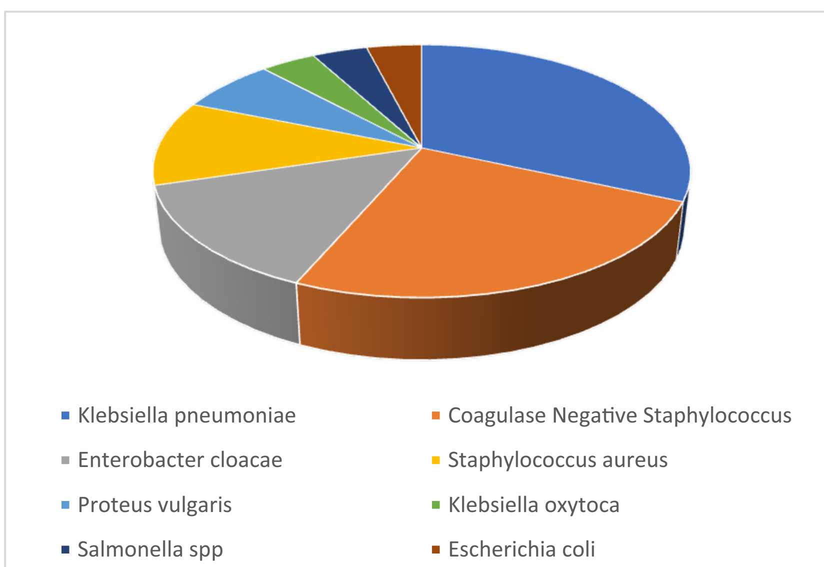

Fig. 2 Distribution of isolated bacteria

Table 6 Antibiotic sensitivity of Gram-negative bacillus strains

	AMP		AMC		CTX		ATM		ERT		IPM		CT	
	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)
Escherichia coli	100	100	75	50	82	67	54.5	33	0	0	0	0	0	0
Klebsiella pneumoniae	100	100	75	50	62.5	50	37.5	50	0	0	0	0	0	0
Citrobacter freundii	50	–	50	–	50	–	50	–	0	–	0	–	0	–
Enterobacter cloacea	100	100	80	50	67	50	33	0	0	0	0	0	100	100
Proteus vulgaris	67	100	34	100	75	50	50	50	50	0	0	0	0	0
Pseudomonas aeruginosa	100	100	100	100	50	50	50	0	0	0	0	0	0	0
Klebsiella oxytoca	100	–	100	–	100	–	0	0	0	–	0	–	0	–

disposable gloves, the bandages and the catheters were removed without performing antisepsis. Five centimeters from the inner end of the catheter was then cut with sterile scissors and placed in a sterile vial containing 4 ml of Tripcase soy broth. All samples were sent to the laboratory and incubated at 37 °C for 16 h.

Bacteriological Examination and Data Analysis

The incubated broths were analyzed using standard bacteriology techniques described by [19]. Isolated strains were then tested for antimicrobial susceptibility according to the recommendations of the Antibiotic Committee of the French Microbiology Society [20]. Antibiotic discs tested are presented in Table 1.

A comparison of the resistance profiles between the strains isolated from the wounds and those isolated from the dressing room and materials served to assess the implication of the hospital strains in the postoperative infection of the wounds.

Hygiene practices observed by the personnel during catheterization and the presence of inflammation (thrombosis) were recorded as risk factors and analyzed along with bacteriological analyses of the catheter samples. The practices observed related to the contact time of the antiseptic with the skin (30 s) and the drying time (30 s) of the antiseptic before the installation of the catheter. Correlation between risk factors and the occurrence of catheter-related infections was determined with Chi^2 test using the GraphPad 6 software.

Results

We identified eighty-five (85)-positive wound swabs out of the cent (100) and twenty-five (25)-positive swabs from the dressing room and materials out of forty (40) (Table 2). There was no significant statistical association between the hospitalization status and the occurrence of healthcare-associated infections ($p = 0.084 > 0.05$) (Table 3) nor between the appearance of the wounds and occurrence of infections ($p = 0.759 > 0.05$) (Table 4). However, a statistically significant association was noted between the number of dressings and the occurrence of healthcare-associated infections ($p = 0.034 < 0.05$) (Table 5). Ninety-six (96) bacterial strains were isolated from eighty-five (85)-positive wound swabs, and twenty-nine (29) bacterial strains were identified from twenty-five (25) environmental swabs. *Staphylococcus aureus* were the most isolated bacteria in both wound swabs and environmental swabs. It is followed by strains of coagulase-negative Staphylococci

Table 7 Antibiotic susceptibility of Gram-positive cocci strains

	AMP		OX		FOX		VAN		DA		E		GEN	
	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)	Wounds (%)	Env. (%)
Staphylococcus aureus	82	100	80	60	60	50	0	0	0	0	0	0	63	40
Coagulase-negative *Staphylococcus*	75	100	60	75	40	37.5	0	0	0	0	0	0	50	67

Table 8 Distribution of antibiotic resistance profiles

Species	Profiles	Wounds	Environment
Escherichia coli	AMP ^{R} AMC ^{R} CTX ^{R} ATM ^{R} ERT ^{S} IMP ^{S} CS ^{S}	3	1
	AMP ^{R} AMC ^{S} CTX ^{S} ATM ^{S} ERT ^{S} IMP ^{S} CS ^{S}	2	1
Klebsiella pneumonia	AMP ^{R} AMC ^{R} CTX ^{R} ATM ^{R} ERT ^{S} IMP ^{S} CS ^{S}	7	1
	AMP ^{R} AMC ^{S} CTX ^{S} ATM ^{S} ERT ^{S} IMP ^{S} CS ^{S}	3	1
Enterobacter cloacea	AMP ^{R} AMC ^{R} CTX ^{R} ATM ^{R} ERT ^{S} IMP ^{S} CS ^{S}	5	1
Pseudomonas aeruginosa	AMP ^{R} AMC ^{R} CTX ^{R} ATM ^{R} ERT ^{S} IMP ^{S} CS ^{S}	1	1
Staphylococcus aureus	AMP ^{R} OX ^{R} FOX ^{R} VAN ^{S} DA ^{S} E ^{S} GEN ^{R}	4	2
	AMP ^{R} OX ^{R} FOX VAN ^{R} DA ^{S} E ^{S} GEN ^{S}	2	1
	AMP ^{R} OX ^{R} FOX ^{S} VAN ^{S} DA ^{S} E ^{S} GEN ^{R}	1	1
Coagulase-negative *Staphylococcus*	AMP ^{R} OX ^{R} FOX ^{R} VAN ^{S} DA ^{S} E ^{S} GEN ^{R}	3	1
	AMP ^{R} OX ^{R} FOX ^{S} VAN ^{S} DA ^{S} E ^{S} GEN ^{R}	3	2

and *Klebsiella pneumoniae* in wound swabs, and coagulase-negative Staphylococci and *Escherichia coli* were predominant in samples of the environment (Figs. 1 and 2). The study of antibiotic susceptibility showed a strong resistance to betalactam antibiotics for both Gram-negative bacilli (Table 6) and Gram-positive cocci (Table 7) except for carbapenems. Similar resistance patterns were recorded in isolates obtained from wounds and those isolated from the hospital environment and materials from the wound dressing room (Table 8).

Bacteriological analyses of catheter specimens showed twenty-eight (28)-positive catheter tips out of eighty three (83). We recorded forty-seven (47) cases of proper disinfection in the eighty-three (83) cases containing thirty-seven (37)-positive samples (44.6%) with a significant correlation ($p = 0.01 < 0.05$) (Table 9). The catheter was placed for more than 48 h in forty-nine (49) children of whom thirty-eight (38) were contaminated (43.4%). The duration of catheterization and catheter infection showed significant correlation ($p = 0.037 < 0.05$) (Table 10). Sixty-eight (68) children presented inflammation on the catheter site with 47 positive cases. The presence of inflammation was however not significantly correlated to catheter infections ($p = 0.365$) (Table 11).

All twenty-eight (28) bacterial-positive specimens of catheter tips were monomicrobial with predominance of *Klebsiella pneumoniae* (32%), coagulase-negative Staphylococci (25%), and *Enterobacter cloacea* (14%) (Fig. 1). Antimicrobial susceptibility tests show strong resistance to ampicillin. No resistance to carbapenem and colistin or glycopeptides was noted for Gram-positive cocci and Gram-negative bacilli, respectively (Tables 12 and 13).

Table 9 Distribution of samples according to the quality of the disinfection

	Negative	Positive	Total	Chi^2 test
Good disinfection	37 (44.6%)	10 (12%)	47 (56.6%)	0.010
Bad disinfection	18 (21.7%)	18 (21.7%)	36 (43.4%)	
Total	55 (66.3%)	28 (33.7%)	83 (100%)	

Discussion

Surgical operative sites and catheter infections are common nosocomial infections. Many studies reveal incidence of nosocomial infections in Benin, generally [21], and in hospitals, particularly [22]. The present study wanted to explore these two particular types of nosocomial infections in CHU MEL of Cotonou City.

We observed a prevalence of 33.7% of contaminated catheter. This result is above the 14% found in Morocco by [23, 24] for catheter-related bacteremia. In Algeria, [25] reported 26.6% for suspected patients and 2.2% for all patients aged 15 to 60 years. However, catheter-related bacteremia are more common in newborns and children [26] and in adults, which can be attributed to their poor immunity [26]. A significant correlation was reported between the occurrence of catheter-related bacteremia and the duration of catheterization. Many authors have come to the same conclusion for catheter-related bacteremias [25, 27, 28]. Merrer [27] explains this fact by the multiplication of potentially septic manipulations from the connector (faucets, infusions …). For Douard [29], the occurrence of catheter-related bacteremia remains constant during the period of hospitalization. It is therefore at the time of the catheter placement that the bacteria are inserted. This leads

Table 10 Distribution of samples according to catheterization time

	Negative	Positive	Total	Chi^2 test
> 48 h	17 (20.5%)	17 (20.5%)	34 (41%)	0.037
< 48 h	38 (43.4%)	11 (15.7%)	49 (43.4%)	
Total	55 (66.3%)	28 (33.7%)	83 (100%)	

Table 11 Distribution of samples according to the presence of inflammation

	Negative	Positive	Total	Chi^2 test
Presence of inflammation	47 (56.6%)	21 (25.3%)	68 (41%)	0.365
Absence of inflammation	8 (9.6%)	7 (8.4%)	15 (18%)	
Total	55 (66.3%)	28 (33.7%)	83 (100%)	

Table 12 Antibiotic sensitivity of Gram-negative bacillus strains

	AMP (%)	AMC (%)	CTX (%)	ATM (%)	ERT (%)	IPM (%)	CS (%)
Escherichia coli	100	100	0	0	0	0	0
Klebsiella pneumoniae	100	100	33	22	0	0	0
Enterobacter cloacea	50	0	50	25	0	0	0
Proteus vulgaris	50	50	50	0	0	0	100
Klebsiella oxytoca	100	100	0	0	0	0	0

back to the second factor related to the occurrence of catheter-related bacteremia in our study: the quality of disinfection. This includes hand hygiene, the quality of the antiseptic used for hand hygiene, and the disinfection of the operation site [30, 31]. After bacteriological examination, the most isolated bacterial species from catheter tips are *Klebsiella pneumoniae* (32%), Staphylococci coagulase-negative (25%), and *Enterobacter cloacea* (14%). Numerous studies have shown the important role of coagulase-negative Staphylococci in nosocomial bacteremia [25, 32, 33]. Moreover, the antimicrobial susceptibility pattern of the strains reveals the presence of multidrug-resistant strains in these healthcare-associated infections.

On the other hand, wounds from cesarized women, the hospital environment, and materials used in the wound dressing were all infected by similar types of bacteria. These results are in agreement with those found by Kouchica [34] and Sossa [35] which show poor quality of material sterilization. *Staphylococcus aureus* were the most isolated bacteria in both wound swabs and environmental swabs, followed by coagulase-negative Staphylococci and *Klebsiella pneumoniae* in swabs of wounds, and coagulase-negative Staphylococci and *Escherichia coli* in samples from the environment. Studies have shown that *Staphylococcus aureus* remains the number 1 germ in all healthcare-associated infections, apart from abdominal surgery where Gram-negative bacilli prevail [36–38]. Considering the results with individual species, our results are also consistent with those of Coello and Guetarni [39, 40] who showed the prevalence of *Staphylococcus aureus* and negative-coagulase Staphylococci in abdominal healthcare-associated infections. These authors explain this fact by the presence of these species as commensals of the skin and mucous membranes, which are at high risk of contaminating the skin and mucous membranes during incision and manipulation. We moreover observed that higher number of wound dressing increases the level on wound infection, which supports the idea that the wounds are infected during dressing due to poor hygiene practices and poor sterilization of the materials. The strains found in the wounds were multidrug resistant with the same pattern as those found in the hospital environment, which further suggests an implication of the hospital environment in wound reinfection as previously described [41, 42].

Conclusion

Catheter-related bloodstream infections are major concern for the general population and children in particular. The results of the present study show the implication of the strains from the hospital environment and equipment in the occurrence of

Table 13 Antibiotic susceptibility of Gram-positive cocci strains

	AMP (%)	OX (%)	FOX (%)	VAN (%)	DA (%)	E (%)	GEN (%)
Staphylococcus aureus	100	67	0	0	0	0	86
Coagulase-negative *Staphylococcus*	74	57	33	0	0	0	57

healthcare-associated infections in cesarized women. The bacteremia associated with catheters is related to the duration of catheterization and the quality of disinfection. The most isolated strains were *Staphylococcus aureus*, coagulase-negative Staphylococci, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacea*, which were all multidrug resistant. It is important that good hygiene practices be promoted to limit healthcare-associated infections for the betterment of health and well-being.

Limitations Sequencing of *Salmonella* genome is not possible in Benin.

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Author Contributions VD, HK, and L B-M wrote the protocol.
KF, AO, IC, VD, and HK processed the samples.
DV did the statistical analyses.
HK and DV wrote the draft of the manuscript.
DV, HK, and L B-M reviewed the manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Compliance with Ethical Standards

Ethical Approval and Consent to Participate The Benin National Ethical Committee for Health Research has reviewed and approved this study under No 65/MS/DC/SGM/DRFMT/CNERS/SA. A written approval was taken from each patient involved in the study.

Consent for Publication All authors have read and gave their consent for publication. Written informed consent was obtained from the patients for publication of their individual details and accompanying images in this manuscript. However, the data submitted for this publication cannot permit to reveal their identity. The consent form is held by the authors' institution (Polytechnic School of Abomey-Calavi) and is available for review by the Editor-in-Chief.

Competing Interests All the authors declare that they have no competing interests.

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