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Antibiotics Susceptibility Profile of *Staphylococcus aureus* Clinical Isolates Collected in Hospitals in the City of Douala (Cameroon)

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Abstract

Introduction: *Staphylococcus aureus* is one of the most important agents involved in community and hospital-acquired infections. Due to the multi-drug resistance of strains to antibiotics, treatment to eradicate it is becoming more difficult and poses a global public health problem. **Methodology**: This was a cross-sectional study conducted from March to August 2020 in hospitals in the city of Douala, including all *S. aureus* isolates from diagnostic samples. Strain identification and antibiotic susceptibility testing were performed using the Vitek2 Compact™ (BioMerieux). **Results**: During the study period, 136 non-repetitive *S. aureus* strains were identified with a high frequency of methicillin-resistant *S. aureus* of 78.7%. The majority of the strains originated from the Douala General Hospital (66.9%) and was most frequently isolated from blood culture samples (55.1%). The study of biochemical characteristics showed that most of the strains identified had between 87% and 99% homology with the reference strain. The most active antibiotics were Quinupristin/Dalfopristin (94.2%), Linezolid (87.8%) and Vancomycin (84.2%). Methicillin resistance was associated with decreased susceptibility of *S. aureus* to other antibiotics such as Gentamycin (44.9%), Erythromycin (38.2%), Tetracycline (38.3%), Trimethoprim (21.4%), Ciprofloxacin (19.1%) and Levofloxacin (24.0%). Inducible MLSb and constitutive resistance phenotypes were

identified with 26.7% and 22.8% respectively. **Conclusion:** The sensitivity of *S. aureus* strains differs from one antibiotic family to another, and remains good for molecules that are not available in our context. The high frequency of Methicillin-Resistant *S. aureus* shows the continuous progression of multi-resistant strains of *S. aureus* and their decreased sensitivity to usual antibiotics becomes more and more alarming.

Keywords

Staphylococcus aureus, Multidrug Resistance, MRSA, Douala

1. Introduction

Staphylococci are bacteria that have been implicated in pathologies of varying severity and *Staphylococcus aureus* ranks first among opportunistic Gram-positive cocci responsible for nosocomial infections [1] [2]. Indeed, *S. aureus* has developed over the years several mechanisms of resistance to antibiotics and actually over 90% of strains produce a penicillinase, PLP2a (penicillin-binding protein) that subsequently leads to cross-resistance between the beta-lactam family (penicillin, carbapenem, cephalosporin) and other families of associated antibiotics [3] [4]. Due to the continuous progression of methicillin-resistant *S. aureus* (MRSA) infections, particularly in hospital settings, many studies are being carried out in order to better monitor them, understand their evolution and establish appropriate control strategies [5]. According to the WHO 2021 report on global antibiotic resistance, people with MRSA have a 64% higher risk of death compared to people with a non-resistant form of the infection; for this purpose, 25 countries have reported *S. aureus* resistance to Methicillin in 2019 as part of the Global Antimicrobial Resistance Surveillance System (GLASS) [6]. In Africa, the WHO reports major gaps in the monitoring of antibiotic resistance due to the lack of data, and the data available is worrying; in some countries, up to 80% of *S. aureus* resistant to methicillin is noted, which means that the treatment of infections of these strains with conventional antibiotics is ineffective [6] [7].

It is in this context that we carried out this study, the aim of which was to investigate the sensitivity to antibiotics of *S. aureus* strains isolated in two hospitals in the city of Douala (Cameroon), in order to specify the characteristics of this microorganism.

2. Methodology

Type and location of the study

This was a cross-sectional study conducted over a period of 6 months from March 1 to August 31, 2020. The strains were collected in the laboratories of seven public hospitals in Douala city, including: Laquintinie Hospital, General Hospital, Gyneco-Obstetric and Pediatric Hospital, Military Hospital, Deido, Nylon-Brazzaville and Bonassama District Hospitals. All sample analyses were

performed at the bacteriology unit of the clinical biology laboratory of the Douala General Hospital (DGH).

Strains studied

Non-repetitive strains of *S. aureus* isolated from diagnostic specimens of patients who had undergone bacteriological examination in the laboratories concerned were included. Isolates considered as contaminants were excluded.

Data collection, transport and storage of strains

Data concerning the patient (sex, age, department of origin) and the strain (date and type of sampling, bacterial species) were collected from the records in strict observance of confidentiality. The tubes containing the *S. aureus* isolates were transported in a closed cooler at room temperature to the laboratory.

Strain identification

Isolation of the strains in order to obtain pure isolates was performed by plating the samples on Mannitol Salt Agar (BioMérieux reference 610029). After macroscopic analysis of the colonies (appearance, texture, size), microscopic analysis was performed on Gram stained smears for confirmation of morphology (Gram positive cocci in clusters).

Using GP identification cards (BioMerieux reference 21342) the *S. aureus* strains were identified by the Vitek 2 compact™ automated system (BioMerieux).

The biochemical profile obtained was compared to those of the taxa contained in the database and the Vitek2™ performed a calculation of probability of correct identification automatically. Different confidence levels were associated.

Antibiotic sensitivity

The antibiotic susceptibility study was determined by the Vitek2 compact™ dilution method using AST-GP75 cards (BioMérieux reference 415670) and the results interpreted according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (EUCAST/CASFM 2019) [8].

Panel of tested molecules: Benzylpenicillin (BEZ), Oxacillin (OXA), Cefoxitin (CFX), Gentamycin (GEN), Ciprofloxacin (CIP), Levofloxacin (LVX), Moxifloxacin (MXP), Clindamycine (CLI), Erythromycin (ERY) Quinupristin/Dalfopristin (QDA), Vancomycin (VAN), Tetracycline (TET), Tigecycline (TIG), Trimethoprim/Sulfamethoxazole (TSU), Linezolid (LNZ).

Interpretation of results

For the interpretation of the susceptibility tests, two clinical categories were retained based on the minimum inhibitory concentrations of the antibiotics tested:

- Susceptible (S) strains to antibiotics for which the probability of therapeutic success is high.
- Resistant strains (R) for which there is a high probability of therapeutic failure.

Cefoxitin was considered as a screening antibiotic for MRSA. Results were interpreted as positive (+) for MRSA (Methicillin-Resistant *Staphylococcus aureus*) and negative (−) for MSSA (Methicillin-Sensitive *Staphylococcus aureus*).

Inducible resistance to Macrolides, Lincosamines and Streptogramines (MLS) was detected by the Muller Hinton agar diffusion method with Clindamycin disk in the presence of Erytromycin disk. The results were interpreted as positive (+) when induction was present and negative (−) when induction was absent.

Statistical analysis

Microsoft office Excel 2010 software was used to record the data and calculate the frequencies. The qualitative variables were represented in frequency and for the comparison of the variables we used the Chi-square test. The difference was considered statistically significant for a P-value less than 0.05.

3. Results

During the study period, 136 strains of *S. aureus* were selected from the laboratories of 7 hospitals in the city of Douala (Table 1).

The strains were most frequently isolated at the Douala General Hospital (66.9%), regardless of the type of sample, and the majority was from the departments of Medicine, Surgery and Pediatrics (36.8%, 21.3% and 18.4% respectively) (Table 1). Most of the strains came from blood cultures (55.1%) and pus (35.3%). The age range of the patients most represented was 30 to 40 years, with a male predominance (Sex ratio = 1.6) (Table 1).

Biochemical profile

The biochemical characteristics studied revealed 10 different phenotypic profiles ranging from 87% to 99% homology with the reference strain.

Antibiotic susceptibility profile

- **Overall susceptibility**

Benzylpenicillin, oxacillin and cefoxitin showed low activity among all strains with 3.2%, 17.4% and 21.3% respectively. Quinupristin/Dalfomipristin, Linezolid and Vancomycin however, showed better activity on all strains with 94.2%, 87.8%, and 84.2% respectively.

- **According to the hospitals (Figure 1)**

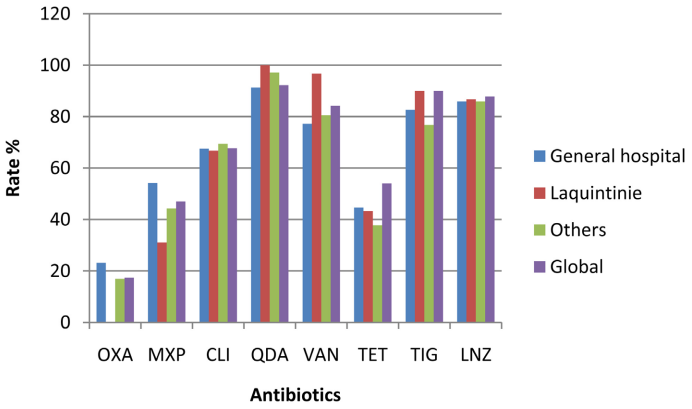

Figure 1. Susceptibility profile of *S. aureus* according to healthcare facilities. OXA = Oxacilline; MXP = Moxifloxacin; CLI = Clindamycine; QDA = Quinupristin/Dalfopristin; VAN = Vancomycin; TET = Tetracycline; TIG = Tigecycline; LNZ = Linezolid. Others: Military Hospital, Deido, Nylon-Brazzaville and Bonassama District Hospitals.

Table 1. Distribution of *Staphylococcus aureus* strains.

Variable		MRSA	MSSA	Total		p-value
		n	n	n	%	
Healthcare facility	General hospital	70	21	91	66.9	0.25
	Laquintinie	26	3	29	21.3	
	Deido	1	2	3	2.2	
	Gyneco-Obs-Pediatric	2	1	3	2.2	
	Military Hospital	4	0	4	2.9	
	Nylon-Brazzaville	2	1	3	2.2	
	Bonassama	2	1	3	2.2	
Service	Surgery	23	6	29	21.3	0.09
	Outpatient	15	1	16	11.8	
	Gynecology	1	1	2	1.5	
	General Medicine	37	13	50	36.8	
	Neonatology	14	0	14	10.3	
	Pediatric	17	8	25	18.4	
Gender	Female	40	9	49	36	0.53
	Male	67	20	87	64	
Age groups (Year) Mean: 32.5 ± 23.5 Min: 1 day Max: 89 years	0 - 1	20	2	22	16.2	0.11
	1 - 10	7	5	12	8.8	
	10 - 20	12	1	13	9.6	
	20 - 30	8	7	15	11.0	
	30 - 40	21	3	24	17.6	
	40 - 50	11	5	16	11.8	
	50 - 60	13	2	15	11.0	
	60 - 70	9	3	12	8.8	
	>70	6	1	7	5.1	
Type of sample	Urine	7	2	9	6.6	0.4
	Blood culture	60	15	75	55.1	
	PUS	38	10	48	35.3	
	Genital	2	2	4	2.9	
Total		**107 (78.7%)**	**29 (21.3%)**	**136**	**100**	

MSSA: Methicillin susceptible *Staphylococcus aureus*; MRSA: Methicillin resistant *Staphylococcus aureus*.

The most active antibiotics at the Douala General Hospital were Quinupristin/Dalfomipristin, Tigecycline and Linezolid with 91.3%, 82.6%, and 85.9% susceptibility rates respectively.

At Laquintinie Hospital, Oxacilin, Benzylpenicillin and Cefoxitin had low activity on all strains with 0%, 3.3% and 10.3% respectively. In comparison, Quinupristin/Dalfomipristin, Vancomycin and Tigecycline were 100%, 96.7% and 90% active respectively.

In the other hospitals (Gyneco-Obstetric and Pediatric Hospital, Military Hospital, Deido, Nylon-Brazzaville and Bonassama District Hospitals), Quinupristin/Dalfomipristin, Linezolid and Vancomycin were the most active with rates of 97.1%, 85.9% and 80.5% respectively. Benzylpenicillin, Oxacilin and Cefoxitin were the most inactive with 1.4%, 16.9% and 20.0% respectively.

- **According to gender**

Quinupristin/Dalfomipristin and Tigecycline were the most active in women with 93.2% and 89.5% respectively. Linezolid and Vancomycin were slightly more active in males (88.8% and 84.3%). Benzylpenicillin, Trimethoprim/Sulfamethoxazole and Oxacillin were the least active on both sexes.

- **According to age**

Quinupristin/Dalfomipristin, Tigecycline and Linezolid were 100% active in the 1 - 10 and 40 - 50 age groups. Over 70 years of age, Quinupristin remained 100% active, but the sensitivity decreased with Vancomycin and Tigecycline, with 57.1% and 40.0%.

- **According to the type of specimens (Figure 2)**

Quinupristin/Dalfomipristin and Vancomycin had good activity on urine culture (100%). Quinupristin/Dalfomipristin also had good activity in blood cultures (97.1%) and Tigecycline in pus (97.8%).

Frequency of MRSA

MRSA predominated (78.7%) compared to MSSA regardless of collection site, type of specimen, age group or sex.

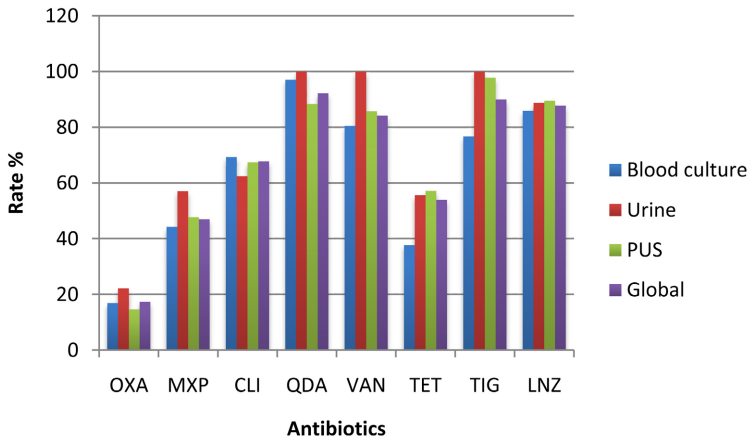

Figure 2. Susceptibility profile of *S. aureus* according to type of sample. OXA = Oxacilline; MXP = Moxifloxacin; CLI = Clindamycine; QDA = Quinupristin/Dalfopristin; VAN = Vancomycin; TET = Tetracycline; TIG = Tigecycline; LNZ = Linezolid.

MRSA were more present at the General Hospital and Laquintinie Hospital compared to the other health facilities, but this result was not statistically significant (p-value > 0.05); similarly the 30 to 40 year old age group was the most affected by MRSA (p-value > 0.05). MRSA strains were predominantly isolated from blood cultures and pus that is 80.0% and 79.2% respectively (Table 1).

Comparison of antibiotic susceptibility patterns of MRSA and MSSA (Figure 3)

Benzylpenicillin, Cefoxitin and Oxacilin were not active on MRSA strains. Erythromycin was less active on MRSA (38.2%), while Quinupristin/Dalfomipristin and Clindamycin showed better activity with sensitivity rates of 92.7% and 64.3% respectively. Tetracycline showed a low activity on MRSA strains with 38.3% contrary to Tigecycline that showed a good activity on strains with 83.9%. All the Fluroquinolone group antibiotics showed low activity on MRSA strains with 19.1% for Ciprofloxacin, 24.0% for Levofloxacin and 33.3% for Moxifloxacin. A good sensitivity of strains was observed for Vancomycin and Linezolid with rates of 80.2% and 85.8% respectively. A low sensitivity of strains was observed for the Trimethropine-Sulfamethoxazole combination (21.4%).

The difference in profile between MRSA and SASM was:

- Significant for Oxacilin, Cefoxitin, Gentamycin, and Ciprofloxacin (p-value < 0.05).

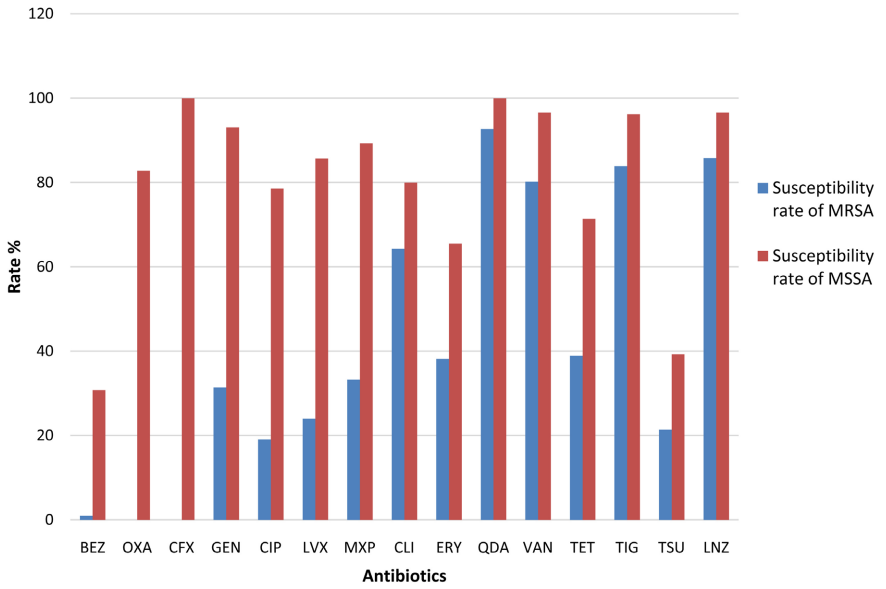

Figure 3. Susceptibility profile of *S. aureus* comparing MRSA and MSSA. MRSA = *Meticillin Resistant S. Aureus*; MSSA = Meticillin Susceptible *S. aureus*; BEZ = Benzylpenicillin; OXA = Oxacilline; CFX = Cefoxitin; GEN = Gentamycin; CIP = Ciprofloxacin; LVX = Levofloxacin; MXP = Moxifloxacin; CLI = Clindamycine; ERY = Erytromycin; QDA = Quinupristin/Dalfopristin; VAN = Vancomycin; TET = Tetracycline; TIG = Tigecycline; TSU = Trimethoprim/Sulfamethoxazole; LNZ = Linezolid. p-value ≤ 0.001 for Benzylpenicillin, Oxacilin, Cefoxitin, Gentamycin, Ciprofloxacin, Levofloxacin and Moxifloxacin; p-value > 0.05 for Quinupristin/Dalfomipristin, Vancomycin, Tetracycline, Tigecycline, and Linezolid.

- Not significant for Quinupristin/Dalfomipristin, Vancomycin, Tigecycline, and Linezolid (p-value > 0.05).

Resistance phenotypes

Inducible Clindamycine resistance associated with MRSA were found in 26.7% of the strains isolated and constitutive MLSBs in 22.8%.

4. Discussion

Nature of the sample

At the end of this study we observed that *S. aureus* strains were found mostly in blood culture samples with 11.8% from outpatients; Frikh *et al.* in 2015 had isolated *S. aureus* mostly in pus (29.1%) and in blood cultures (22.2%) over a longer period (8 years) on a sample of 1124 specimens of which the majority (77%) were of hospital origin [7].

Biochemical profile

The analysis of the biochemical profiles showed an 87% - 99% homology with the reference strain, which reveals a good identification of staphylococci in the different health facilities, but also a large diversity that makes epidemiological monitoring difficult. This variability could be caused by mutations due to the selective pressure of antibiotics, thus favoring the presence of multiple clones [5].

Antibiotic susceptibility profile

Antibiotic susceptibility testing of strains isolated from patients and medical devices in hospitals is very important in directing the choice of treatment and reducing the selection risk exerted by antibiotics [9].

The susceptibility profile of all *S. aureus* strains showed good activity of Quinupristin/Dalfopristin, Linezolid and Vancomycin regardless of origin, patient gender or age. This result is comparable to the results of Njoungang *et al.* in 2015 at the Military Hospital of Yaoundé; however, this good sensitivity towards these antibiotics could be explained by the limited use and unavailability in the local market of these molecules [10].

Low activity on the strains was observed for Benzylpenicillin (3.2%) and Oxacillin (17.4%); Koinan *et al.* in 2017 in Burkina Faso had observed also low sensitivity rates for these two molecules, *i.e.* 8% and 16% respectively [11]. This decrease in sensitivity could be the consequence of a hyper production of penicillinase responsible for the hydrolysis of penicillin M and cephalosporins; the most likely cause of all these mechanisms would be the genetic plasticity of *S. aureus* and certainly the empirical or inappropriate consumption of these antibiotics [12] [13].

Frequency of MRSA

This study showed a high rate of MRSA previously observed by some other authors in Cameroon, in particular Njoungang *et al.* in 2015, Gonsu *et al.* in 2013 and Kengne *et al.* in 2019 who had found rates of 72%, 76% and 80.4% on strains isolated at the Central and Military Hospitals of Yaoundé [10] [14] [15].

The presence of MRSA is often associated with resistance to many antibiotics as noted by Kengne *et al.* with Vancomycin (79.7%), Tobramycin (70.3%), Doxycycline (68%) and Erythromycin (55.7%); therefore, there is a need to improve surveillance systems in order to reduce the spread of these multi-resistant bacteria [15]. The frequencies vary from one country to another but are generally high: 16% in Senegal and Niger, 20% to 47% in Nigeria, 36% in Benin and 35.7% in Togo [16]. In Ivory Coast, a study conducted in three referral hospitals reported a rate of 39%, reflecting the extent of the MRSA problem in West Africa [9].

The spread of resistant strains in our environment could be impacted by a number of factors among which the lack of monitoring of *S. aureus* infections in hospitals and uncontrolled over prescription of antibiotics; this situation requires the implementation of a policy to control the clonal diffusion of strains [5].

The identification of MRSA in hospitalized and outpatients suggests the presence of these strains in the community and in hospitals of the city of Douala with frequencies different from those reported by Atshan *et al.* in 2016 in Mauritania and Njoungang *et al.* in 2015 in the city of Yaoundé [5] [10]. Actually, the frequency of MRSA varies according to activity, nature of infections, type of prophylaxis and antibiotic therapy and by country [17]. However, other factors such as presence of epidemic strains, period of the study, transfer of patients between different departments, hospitalizations in intensive care units could determine this frequency [3].

Resistance to Methicillin is often associated with a decrease in the sensitivity of *S. aureus* to other families of antibiotics such as the aminoglycosides represented by gentamicin; in Turkey in 2014 the research of Yildiz *et al.* had shown high rates of resistance to gentamicin (81.0%), erythromycin (63%) and clindamycin (50%) [18].

The family of fluroquinolones represented by 3 molecules (Ciprofloxacin, Levofloxacin, Moxifloxacin) also showed a very low activity against MRSA strains. These results are similar to those of Frikh *et al.* in Morocco who found a high resistance rate of 68.3% [7].

Decreased sensitivity to macrolides was observed for Erythromycin, *i.e.* 38.2%; Abbas *et al.* in 2015 found a rate of resistance to this antibiotic of 40.2% in *S. aureus* strains isolated in several bacteriological samples [19].

Although Vancomycin has good activity on MRSA strains, 21 strains were resistant. Kengne *et al.* also observed this resistance at the Central Hospital of Yaoundé with a high rate of 79.7% [15]. Vancomycin-resistant (VRSA) and intermediate-resistant (VISA) strains of *S. aureus* have been described since 1997 and the next few years in Japan and other Asian countries, the United States and Africa [20]. This reflects a metabolic reorganization of the peptidoglycan linked to mutations in several genes or in the expression of these genes that can prevent the accessibility of Vancomycin to its target [19]. The decreased susceptibility of

S. aureus to Vancomycin is a current problem as this molecule has been a drug of choice for the treatment of MRSA infections [21].

Associated risk factors

The predominance of *S. aureus* strains has been observed in male patients with a rate of 64%; Frikh *et al.* observed the same tendency, however, the difference is not always statistically significant [7].

High-risk factors for staphylococcal infections are hospitalization, prolonged stay in healthcare facilities, chronic renal failure, HIV infection, diabetes, and young age (newborn and elderly) [22]. We observed the highest frequencies in the age groups 0 to 1 year and 30 to 40 years.

Resistance phenotypes

The study of resistance to Clindamycin revealed 26.7% of strains with inducible MLSb and 22.8% of constitutive MLSb. These results are similar to those of Rebiahi *et al. in* 2011 and Ouchenane *et al.* in 2011 [23] [24]. The frequency of these phenotypes varies according to geographical region and proves that MRSA expresses the more advanced macrolide resistances usually related to methylase production [25].

5. Conclusion

The MRSA strains show a decreased sensitivity towards several families of antibiotics. In general, Linezolid, Quinupristin and Vancomycin maintain a good activity on all strains. However, this sensitivity is not the same in all hospitals in the city of Douala. The progression of these strains is a phenomenon to be monitored because the transmission of MRSA is not controlled. The implementation of new strategies to control multi-resistant strains of *S. aureus* is of major importance in order to limit their spread within hospitals.

Acknowledgements

We express our gratitude to the staff of all the Laboratories concerned for their support and cooperation during this study.

Availability of Data

All data supporting these findings can be found in the bacteriological Unit of the Laboratory of Clinical Biology of the DGH.

Authors' Contributions

COE and DA coordinated the study, COE, JPNM and YS drafted the manuscript, YS, ERM, GPN, NH and GN collected data and participate in its design, and IPNM, NH and ERM performed the statistical analysis. All authors read and approved the final manuscript.

Ethics

The study was conducted in accordance with ethics directives related to research

in Cameroon. We obtained the research authorizations of the Directors of the concerned hospitals, ethical clearance from the Institutional Ethics Committee of Research for Human Health of the University of Douala, and the approval of the General Hospital of Douala institutional review board.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviation

DGH: Douala General Hospital