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Prevalence and antibiotic resistance patterns of strains of *Staphylococcus aureus* isolated at the Yaounde Military Hospital, Cameroon

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ABSTRACT

Staphylococcus aureus is a ubiquitous pathogen involved in various pathologies. It has become a health problem worldwide due to the emergence of multidrug-resistant strains. This study was conducted to determine the prevalence and resistance patterns of *S. aureus* isolates from clinical specimens at the Yaounde Military Hospital, Cameroon. A total of 201 specimens were collected from both in and outpatients during a period of 6 months (June to December, 2013). Antibiotic drug susceptibility testing was performed using the disk diffusion method of Kirby-Bauer, according to the recommendations of the French Society of Microbiology. Data was analyzed using SPSS version 17. A total of 39 strains of *S. aureus* were isolated from various clinical specimens; 28 (72%) were resistant to methicillin. The results showed that all (100%) MRSA strains were multidrug resistant (resistance to one or more antibiotics classes), with 18 different resistance patterns. High resistance was recorded for penicillin (100%), cefoxitin (100%), doxycycline (61%), ofloxacin (50%), and erythromycin (50%). In contrast, the strains showed a high sensitivity to vancomycin (100%), pristinamycin (100%), followed by gentamicin (68%), netilmicin (68 %) and lincomycin (61%). Also, one wild type (susceptible to all antibiotics tested) was identified, suggesting that a rational drugs use could reduce the threat posed by the emergence of antimicrobial resistance. This study has highlighted the large number of staphylococcal infections; especially MRSA phenotype. There is an urgent need to monitor *S. aureus* to develop therapeutic guidelines for better treatment and to implement antimicrobial stewardship programs in communities as well as in hospitals.

Keywords: Prevalence, *Staphylococcus aureus*, antibiotics, resistance, MRSA.

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INTRODUCTION

Staphylococcus aureus is one of the organisms most frequently encountered in human infections, especially in elderly individuals (Boucher and Corey, 2008; Bradley, 2002). It has emerged over the years, as a universal and versatile dangerous pathogen in humans (Lowy, 1998). It is responsible for a large variety of infections, ranging from simple minor skin infections to severe and generalized ones (Tiwari et al., 2009). *S. aureus* plasmid

acquired resistance to penicillin barely about ten years after its discovery. A new antibiotic, methicillin, was introduced in 1959 to deal with this problem of penicillin resistance. However, a new resistance mechanism (chromosomal this time) emerged due to methicillin resistant strains of *S. aureus* (MRSA) (Loffler and Macdougall, 2007; Maple, 1989).

The frequency of both hospital and community acquired infections has gradually increased, leading to changes in global mortality rate (Lowy, 1998; Johnsson et al., 2004). MRSA has become a human health problem, requiring multidisciplinary efforts worldwide because of the emergence of MRSA pandemics in hospitals, and strains with reduced sensitivity to glycopeptides (Cosgrove et al., 2003; Kopp et al., 2004). The proliferation of international contacts through travel and trade has simplified the rapid spread of new resistant strains throughout the world. Infections due to multidrug-resistant bacteria are difficult to treat and are frequently associated with high mortality rate, increased costs and longer duration of treatment. Broad spectrum antibiotics used in the routine treatment of various infections caused by *S. aureus* are no longer effective so: it is necessary to use a new generation of antibacterial substances like mupirocin or moxifloxacin that are considered the last resort in MRSA treatment and also are very expensive. The objective of this study was to determine the prevalence and resistance patterns of strains of *S. aureus* isolated from clinical samples at the Yaounde Military Hospital, Cameroon.

MATERIALS AND METHODS

Study population

This study was conducted during a period of 6 months (June to December, 2013), at the Yaoundé Military Hospital. This tertiary hospital is located in Yaoundé – the capital of Cameroon. Permission to conduct this study was obtained from the chief medical officer of the Hospital. Informed consent was obtained from all subjects before participation. A total of 201 specimens were collected from both in- and out-patients. These included genital swabs, pus swabs, bone fragments, urine samples, and ear discharge. The samples were collected using the methods described by Cheesebrough (2000).

Isolation and identification of *Staphylococcus aureus*

The samples were seeded on Columbia agar and Mannitol salt medium. Formed colonies were purified on blood agar containing poly- vitamin supplement, and stored at -20 and -80°C in nutrient broth 20% glycerol. The isolated bacteria were identified based on their morphology after Gram staining, the appearance of colonies, and biochemical tests (catalase, mannitol fermentation and DNase production).

Antibiotic sensitivity testing and interpretation

The Kirby- Bauer method was used for the antibiotic sensitivity testing (Bauer et al., 1966). Using an 18 to 24 h fresh culture, 1 to 2 colonies were mixed with 2.5 ml of sterile distilled water, resulting in a 0.5 turbidity on the McFarland scale. Mueller Hinton agar plates were inoculated with 0.5 ml McFarland suspension and then dried at room temperature for 10 min. Thereafter, antibiotic discs were carefully placed onto the previously inoculated plates, followed by incubation at 35 to 37°C for 18 to 24 h in normal atmosphere. The inhibition zone was measured for each antibiotic disc and interpreted according to the Committee on Antibiotic susceptibility of the French Society of Microbiology (CA-SFM) (CA-SFM, 2013). The following antibiotics were tested: Penicillin (P - 10 UI), Cefoxitin (FOX - 30 µg), Gentamicin (G - 15 µg), Tobramycine (TOB - 10 µg), Amikacin (AK - 30 µg), Netilmycin (NET – 30 µg), Doxycycline (DOX - 30 µg), Vancomycin (VA – 30 µg), Trimethoprim-sulfamethoxazole (cotrimoxazole) (SXT - 1.25/23.75 µg), Erythromycine (E - 15 µg), Lincomycine (L - 15 µg), Pristinamycine (PT - 15 µg), Pefloxacine (PEF - 5 µg) and Ofloxacine (OFL - 5 µg). The phenotypic detection of methicillin resistance was performed using a cefoxitin disk (30 µg) incubated for 18 to 24 h in normal atmosphere at 37°C. Any strains with a diameter of inhibition less than 25 mm around the cefoxitin disk were interpreted as MRSA (CA-SFM, 2013). In addition, the phenotypic macrolide, lincosamides and Streptogramine B (MLSB) resistance profile were identified using the D-test. Briefly, lincomycine (used as surrogate of clindamycin) and erythromycin discs were placed at 15 mm (edge to edge) following the CLSI guidelines (2009) and alongside with the routine antibiotic sensitivity testing (CLSI, 2009). Inducible resistance to lincomycine were characterized by a flattening or blunting of the lincomycine zone adjacent to the Erythromycin disc, giving a D shape and confirming the phenotype MLSB inducible. Single resistance to erythromycin or lincomycine were identified as MLSB via efflux pump and resistance to erythromycin, lincomycine and pristinamycine concomitantly were characterized as MLSB constitutive.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS version 17.0; Chicago, USA). Significant differences between categorical variables were determined using the Friedman test. A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 201 clinical samples were analyzed for the detection of *S. aureus* strains. This pathogen was detected in 39 of the samples collected with a prevalence rate of 19.4%. Of these, 28 (72%) were resistant to methicillin. The prevalence rate of *S. aureus* according to sex and isolation site is detailed in Tables 1 and 2. The most frequent infection sources are urogenital swabs with 21 strains (10.4%) of *S. aureus*; the majority being from women (57%) and including 17 MRSA (81%) (Table 2).

The results of the antibiotic resistance patterns are shown in Table 3. A total of 11 strains (28%) were resistant to at least one antibiotic (Penicillin G (MSSA); while 28 (72%) were resistant to at least two antibiotics (penicillin G and cefoxitin (MRSA). All isolated strains expressed a variable sensitivity for all 14 antibiotics tested. The MRSA isolates expressed a high level (100%) of sensitivity to pristinamycine and vancomycin. In contrast, a high level of resistance was expressed for: doxycycline (61%), ofloxacin (50%), pefloxacin (50%),

Table 1. *S. aureus* isolation by sample type.

Sample type	*S. aureus* (%)	MRSA (%)	MSSA (%)
Uro-genital swabs	21 (54)	17 (81)	4 (19)
Urine samples	12 (31)	6 (50)	6 (50)
Bone fragments	2 (5)	1 (50)	1 (50)
Pus swabs	2 (5)	2 (100)	0 (0)
Ear discharge	2 (5)	2 (100)	0 (0)
Total	39	28 (72)	11 (28)

$X^2 = 10$, df = 1, p = 0.01. MRSA: methicillin resistant *S. aureus*, MSSA: methicillin susceptible *S. aureus.*

Table 2. Distribution of *S. aureus* by sex and sample type.

Sample	Male		Female	
	Number	Percentage	Number	Percentage
Uro-genital swabs	12	57	9	43
Urine samples	5	42	7	58
Pus swabs	0	0	2	100
Bone fragments	0	0	2	100
Ear discharge	1	50	1	50
Total	18	46	21	54

$X^2 = 1.8$, df = 1, p = 0.18.

Table 3. Antibiotic resistance patterns of *S. aureus* isolates.

Antibiotics tested	MRSA isolates (n = 28)		MSSA isolates (n = 11)	
	Number	Percentage	Number	Percentage
Penicillin	28	100	9	82
Cefoxitin	28	100	0	0
Gentamicin	9	32	4	36
Netilmycine	9	32	4	36
Tobramycine	12	43	6	55
Amikacine	12	43	6	55
Erythromycin	14	50	6	55
Lincomycine	11	39	4	36
Pristinamycine	0	0	0	0
Pefloxacin	14	50	6	55
Ofloxacin	14	50	6	55
Co-trimoxazole	10	36	7	64
Doxycycline	17	61	8	73
Vancomycin	**0**	0	0	0

$X^2 = 12$, df = 1, p = 0.01.

and erythromycin (50%).

Figure 1 shows the distribution of *S. aureus* phenotypes per ward. Of the 39 *S. aureus* isolated from various units, 24 (62%) were from out-patients; among these, 18 (46%) were MRSA. The gynecology, surgery and ORL wards displayed 5 (13%), 3 (8%) and 2 (5%) isolated MRSA, respectively.

A high level of multidrug resistance (ability of bacteria to resist to 3 or more antibiotics) was observed for MRSA and MSSA strains (Figures 2 and 3). Twenty nine percent of MRSA were resistant to 8 antibiotics, 18% were resistant to 10 antibiotics, followed by the resistance to 9 and 7 antibiotics with a prevalence of 11% each. Although methicillin susceptible, 81% of MSSA strains

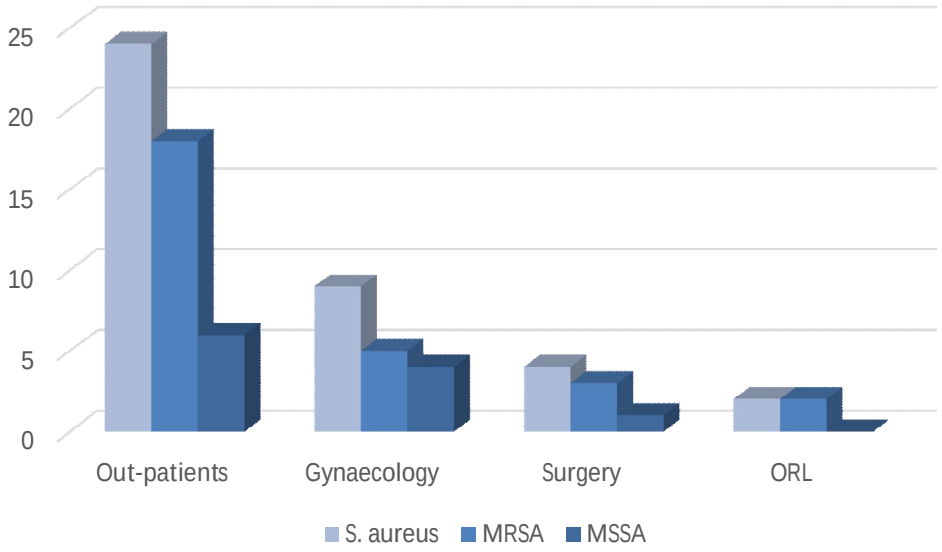

Figure 1. Distribution of *S. aureus* phenotypes per hospital ward.

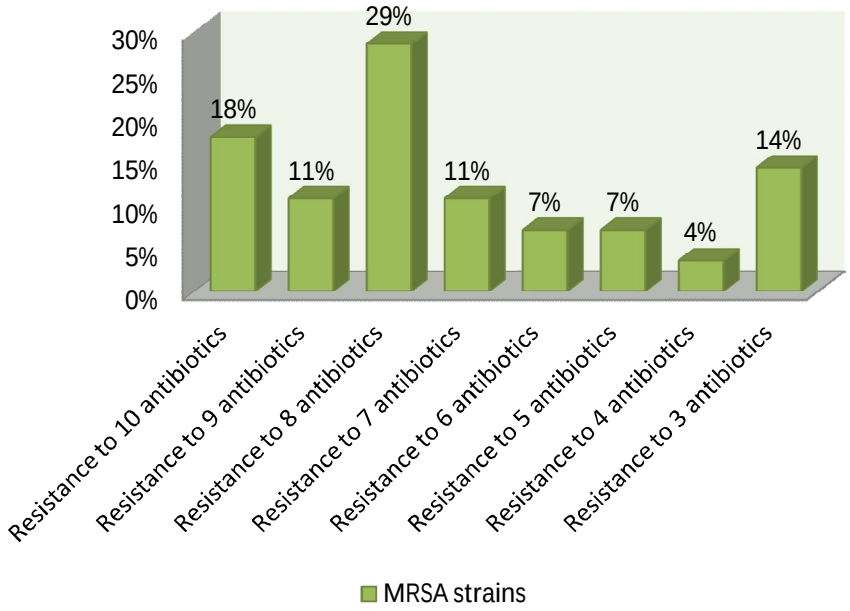

Figure 2. Multidrug resistance of MRSA strains.

were multidrug resistant. The resistance to 3 antibiotics were most common (45%) followed by the resistance to 7 (36%) and 5 (9%) antibiotics respectively. One wild type strain was identified with natural sensitivity to all antibiotics tested.

MRSA strains were commonly resistant to 10 antibiotics P.FOX.AK.TOB.CN.OFL.PEF.E.L.DXT (14%), followed by the profile P.FOX.DXT (11%), and P.FOX.OFL.PEF.E.L.DXT.SXT, P.FOX.AK.TOB.CN.OFL.PEF.DXT.SXT, P.FOX.AK.TOB.OFL.PEF.DXT.SXT, P.FOX.AK.TOB.E.L.DXT and P.FOX.AK.TOB.OFL.PEF.E.L for a prevalence of 7% each (Figure 4). MSSA strains were frequently concomitantly resistant to P.DXT.SXT (36%), to P.AK.TOB.CN.E.DXT.SXT (18%) and AK.OFL.PEF.E.L.DXT.SXT (18%) (Figure 5).

Four resistance phenotypes (Tables 4 and 5) were identified among the macrolides and within the aminoglycosides among the 28 MRSA strains. Seven (25%) were MLSB inducible, 6 (21%) MLSB constitutive and 4 (14%) resistant to erythromycin via efflux pumps.

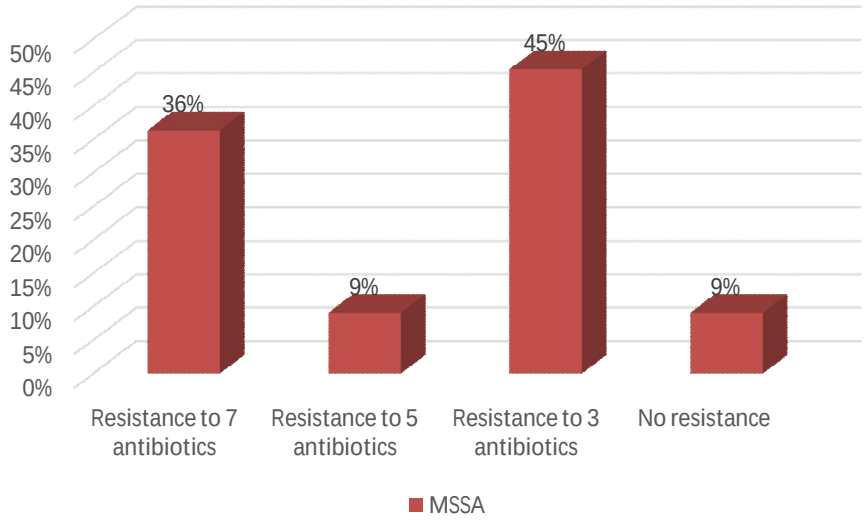

Figure 3. Multidrug resistance of MSSA strains.

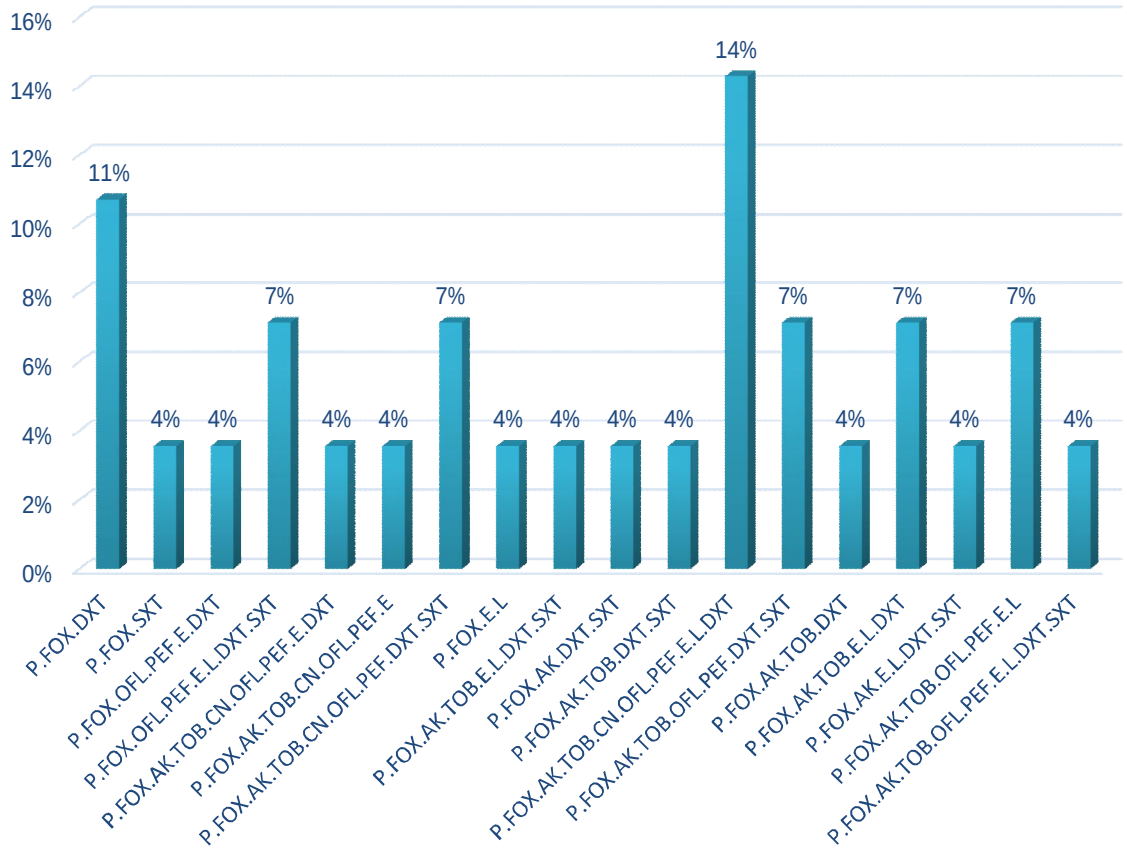

Figure 4. Antibiotic resistance profiles of MRSA strains.

The resistance phenotypes of the MRSA with aminoglycosides revealed that 9 (32%) strains were phenotype KT, 8 (29%) KTG and 3 (11%) were K phenotype.

DISCUSSION

S. aureus is one of the most common species responsible for human infections. It has become a human

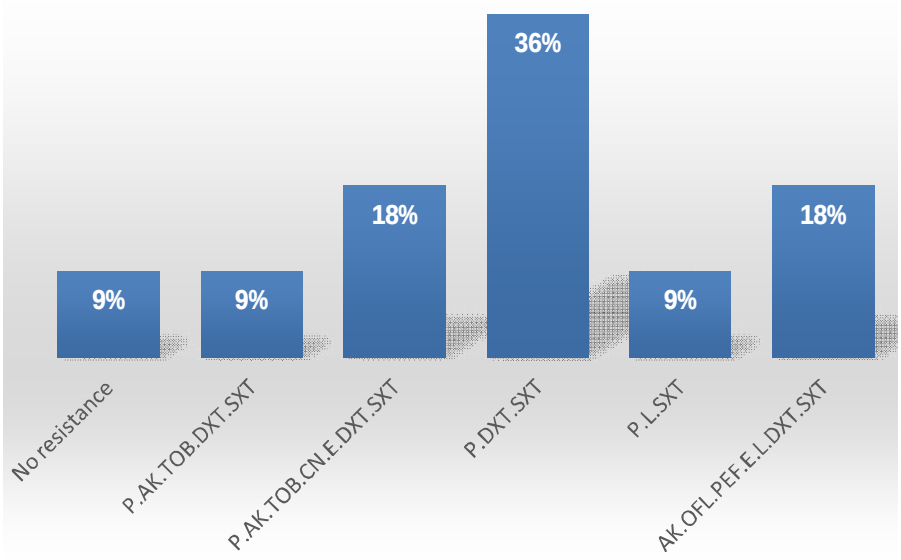

Figure 5. Antibiotic resistance profiles of MSSA strains.

Table 4. Resistance phenotypes of MRSA against macrolides.

Erythromycin	Lincomycine	Pristinamycine	Phenotype/mechanisms	Number of strains	Percentage
S	S	S	Wild type	11	39
R	S	S	Erythromycin resistant (Efflux pump)	4	14
R	S	S	MLSB inducible	7	25
R	R	S	MLSB constitutive	6	21

S: D-zone around the lincomycine disk (inducible lincomycine resistance).

Table 5. Resistance phenotypes of MRSA against aminoglycosides.

Amikacin	Tobramycine	Gentamicin	Phenotypes	Number of strains	Percentage
S	S	S	Sensitive (Wild type)	8	29
R	S	S	K	3	11
R	R	S	KT	9	32
R	R	R	KTG	8	29

health problem, requiring multidisciplinary efforts at a global level because of the emergence of MRSA pandemics in hospitals (Cosgrove et al., 2003; Kopp et al., 2004). This study assessed the prevalence and antibiotic resistance patterns of *S. aureus* in a healthcare setting to provide epidemiological data and knowledge about this pathogen in Yaoundé, Cameroon.

Of the 201 samples tested, 39 (19%) strains were identified as S. *aureus*. The high prevalence of *S. aureus* among clinical samples could be as a result of the widespread of the pathogen in nature (Murray et al., 2003). Similar results were reported by Opere et al. (2013) in Nigeria, where the prevalence of *S. aureus* was 18.06%. The prevalence obtained in this study is higher than that reported by Benouda and Elhamzani (2009) in Morocco, where a prevalence rate of 6.7% was obtained. However, it is lower than that obtained in India where the prevalence of this organism was 85.7% (Bilal and Srikanth, 2013).

Eleven strains (28 %) were considered as methicillin susceptible *S. aureus* (MSSA) strains; while 28 (72%) were identified as MRSA. The majority (46%) were from out-patients, followed by the gynecology (13%), surgery (8%) and ORL (5%) hospital wards. The isolation of MRSA strains among in- and out-patients suggests that MRSA is present in communities and hospitals in Cameroon, with prevalence consistent with that reported elsewhere. In 2004 and 2007 in the USA, the LEADER program, found a prevalence of 54.2 and 58.1%, respectively in the case of MRSA strains (Draghi et al.,

2005; Jones et al., 2008). In a study on MRSA inducing bacteraemia in the United Kingdom, the prevalence of this organism ranged between 40 and 45% from 2001 to 2005, and in 2007 it dropped to 36% through the implementation of target strategies (Johnson, 2005). Across Europe, the prevalence of MRSA isolates exceeds 25%, with rates close to 50% reported from Greece, Portugal, Cyprus and Malta in 2007 (Woodford and Livermore, 2009). MRSA prevalence is still low (<2%) in the Netherlands and Scandinavia, thanks to the success of the "Search and Destroy" program Infection Control (Wertheim et al., 2004). The Indian Network for Surveillance of antimicrobial resistance (2013) and Mukhiya et al. (2013) reported that in India, Nepal and Pakistan, the incidence of the MRSA strains were found to be 40, 62 and 23.9%, respectively. The prevalence of MRSA found in our study is higher to that reported over the past decade in Africa, where MRSA prevalence deriving from the basin of the Mediterranean, South Africa, Nigeria, ranged from 25 to 50% for many countries, and less than 25 % for other countries (Benouda and Elhamzani, 2009). In Morocco, the incidence was 13.5% (Benouda and Elhamzani, 2009). Recently in Cameroon, a prevalence rate of 76% was reported in a study on carriage of MRSA in the case of the medical staff at the university teaching hospital in Yaoundé (Gonsu et al., 2013). The differences are clear by comparison with the European countries that have been committed for a long time in surveillance programs and prevention of nosocomial infections. A different management of nosocomial infection and a better control of antibiotic therapy could be key elements in these variations. Regional differences in the availability and consumption of antibiotics, the spread of HIV and tuberculosis, may explain these differences in the sub-Saharan Africa countries (Falagas et al., 2013).

The analyses of resistance profiles confirmed that all the 28 (100%) MRSA isolates were multidrug resistant strains (Siegel et al., 2010). This high level of multidrug resistance could be associated with the frequent resistance to the aminoglycosides and macrolides. The phenotype KTG (29%) showing resistance to 3 antibiotics (Kanamycin, Tobramycin and Gentamicin) found in our study is comparable to reported studies in Algeria (22%) and higher than data generated from a study in Morocco (1%) (Rahima et al., 2015; Elazhari et al., 2010). Twenty five percent of MRSA strains were MLSB inducible (resistance to erythromycin and flattening of the lincomycine zone adjacent to the erythromycin disk); 21% had the phenotype MLSB constitutive (resistance to both erythromycin and clindamycin) and 39% were MLSB sensitive phenotype. The rate of MLSB constitutive resistance is rather inferior to other studies in India (50%) and Algeria (40%) (Saikia et al., 2009; Rahima et al., 2015). Our results concerning the MLSB inducible resistance and sensitive phenotypes are however higher than that reported from India (9% MLSB inducible and 19% MLSB sensitive) (Saikia et al., 2009). More specifically, the resistance profile P.FOX.AK.TOB.CN.OFL.PEF.E.L.DXT (resistance to 10 antibiotics tested) were the most common (14%); followed by the resistance profiles P.FOX.DXT (11%), and P.FOX.OFL.PEF.E.L.DXT.SXT, P.FOX.AK.TOB.CN.OFL.PEF.DXT.SXT, P.FOX.AK.TOB.OFL.PEF.DXT.SXT, P.FOX.AK.TOB.E.L.DXT and P.FOX.AK.TOB.OFL.PEF.E.L with 7% of prevalence each. The high prevalence of multidrug resistance to beta-lactam antibiotics, aminoglycoside, fluoroquinolones, cyclines and trimethoprim-sulfamethoxazole could likely be related to the irrational drug use (misuse, overuse and inappropriate use) in the country (Asongalem et al., 2015). The co-resistance could also be explained by the presence of other mobile genetic elements (MGEs) such as plasmids, integrons, transposons and insertion sequences carried by the *S. aureus* strains with or without the methicillin resistance gene (mec gene) and responsible of further resistance (IWG-SCC, 2009; Abdulgader et al., 2015). Similar results have also been reported by Schaumburg et al. (2014) rather in MSSA strains (Schaumburg et al., 2014).

Although, methicillin susceptible, the majority of MSSA strains (81%) were also resistant to various antimicrobial drugs. The resistance to 3 antibiotics P. DXT.SXT (36%) were frequently identified, followed by the resistance to 7 antibiotics AK.OFL.PEF.E.L.DXT.SXT and P.AK.TOB.CN.E.DXT.SXT 18% each. These results are similar to other studies such as that of Schaumburg et al. (2014). They could also be explained by the fact that antibiotics are probably widespread and irrationally used in the country.

Finally, despite the global resistance found in our study, vancomycin and pristinamycine were the most active antibiotics with 100% of sensitivity each. These drugs could thus be used as drugs of last resort for the treatment of MRSA infections in Cameroon. One strain expressed a wild type phenotype which is becoming rare in the population with the availability of penicillin and methicillin; and suggests that it is possible to contain the emergence and spread of resistant bacteria with the prudent use of antibiotics.

Conclusion

This study has highlighted the high prevalence of MRSA infections. It has pointed out the need to initiate epidemiological studies of staphylococcal infections and the need to create a network for the prevention and control of multidrug-resistant bacteria in general and MRSA in particular. It has further shown the importance of defining strict therapeutic guidelines for use at a national level, so as to achieve better treatment. It is necessary to implement epidemiological studies on

staphylococcal infections and to create a national network for the prevention and control of multidrug-resistant bacteria in Cameroon. Studies on the molecular epidemiology of *S. aureus* should be carried out in the future, to emphasize the understanding of the public health threat posed by this pathogen.

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