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Full Length Research Paper

Nasal carriage of methicillin resistant *Staphylococcus aureus* and its antibiotic susceptibility pattern in adult hospitalized patients and medical staff in some hospitals in Cameroon

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Methicillin-resistant *Staphylococcus aureus* (MRSA) have evolved as a major health care-acquired pathogen worldwide during the last three decades. A prospective study was carried out to ascertain the prevalence of nasal carriage of MRSA in health care workers and in hospitalized adult patients and their antibiotic susceptibility profile in Cameroon. The bacterial strains were identified by conventional method and the antibiotic resistance was carried out by the Kirby Bauer disc diffusion method. Of the 295 samples analysed, 120 (40.6%) were positive for *S. aureus*, 102 (34.6%) were MRSA. MRSA constituted 85% of all the *S. aureus* identified. The prevalence of nasal carriage of MRSA in medical staff was 41.3 and 32% for hospitalized patients. The MRSA carriage rate at the regional hospital, Limbe was 38%, 37.1% at the Yaoundé University Teaching Hospital and 32.1% at Laquintinie Hospital, Douala. MRSA was identified in 34.2% of males and 35% of females. Most MRSA strains were highly sensitive to vancomycin and teicoplanin in patients; while in medical staff, most strains were sensitive to clindamycin. The highest rate of resistance in medical staff was recorded with penicillin G, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid; while in hospitalized patients, gentamicin and erythromycin had the highest rate of resistance.

Key words: Health care personnel, adult patients, nasal carriage, methicillin-resistant *Staphylococcus aureus* (MRSA), antibiotic susceptibility.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a highly infectious strain of the ordinary *S. aureus* bacteria that is able to withstand the curative powers of ordinary antibiotics. *S. aureus* was the most common cause of hospital acquired infections reported in 1990-1996 (CDC, 1996). MRSA is found worldwide with an estimated colonization rate ranging from 11 to 40% in specific populations with more than 50% of these estimated to develop infection. However, it is very common to find a high rate of nasal carriage of MRSA in health care workers where MRSA is endemic (Boyce, 1994). *S. aureus* is still one of the five most common causes of hospital acquired infections *and* because its primary habitat is moist squamous epithelium of the anterior nares. Most invasive *S. aureus* infections are assumed to arise from nasal carriage (Duran et al., 2006). The inci-

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dence of hospital-acquired *S. aureus* infections has been rising with increasing emergence of MRSA (Emori and Gaynes, 1993; Steinberg et al., 1996; Fluit, 2001; Ryan and Ray, 2004; Deresinski, 2005). MRSA is an established pathogen in most health care facilities. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection (Herold et al., 1998). A joint study conducted in 8 African hospitals and Malta, between 1996 and 1997 revealed that the proportion of isolated MRSA was 30% in Nigeria, 21% in Cameroon, 14% in Morocco, 13% in Senegal and below 10% in Tunisia, Malta and Algeria (Kesah, 1996).

Resistance to methicillin and other β-lactam antibiotics is caused by the *mecA* gene, which is situated on a mobile genetic element, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) (Ito et al., 2003). Six SCC*mec* types (I to VI) have been distinguished (Deurenberg et al., 2007). The molecular epidemiology of MRSA can be studied using, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing and SCC*mec* typing (de Sousa and de Lencastre, 2004). Several MRSA clones have emerged and disseminated worldwide. Although, in the past, MRSA strains were mainly hospital acquired (HA-MRSA), from the late 1990s, community-acquired MRSA (CA-MRSA) emerged. CA-MRSA harbours SCC*mec* type IV or V and is often associated with Panton-Valentine leukocidin (PVL) (Deurenberg et al., 2007).

Many clinical infections arise from spread from a healthy carrier. Therefore, an understanding of the risk factors for carriage of S*. aureus* is crucial to understand the potential for invasive infections and transmission of MRSA. However, most surveillance of *S. aureus* and MRSA has focused on individuals with invasive infections rather than on hospitalized patients (Salgado et al., 2003; Weber et al., 2003; Harbarth et al., 2005; Kuehnert et al., 2005; Ma et al., 2005; Ochoa et al., 2005). Focusing on individuals with invasive infections provides a good indication of severe disease but does not provide an accurate assessment of the reservoir of *S. aureus* and potential for transmission.

Data on carriage of MRSA of both hospitalized patients and medical staff is limited in Cameroon, we deemed it of great importance to carry out this study to enable health policy makers develop and implement an effective MRSA control policy in hospitals in Cameroon.

MATERIALS AND METHODS

A prospective cross-sectional and analytical study was carried out from January to April 2011. Hospitalized patients and medical staff age 18 years and above who consented to participate in the study were recruited from three units; surgery, medicine and intensive care of three selected hospitals; Yaoundé University Teaching Hospital (UTHY), Laquintinie Hospital Douala (LHD) and Regional Hospital Limbe (RHL). Nasal swabs were collected using sterile cotton swabs previously moistened with 2 to 3 drops of 5% normal saline from the anterior nares of each nostril from each participant. Swabs were carefully inserted into each nostril so that the tip is entirely at the nasal osteum level (about 2.5 cm from the edge of the nare) and gently rolled 5 times and placed into a sterile tube containing amies transport media. The collected samples were transported in a cold chain within two hours to the microbiology laboratory of YUTH, LHD and LRH.

Nasal swabs from both nostrils were streaked on blood agar (BA) after primary enrichment on nutrient broth for 24 h at 37°C. Verification of *Staphylococci* was performed by i) characteristic phenotypical growth on BA plate, ii) Gram stain, iii) positive catalase reaction. Suspected colonies were incubated on Mannitol Salt Agar (MSA) plates. Isolates displaying yellow growth in MSA plates were identified as *Staphylococcus aureus* (*S. aureus*) and subsequently verified by Analytical Profile Index (API). Identified isolates of *S. aureus* were screened for oxacillin resistance by 24 h incubation on Oxacillin Screen Agar (Mueller Hinton Agar, Oxacillin 5 µg/ml, 4% NaCl).

Staphylococci isolates not displaying yellow growth on MSA plates were deemed as coagulase-negative *Staphylococci* (CoNS). Screening for oxacillin-resistance was performed as above. Further analysis was only executed in the case of positive oxacillin-resistance screening. The same test was done with the control strain (ATCC 25923).

Antibiotic susceptibility was tested by the Standardized Kirby-Bauer disc diffusion method with OSIRIS system (Biorad, Marne la Coquette, France) on Mueller-Hinton agar. The quality control of discs used was performed using the following reference strains : *S. aureus* (ATCC 25923). Phenotypic disc confirmatory test was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2009) except for Fusidic acid where the French Society of Microbiology recommendations were used. It is important to note that molecular analysis were not carried out to confirm the different phenotypes due to limited resources. The identified MRSA were stored in brain heart infusion broth supplimented with 15% glycerol at -20°C.

Data analysis

Data was analyzed using Microsoft excel and Epi info v3.4.3. Prevalence was expressed as percentages. The Chi square (X^2) test was used to analyze categorical variables and the Yates correction test where appropriate. A 95% confidence interval (CI) was calculated and values of $P < 0.05$ were considered significant.

Ethical issues

An ethical clearance was obtained from the Cameroon National Ethics Committee, informed consent was obtained from the participants and an administrative authorization was obtained from the different participating hospitals.

RESULTS AND DISCUSSION

A total of 295 samples were collected in this study and *S. aureus* was identified in 40.6% (120/295) of these samples. This is higher than the WHO reported range of 21 to 30% of *S. aureus* for Central Africa in the general population (Brown et al., 2005), and that of Saudi Arabia with an overall nasal carriage of 38% (Saxena and Panhotra, 2003), but lower than that obtained in a study conducted in Gangtock East Sikkim India, which showed a prevalence rate of 52.2% (Devjyoti et al., 2008). Among

Table 1. Distribution of MRSA according to duration of hospitalisation.

Length of hospital stay	Number of patient	Frequency of MRSA	Percent (%)	P-value
<8 days	112	29	25.8	
8-14 days	45	14	31.1	
15-30 days	27	11	40.7	
>30 days	35	17	48.5	0.86
Total	220	71	32.2	

the *S. aureus* positive, 37 (49.3%) were found in medical staff and 83 (37.7%) in hospitalized patients which is lower than the result obtained in another study on hemodialysis patients which recorded 53% (Lederer et al., 2007). 28.3% of *S. aureus* were identified in the YUTH, 51.6% in LHD and 20% in RHL.

It was observed that one of the ecological nitche for the colonization of *Staphylococci* is the anterior nares, as most of the nasal specimen yielded Staphylococcal growth on culture. This confirms that *Staphylococci* are part of normal flora of the anterior nares.

The present study showed a prevalence of 34.6% (102/295) of nasal carriage of MRSA which is higher than that obtained in each of the two Nord West fire district in USA (22.5%) (Merilyn et al., 2011). In Europe, MRSA prevalence varied almost a 100 fold in a study, from <1% in northern Europe to ≥50% in south Western Europe (ECDC, 2011). In the United States, the proportion of MRSA rapidly increased from below 5% in the 1980's to 29% in 1991. From the foregoing, it is clear that there is considerable variation of MRSA carriage within hospital settings, wards of the same hospital, regions and even countries.

Generally, MRSA has become a global nosocomial pathogen with attendant therapeutic problems which could be due to its possible rapid spread and capacity to acquire resistance to commonly used antibiotics. The highest rate of MRSA carriage was found in medical staff, 31 (41.3%), which is higher than results in another study with a carriage rate of 27.2% in medical staff (Kumar et al., 2011). Hospitalized patients had a carriage rate of 32% (71/220) which is higher than that of a study conducted in Kwazulu-Natal which had a rate of 21% (Scolt et al., 2011). There was a significant difference (p=0.01) in rates of MRSA carriage between medical staff and hospitalized patients. The proportions of MRSA carriage in both hospitalized patients and medical staff in the three hospital settings were as follows: 37.1% in UTHY, 32.1% in LHD, 38% in RHL, with a significant difference (P=0.01) between the sites of collection and nasal colonization with MRSA.

A prevalence of 31.4% of nasal carriage of MRSA by medical unit highlights the awareness level of the staff on implementation of preventive measures against nosocomial infections. On the other hand, the high prevalence of MRSA (41%) in intensive care unit could be attributed to lack of adequate precautions and very limited infection control applications. This result is lower than that obtained in a study conducted by Moniri and co-workers (2009), who recorded a prevalence of 75% in the intensive care unit. Results from several studies on MRSA in intensive care units have also revealed that MRSA colonization predisposed to MRSA infection during the same hospitalization period. MRSA was identified in 35% of samples from the surgical unit. Similar rate of MRSA colonization in surgical unit was obtained in another study conducted in USA (Reboli et al., 1990).

Although sex, co-morbidities and length of hospital stay did not show any association with nasal carriage of MRSA in the study population, highest rates of colonization were found in female; 53 (35%), long stay in hospital: ≥31 days; 17 (48.5%) (Table 1), and in medical staff that have been working for 20 to 25 years (100%).

HIV positive status was the main risk factor (p = 0.01). Incidence and risk factors for clinically significant methicillin-resistant *S. aureus* infection in a cohort of HIV-infected adults suggested that HIV-infected patients have increased rates of methicillin-resistant *S. aureus* colonization and infections (Mathews et al., 2005). HIV infected patients also appeared to be at increased risk for persistent *S. aureus* nasal colonization and for recurrent *S. aureus* infections at low $CD4^+$ cell counts (Mathews et al., 2005).

A high prevalence of MRSA resistance to most of the antibiotics used is of primary importance particularly among health care providers who may transmit these strains to patients during care delivery. The high frequency of MRSA resistance to antibiotics in this study was in accordance with other studies (Figure 1). As reported, the highest rates of antibiotic resistance was observed in penicillin G (84%) and trimethoprim/sulfamethoxazole (76%), followed by erythromycin (55%) and gentamicin (53.5%). The high level of resistance to these drugs in this study may be attributed to the fact that they have a wide clinical application, are inexpensive and are available from diverse sources where they are sold with or without prescription and therefore available for abuse.

The glycopeptides teicoplanin and vancomycin exhibitted a relatively high level of sensitivity ranging from 70.4 to 84.5%. The high susceptibility observed in vancomycin and teicoplanin, may be due to the fact that they are relatively expensive and newer antimicrobial drugs in the

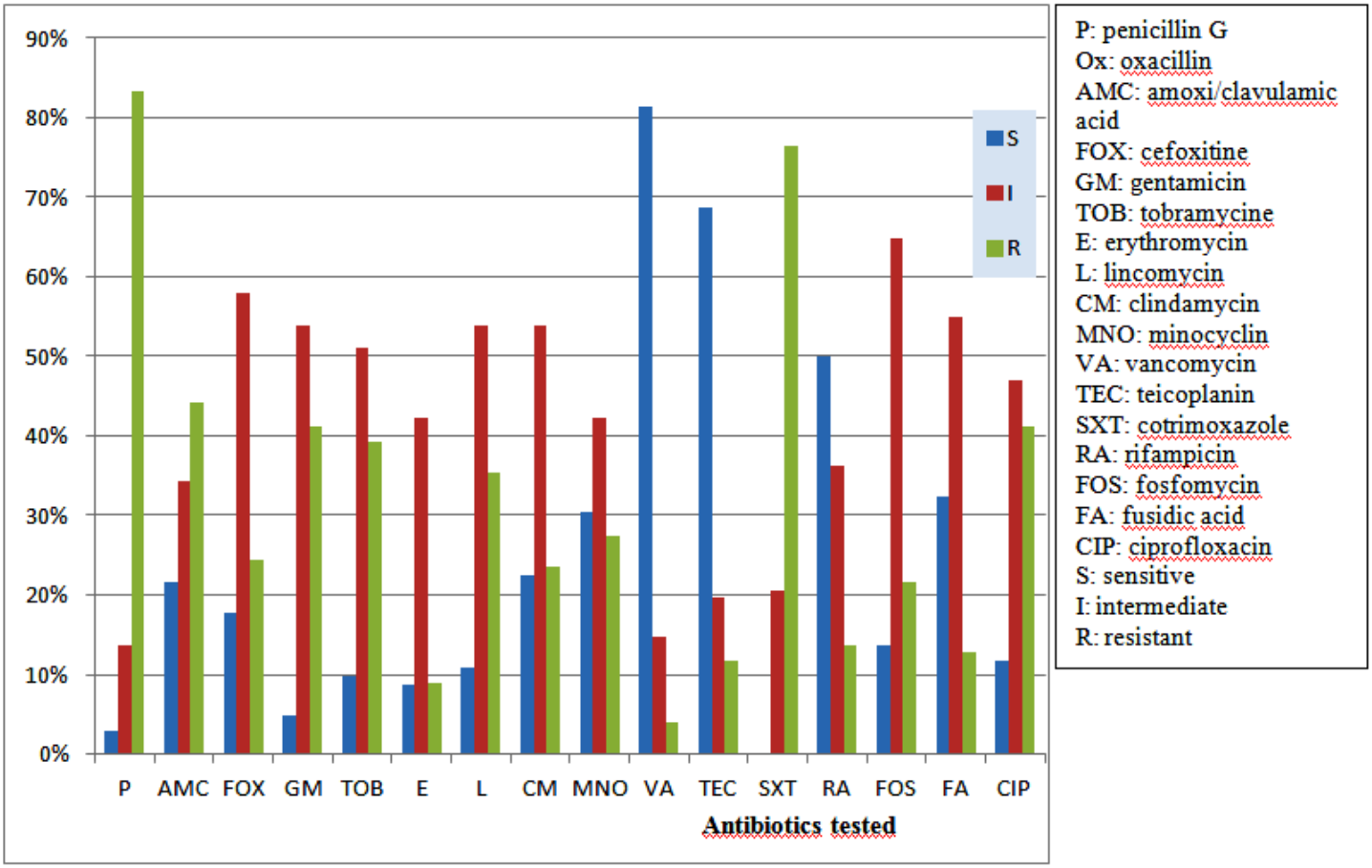

Figure 1. Susceptibility pattern of identified MRSA.

Table 2. Comparative resistance profile of MRSA isolated in hospitalized patients and medical staff at the three different hospitals.

Antimicrobial	UTHY		LHD		RHL		P-values
	HP (%)	MS (%)	HP (%)	MS (%)	HP (%)	MS (%)	
SXT	87	00	00	68	00	62	0.01
Cefoxitin	48	36	23	00	08	25	0.13
Ciprofloxacin	60	36	55	32	00	00	0.07
Fusidic acid	08	22	15	11	13	13	0.75
Penicillin G	94	100	80	55	78	88	0.01
Amoxicillin/clavulanic acid	68	50	23	12	65	00	0.59
Gentamicin	68	50	48	45	08	00	0.29
Tobramycin	68	50	50	12	08	00	0.01
Erythromycin	60	43	58	33	42	25	0.17
Lincomycin	56	50	30	45	21	13	0.37
Clindamycin	27	36	17	22	21	13	0.38
Minocycline	68	43	15	11	29	13	0.46
Vancomycin	00	00	05	11	00	13	0.43
Teicoplanin	00	22	12	11	08	25	0.27
Rifampicin	14	00	22	00	14	13	0.01
Fosfomycin	20	18	19	34	36	13	0.22

HP: Hospitalized patients, MS: medical staff, SXT: trimethoprim/sulfamethoxazole.

present context, therefore less available for abuse. The susceptibility pattern of the two groups differ statistically with penicillin G (p = 0.01), tobramycin (p = 0.01), trimethoprim/sulfamethoxazole (p = 0.01) and rifampicin (p = 0.01).

The isolates tested were highly resistant to most classes of antibiotics; 17 (66.19%) in hospitalized patients and 47 (54.83%) in medical staff with an overall prevalence of 62.74%. Table 2 shows the resistance pattern of isolated MRSA in hospitalized and medical staff in the three different hospitals. Multiple resistances are a common feature among commonly used antimicrobial agents.

Nasal carriage of MRSA among hospitalized patients and medical staff is significant not only in terms of predisposing to subsequent infections, but also plays an important role in transmission among medical staff, patients and the community.

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