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Staphylococcus aureus isolates from clinical and environmental samples in a semi-rural area of Cameroon: phenotypic characterization of isolates

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This study was undertaken to determine the prevalence of *Staphylococcus aureus* in clinical and environmental samples and their susceptibility pattern to antibiotics. A total of 231 samples comprising 127 clinical specimens obtained from patients and health personnel of various health facilities and 104 environmental samples from these facilities were screened for *S. aureus* using standard microbiological and biochemical methods (API Staph). Antimicrobial susceptibility of isolates was determined by the Kirby-Bauer disk diffusion assay. Results were analyzed by the chi-square test and p values < 0.05 were considered significant. Of the 231 samples screened, 85 (36.8%) were positive for *S. aureus*. Fifty-three (23%) and thirty two (14%) represented positive cultures from clinical and environmental samples respectively. Biochemical characterization grouped the isolates into five biotypes with biotype UB I being the most prevalent (49.9%) and biotype UB IV the least (5.9%) . There was a significant difference (p < 0.05) in the susceptibility of the isolates to vancomycin (80%); ofloxacin (72.9%) and ciprofloxacin (71.8%). Marked resistance was observed for methicillin (94.1%), gentamicin (83.5%) and oxacillin (75.3%). Twenty one antibiotypes were identified, with resistance to three or more antibiotics being common. Healthcare personnel and the hospital environment could serve as potential reservoirs of *S. aureus* in the study locality. These findings have clinical and epidemiological significance.

Key words: *Staphylococcus aureus*, biotypes, antibiotypes, public health, environmental health, epidemiology.

INTRODUCTION

Staphylococcus aureus is a ubiquitous pathogen. Its role in a variety of clinical conditions has been recognized to include nosocomial infections, septicaemia, pneumonia, wound sepsis, septic abortion, osteomyelitis, septic arthritis, post surgical infections and toxic shock syndrome. *S. aureus* infection is one of the most common bacterial infections in AIDS patients (Moore and Lindsay, 2001; Warner and Onderdonk, 2004; Augenbraun, 2000). Bacteraemia caused by this pathogen is a common cause of morbidity and mortality in humans' worldwide (Murray et al., 2004). When not properly handled it may extend the length of hospital stay, increase antibiotic use, costs and mortality (Heiman et al., 2004). Over the last 20 years, the incidence of both hospital and community acquired *S. aureus* infections have increased, and antibiotic treatment is increasingly being hampered by the spread of strains resistant to multiple drugs including methicillin (MRSA). The increasing prevalence of benzyl penicillin resistant *S. aureus* was initially overcome by the introduction of the semi synthetic penicillin, methicillin.

However, MRSA has rapidly emerged and become a major clinical problem (Moore and Lindsay, 2001). With

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the increased incidence of MRSA, the effectiveness of penicillin and cephalosporins is questioned. In fact many strains of MRSA exhibit resistance to both lactams and aminoglycosides (Anupurba et al., 2003).

Epidemiological studies on high level MRSA resistant to numerous antibiotics and antiseptics have been reported in out breaks with clones disseminating nationally and internationally (O'sullivan and Keane, 2000; Felten et al., 2002; Darini et al., 2004). However, worldwide community acquired MRSA has been limited to high risk groups such as intravenous or drug abusers. Typing of *S. aureus* is a necessary procedure for monitoring the transmission of the organism among carriers and in epidemiological follow-up which may enhance effective eradication (Miedzobrodzki et al., 2008). Several methods including biotyping, antibiogram, phage typing, pulsed-field gel electrophoresis (PFGE), plasmid profile analysis, protein electrophoresis as well as various PCR- based techniques have been used to characterize the organism (Tenover et al., 1994). However, though phage typing, PFGE, and PCR-based techniques have a high discriminatory power, they are more complex, cumbersome and expensive making them not suitable for routine investigation or for laboratories with limited resources as is common in developing countries. Other investigators have employed biotyping as an effective means to the investigation and surveillance of *S. aureus* infection (Marsou et al., 2001; Blaiotta et al., 2006). In the present study, we employed biotyping and antibiogram previously reported in our laboratory (Ndip et al., 2005) as they offer advantages to smaller laboratories such as ours, which are not optimally equipped.

Several reports have indicated the existence and emergence of MRSA in several African countries including Cameroon (Kakai and Womola, 2002; Kesah et al., 2003; Taiwo, 2004). However, the study in Cameroon was done in Yaounde, about 500 km from Buea and over three to four years ago. Acknowledging that the susceptibility pattern of microorganisms has been reported to vary with time and geographical location (Ndip et al., 2005), and noting that such studies seem to be lacking in the Buea health district, knowledge on the prevalence of S*. aureus* and their antimicrobial profile therefore becomes necessary in the selection of appropriate antibiotics for empiric treatment. We therefore sought to determine the prevalence and antibiogram of S*. aureus* in clinical and environmental samples in an attempt to provide baseline data for clinical management and epidemiological surveys.

MATERIALS AND METHODS

Study design and subjects

A total of 231 swab samples were investigated (127 clinical and 104 environmental). The clinical specimens constituted swabs from wounds and health personnel (finger nails and nostrils), while the environmental samples were formites, floors, benches, furniture (cupboards, beds), sinks, taps, switches, routine laboratory and surgical equipment. The clinical specimens were consecutive samples from the various tissue sites sent to the laboratory as well as those of personnel of these hospitals. Specimens from health personnel and the environmental samples were selected based on the local hospitals infection control programmes. Demographic data including age and sex of patients and health workers were recorded. The aim of the study was explained to the subjects and their consent to participate solicited. Ethical clearance was obtained from the management boards of the various hospitals involved in the study and from the Provincial Delegation of Public Health for the South West province of Cameroon. Specimens were collected and transported to the laboratory following previously described methods (Forbes et al., 1998).

Bacteriological analysis

Specimens were plated onto sterile manittol salt agar (MSA) and incubated at 37°C for 24 h. Suspect colonies were subcultured once on MSA and twice in blood agar for purification. Purified colonies were maintained on nutrient agar slants at 4°C until used. Isolates were identified using sugar fermentation tests and other biochemical characteristics using the API Staph (Biomerieux, France) as earlier described (Cheesbrough, 2000; Koneman et al., 1992).

Antibiotic succeptibility testing

The Kirby-Bauer disk diffusion test, which conforms to the recommended standard of the National Committee for Clinical Laboratory Standards (now Clinical and Laboratory Standards Institute) (NCCLS, 2002) was used as previously described (Bauer et al., 1966). Briefly, a small inoculum of each bacterial isolate was emulsified in 3ml sterile normal saline in Bijou bottles and the density compared to a barium chloride standard (0.5 McFarland) . A sterile cotton swab was dipped into the standardized solution of bacterial cultures and used to evenly inoculate Mueller-Hinton plates (Biotec, England) and allowed to dry. Thereafter, antibiotic disks with the following drug contents: vancomycin (30 μg); ofloxacin (5 μg); ciprofloxacin (5 μg); ceftriaxone (30 μg); doxycycline (30 μg); trimethoprim-sulfamethoxazole (5 μg); augmentin (10 μg); oxacillin (10 g); gentamicin (10 g); methicillin (5 g); ampicillin (10 g); penicillin (5 IU); and erythromycin (15 g) (Oxoid, England) were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. The plates were incubated at 37°C for 24 h and the diameters were compared with recorded diameters of a control organism*, E. coli* ATCC 25922 to determine susceptibility or resistance (Ndip et al., 2005).

Statistical analysis

Data obtained were analysed using Epi info5 (Centers for Disease Control and Prevention, USA). Categorical data were compared by chi-square. $P < 0.05$ was considered statistically significant.

RESULTS

Of the 231 specimens examined, 85 (36.5%) yielded *S. aureus* growth. Fourteen percent of the positive isolates were from environmental samples while the clinical

Table 1. Biotype distribution of *Staphylococcus aureus* isolates based on their biochemical characteristics.

Biotype*	Haemolysis on blood agar	Sugar Fermentation									Nitrate reduction	Total (%)
		Mannitol	Sucrose	Lactose	Fructose	Ribose	Maltose	Glucose	VP	Urease		
I	+	+g	+	+g	+	+	+g	+g	+	+	+	42 (49.4)
II	-	+	+	-	+	+	+	+	+	+	+	20 (32.5)
III	+	+g	+g	+	+	-	+	+	+	+	+	11 (12.9)
IV	+	+	+	-	+	-	+	+g	+	+	+	5 (5.9)
V	-	+	+	+	+	-	+	+g	+	+	+	7 (8.2)
Total	75	85	80	65	85	60	85	85	85	85	85	85 (100)
(%)	(88.2)	(100)	(94)	(77)	(100)	(70)	(100)	(100)	(100)	(100)	(100)	

+, Positive reaction with acid production; +g, positive reaction with acid and gas production; VP, Voges Proskaeur; *, UB.

Table 2. Antimicrobial sensitivity results of *S. aureus* isolates from clinical and environmental samples.

Drugs	Number of samples susceptible (%)	Number of samples with intermediate susceptibility (%)	Number of samples resistant (%)	Total
Vancomycin (VAN) [30 g]	68 (80)	10 (11.8)	7 (8.2)	85
Ofloxacin (OFLX) [5 g]	62 (72.9)	12 (14.1)	11 (12.9)	85
Ciprofloxacin (CIP) [5 g]	61 (71.8)	7 (8.2)	17 (20.0)	85
Doxycycline (DOX) [30 g]	43 (50.6)	9 (10.6)	33 (38.8)	85
Erythromycin (ERY) [15 g]	35 (36.5)	31(36.5)	19(22.4)	85
Trimethoprim/sulfamethaxazole (STX) [1.25/23.7[5 g]	25 (29.4)	41 (48.2)	19 (22.4)	85
Ceftriaxone (CRO) [30 g]	15 (17.6)	11 (12.9)	59 (69)	85
Oxacillin (OXA) [1 g]	13 (15.3)	8 (9.4)	64 (75.3)	85
Gentamicin (GEN) [10 g]	9 (10.6)	5 (5.9)	71 (83.5)	85
Methicillin (MET) [5 g]	3 (3.5)	2 (2.4)	80 (94.1)	85
Ampicillin (AMP) [10 g]	0 (0.0)	_____*	85 (100)	85
Penicillin (P) [5 units]	0 (0.0)	_____*	85 (100)	85

*, No values for diameter of zone of inhibition were reported according to NCCLS[21].

specimens accounted for 23% with genital specimens from both males and females accounting for 50.9% of the isolates.

The result on the distribution of isolates based on biochemical characterization is presented in Table 1. Five biotypes were distinguished namely UB I, UB II, UB III, UB IV, and UB V. Biotype UB I was the most prevalent (49.4%) and biotype UB IV the least (8.2%). All the 5 biotypes were detected in in-patients (100%). The other sample sources (environmental, health personnel and out-patients) were each contaminated by 4 biotypes (75%) though in different combinations. However, the distribution of the biotypes in the various sample sources was not statistically significant ($p > 0.05$).

Antimicrobial results exhibited by isolates are shown in Table 2. Vancomycin exhibited the highest activity (80%). This was followed by ofloxacin (72.9%) and ciprofloxacin (71.8%). Although ampicillin and penicillin recorded 0% susceptibilities, isolates were also markedly resistant to methicillin (94.1%).

The resistance patterns (antibiotypes) of isolates are presented in Table 3. Of the 21 antibiotypes, the predominant resistance pattern was trimethoprim / sulfamethoxazole, gentamicin, ampicillin and penicillin (STX^R GEN^R AMP^R P^R) occurring in 34.1% of the isolates. The least resistance patterns corresponding to resistance index A1, A2, A3, A4, A5, A7, A12 and A18 were exhibited respectively by one (1.2%) isolate.

Table 3. Antimicrobial resistance pattern of *S. aureus* isolates.

Number	Antibiotype Resistance pattern	Number of positive isolates showing pattern (%)
A1	CRO^R AMP^R P^R	1 (1.2)
A2	CRO^R ERY^R GEN^R AMP^R P^R	1 (1.2)
A3	CRO^R ERY^R DOX^R CIP^R GEN^R AMP^R P^R	1 (1.2)
A4	CRO^R ERY^R CIP^R DOX^R AMP^R P^R	1 (1.2)
A5	CRO^R DOX^R GEN^R AMP^R P^R	1 (1.2)
A6	$OFLX^R$ ERY^R DOX^R CIP^R GEN^R AMP^R P^R	3 (3.5)
A7	$OFLX^R$ GEN^R STX^R AMP^R P^R	1 (1.2)
A8	$OFLX^R$ DOX^R AMP^R P^R	6 (7.1)
A9	CIP^R DOX^R GEN^R AMP^R P^R	4 (4.7)
A10	CIP^R DOX^R ERY^R GEN^R AMP^R P^R	3 (3.5)
A11	CIP^R GEN^R AMP^R P^R	6 (7.1)
A12	CIP^R ERY^R GEN^R AMP^R P^R	1 (1.2)
A13	DOX^R AMP^R P^R	2 (2.4)
A14	DOX^R GEN^R AMP^R P^R	8 (9.4)
A15	DOX^R GEN^R ERY^R AMP^R P^R	3 (3.5)
A16	DOX^R GEN^R STX^R AMP^R P^R	6 (7.1)
A17	DOX^R GEN^R STX^R ERY^R AMP^R P^R	2 (2.4)
A18	DOX^R STX^R AMP^R P^R	1 (1.2)
A19	ERY^R STX^R GEN^R AMP^R P^R	2 (2.4)
A20	ERY^R GEN^R AMP^R P^R	3 (3.5)
A21	STX^R GEN^R AMP^R P^R	29 (34.1)
Total		85

$X^2 = 183.06$; df = 20; $p<0.05$; R, resistant.

The occurrence of *S. aureus* antibiotype according to status of individuals and source of samples is presented in Table 4. Nine of the 21 antibiotypes (42.9%) were detected in out- patients as against 7(33.3%) in in-patients ($p < 0.05$); environmental samples had the least number of antibiotypes, 1 of 21(4.8%). There was however, no significant difference ($p > 0.05$) in the distribution of antibiotypes obtained from the various sample sources.

DISCUSSION

The isolation of *S. aureus* in Buea reflects it's ubiquitous occurrence as previously reported (Moore and Lindsay, 2001). In a study in the USA, MRSA was found in 100% of formites in subways, buses and trains (Sexton et al., 2007) revealing the need for constant monitoring of the organism to trace reservoirs, which may enhance effective eradication. The isolation rate of 36.8% compares with that obtained (30%) in earlier studies from clinical specimens in some Cameroonian hospitals (Taiwo, 2004) . The high isolation rate in genital samples (50.9%) might be attributed to the fact that the anatomy of females exposes them to easy contamination as this pathogen is endogenous colonizing the vagina vault of healthy women (Warner and Onderdonk, 2004). The occurrence of the pathogen in environmental samples (13.85%) may indicate poor infection control (sanitation) in the hospital environment, which could serve as a reservoir of the organism. This may also account for the high incidence of the organism observed in health personnel.

In spite of the clinical importance of *S. aureus*, there is no scheme developed locally for biotyping the organism in our environment. The biotyping scheme used in this study made use of sugar fermentation and other miniaturized test in the API Staph kit that identifies mostorganisms with up to 99% accuracy (Koneman et al., 1992). We had previously observed (Ndip et al., 2005) that biotyping could be an easy and cheap method of typing organisms, especially in resource limited laboratories in the developing world. Consequently we employed a panel of biochemical tests to further characterize our isolates. Of the 5 biotypes identified biotypes UB I, UB II, and UB III were encountered in out- and in-patients, health personnel and the hospital environment. This may suggest that they were acquired from the community. This is in line with previous studies (Murray et al., 2004; O'sullivan and Keane, 2000) which equally documented the spread of community acquired *S. aureus*. It is also likely that biotype UB V was probably of community origin as it was found in all the sample sources except health personnel. Biotype UBIV was

Table 4. Occurrence of *S. aureus* antibiotypes according to status of individuals and source sample.

Status/Source	Antibiotype resistance index	Number of antibiotype (%)	Number of positive samples (%)
Environmental	A21	1 (4.8)	26 (34.1)
Health workers	A1, A8	2 (9.5)	7 (8.2)
Health workers and environmental	A14	1 (4.8)	6 (7.1)
In-patients	A3, A5, A7, A9, A13, A18, A19	7 (33.3)	12 (14.1)
Out-patients	A3, A4, A6, A10, A12, A15, A16, A17, A20	9 (42.9)	23 (27.1)
	Total	21	85

$X^2 = 8.23$ df = 5, $p > 0.05$.

absent in out- patients suggesting its nosocomial origin. This supports previous reports that incriminated the organism in nosocomial outbreaks (Heiman et al., 2004; O'sullivan and Keane, 2000; Kesah et al., 2003). Other investigators have used a combination of biotyping, antibiogram, and DNA typing to characterize *S. aureus* (Tenover et al., 1994; Herbert et al., 1988). They however, concluded that biotyping has a low discriminatory power which is in line with our results. We therefore think that elaborate studies need to be carried out with more discriminatory typing methods such as pulse-field gel electrophoresis, multi-locus sequence typing, restriction fragment length polymorphism analysis and bacteriopahge typing to be able to draw definitive conclusion because other studies have also shown that some conventional methods like biochemical profiles for strain identification can only detect unstable differences between clonally related strains or maybe applied to just few isolates (Gallego et al., 2000). This could be linked to variations in certain phenotypic characteristics of genetically related strains.

Our isolates showed high susceptibility to vancomycin (80%) and this was statistically significant ($p < 0.05$) compared with the other drugs used in the study. This is in agreement with previous studies (Kesah et al., 2003; Shittu and Lin, 2006). We speculate that the effectiveness observed with the drug might be due to its high cost in our environment making it less readily available and hence less misused. However, regular monitoring of the drug's sensitivity is of importance because resistance has been reported in the USA, Japan and Korea (Shittu and Lin, 2006; CDC, 2002; Classen et al., 2005). Susceptibilities of 72.9 and 71.8% were also noted for ofloxacin and ciprofloxacin respectively corroborating previous results (Shittu and Lin, 2006; Saxena et al., 2003).

S. aureus resistance to antibiotics, especially methicillin is a serious problem worldwide (Felten et al., 2002; O'sullivan et al. 2003; Darini et al., 2004). The high rate of methicillin resistance and that observed with other beta-lactams with our isolates notably ampicillin and penicillin corroborates earlier reports (O'sullivan and Keane, 2000; Kesah et al., 2003; Taiwo, 2004; Mi-Na et al., 2002; Liu and Chambers, 2003). The pattern of resistance observed might be due to the fact that beta-lactams are used in auto- therapy in this locality, which may result in a multitude of antibiotics used at sub-therapeutic levels heralding the emergence of resistant strains. Although we did not investigate the production of ß-lactamase by our strains, it is also likely that the enzyme maybe playing a role in the observed resistance.

Antimicrobial resistance patterns revealed a total of twenty-one antibiotypes of which the most prevalent was trimethoprim/ sulfamethoxazole, gentamicin, ampicillin and penicillin (STX^R GEN^R AMP^R P^R) and they accounted for 29 (34.1%) of the isolates. The least resistance patterns were noted for the resistance indices designated A1, A2, A3, A4, A5, A7, A12 and A18 (with erythromycin common in most of the patterns) exhibited by 1.2% of the isolates respectively. Approximately 62% of the isolates were resistant to three or more antibiotics. Although the majority of these multidrug- resistant isolates originated from clinical samples, 4.8% of the isolates were of environmental origin. We previously documented (Ndip et al., 2005) multidrug-resistant environmental strains of *P. aeruginosa* and linked it to the uncontrolled disposing of antibiotics and chemicals into the hospital environment, which turns to create selective pressure on the drugs. Pellegrino et al. (2002) also reported that the use of antibiotics in hospital and the community at large serves as a major selective pressure for antibiotic resistant bacteria. We are therefore constrained to speculate that the same situation might have been responsible for the multidrug - resistance pattern observed in the present study.

Conclusion

Based on our findings, patients, health personnel as well as the hospital environment could serve as reservoir of *S. aureus* in the environment of Buea, Cameroon. Multidrug - resistant strains are common, but vancomycin may present a unique opportunity in the management of infections. Regular surveillance of hospital and community

associated *S. aureus* infections and their susceptibility to antibiotics is necessary to prevent an outbreak and spread of resistant strains, especially MRSA in the locality. We recommend a combination of biotyping and antibiogram, which are cheap and routinely available methods in most laboratories in resource poor areas as useful tools for clinical and epidemiological studies

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