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Prevalence Of Multi-Drug Resistant *Staphylococcus Aureus* In Clinical Specimens Obtained From Patients Attending The University Of Benin Teaching Hospital, Benin City, Nigeria.

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Abstract
Methicillin resistant *Staphylococcus aureus* (MRSA) is a source of hospital required infection (HAI) world-wide and an important human pathogen that is found in most communities in Nigeria. The study was carried out to determine the prevalence and resistance pattern of methicillin-resistant strains of *Staphylococcus aureus* in specimens of pus, wound, aspirates and swabs obtained from patients attending the University of Benin Hospital, Benin City. A total of 3612 samples collected by clinical staff and sent for routine examination were bacteriologically processed using standard methods. Sensitivity tests were carried out by the disc diffusion method and minimum inhibitory concentration (MIC) was determined with graded concentrations of oxacillin in Mueller-Hinton agar. A total of 3,533 (97.2%) isolates were obtained of which 1315 (37.2%) were *Staphylococcus aureus.* Methicillin-resistant stains of the *Staphylococcus aureus* isolates were 1039 (79%). MRSA was isolated at a significantly higher ($p<0.05$) rate from pus and wounds of women than men. All the MRSA were resistant to ampicillin (100%), 99.8% to streptomycin and 99.9% to tetracycline. Resistance to amoxycillin-clavulanate (28.6%) was significantly lower ($p<0.001$) compared to other agents except for vancomycin resistance (0.007%). Therefore, the use of antimicrobial agents other than vancomycin can be successful after sensitivity tests have been carefully carried out. The need to improve on the public health awareness of the use of antimicrobial agents is further encouraged as this would reduce the incidence and prevalence of resistance among clinical isolates especially MRSA.
Key words: *Staphylococcus aureus,* prevalence, multi-drug resistance, antimicrobial agents .

1.0:Introduction

Staphylococcus aureus is a commensal and major pathogen of human . The bacterium is important in human infections ranging from minor skin-infections to serious life threatening infections that may include endocarditis, deep seated abscesses, septicaemia, catheter-associated bacteremia, ventilator-associated pneumonia, food borne-illness, toxic shock syndrome (TSS) and many other infections(Chambers,2001). Studies in Nigeria have shown that *Staphylococcus aureus* is the commonest micro-organism isolated from many wounds and pus samples (Ako-Nai *et al*.,1995;Emele and Izomoh,1999).

Infections caused by multi-resistant strains of *Staphylococcus aureus* are identified by their resistance to methicillin or oxacillin (MRSA/ORSA). MRSA by definition is any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics which include beta-lactam stable formulations such as methicillin, oxacillin, flucloxacillin, nafcillin and cephalosporins. These MRSA strains are often responsible for several difficult to treat infections in humans (Lowry,2003; Taiwo *et al*., 2004;Shittu and Lin,2006). MRSA strains have also been reported to be implicated in causing progressively increased mortality, morbidity and increased heath care costs (French, 1996; Cosgrove *et al*., 2003;Tiwari *et al*., 2008).

Multi-drug resistant strains of *Staphylococcus aureus* or MRSA are characteristically resistant to three or more classes of antimicrobial agents other than beta-lactams. These stains have been recognized as the most common pathogen identified in wound infections (Bell and Jounidge, 2002). Infections caused by *Staphylococcus aureus* have a poorer prognosis when the strain is MRSA (Cosgrove *et al*., 2003;Tiwari *et al*., 2008). MRSA was first recognized in the hospital in 1961(Jevons,1961), but the strain is now increasing recognized in the community(Chambers,2001). The prevalence of MRSA varies within countries and in hospitals. The prevalence is constantly increasing in many countries and in some hospitals more than half of all *Staphylococcus aureus* isolates are MRSA (Voss,2006). In Nigeria, a prevalence of MRSA that varies between 34.7% and 71.2% has been reported(Taiwo *et al*., 2004; Onanuga *et al*.,2005). The current prevalence of MRSA is not precisely known especially in Benin City. The study was carried out to determine the prevalence, and resistance pattern of MRSA isolates in pus, wound and swab samples.

2.0 *Materials and Methods*

2.1 *Sample Collection:* A total of 3612 clinical samples of pus, wounds, aspirates and discharge swabs collected for routine investigation in different wards and clinics of the University of Benin Teaching Hospital, Benin City, Nigeria were transported to the laboratory for examination. The specimens were processed within one hour of collection. 2.2: *Incubation of Samples:* Samples were inoculated onto two blood agar plates (Oxoid CM 55) and one incubated anaerobically. Also inoculated were MacConkey agar (Oxoid CM 7) sabouraud dextrose slants (Oxoid CM 81), mannitol salt agar (Oxoid CM 85), heated blood agar (Oxoid CM 55) was also inoculated for genital swabs. All inoculated media were incubated in air at 37 ^{0}C, and examined after 18 h. Cultures without or insufficient growth were re-incubated for a further 18 h while the cooked meat medium was sub-cultured onto two blood agar plates and MacConkey agar for incubation to recover micro-organisms that may have been very scanty in the original specimen.

2.3. *Identification and antibiogram of microbial isolates:*Discrete colonies were picked up for characterization and identification tests using the scheme outlined (Cowan and Steel, 1974). Sensitivity tests were set up by the disc diffusion method (Baker and Beach,1980) in Mueller-Hinton agar (Lab 39). Inoculum size was standardized by matching the organisms emulsion in physiological with 0.5 McFarland (BSAC,2009) and the following antimicrobial agents discs from Abtek Biologicals (Liverpool, U.K.) were placed 25 mm apart from each other: ampicillin 10 µg, amoxycillin-clavulanate 30 µg, cloxacillin 5 µg, oxacillin 1µg, cefuroxime 30µg, ceftazidime 30 µg, cefotaxime 30 µg, ofloxacin 5 µg, ciprofloxacin 5 µg, gentamycin 10 µg, streptomycin 10 µg, azithromycin 15 µg, erythromycin 5 µg and tetracycline 10 µg. *Staphylococcus aureus* isolates were identified by Gram stain, calalase test, slide and tube coagulase tests, growth in salt and fermentation of mannitol and phosphatase production. Oxacillin was diluted in 2 % sodium chloride in concentrations ranging from 0.2-128 µg/ml in 20 ml of molten Mueller-Hinton agar. An 18 h culture in Mueller-Hinton broth of each *Staphylococcus aureus* isolate was in diluted sterile physiological saline and turbidity adjusted until it matched 0.5 McFarland standard of 10^4 cfu/ml. Each plate was inoculated at discrete points with a sterile swab stick and incubated at 37 ^{0}C for 18 h. Oxacillin resistance was recorded when the minimum concentration that inhibited an isolate was $\geq$ 2 µg/ml. Resistance to vancomycin was determined in serial dilutions of vancomycin (1, 2….32µg/ml) in Mueller-Hinton agar. An 18 h Mueller-Hinton broth culture of each isolate was adjusted to 10^4 cfu/ml. MacFarland 0.5 standard. This was spot inoculated and incubated at 37 ^{o}C for 18 h. Susceptibility breakpoints were recorded according to CLSI guidelines(CLSI,2006).

Data analysis was done using Chi- Square X^2 and the degree of confidence was set at 95% ($p<0.05$)

3.0:Results

Examination of 3612 pus, wounds and swab samples resulted in isolation of 3533 (97.2%) significant microbial growth. *Staphylococcus aureus* 1315(37.2%) was the most frequently isolated micro-organisms as shown in Fig. 1, *Klebsiella* species, *Pseudomonas aeruginosa* and other micro-organisms isolated were each less than a third of the proportion of *Staphylococcus aureus* (37.2%) rate of isolation.

3.1. *Antibiogram of Staphylococcus aureus :* The resistance pattern of *Staphylococcus aureus* isolates to antimicrobial agents is shown in Table 1. Of the 1315 *Staphylococcus aureus,* 1039(79%) were methicillin resistant (MRSA). The lowest resistance ratio of 28.6% was with amoxycillin-clavulanate and the highest rates of 100%, 99.9% and 99.8% were recorded with ampicillin, streptomycin and tetracycline respectively. The resistance of the *Staphylococcus aureus* varied with other antimicrobial agents.

The distribution of MRSA in sample according to gender is shown in Table 2. A significantly higher ($P<0.05$) number of pus, wounds and aspirates from female sources (44.1%) yielded MRSA. MRSA isolates from the ear, nose and throat of females were also higher (39.9%) in comparison to 27.9% from males. There was no significant difference ($p > 0.05$) in the isolation rates of MRSA from genital and eye swabs from both sexes.

The resistance pattern of MRSA is presented in Table 3. A total of 868 (83.5%) MRSA isolates were resistant to three or more antimicrobial agents. Twenty-nine (2.8%) were resistant to all antimicrobial agent except vancomycin and amoxycillin-clavulanate. Resistance to all antimicrobial agents except vancomycin was recorded in 29 (2.8%) of the MRSA isolates.

Vancomycin MIC for 2(0.002%) of MRSA isolates were 4 and 8µg/ml respectively (or vancomycin intermediate resistant *Staphylococcus aureus*, VISA) and 5(0.005%) of the isolates had MIC that ranged from 16 to 64µg/ml (vancomycin resistant *Staphylococcus aureus*, VRSA)

4.0: Discussion

This study has revealed that *Staphylococcus aureus* is the most predominant micro-organism from pus, wounds and swab samples as earlier studies have indicated (Bells and Jounldge,2002). The resistance of *Staphylococcus*

aureus to many groups of antimicrobial agents represents a serious concern in therapeutic option available to the clinician in managing such infections. Methicillin resistance - the marker of multi-drug resistance showed a high MRSA prevalence (79%) amongst isolates of *Staphylococcus aureus.* Previous study in Nigerian women recorded 71.2% (Onanuga *et al*.,2005). The higher rate from this study is not unexpected because it has been reported that MRSA prevalence is ever increasing (Voss, 2006).

The lowest resistance of 28.6% was observed with amoxycillin-clavulanate. This may suggest that this agent remains important in the management of MRSA in this community. The occurrence of MRSA within the genders indicated that females are at a significantly higher ($p < 0.05$) risk of harbouring or being infected with MRSA strains in pus, wounds and ENT samples. The exact reason for this disparity in MRSA isolation is not clearly known. It is however, possible that the dressing style of females that encourages more flowing garments may facilitate contamination and transference of MRSA to skin breaches or open wounds. The rates of MRSA isolation from genital and eye swabs was not significantly different ($p > 0.05$) in both sexes.

MRSA isolates were resistant to three or more groups of antimicrobial in 83.5% of the cases. The misuse and misapplication of many antimicrobial agents in many parts of Nigeria may contribute to the high MRSA rate in this community. This poses a significant difficulty in antimicrobial agent choice for patients with this variety of infections. The third generation cephalosporins, quinolones and improved macrolides indicated for serious infections may have substantially lost their place in treatment of many MRSA infections in this community. This indirectly may increase costs of treatment and the additional difficulty in control (Tiwari *et al*., 2008).

5.0: *Conclusion:* Resistance to vancomycin - a reserved drug for MRSA observed in this study highlights the magnitude of the burden of MRSA in this community. A renewed effort must therefore be put in place at control measures that should include a renewed awareness, isolation of MRSA infected patients in hospitals and multi-drug resistance surveillance and enforcement of empiric use of antimicrobial agents to stem the tide of MRSA.

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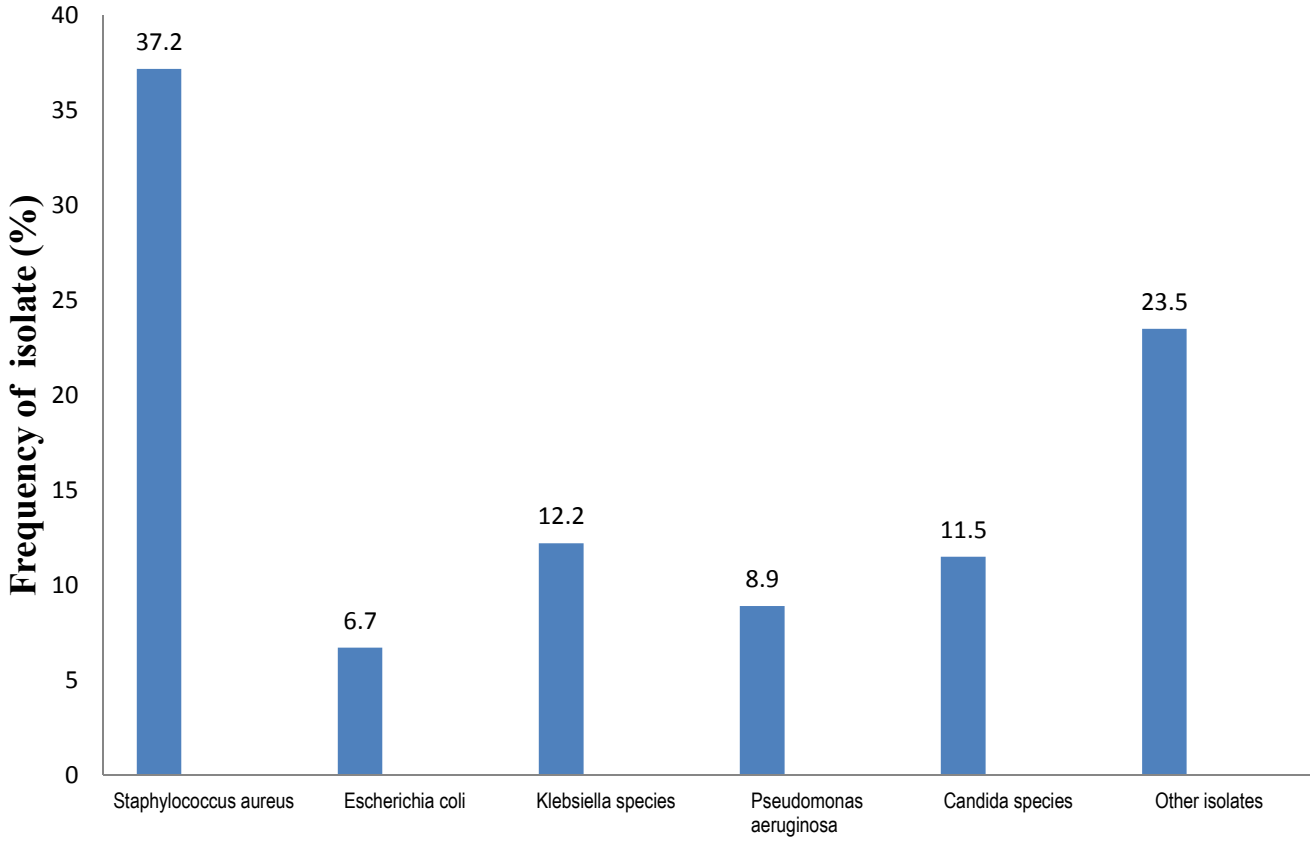

Fig 1: Microorganisms isolated from clinical samples.

Table 1: Resistance pattern of *Staphylococcus aureus* isolates to antimicrobial agents

Antimicrobial agent s	Resistance (%)
Amplicillin	1315 (100%)
Amoxycillin-clavulanate	376 (28.6%)
Cloxacillin	1044 (79.4%)
Oxacillin	1039 (79.0%)
Cefuroxime	575 (43.7%)
Ceftazidime	714 (54.3%)
Cefotaxime	546 (41.5%)
Ofloxacin	558 (42.4%)
Ciprofloxacin	796 (60.5%)
Gentamycin	696 (52.9%)
Streptomycin	1312 (99.8%)
Azithromycin	601(45.7%)
Erythromycin	894 (68.0%)
Tetracycline	1314 (99.9%)

Table 2: Distribution of methicllin resistant *Staphylococcus aureus* in samples according to gender

Nature of specimen	No. of cases (%)	
	Male	**Female**
Pus/wounds/aspirates	237/993 (23.9%)	263/597 (44.1%) ($p < 0.05$)
Genital	92/357 (25.8%)	236/912 (25.9%) ($p > 0.05$)
Ear, nose and throat	66/237 (27.9%)	79/198 (39.9%) ($p < 0.05$)
Eye	26/90 (28.9%)	40/149 (26.8%) ($p > 0.05$)

Table 3: Resistance pattern of MRSA isolates

Resistance to antimicrobial agents	MRSA (%)
Three or more non-vancomycin	868 (83.5%)
All antimicrobial except vancomycin and amoxycillin –clavulanate	29 (2.8%)
All antimicrobial except vancomycin	29 (2.8%)
All anti-microbial agents including vancomycin	7 (0.007%)

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