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Occurrence of Methicilin-resistant *Staphylococcus aureus* in a Nigerian tertiary Hospital

Ogunleye, V.O[1]., Ogunleye, A.O[2]*., Ajuwape, A.T.P[2]., Akande, K.A[1]and Adetosoye, A.I[2]

[1]*Department of Medical Microbiology University College Hospital, Ibadan, Nigeria*
[2]*Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan. Nigeria.*

ABSTRACT: A total of 3,692 clinical samples consisting 1,969 urine samples, 535 blood culture, 362 high vagina swabs, 207 endocervical swabs, 125 cerebrospinal fluid, 136 ear swabs, 200 eye swabs and 158 wound swabs were microbiologically investigated for *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* at the Department of Medical Microbiology, University Teaching Hospital Ibadan. Two hundred and eight *Staphylococcus aureus* (5.6%) were isolated, twenty two, (10.6%) of which were methicilin resistant. Nine isolates 9/22(40.9%), were resistant to nine antibiotics having, R-type aug.amg.amx.pef.tet.cxc.e.nit.nal, two isolates 2/22 (9.1%) had octuple resistant pattern ; R-type aug.amg.amx.pef.cxc.e.nit.nal, four isolates 4/22 (18.2%) had septuple ; R-type aug.amg.amx.pef.cxc.e.nit resistant pattern, two isolates 2/22(9.1%) had quintuple ; R-type aug.amx.cxc.e.nit, one isolate 1/22 (4.5%) quadruple ; R-type aug.amx.cxc.nit, and another four isolates 4/22 (18.2%) had triple; R-type amx.cxc.nit. Seventeen, (77.3%) of the methicilin resistant *Staphylococcus aureus*, were resistant to 30mcg Ceftriaxone, while nineteen, (86.4%) were resistant to 30mcg Ceftazidime. All the twenty two methicilin resistant *Staphylococcus aureus* were resistant to Amoxycillin (25mcg), Cloxacillin (10mcg), Nitrofurantoin (300mcg), and Gentamycin (10mcg). The MIC of Ceftazidime for 3 isolates, 3/19(15.8%) was 120mcg/ml and the MIC of Ceftazidime for 16 isolates, 16/19 (84.2%) was 60mcg/ml, while the MIC of Ceftriaxone for 1 isolate, 1/17 (5.9%), 6 isolates, 6/17 (35.3%) , 10 isolates, 10/17 (58.8%) were >150mcg/ml, 150mcg/ml, 75mcg/ml respectively and the MIC of Ceftriaxone for the remaining 6 isolates, 6/17(35.3%) was 37.5mcg/ml . Taking into consideration the danger associated with methicillin resistant *Staphylococcus aureus*, the findings from this study underscores the need for public enlightenment of both the hospital workers and the general public on the risk associated with this group of globally important pathogens and the necessary precautions for its control both in the hospitals and the communities in Nigeria.

Key Words: Methicilin resistant *Staphylococcus aureus*, Ibadan, Nigeria

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is commonly associated with nosocomial infection, but it has been reported in community acquired MRSA infections producing skin and soft tissue diseases among otherwise healthy persons having little or no contact with healthcare settings (Johnston. 1994; CDC.1999; Naimi *et al*.,2003; Kazakova *et al*.,2005). The main clinical implication of MRSA infection is that the organism produces multiple-resistance to many known antimicrobial drugs (Michel and Gutmann. 1997; Kluytmans**,** *et al*.1997). Most MRSA have epidemiological significance: showing multi-drug resistant pattern to macrolides, aminoglycosides and virtually all Beta-lactams by the production of penincillinlase and low affinity penincillin binding protein (Hirano and Bayer.1991; Greenwood. 2002).
Its prevalence varies significantly from hospital to hospital and in different countries. High and increasing prevalence of MRSA have been documented from

Manuscript received: February 2009; Accepted: June 2009

Correspondence:
E-mail: peculiarj@yahoo.com; ao.ogunleye@mail.ui.edu.ng

United States, Latin America and several regions of Europe (Diakema *et al*., 2001; EARSS. 2003). However, Netherland interestingly recorded the lowest MRSA prevalence of 1% in clinical isolates in Europe (EARSS.2006; Mireille *et al*., 2006).

MRSA, is known to be a problematic pathogen in human medicine, it is also emerging as a problem in Veterinary Medicine (Osawa *et al.,* 2003; Mashall *et al.,* 2004; Tiersma *et al*., 2004; Kuehnert *et al*.,2005 ; Xander *et al*.,2006). In the past ten years, the epidemiology of the infection caused by MRSA have rapidly changed, with the emergence of the organism as a community acquired infections (Cynthia. 2007). The current work focuses on occurrence of methicillin-resistant *Staphylococcus aureus* from clinical samples in the University Teaching Hospital Ibadan, Nigeria.

MATERIALS AND METHODS

Sample collection: The study was carried out between May 2004 and July 2004, clinical samples totaling 3,692 consisting of : 1969 urine samples, 535 blood culture, 362 high vaginal swabs, 207 endocervical swabs, 125 cerebrospinal fluid, 136 ear swabs, 200 eye swabs, and 158 wound swabs. The samples were collected aseptically using sterile swab and sent to the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria for Microbiological study.

Bacteriological analysis:

All the urine samples were cultured on blood agar and Cistein Lactose Electrolyte Deficient Medium (CLED). The high vaginal swabs, endocervical swabs, eye swabs and cerebrospinal fluids were respectively cultured on blood agar, chocolate agar and MacConkey agar, while the wound swabs were inoculated respectively onto blood agar and MacConkey agar. The blood samples were respectively cultured in brain heart infusion broth, incubated for 48hours, and subsequently sub-cultured onto blood agar and MacConkey agar.

All the samples inoculated onto blood agar, CLED, and MacConkey agar were incubated at 37^{o}C for 24hours. The chocolate agar cultures were incubated in a candle jar at 37^{o}C overnight. Colonies isolated were Gram stained, all colonies that yielded Gram positive cocci, that were catalase positive and oxidase negative were subjected to slide and tube coagulase test using human and sheep plasma (Langlois *et al.,* 1990; Ajuwape *et al*., 2006). The tube coagulase test was carried out according to the method described by Langlois *et al.* (1990) as employed by Ajuwape *et al*. (2006), using *Staphylococcus aureus* (ATCC25923) and sterile human and sheep plasma in broth as positive control. Any tube that did not form a clot at 24hours was regarded as negative. Acid production by the *Staphylococci* inoculated into carbohydrates like glucose, mannose, lactose, sucrose, xylose was detected by agar plate method as described by Cruickshank *et al.* (1975).

Antibiotic sensitivity test:

The *in-vitro* antibiotic sensitivity test of each isolated *Staphylococcus aureus* was carried out as described by Walton (1972) as modified by Adetosoye (1984). A colony of each isolated *Staphylococcus aureus* was inoculated into 5ml sterile nutrient broth and incubated at 37^{o}C for 8hours. The 0.01ml portion of the culture was delivered into 4ml of sterile nutrient broth and the mixture was vigorously shaken to give a 1:2000 dilution. Subsequently a diagnostic sensitivity test agar plate was inoculated by flooding with the 1:2000 diluted broth. The excess broth was drained off and the plate was allowed to stand on the bench for 1hour after which the Oxoid® antibiotic sensitivity discs of Augumentin (30mcg) Ampicillin (25mcg), Cloxacillin (10mcg), Amoxycillin (25mcg), Pefloxacin (30mcg), Tetracycline (30mcg), Erythromycin (10mcg) and Gentamycin (10mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Nitrofurantoin (300mcg), Nalidicic acid (30mcg) and ciprofloxacin (5mcg) were aseptically and respectively applied. The test plates were allowed to stand on the bench for 1 hour to allow the antimicrobial agents to diffuse into the agar. The plates were then incubated at 37^{o}C for 18hours after which the results were recorded. For each set of the test *Staphylococcus aureus* ATCC 25923 was used as control. The zone of inhibitions around each antibiotic disc in each test was compared with the corresponding zone of inhibition around the antibiotic disc for the control organism.

Minimum Inhibitory Concentration of the Multi drug resistant *Staphylococcus aureus*.

The minimum inhibitory concentration (MIC) of Ceftazidime was determined for nineteen isolates that were resistant to the 30mcg of Ceftazidime. The 19 isolates were also resistant to between three and nine antibiotic tested by disc diffusion method. Likewise the MIC of Ceftriaxone was also determined for seventeen isolates that were resistant to 30mcg of Ceftriaxone by disc diffusion method. The seventeen isolates were also resistant to between three and nine antibiotic tested by disc diffusion method. Broth dilution method as recommended by (CLSI.2002), with slight modification was used for the determination of the minimum inhibitory concentration of the isolates. Known weight of antibiotic powder of Ceftazidime and Ceftriaxone powder were respectively dissolved in sterile distilled water to a final concentration of 300mcg/ml and 240mcg/ml for Ceftazidime and Ceftriaxone respectively. One milliliter of sterile nutrient broth was delivered into each of two sets of khan tubes. One milliliter of 300mcg/ml of Ceftazidime and 1ml of

240mcg/ml of Ceftriaxone were added to the respective first tubes of the two sets. From the respective first tube a serial dilution was made until the last tubes when 1ml was respectively discarded for the two antibiotics. Thus given antibiotic concentration range of 150-0.07mcg/ml for Ceftazidime and 120-0.234mcg/ml for Ceftriaxone . A volume of 0.02ml each of the overnight broth culture of the multi-drug resistant *Staphylococcus aureus* were inoculated to the Khan tubes containing different concentrations of the antibiotic using sterile Pasteur pipettes. They were all incubated at 37°C for 24hours. *Staphylococcus aureus* ATCC25923 was used as control. The lowest concentration of the antibiotic that showed no turbidity after incubation at 37°C overnight was taken as the MIC of the isolate.

RESULTS

Two hundred and eight *Staphylococcus aureus* were isolated from the 3,692 clinical samples processed, 208/3692(5.6%) of which 22/208(10.6%) of isolated *Staphylococcus aureus* were methicilin resistant, that is 22/3692(0.6%) of the total samples subjected to bacteriological studies as shown in Figure 1.

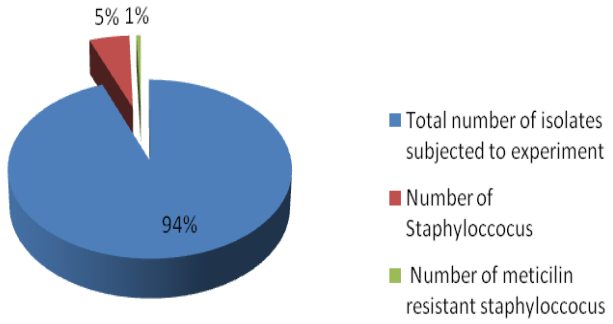

Figure 1:
Percentage of *Staphylococcus aureus* isolated from the total samples collected and the percentage that was methicilin resistant.

The antibiotic sensitivity pattern of the 208 *Staphylococcus aureus*, to antibiotics is shown in Table 2 and Figure 2, with Augumentin and Ciprofloxacin having the highest percentage of sensitivity of 186/208 (89.4%) and 186/208 (89.4%) respectively, whereas the highest resistance pattern of 208/208 (100%) was observed for Gentamycin, followed by 124/208 (59.6%) for Nalidicic acid, 116/208 (55.8%) for Nitrofurantoin, 103/208 (49.5%) for Ampicillin, 102/208 (49%) for Tetracycline, 57/208 (27.4%) for Pefloxacin, 47/208 (22.6%) for Erythromycin and 36/208 (17.3%) for Cloxacillin respectively.

Table 1:
The number and percentage resistance of *Staphylococcus aureus* to 30mcg disc of ceftriaxone and Ceftazidime discs

Antibiotics	Total number of multidrug resistant isolate	Number resistant to the antibiotic	Percentage resistant to the antibiotic
Ceftriaxone	22	17	77.3
Ceftazidime	22	19	86.4

Table 2:
The sensitivity pattern of the *Staphylococcus aureus* to the antibiotic discs

Antibiotic disc	Total isolate tested	Total isolate resistant	Total isolate sensitive
Augumentin	208	22	186
Ampicillin	208	103	105
Amoxycillin	208	22	186
Pefloxacin	208	57	151
Tetracycline	208	102	106
Cloxacillin	208	36	172
Erythromycin	208	47	161
Gentamycin	208	208	0
Ciprofloxacin	208	22	186
Nitrofurantoin	208	116	92
Nalidicic acid	208	124	84

Twenty-two of the total two hundred and eight, 22/208(10.6%) *Staphylococcus aureus* were resistant to three to nine of the antibiotics tested (multi-resistant). Nine isolates 9/22(40.9%), were resistant to nine of the antibiotic tested, presented with R-type aug.amg.amx.pef.cxc.e.nit.nal, two isolates 2/22 (9.1%) had octuple resistant pattern ; R-type aug.amg.amx.pef.cxc.e.nit.nal, four isolates 4/22 (18.2%) have septuple, R-type aug.amg.amx.pef.cxc.e.nit resistant pattern, two isolates 2/22(9.1%) had quintuple R-type aug.amx.cxc.e.nit, one isolate 1/22 (4.5%) quadruple R-type aug.amx.cxc.nit, and another four isolates 4/22 (18.2%) had triple R-type amx.cxc.nit. All the 22 multi-drug resistant *Staphylococcus aureus* isolates were resistant also to Gentamycin (10mcg) and to Amoxycillin (25mcg), Cloxacillin(10mcg), Nitrofurantoin (300mcg).

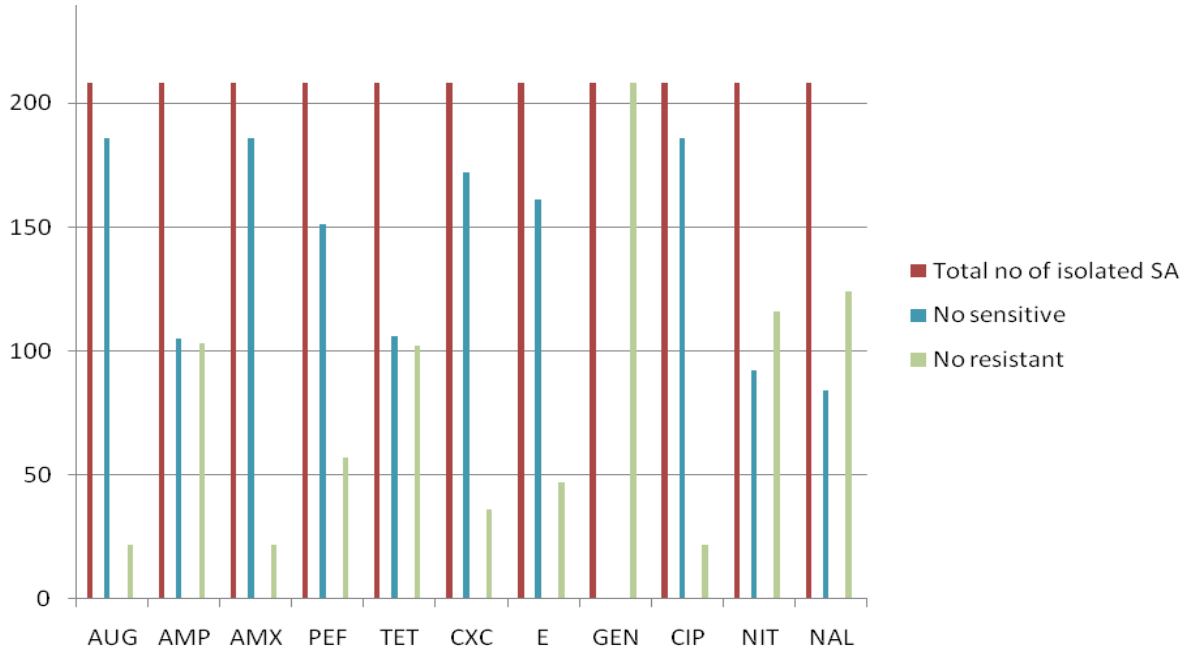

Figure 2:
The antibiotic sensitivity pattern of *Staphylococcus aureus* to antibiotic discs of Augumentin (AUG), Ampicillin (AMP), Amoxycillin (AMX), Pefloxacin (PEF), Tetracycline (TET), Cloxacillin (CXC), Erythromycin (E), Gentamycin GEN), Ciprofloxacin (CIP), Nitrofurantoin (NIT) and Nalidicic acid (NAL).

However only seventeen of them 17/22 (77.3%), were resistant to 30mcg Ceftriaxone, and 19/22 (86.4%) were resistant to 30mcg Ceftazidime Table 1.

The MIC for the *Staphylococcus aureus* ATCC25923 to Ceftazidime was 7.5mcg/ml, whereas the MIC of Ceftazidime for 3 isolates, 3/19(15.8%) was 120mcg/ml , that of 16 isolates, 16/19 (84.2%) was 60mcg/ml. The MIC of Ceftriaxone for the control *Staphylococcus aureus* ATCC25923 was 4.69mcg/ml. The MIC of Ceftriaxone for 1 isolate, 1/17 (5.9%) was >150mcg/ml, for 6 isolate, 6/17 (35.3%) was 150mcg/ml, for 10 isolates, 10/17 (58.8%) was 75mcg/ml and for another 6 isolates, 6/17(35.3%) was 37.5mcg/ml respectively.

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is characterized by resistance against almost all clinically available antibiotics including the aminoglycosides, macrolides, sulphamethoxazole, trimethoprim, tetracycline, rifampicin and fluoroquinolones (Patterson.2000). In this investigation 22 *Staphylococcus aureus*, 22/208(10.6%) *Staphylococcus aureus* were resistant to three to nine of the antibiotics tested (multi-resistant). Nine isolates 9/22(40.9%), were resistant nine of the antibiotic tested showing; R-type aug.amg.amx.pef.tet.cxc.e.nit.nal, two isolates 2/22 (9.1%) had octuple resistant pattern R-type aug.amg.amx.pef.cxc.e.nit.nal, four isolates 4/22 (18.2%) have septuple R-type aug.amg.amx.pef.cxc.e.nit resistant pattern, two isolates 2/22(9.1%) had quintuple R-type aug.amx.cxc.e.nit, one isolate 1/22 (4.5%) quadruple R-type aug.amx.cxc.nit, and another four isolates 4/22 (18.2%) had triple R-type amx.cxc.nit. All the twenty two isolates were resistant to 25mcg Amoxicillin, 10mcg Cloxacillin and 300mcg nitrofurantoin. Furthermore, 17/22 (77.3%) were also resistant to 30mcg of Ceftriaxone, and 19/22 (86.4%) were resistant to 30mcg of Ceftazidime when disc diffusion method was performed. The MIC of Ceftriaxone for all the seventeen, 17/17(100%) resistant to 30mcg of Ceftriaxone was higher than 2mcg/ml for Ceftriaxone and the MIC of Ceftazidime for all the 19 isolates,19/19(100%) resistant to 30mcg of Ceftazidime was above 2mcg/ml. These findings conform with earlier observation of Hallander and Laurell. (1972), who found close association between methicillin and cephalosporin resistance in *Staphylococcus aureus* as heteroresistance trait especially for isolates having MIC of up to 2mcg/ml for both group of antibiotics. This also agrees with the recommendation of the World Health Organization sponsored group that strains with

the MIC of 2mcg/ml and above should be presumed heteroresistant with an all-or none interpretation in disc diffusion test (Ericsson and Sherris, 1971; Greenwood,2002).

The high MIC of Ceftazidime and Ceftriaxone to these resistant isolates further confirms the identity of the isolates as MRSA especially when compared with the respective MIC of the respective antibiotics to the reference *Staphylococcus aureus* ATCC 2593. For instance, the MIC of Ceftazidime for the control organism was 7.5mcg/ml whereas the MIC of Ceftazidime, for 3 isolate, 3/19(15.8%) was 120mcg/ml, for 16 isolates 16/19(84.2%) was 60mcg/ml. Also the MIC of Ceftriaxone, was 4.69mcg/ml for the control organism compared with those where MIC of Ceftriaxone for 1 isolates, 1/17(5.9%) was >150mcg/ml, for 6 isolates, 6/17(35.3%) was 150mcg/ml, for 10 isolates, 10/17(58.8%) was 75mcg/ml and the MIC of another 6 isolates, 6/17(35.3%) was 35.5mcg/ml.

MRSA, is a common cause of nosocomial infections, and has also emerged as an increasingly cause of community associated infection (Moran *et al*., 2006). In the United States of America, between 1999-2005, the number of *Staphylococcus aureus* related hospitalizations increased to 62%, from 294,570 to 477,927 and the estimated number of MRSA related hospitalization was more than doubled, from 127,036 to 278,203 (Klein et al., 2007). MRSA infection has been reported as major cause of illness and death as well as imposing serious economic cost on patients and hospitals (Klein *et al*., 2007). More than 40% of all *Staphylococcus aureus* isolates from patients from Southern Europe were MRSA from patients with bacteraemia, while in the Northern Europe MRSA isolate comprises <5% of the patients with bacteraemia (Bloomfield *et al*., 2006).

Against the backdrop of danger associated with MRSA, the need for public health educational programs in electronic and print media, schools and market places, cannot be overemphasized. The general public should be adequately informed about the importance of good hygienic practices in controlling the spread of MSRA and the risk associated with antibiotics self medication which predisposes to antimicrobial resistance. The multiple-drug resistance profile of most *Staphylococcus aureus* strains has drastically reduced the available therapeutic antimicrobial options (Camargo *et al.*, 2008). The implication of MRSA infection calls for regular surveillance of this organism and there is the need to develop educational packages for the hospital workers, general public, as well as sick patients, their friends and relations.

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