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Evaluation of antibacterial profile of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from hospitals in Imo state, Nigeria

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Awareness of the threat of methicillin resistant *Staphylococcus aureus* (MRSA) is growing. Oxacillin and methicillin are penicillinase-stable penicillins, and strains that are oxacillin and methicillinn resistant are historically termed MRSA. To determine the existence of MRSA strains patients attending treatments in hospitals in Imo state, 200 clinical specimens were examined using conventional method such as culture and sensitivity. The specimens include urogenital swabs, nasal swabs, wound swabs, pus and blood. Out of the 200 samples collected, 23.68% showed resistance to oxacillin, 25.00% of the isolates from Owerri Zone were MRSA, while 23.81% of isolates from Okigwe Zone yielded MRSA, and 20.00% from Orlu Zone yielded MRSA. The mean for zones is 25.33 ± 13.05. Children under the age of 10 have the highest incidence of (57.14%) of *S. aureus* isolates resistant to oxacillin, followed by the elderly people of age group 51 to 60 years (27.27%). The young adults between the ages of 21 to 30 have the least incidence (10.00%) and the mean for age groups is 43.66 ± 6.26. The mean for clinical specimens is 15.20 ± 22.16. Wound specimens produced the highest incidence (40.00%) among clinical specimens followed by pus (33.33%) and blood has the least occurrence (00.00%). Males produced the highest incidence between the sexes (26.67%) and women (21.74%), and the mean for sexes is 38.00 ± 11.31. This shows that MRSA exists in hospitals in Imo state and considering the danger, it portends to healthcare setting, and efforts are needed to contain its spread.

Key words: Methicillin, antibiotics, zones, sex, children, specimen, infections.

INTRODUCTION

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Genes can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation. Thus a gene for antibiotic resistance which had evolved via natural selection may be shared.

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Evolutionary stress such as exposure to antibiotics was then selected for the antibiotic resistant trait. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistant genes it is called multi-resistant or informally, a superbug or super bacterium (Noskin et al., 2005).

The primary cause of antibiotic resistance is genetic mutation in bacteria. The prevalence of antibiotic resistant bacteria is a result of antibiotics use both within medicine and veterinary medicine. The greater the duration of exposure, the greater the risk of the development of resistance irrespective of the severity of the need for antibiotics. As resistance towards antibiotic becomes more common, a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies, there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem.

During the late 1950s and early 1960s, *Staphylococcus aureus* caused considerable morbidity and mortality as a nosocomial, or hospital acquired, pathogen. Since then, penicillinase-resistant, semi synthetic penicillins have proved to be successful antimicrobial agents in the treatment of Staphylococcal infections. Unfortunately, methicillin-resistant *S. aureus* (MRSA) strains have recently emerged as major nosocomial problem. One way in which Staphylococci become resistant is through acquisition of a chromosomal gene (*mecA*) that encodes an alternate target protein which is not inactivated by methicillin (Proctor, 2008).

S. aureus is a non-motile gram-positive cocci that appears in clusters. It is found worldwide and is a leading cause of infectious disease. It can normally only transiently colonize the outside and entry portals of the human body (skins, ears, eyes, nasal passages etc.) but it is estimated that 20% of humans are carriers (asymptomatic permanent colonizatiion) (Kluytmans et al., 1997). However, even transient colonization can lead to infection if the conditions are right, such as a breach in the protective layer of epithelial cells, or a compromised immune system. The ability to cause disease is via two mechanisms, namely; toxin production and proliferation of the organism, which causes tissue destruction. Most infections remain localized at entry portals and are usually self-limiting and non-life threatening. Much less frequently, more serious infections may occur when the organism is able to invade deeper into the body (osteomyelitis, septicemia, pneumonia etc). These deeper infections may be extremely serious and even fatal because infections of *S. aureus* occur at a higher rate than that of many bacteria. The costs that are incurred for hospitalization and treatment can be tremendous.

In just one year, 1995 in New York City, it was estimated that there were at last 13,550 cases of *S.aureus* infections resulting in an estimated cost of about $435.5 million (Rubin et al., 1999). In a study that was published in 2007 with data from the year 2003, it was found that nearly 390,000 people in the US were hospitalized with *S. aureus* infections, with the average hospital stay for this type of infection costing $37,251. The total cost of *S. aureus* infections in the US for 2003 was $14.5 million (Noskin et al., 2007).

Before the advent of antibiotics, the mortality rate from *S. aureus* infections was near 80%. When pencillin therapy was introduced in the 1940s, it seemed that *S. aureus* (along with many other bacteria) infections could now be easily treated. However, within a short amount of time penicillin – resistant *S. aureus* had appeared, with approximately 80% of *S. aureus* now being penicillin resistant (Deurenberg et al., 2007). This led to the discovery and use of other antimicrobials such as methicillin in the 1960s.

Once again, the organism seemed to be under control. It was not long however before methicillin – resistant *S. aureus* (MRSA) appeared, and strain that were sensitive to methicillin became known as methicillin-susceptible *S. aureus* (MSSA) (Chambers, 2001). Then clinicians turned to the use of vancomycin for serious MRSA infections, and recently we have seen that MRSA strains are evolving that are able to overcome vancomycin. A few vancomycin-intermediate resistant *S. aureus* (VISA) have been isolated (Smith et al., 1999).

Oxacillin is a parental second generation penicillin antibiotic that is used to treat moderate-to-severe penicillinase-resistant Staphylococci infections. It was approved for use in United State in 1989 and is still in common use (CDC, 2010). Oxacillin and methicillin are penicillinase-stable penicillins and strains that are oxacillin and methicillins resistant and are historically termed methicillin-resistant *S. aureus* (MRSA) (Clinical and Laboratory Standard Institute, US, 2007). Because methicillin is no longer commercially available in the United State, oxacillin maintains its activity during storage better than methicillin and is more likely to detect hetero-resistant strains. Hence oxacillin is tested instead of methicillin (CLSI, 2007).

The isolate tested with oxacillin is called MRSA instead of ORSA because when resistance was first described in 1961, methicillin was used to test and treat infections caused by *S. aureus.* However, oxacillin which is in the same class of drugs as methicillin was chosen as the agent of choice for testing *Staphylococcus* in the early 1990s. The acronym MRSA is still used by many to describe these isolates because of its historic role (Bannerman, 2003).

MRSA was discovered in 1961 in the United Kingdom. It made its first major appearance in the United States in 1981 among intravenous drug users. MRSA is often referred to in the process as a SUPERBUG. The number

of MRSA infection in the United States has been increasing significantly. A 2007 report in Emerging Infectious Disease, a publication of the Centres for Disease control and Prevention (CDC 2007), estimated the number of MRSA infection in hospitals doubled nationwide, from approximately 127,000 in 1999 to 278,000 in 2005, while at the same time annual deaths increased from 11,000 to more than 17,000 (Klein et al., 2007) and estimated that MRSA would have been responsible for 94,360 serious infections and associated with 18,560 hospital stay-related deaths in the United State in 2005 (Klevens et al., 2007; CDC, 2007). These figures suggest that MRSA infections are responsible for more death in the US each year than AIDS (Stein, 2007). Kleven (2007) also suggested that in the incidence of invasive infections caused by USA, 300 remains more in rural than in the urban centers.

Though MRSA since its emergence has become a major cause of illness and death in the healthcare settings, no serious previous attempt has been made to study its profile in the hospitals situated in Imo State. Before now Imo inhabitants have the belief that patients only go to hospitals to be treated and not to contact infections. This research shall therefore assess the prevalence of this MRSA amongst the hospitals in Imo State, determine the gender, zone, age-group and clinical specimen that yielded most of the MRSA and proffer solution to prevent epidemics due to hospital acquired MRSA.

MATERIALS AND METHODS

Discription of the study area

The study area is Imo State, located at the South Eastern part of Nigeria. Lies within latitudes 4° 45' N and 7° 15' N and longitude 6° 50' E and 7° 25' E. It is bounded in the South by Rivers State, in the North and East by Abia State and in the West by Anambra State. It has many primary, secondary and tertiary health institutions among which are the health centres at the community levels, the General Hospitals under the State Hospital Management Board (HMB) at every Local Government Area and health institutions like Federal Medical Centre Owerri and Imo State University Teaching Hospital Orlu which serve as referral centres for Imo State and neighbouring States.

Sample and sampling technique

A total of two hundred (200) samples were collected from patients who have been admitted for about 2 to 4 days from the selected hospitals between the month of October, 2010 and August, 2011, using random sampling method on both manitol salt agar and blood agar medium. All specimens were cultured within 3 h of collection. The selected hospitals include: General Hospitals, Okigwe in Okigwe zone, General Hospital Uguta in Orlu zone and General Hospital Owerri in Owerri zone, to represent the three geographical zones in Imo State. Samples were obtained from the following clinical specimens: Urogenital specimens (including urine, urethral swabs, vaginal swabs, and semen), nasal swabs, wound swabs pus and blood.

Ethical consideration

Permission was sought from Imo State Ministry of Health under which the Health Management Board operates. Letter of authority from this ministry was presented to each of these hospitals before samples were allowed to be collected from in-patients.

Innoculation, isolation and identification of organisms

The specimens were inoculated on manitol salt agar and blood agar plates using the streak technique to obtain discrete colonies while blood samples were first inoculated into brain heart infusion broth, incubated for 24 h before being transferred to the solid media. This transfer was repeated every day for 7 days, before it was considered "no bacterial growth". The plates were incubated at 37 and 30°C for manitol salt and blood agar, respectively for 24 h under aerobic conditions. The culture plates were examined recording to appearance, size, colour and morphology of colonies. Gram stain reaction, catalase and coagulase test were carried out on the isolates. Isolates that were gram positive cocci, catalase positive and coagulated human plasma were considered as *S. aureus* (Chigbu and Ezeronye, 2003; Uabi-Egbeni, 2003).

Susceptibility of isolates to oxacillin

Antimicrobial susceptibility of isolates to oxacillin was carried out on *S. aureus* isolates using the paper disc diffusion technique. A. 0.06 ml of overnight culture of the test organism (Mc Ferland standard 0.5) was seeded on Mueller Hinton agar plate. This was spread over the entire surface of the agar plate using a sterile glass spreader and allowed to dry for about 15 to 30 min. The 5 µg oxacillin antibiotic disc (oxoid) was then placed on the agar plates using sterile forceps. The inhibition zone diameter (IZD) was measured and recorded in millimetres.

Minimum inhibitory concentration (MIC)

To confirm the resistivity of the *S. aureus* isolates to oxacillin, minimum inhibitory concentration (MIC) of oxacillin for the isolates by agar dilution method was determined. Antibiotic stock solution (10,000 mg/l) of oxacillin powder obtained from oxoid was prepared according to manufacturers instruction. A two-fold serial (double) dilution for series of the antimicrobial agent was prepared in 30 ml containers, including a drug free control. 19 ml of molten Mueller-Hinton agar at 50°C was added to each of the containers, mixed thoroughly and poured into pre-labelled sterile petri dishes on a level surface. It was allowed to solidify at room temperature and was dried in the incubator to remove moisture. Then, from a 10^4 cfu density (Mc Farland standard) of *S. aureus* solution already prepared, the plates including the control were inoculated using a standard wire loop. The inoculum spots were allowed to dry at room temperature before inverting the plates for incubation at 30°C for 18 h under aerobic condition. The trailing end point was investigated by sub-culturing and re-testing to confirm β-lactamase production.

Statistical analysis

The parameters estimated are the proportion resistant to oxacillin,

Table 1. Distribution of respondents who yielded *Staphylococcus aureus* according to Zone.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	n i2/f (pi)	% pi
Orlu	12	3	15	0.2000	20.00
Okigwe	16	5	21	0.2381	23.81
Owerri	30	10	40	0.2500	25.00

Mean (X) = 25.33 ± 13.05. Std. Deviation (S) = 13.051.

Table 2. Distribution of respondents who yielded *Staphylococcus aureus* according to age group.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	Mid point	fx	Fx^2	n i2/f (pi)	% pi
1-10	3	4	7	5.5	38.5	211.75	0.5714	57.14
11-20	6	1	7	15.5	108.5	1681.75	0.1429	14.29
21-30	9	1	10	25.5	255.0	6502.50	0.1000	10.00
31-40	5	1	6	35.5	213.0	7561.50	0.1667	16.67
41-50	8	2	10	45.5	455.0	20702.50	0.2000	20.00
51-60	8	3	11	55.5	610.5	33882.75	0.2727	27.27
61-70	19	6	25	65.5	1637.5	107,256.25	0.2400	24.00
Total	-	-	76	-	3318.0	177,799.00	-	-

Mean (X) = 443.66 ± 6.26. Std. Deviation (S) = 6.263

Table 3. Distribution of respondents who yielded *Staphylococcus aureus* according to clinical specimen.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	n i2/f (pi)	% pi
Urogenital	41	13	54	0.2407	24.07
Nasal	11	2	13	0.1538	15.38
Wound	3	2	5	0.4000	40.00
Pus	2	1	3	0.3333	33.33
Blood	1	0	1	0.0000	00.00

Mean (X) = 15.20 ± 22.16. Std. Deviation (S) = 22.163

the mean and the standard deviation of both resistivity and sensitivity from the mean. The methods of analysis are: (1) Chi – square test statistic for contingency; (2) Pair wise comparison was used to compare the proportions.

RESULTS

The distribution of samples among the three geographical zones of Orlu, Okigwe and Owerri, respectively showed that Owerri Zone had the highest incidence (25.00%) of *S. aureus* isolates resistant to oxacillin. This was followed by Okigwe Zone (23.81%) and lastly Orlu Zone (20.00%) as shown in Table 1. Table 2 shows the incidence of MRSA among different age groups. It was observed that children under the age of 10 had the highest incidence (57.14%) of *S. aureus* isolates resistant to oxacillin, followed by the elderly of 51 to 60 years of age (27.27%). The young adults between the ages of 21 to 30 has the least incidence (10.00%). The distribution among different clinical specimens collected as shown in Table 3 indicated that wound specimen has the highest incidence (40.00%) of *S. aureus* isolates resistant to oxacillin, followed by pus specimen (33.33%). Blood has the least occurrence (00.00%). And Table 4 shows the incidence of MRSA between sexes. It was observed that males were more infected with incidence rate (26.67%) of *S. aureus* isolates resistant to oxacillin.

The mean are as follows; zones = 25.33 ± 13.05, age groups = 43.66 ± 6.26, clinical specimens = 15.20 ± 22.16 and sexes = 38.00 ± 11.31, and the standard deviations (S): zone = 13.015, age group = 6.263, clinical specimen = 22.163 and sex = 11.314.

Table 4. Distribution of respondents who yielded *Staphylococcus aureus* according to sex.

Characteristic	Sensitive (ni1)	Resistance (ni2)	No. that yielded *Staphylococcus* (f)	n i2/f (pi)	% pi
Male	22	8	30	0.2667	26.67
Female	36	10	46	0.2174	23.68
All	58	18	76	0.2368	23.68

Mean (X) = 38.00 ± 11.31. Std. Deviation (S) = 11.314.

DISCUSSION

This study was done primarily to determine the level of resistant of *S. aureus* species isolated from hospitals in Imo State. It is also aimed at demonstrating the frequency and pattern of distribution among zones, specimen, gender and ages in the State. Much has been written in recent years about the methicillin resistance *S. aureus* but not much work has been done on that in this part of Nigeria.

All together, 200 individuals were screened in this study. These include 100 males and 100 females from the three geographical zones of the State and of different age groups. Also different clinical specimens were used which include; nasal, urogenital, wound, pus and blood. According to the zones of location, the percentage of *S. aureus* growth that is resistant to oxacillin is least in Orlu (with 20%) and highest in Owerri (with 25 %). The result of this therefore is in contrast with (Klevens et al., 2007) who suggested that in the incidence of invasive infections caused by USA, 300 remains more in rural areas than in the Urban center. This contradiction may be because of the central nature of Owerri which made it possible for people to be operating from every part of the State to the Capital hence are having access to hospital services at urban area even when they are living in the rural areas.

Percentage of *S. aureus* growth resistant to oxacillin decreased from about 57.14% among respondents aged 1 to 10 years to about 10.0% among those aged 21 to 30 years and thereafter, increased to about 27.27% among those aged 51 to 60 years of age. This is in line with a 2007 study by the “Archives of disease in children including hospital nurseries”. This study incriminated MRSA as also becoming a problem in pediatric settings. The U curve incidence among the ages could be attributed to the low immunity of the children and the elderly. While the children have not built up enough immunity to fight incoming infections, the elderlys’ immune systems may have been affected by disease like diabetes, cancer, ashma or, the many have been transplant recipients. This is in agreement with Reuters (2009) who included these categories of people among the risk population for MRSA. It is also believed that the young children and the elderly are the ages that visit hospitals more regularly for admission. Hence the high incidence found among their age groups can be understood as a result of their frequent admissions in hospitals.

By their sexes, the percentage of *S. aureus* resistant to oxacillin is slightly higher for males (with about 26.67%) than for females (with about 21.74%). This relatively high rate in male cannot be easily explained except that the reign of motor bikes in the State made it possible for males to be visiting hospitals more frequently than the females. This is as a result of frequent accident that are occurring among the bike users which males are the major culprits.

According to the clinical specimen, *S. aureus* growth from wound showed the greatest resistance to oxacillin (with about 40%) while growth from blood and nasal specimen showed the least resistance (with about 00.00 and 18.38%, respectively). The observed high incidence rate in wound specimens can be attributed to its exposure, which makes it more possible for infections to be attracted to it. It can also be attributed to the use of antibiotic in dressing the wounds which makes it possible also for the infections to be resistant to other antibiotics. This work agreed with the findings of a previous work by Chambers (1997) who observed that the usual source of staphylococci in a ward is from septic wounds, particularly several fistulate and colostomies. If dressings from such wound are improperly handled, they could contaminate the ward, air, dust and hands of nurses and then be transferred to others. Even though nasal specimens yielded the highest incidence of *S. aureus* growth, it did not produce the highest incidence of *S. aureus* resistance to oxacillin. This can be attributed to *S. aureus* being a normal flora of the nasal passage. The zero value associated with the blood specimen may be more due to the number of cases than actual resistance. Again blood is expected to be sterile. These specific values of percentage of growth of *S. aureus* resistance to oxacillin differ greatly from the overall value (23.68%). This calls for further investigation on the degree of variation among categories of the different characteristics. This study has several limitations, among which is that considerations were not made of those who have acquired the infections before their admissions to

the hospitals, since the MRSA can also be acquired at community level. Also, this work did not involve typing of the invasive MRSA isolates.

Another is that *S. aureus* isolates resistant to other antibiotics were not considered so as to know if they are more multi drug resistance rather than oxacillin alone. Based on these limitations, some might argue that MRSA is not acquired from the hospitals in Imo State. However, MRSA certainly has become a hot topic in Nigeria, Imo State not excluded. It has been isolated at the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeriia (Taiwo et al., 2004).

CONCLUSION AND RECOMMENDATIONS

It has been confirmed that MRSA has become a major cause of illness and death in the society. Also, this research has proved that this MRSA exists in hospitals in Imo State, that children and elderly are more vulnerable. This work also proved that the choice of methicillin in treatment of *S. aureus* infection is no more fashionable and that even the use of varcomycin is becoming obsolete. We therefore recommend that a surveillance system be established by the State Government to monitor the number and incidence of infections, that there should be a monitoring body for changes in their antimicrobial susceptibility. Also, our hospital must screen the patients upon admission to prevent the cohabitation of MRSA carriers with non-carriers. Finally, education of the hospital staff as well as the patients is very vital to enable each to maintain the measures required to prevent the spread of the infections

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