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METHICILLIN-RESISTANT STAPHYLOCOCCUS aureus (MRSA) AS A CAUSE OF NOSOCOMIAL INFECTION IN IBADAN, NIGERIA

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be one of the most important causative agents of nosocomial infections. MRSA is multidrug resistant and immense clinical problem, all things considered, penicillins and other groups of antibiotics routinely utilized against *S. aureus* has turned out to be useless as treatments. MRSA infections are treated with glycopeptide (vancomycin and teicoplanin) which can prompt new resistance to these drugs. Clinicians and researchers need to understand the organism, the patients and the trends in the antibiotic-resistance patterns, so as to have sufficient data for treatment of methicillin resistant *S. aureus* infections in hospital. A total of one hundred and twenty (120) samples (from nasal swab, inanimate Objects; such as beds, linens, clinical samples such as wound, urine, blood) were examined. A total of eighty six (86) isolates were confirmed to be *S. aureus* from the three categories of samples. Methicillin resistant *S. aureus* (MRSA) was differentiated from methicillin sensitive S. *aureus* (MSSA) by carrying out antibiotic susceptibility pattern using disk diffusion method with different antibiotics (methicillin, oxacillin, cefoxitin, erythromycin, clindamycin and vancomycin). The prevalence of methicillin resistance among *S. aureus* isolates was 48.8%. Resistance to cefoxitin was the highest (55.8%) followed by; oxacilllin (53.5%), methicillin (48.8%), erythromycin (34.9%), clindamycin (23.3%) and vancomycin (2.3%). Two vancomycin resistant isolates were obtained among clinical samples (blood and urine). The gathered information affirms that a consistent and dynamic observation of this kind of nosocomial infection is important. The outcomes are valuable for correlation of recurrence of MRSA contamination in other medical clinics. Thorough training, great participation and backing from the hospital board are critical for the fruitful utilization of control measures.

INRODUCTION

Staphylococcus aureus is a Gram-positive bacterium belonging to the family *Staphylococaceae* and is often found as a commensal on the skin, skin glands and mucous membranes particularly in the nose of healthy individuals (Plata *et al.,* 2009). Methicillin-resistant *Staphylococcus aureus* (MRSA) are isolates of the bacterium *S. aureus* that have acquired genes encoding antibiotic resistance to all β-lactam antibiotics, including methicillin, oxacillin, flucloxacillin, nafcillin and cephalosporins (Foster, 1996). However, the term has increasingly been used to refer to multi-drug resistant *S. aureus*, as MRSA isolates also frequently carry resistance genes to other antibiotics that have traditionally been used against *S. aureus* (Franklin, 2003). The Centre for Disease Control and Prevention (CDC) defines Nosocomial Infection (or Hospital-Acquired Infection HAI) as any infection, which is usually acquired 48 hours or more after admission to hospital or after contact with a healthcare facility, such as a day care unit or a nursing home, etc. Hospitalized patients are particularly susceptible to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections (Lindsay and Holden, 2004). MRSA is considered to be one of the most important causative agents of nosocomial (Hospital-Acquired) infection worldwide, predominantly in hospitals and institutions such as nursing homes (Goettsch *et al.,* 2000), while, less commonly, in the general community than many other bacteria such as *Escherichia coli*, *Clostridium difficile*, *Pseudomonas aeruginosa* etc. Community Acquired (CA-MRSA) was defined as MRSA strains isolated in an outpatient setting, or isolated from patients within 48 hours of hospital admission. Furthermore, these patients must have no medical history of MRSA infection or colonization, and no medical history in the past year of either hospitalization (e.g. surgery), admission to a nursing home, or dialysis. Moreover, the patient should not have permanent indwelling devices, such as catheters or a percutaneous device at the time of culture or previous isolation from the patient (Morrison *et al.*, 2006; Naimi *et al.*, 2003).

There are three main reservoirs (and thus sources of spread and infection) for MRSA in hospitals and

institutions, i.e. staff, patients and inanimate objects such as beds and linens. By far the most important reservoirs are patients who may be colonized with MRSA without evidence of infection, especially since MRSA may be carried for an extremely long period of time. Hospital-acquired infection is often caused by antibiotic-resistant strains (such as MRSA) and is treated with vancomycin or teicoplanin (Helisangela *et al.,* 2003). Infections caused by *Staphylococcus aureus* resistant to methicillin (MRSA) are increasing in prevalence in adults and children. Nosocomial infections account for morbidity and mortality of millions of patients annually, worldwide. *Staphylococcus aureus*, especially Methicillin-resistance *S. aureus* (MRSA) is relatively ubiquitous and is the cause of many community's endemic and epidemic nosocomial colonization and infections (Mansouri *et al.,* 1997). This microorganism can become versatile pathogen causing a broad spectrum of infections due to a large arsenal of virulence factors which ranges from common skin infections(sores or boils), to deep-seated infections(heart, lungs, kidney, spine, and liver). It ranks first among bacterial pathogen causing bloodstream infections and is the leading cause of nosocomial pneumonia (Biedenbach *et al.,* 2004; Wisplinghoff *et al.,* 2004). In addition, it causes infections of surgical wounds, septicemia, catheter-associated bacteremia, prosthetic implants and the urinary tract. Though most MRSA infections are not serious, some can be life-threatening. Many public health experts are alarmed by the spread of tough strains of MRSA (Hoban *et al.,* 2003). This study is therefore aimed at evaluating the prevalence of MRSA in nosocomial infections and determination of the susceptibility profile of the isolated *S. aureus* to methicillin and cefoxitin and other specifically used antibiotics for effective diagnosis and accurate drug prescription to patients.

MATERIALS AND METHODS

Collection of samples

The clinical samples were collected from inpatients in Hospital (UCH), Ibadan, Oyo state. Samples were collected from hospital personnel and inanimate objects also deep and superficial wound samples were collected from different body sites. A total of 120 samples were obtained, stored and carefully transported to the laboratory for examination.

Preservation of samples

Swab sticks were preserved in screwed cap tubes containing normal saline. Boric acid was added to urine sample, which can preserve it for as long as 16hours before examination.

Culture Media

The media used includes Peptone Water, Nutrient Agar, 5% Sheep or Horse Blood Agar, Chocolate Agar, Mannitol Salt Agar (MSA), Mueller-Hinton Agar (Oxoid, UK). All media were prepared according to the manufacturer's specifications and then sterilized in an autoclave at 121^0C for 15 minutes.

Isolation of microorganisms

Sterile swab sticks moistened with Normal Saline was used to collect samples from skin infection like abscess and other pus-producing tissue infection. Blood and urine samples from in-dwelling catheter were collected using Sterile EDTA and Universal Bottles respectively. More samples were obtained from the anterior nares (Nasopharynx) of hospital personnel and other asymptomatic carriers and from hospital wards; floors, windows, beddings, equipment and other inanimate objects using sterile swab stick. The samples were properly shaken to homogenize and then aseptically introduced onto already prepared, well cooled and dried-surface duplicated agar plates. The inoculated plates were then incubated at 37^0C for 24 hours in an Incubator.

Culture Examination

The inoculated cultures were checked for visible and discrete colonies after 24hours of incubation.

Subculture

Selected colonies were picked from previously incubated plates and aseptically streaked onto fresh duplicated agar plates in order to obtain a pure colony. These were incubated at 37^0C for 24 hours.

Culture Preservation

Pure colonies were picked from the sub cultured plates and inoculated into already prepared sterile nutrient agar slants/slopes; these were then incubated at 37^0C for 24 hours. Afterwards, the isolates were preserved in the refrigerator at 4^0C.

Characterization of the bacterial isolates

Identification of isolates was by standard microbiological procedures such as phenotypic characteristics (elevation, mucous production), microscopy, biochemical tests etc.

Antimicrobial susceptibility testing by disk diffusion (kirby-bauer method)

Inoculum preparation

The direct colony suspension method was used by selecting at least three to five well-isolated colonies of the same morphological type from an 18 to 24hour mannitol salt agar plate. The top of each colony was touched with sterile wire loop and transferred the growth into a tube containing 5ml of peptone water. The density of the suspension was then adjusted to match the 0.5 McFarland's turbidity standard.

Inoculation of agar plates

A sterile cotton swab was dipped into the adjusted suspension and excess inoculum was removed by pressing the swab firmly on the inside wall of the tube above the fluid level. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire surface. This procedure was repeated by

streaking two more times, rotating the plate approximately 60^0 each time to ensure an even distribution of inoculum. The lid of the plate was left slightly opened for 3 to 5minutes to allow surface moisture to be absorbed before applying the antibiotic-impregnated disks.

Application of disks to inoculated agar plates

The commercially prepared, single antimicrobial disks and their antibiogram used in the disk diffusion technique are: Methicillin (5µg), Oxacillin (1µg), Cefoxitin (30µg), Clindamycin (10µg), Erythromycin (15µg) and Vancomycin (30µg).were placed firmly on the surface of the inoculated agar plate using sterile forceps. They were evenly distributed with at least 24 mm from center to center. The plates were inverted and incubated at 35^0C overnight within 15 min after the discs were applied.

Reading of plates and interpretation of results

After 18 to 24 h of incubation, the plates were examined and the diameters of the zones of inhibition were measured to the nearest millimeter. Results were classified as sensitive or resistant, according to the Clinical and Laboratory Standards Institute (CLSI, 2011).

RESULTS AND DISCUSSION

Isolates were identified by using conventional microscopy, by which *Staphylococci* appear as round, Gram-positive cocci growing in clusters. The *Staphylococci* are then distinguished from *Streptococci* by a positive catalase test. *Staphylococcus aureus* isolates were distinguished from other *staphylococcal* species, such as coagulase-negative staphylococci (CoNS) by a positive coagulase test. A confirmative test for *S. aureus* was by inoculation of the isolates on Mannitol Salt Agar (MSA), which change the color of the agar from reddish-pink to yellow after >24hours (preferably 48hours) and incubation. One hundred and twenty isolates were recovered in all out of which 30 were from nasal swab, 20 from inanimate objects and 70 from clinical samples but 86 of these isolates were identified as *Staphylococcus aureus* as shown in Table 4.1.

The antimicrobial susceptibility pattern of the 86 *S. aureus* isolates from nasal, inanimate objects and clinical samples to the various commercially available antibiotics used are shown in Tables 2 and 3. The Clinical and Laboratory Standards Institute (CLSI) was used to determine the zone diameter breakpoints for *S. aureus* to the nearest millimeter (mm) as shown in Table 4. The rate of resistance was highest for cefoxitin in the three categories of isolates (nasal, inanimate objects and clinical) by 55.8% followed by oxacillin 53.5%. Of all the *S. aureus* isolates, 42 showed methicillin resistance giving a MRSA prevalence rate of 48.8%. Over the past 20 years, the incidences of both community-acquired (CA) and hospital-acquired (HA) *S. aureus* infections have increased, while antibiotic treatment is increasingly hampered by the spread of *S. aureus* strains that are resistant to multiple antibiotics, including methicillin (Ghebremedhin *et al.*, 2009). According to the results obtained from disk diffusion test, MRSA prevalence in this study was 48.8%. This finding is comparable to that obtained by Olowe *et al.* (2007) who reported 47.8% prevalence of MRSA in Oshogbo, Ladoke Akintola University of Technology College of Health Sciences, South Western Nigeria, but less than that 79% reported by Onemu and Ophori (2013) in University of Benin Hospital, Benin City. Although the prevalence is higher compared to 22.0% reported by Abu-Hujier and Sharif (2008) in Gaza, Palestine.

Under some test conditions, low-level resistance may also be seen in isolates, which produce large amounts of penicillinase (penicillinase hyper producers), and these isolates have been referred to as 'borderline oxacillin-resistant *S. aureus* (Fluit *et al*., 2001). Many laboratories still prefer using oxacillin for detection of MRSA, because oxacillin maintains its activity during storage better than methicillin, and more likely to detect heteroresistant strains (CDC, 2005). The oxacillin disk diffusion test has previously been found to be less reliable, with high numbers of both false-susceptible and false-resistant results. Cefoxitin, however, give clearer endpoints and are easier to read than tests with oxacillin. This explains the close range of resistance to cefoxitin and oxacillin in this study, i.e. 55.8% and 53.4%, respectively. One disadvantage of the cefoxitin disk diffusion test is that the gap between the inhibition zones of isolates is very narrow (sensitive, 22; resistant, 21), and this might affect the results of the cefoxitin disk diffusion test (Skov *et al.*, 1999).

The data shows that the resistance of erythromycin was higher than that of clindamycin among methicillin resistant *Staphylococcus aureus*. Some Staphylococcal strains prevent macrolides and lincosa-mides (erythromycin and clindamycin) from binding to their target site. This explains the higher resistance rate to erythromycin compared with that of clindamycin observed in this study (Joseph *et al.*, 2005).

According to the Physicians in the hospital, many critical cases that were infected with *S. aureus* are treated with glycopeptides (such as Vancomycin or Teicoplanin) which may lead to creation of new strains that are resistant to these antibiotics. Of particular concern are strains of MRSA that are beginning to develop resistance to vancomycin, which is currently the most effective antibiotic against MRSA. This new resistance has arisen because enterococci relatively commonly express vancomycin resistance. In the laboratory, enterococci are capable of transferring the gene for vancomycin resistance to *S. aureus*. Globally, this isolates have been termed Vancomycin-Resistant *S. aureus* (VRSA). Two vancomycin resistant isolates were collected among clinical samples (Stanway, 2004).

The occurrence of MRSA in this study could be attributed to many factors, despite methicillin not being routinely used against *S. aureus* infections. In addition to antibiotic stress, horizontal gene transfer is considered a contributing factor in the occurrence of antibiotic resistance in clinical isolates. Consequently, it has been suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption (Ako-Nai *et al.*, 2005; Brown *et al.*, 2005). The use of antimicrobials in animal food is another contributing factor (Oladipo and Adejumobi, 2010). Antibiotics are commonly added to feed to promote growth in animals, particularly dairy cattle, sheep and poultry (Gin *et al.*, 2001). Frequent traveling is an additional factor for transmitting resistant strains between countries. Misuse of antimicrobials is another contributing factor (Chambers, 1997). Asensio *et al.*, (1996) identified six factors that were independently associated with MRSA infection and colonization, namely increasing age, ward type (particularly intensive care units), coma, previous hospitalization, invasive procedures and length of hospitalization.

The rate of sensitivity was highest for vancomycin (97.7%), followed by clindamycin (76.7%), erythromycin (65.1%), methicillin (51.2%) and oxacillin (46.5%). The least sensitivity was shown by cefoxitin (44.2%). Methicillin resistance in *S. aureus* usually is accompanied by resistance to other groups of antimicrobial agents, so therapeutic options are limited. Therefore, surveillance of the antimicrobial susceptibility patterns of *S. aureus* is of utmost importance in understanding new and emerging resistance trends and in the management of both hospital and community acquired infections.

The prevalence of MRSA in this study is relatively high (48.8%). It is therefore, imperative to establish guidelines for MRSA detection and control in the hospital by daily monitoring of clinical laboratories for MRSA isolates, a programme of monthly prospective culture surveillance of inpatients believed to be at high risk of acquisition of MRSA, screening of hospital personnel, regular hand washing and sanitizing by hospital personnel and policy regulation antibiotics prescription and usage.

New Antimicrobials are needed as alternative agents against the emergence of increasing vancomycin-resistant strains. The most important measures to ensure control of emergence and dissemination of such resistance genes are an adequate antibiotic usage but more important is the application of infection-control practices to prevent transmission of resistant organisms.

Existing guidelines covering MRSA diagnosis and treatment should be used as a basis to standardize diagnostic practices in the hospital. Education is also an important factor in providing consistency of approach to maintenance of MRSA. Microbiology laboratories should participate in the education of medical and healthcare students and workers (such as Seminars, Conference etc.) to perform procedures appropriately and proper regulatory bodies should be set up to provide support and to facilitate the introduction of new techniques in an attempt to limit the scope of this disturbing worldwide problem.

Table 1: Distribution of *S. aureus* isolates among samples examined.

Samples	Number of Isolates (%)	Number of *S. aureus* (%)
Nasal Swab	30(25.0%)	18(20.9%)
Inanimate Objects(Bed, Linen)	20(16.7%)	13(15.1%)
Clinical Samples(deep and superficial wound, blood, urine)	70(58.3%)	55(64.0%)
Total	120	86

Table 2: Distribution of antibiotic resistance among MRSA isolates.

Samples	Isolates	MET	OXA	FOX	ERY	CLD	VAN
Nasal Swab	18	9	10	12	6	3	0
Inanimate Objects	13	6	6	6	3	2	0
Clinical Samples(wound, blood, urine)	55	27	30	30	21	15	2
Total(n)	86	42	46	48	30	20	2
Total(%)=n/86x100		48.8	53.4	55.8	34.9	23.3	2.3

MET: Methicillin, OXA: Oxacillin, FOX: Cefoxitin, ERY: Erythromycin, CLD: Clindamycin, VAN: Vancomycin, MRSA: Methicillin Resistant *S. aureus*,

Table 3: Distribution of antibiotic sensitivity among MSSA isolates.

Samples	Isolates	MET	OXA	FOX	ERY	CLD	VAN
Nasal Swab	18	9	8	6	12	15	18
Inanimate Objects	13	7	7	7	10	11	13
Clinical Samples(wound, blood, urine)	55	28	25	25	34	40	53
Total(n)	86	44	40	38	56	66	84
Total(%)=n/86x100		51.2	46.5	44.2	65.1	76.7	97.7

MET: Methicillin, OXA: Oxacillin, FOX: Cefoxitin, ERY: Erythromycin, CLD: Clindamycin, VAN: Vancomycin, MSSA: Methicillin Sensitive *S. aureus*.

Table 4: CLSI recommended zone diameter breakpoints for *S. aureus*.

Antimicrobial Agents	Sensitive (mm)	Resistant (mm)
Methicillin(5µg)	≥14	≤9
Oxacillin (1µg)	≥13	≤10
Cefoxitin (30µg)	≥22	≤21
Clindamycin (10µg)	≥21	≤14
Erythromycin (15µg)	≥21	≤14
Vancomycin (30µg)	-	-

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