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Multidrug Resistance Pattern of Staphylococcus Aureus Isolates in Maiduguri Metropolis

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Abstract: Multi drug-resistant (MDR) isolates of Staphylococcus aureus are on rise and are becoming a challenge for timely and appropriate treatment. The present study was carried out with an objective to isolate Staphylococcus aureus from clinical samples and determine their sensitivity. Out of 110 samples collected, 44 were shown to contained S. aureus. The isolates were subjected to antibiotic sensitivity tests using 10 different and commonly used antibiotics by modified Kirby- Bauer disc diffusion technique. Out of the total isolates (42) tested, only 7.1% were susceptible to all the antibiotics. Multiple resistance was eminent in over 92% with highest occurrence in 4.8% where the entire antibiotics were resisted. Multiple antibiotic resistance indixes (MAR index) indicated that 0.6 index occurred most (23.8%) followed by 0.5 (19.0%). On the other hand, 0.1 and 0.8 indexes were the lowest with 0.0% and 1.0% occurrence respectively. Ciprofloxacin was resisted by most of the organisms (64.3%) while amoxicillin (64.3%) and streptomycin (61.9%) were most efficacious. With over 90% isolate having MAR index ≥ 0.2, the multiple drug resistance by the S. aureus is quite alarming and might suggest inappropriate antibiotic usage by the sampled population. Therefore, the need to strategize the nature of antibiotic treatment against S. aureus and massive campaign on indiscriminate antibiotic use is urgent.

Keywords: Multiple; Antibiotics; Resistance; Sensitivity; S. aureus.

1. Introduction

The genus *Staphylococcus* includes over 30 species and subspecies, of which *Staphylococcus aureus* is considered medically the most important species worldwide. *S. aureus* is a Gram-positive, catalase positive, coagulase positive, non-motile coccoid bacterium that causes a variety of human [1] and livestock infections [2]. Despite being normal flora of skin and nasopharynx of most humans, it has been an important etiologic agent of life threatening infections, including bacteremia, septicemia, pneumonia, septic arthritis, endocarditis, osteomyelitis, mastitis, wound infection and a number of toxicoses [3]. *Staphylococcus aureus* is one of the common causes of both endemic and epidemic infections acquired in communities and hospitals which result in substantial morbidity and mortality. The ability of the organism to cause disease is attributed to its invasiveness, toxin production or combined effects of the two [1].

Despite the great achievements recorded in antimicrobial chemotherapy, the emergence of multiple drug resistance in *S. aureus* has been an unavoidable setback. Occurrence of antibiotic resistance in this organism has always been empirical rather than theoretical [4]. Multidrug resistant strains of *S. aureus*, including isolate that are resistant to methicillin, lincosamides, macrolides, aminoglycosides, fluoroquinolones or combination of these antibiotic have been reported [5, 6]. For many years, a number of *S. aureus* isolates have evolved resistance to both synthetic and traditional antimicrobial chemotherapy and their prevalence outside the hospital is of potential epidemiological threat [7]. This trend does not only increase morbidity and mortality but also higher cost of healthcare services [8].

The spread of resistance to antimicrobial agents in *Staphylococcus aureus* is largely due to the acquisition of plasmids and or transposons [9]. Plasmid allows the movement of genetic material including antimicrobial resistance genes between bacterial species and genera. Some of the possible predisposing factors that lead to emergence of multiple resistances are prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness and

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consumption of antibiotics before coming to the hospital [4]. Hence, implementation of an appropriate antibiotic policy would reduce the risk of further development of antimicrobial resistance.

In Nigeria and north east in particular, where the populace is bedeviled with insurgency, there is great tendency for rapid emergence of multi drug resistance due to the fact that many injured individuals spend long time in hospitals with poor or inadequate treatment. In addition to that, health care facilities might be inadequate at point in time and thus increase the chances of person-person transmission. For those that could not have access to health facility, indiscriminate use of antibiotics might be the only option. This called for the need to investigate the resistance pattern of *S. aureus* among patient in Maiduguri metropolis (home to many internally displaced persons) in order to update data on which to base selection of appropriate and affordable antibiotics. Hence this research was undertaken.

2. Materials and Methods

2.1. Sample Collection

Samples for this study were collected from University of Maiduguri Teaching Hospital (UMTH) and State Specialists Hospital (SSH) Maiduguri (coordinates: 11.83° N, 13.15° E), Borno State, Nigeria. A total of one hundred and ten (110) clinical samples consisting of urine, stools and wound swabs was collected between July and November 2014. All the samples collected were handled and transported aseptically to Microbiology Laboratory, University of Maiduguri; under controlled temperature. The samples were processed immediately or stored in a refrigerator at 4°C.

2.2. Bacteriological Analysis

The samples collected were inoculated on Mannitol Salt Agar (MSA) and Blood Agar (BA) and incubated at 37°C for 24 hours to selectively isolate *S. aureus* [10]. Colonies with distinct features of small yellow and hemolytic on MSA and BA respectively were subcultured on Nutrient agar (NA) in order to obtain pure colonies. The pure colonies were stored in NA slants for subsequent characterization.

Isolates were characterized based on the methods employed by Olayinka, *et al.* [5]. Morphological characteristics such as Grams reaction, shape and arrangement of cells were determined by Gram's Staining. Ability to ferment Mannitol, and Catalase and Coagulase production were used to determine the biochemical characteristics of the isolates. The isolates were identified based on colonial, morphological and biochemical properties determined above.

In vitro antimicrobial susceptibility testing

2.3. Antibiotics

The modified Kirby-Bauer disc diffusion method was used for the susceptibility testing. Commercially prepared multidisc (POLY-TEST LABS; ENUGU, NIGERIA) comprising ten different antibiotics were used for the study. These include Amoxicillin (30 mg/ml), Ampiclox (30 mg/ml), Chloramphenicol (30 mg/ml), Ciprofloxacin (10 mg/ml), Erythromycin (30 mg/ml), Gentamicin (10 mg/ml), Levofloxacin (30 mg/ml), Norfloxacin (10 mg/ml), Rifampicin (10 mg/ml) and Streptomycin (20 mg/ml) on a single disc.

2.4. Inoculum Preparation

Each of the pure isolates was grown on NA for 24 hours and some colonies picked and inoculated into 2ml sterile peptone water in sterile test tubes. The tubes were plugged with cotton wools and incubated at 37°C for 5 hours. The turbidity of the broths was adjusted to attain McFarland no. 5 (10^5 cfu/ml) [10].

2.5. Sensitivity Testing

Aliquots (0.1ml) of the bacteria suspension were transferred into a well dried surface of NA plates and tilted to spread evenly over the entire surface of the agar plates. The excess fluids were drained off and dried in incubator for 10 min. Antibiotic discs were then placed on the surface of the inoculated plates, placed in a refrigerator to allow proper diffusion of the antibiotics and incubated aerobically at 37°C for 18 to 24 h. Two different readings were taken in each case including the routine sensitive or resistant and zone of inhibition diameter (ZID) measured with a meter rule. The results were interpreted as sensitive or resistant based on the guidelines in manufacturer's manual.

2.6. Determination of Multiple Antibiotic Resistance (MAR) Index

For each of the isolates, MAR index was evaluated using the following relation [5, 11].

$$\text{MAR index} = \frac{\text{Number of antibiotics resisted}}{\text{number of antibiotics tested}} \times 100$$

2.7. Statistical Analysis

The data generated were analyzed using descriptive statistics and chi square. A statistical package INSTAT 3a was used for the analysis.

3. Results

Microbial culture, characterization and identification revealed that, out of 110 samples collected 42 (38.8%) were harboring *S. aureus.* Majority of the isolates were contained in wound swabs 27 (64.3%), followed by urine 9 (21.4%) and stools 6 (14.3%).

Results from Table 1 shows that all the antibiotics tested were resisted by many of the bacterial isolates. Highest resistance by *S. aureus* was observed in response to ciprofloxacin (CPX) with 64.3% resistance. This was followed by ampiclox (AMP) and chloramphenicol (CHL) with 59.5% and 54.8% resistance. The least antibiotics to be resisted were streptomycin and amoxicillin with 38.1% and 35.7% respectively. Similarly, half of the organisms were shown to be resistant to erythromycin while the other half were sensitive. The sensitivity pattern shows that AMX and STR were more efficacious than norfloxacin (NFX), levofloxacin (LEV) and gentamycin (GEN) as shown in Table 1.

Table-1. Resistance pattern of *S. aureus* to antibiotics

Antibiotic	**Number of *Staphylococcus aureus***			**Percentage of resistant (%)**
	Tested	Sensitive	Resistant	
Amoxicilin AMX	42	27	15	35.7
Ampiclox AMP	42	17	25	59.5
Ciprofloxacin CPX	42	15	27	64.3
Chloranphenicol CHL	42	19	23	54.8
Erythromycin ERM	42	21	21	50.0
Gentamycin GEN	42	22	20	47.6
Levofloxacin LEV	42	23	19	45.2
Norfloxacin NFX	42	25	17	40.5
Rifampicin RFP	42	20	22	52.4
Streptomycin STR	42	26	16	38.1

Figure-1. Occurrence of multiple antibiotic resistance (MAR) index

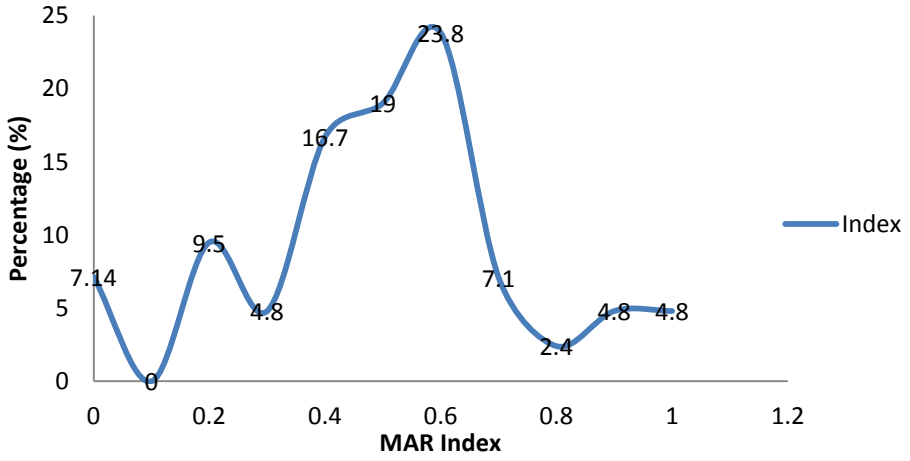

Figure 1 shows the percentage occurrence of multiple antibiotic resistances. Only 7.14% of the isolates had 0.0 resistance index. The remaining isolates had MAR index ranging from 0.1 to 1.0 indicating multiple resistance. Total antibiotic resistance was observed in two isolates with 1.0 MAR index each. However, most species (23.8%) had MAR index of 0.6 followed by 19.0% with 0.5. Statistical analysis showed no significant difference between the antibiotics' activity on the isolates but significant in terms of MAR pattern of the organisms ($p \leq 0.05$).

4. Discussion

In the present study, 38.18% of the samples collected were shown to contain Gram positive cocci in clusters capable of mannitol fermentation, and catalase and coagulase production – some unique features of *S. aureus*; as (or one of) the etiologic agent. This explains the relative versatility of *S. aureus* and its ability to cause diseases in humans. *Staphylococcus aureus* is known to possess a number of virulence factors that contribute to its pathogenicity, thereby infecting different body sites [1]. Most of the *Staphylococcus* species were isolated from wound (64.3%), followed by urine (21.4%) and stool (14.3%) samples. Higher occurrence of the bacteria in wound samples is related to its easy transfer from normal skin surfaces (where it normally thrives) to the injured site where it can cause more damages. In some instances, *S. aureus* could be the sole cause of wound infections due to its invasiveness. This agreed with the findings of Denwe [12] who isolated a substantial number of *S. aureus* from wound samples in Kaduna metropolis, Nigeria.

Results of antibiotic sensitivity showed that most of the antibiotics used were not effective on the bacterial isolates. Only three species (7.14%) were sensitive to all the antibiotics subjected to. This instance presented a clear multiple antibiotic resistance nature of most *S. aureus* isolates. This is in conformity with previous findings that most isolates of *S. aureus* are resistant to large number of commonly prescribed antibiotics [5, 10, 12, 13]. Results from Table 1 showed that amoxicillin and streptomycin were the most efficacious antibiotics. Only 35.7% and 38.1% of the organisms were resistant to these antibiotics respectively as against ciprofloxacin and ampiclox with 64.3% and 59.5% resistance respectively. This might be due to the fact that the sources of the bacteria were engaged in indiscriminate use of ciprofloxacin and ampiclox that led to development of resistance by the organisms. This however, contradicted the findings of Olayinka, *et al.* [5] and Denwe [12] who reported better efficacy of ciprofloxacin as compared to other antibiotics tested. Erythromycin, gentamycin, levofloxacin and norfloxacin were resisted by less than half the total number of the isolates. Resistance of *S. aureus* to aminoglycosides and flouroquinolones has been a subject of discussion in which more findings suggests substantial resistance to the antibiotics [4].

Multiple antibiotic resistance (MAR) index shows that majority of the isolates were resistant to more than two antibiotics. Highest MAR index was observed in 4.8% of the isolates where an MAR index of 1 was recorded. This indicated that the isolates involved do not respond to the effect of all the antibiotics. This could be attributed to possession of multiple resistance genes in the bacterial genome that enable them resist all the antibiotics. This agreed with findings of Kaplan, *et al.* [14] who reported that MAR by *S. aureus* is usually associated with increased expression of multiple antibiotic resistance genes, including those coding for aminoglycoside resistance. Over 50% of the isolates had MAR index ranging between 0.4 and 0.6. This highlighted the fact that, some of the antibiotics are still effective to majority of the isolates despite the MAR. This could be linked to many factors including source of the isolates, its ability to evade antibiotic effects and variation in antibiotic concentration. Many studies have identified bacterial source as an important determinant of MAR especially due to *S. aureus* when it occurred in nosocomial infection. Research findings by Rajaduraipandi, *et al.* [4] showed MAR by clinical isolates of *S. aureus*.

The MAR observed in this study is quite alarming although in accordance with many findings in Nigeria [5, 6] and other parts of the world [10, 15, 16]. Many factors contribute to the emergence of MAR in *S. aureus* among which over prescribing of antibiotics by clinicians, over usage and incomplete course of antibiotics by patients**,** availability of the antibiotics could not be ignored in regions like ours. Environmental and personal hygiene can also contribute to the spread of resistant species among people especially in clinical settings. Mass campaign, regular training, and reformation of drug policies would to greater extent alleviate the increased spread of MAR isolates.

5. Conclusion

This study has shown the occurrence of *S. aureus* as etiologic agent of some infections and its ability to resist most of the commonly used antibiotics. The rate of MAR by the isolates was significant considering the fact that over 80% of the isolates had MAR index greater than 0.2. Some of the antibiotics however, were relatively efficacious especially the amoxicillin and streptomycin. Therefore, there is need for an urgent review of treatment to *S. aureus* infections and public enlightenment against indiscriminate antibiotic usage.

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