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Research Article

Susceptibility Profile and Multiple Antibiotics Resistance Indexes (MARI) Of Clinical Isolates of *Staphylococcus aureus* from Urinary Tract Infections (UTIs)

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Abstract

Background: Urinary Tract Infections (UTIs) affects the urinary tract systems of humans, causing some complications due to microbial infection including those caused by members of the *Enterobacteriaceae* family, Gram positive bacteria and other Gram negative bacteria. A proper susceptibility testing of isolates from UTI patients in Abakaliki, Ebonyi State, Nigeria will help to assuage the poor prognosis of UTI patients as a result of wrong therapy or ineffective therapy due to the presence of drug resistant bacteria. The incidence of multiple antibiotic resistant bacteria especially pathogenic *Staphylococcus aureus* in Urinary Tract Infection (UTI) is alarming mostly in isolates from clinical specimens. This study evaluated the susceptibility profile and Multiple Antibiotics Resistant Index (MARI) of clinical isolates of *Staphylococcus aureus* from UTI patients in a University Teaching Hospital in Abakaliki, Nigeria.

Materials and Methods: A total of 39 non-duplicate clinical isolates of pathogenic *Staphylococcus aureus* was collected from the culture collection unit of the Microbiology department of the University Teaching Hospital. All isolates were re-characterized and subjected to Antimicrobial Susceptibility (AST) suing the modified Kirby-Bauer disk diffusion test on Mueller-Hinton agar plates. The multidrug resistance nature of the *S. aureus* isolates was determined using Multiple Antibiotics Resistance Index (MARI) protocol.

Results: The pathogenic isolates of *S. aureus* investigated in this study for susceptibility to varying antibiotics showed appreciable levels of resistance and susceptibility to the tested antibiotics. Most interestingly, all the *S. aureus* isolates were found to be completely resistant to cloxacillin (100%) - which is a first-line antibiotic used to treat and manage infections caused by *S. aureus*. More so, the *S. aureus* isolates showed reduced susceptibility to bacitracin (92.31%), oxacillin (89.74%), clindamycin (87.18%) and mupirocin (82.05%) – which are also first-line and second-line antibiotics used to treat infections caused by species of *Staphylococcus*. The resistant *S. aureus* isolates were multiply resistant to the tested antibiotics showing MARI in the range of 0.2-1.0, thus implying that the *S. aureus* isolates were multiply resistant to at least 2 or more of the tested antibiotics.

Conclusion: Our study confirms with preliminary data that pathogenic *S. aureus* from UTI patients are multidrug resistant in nature, and may contain some resistance genes or factors that allow them to diminish the efficacy of antimicrobial agents directed towards them *in vivo*. There is therefore need to take more proactive measures in the detection, surveillance and reporting of multidrug resistant *S. aureus* isolates from UTI and other clinical samples in order to forestall any disease outbreak due to such resistant strains.

Keywords: *Staphylococcus aureus*; UTI; Pathogens; Antimicrobial Resistance; Nigeria

Introduction

When antibiotics resistance occurs, there is usually difficulty in treating some common microbial infections including Urinary Tract Infections (UTIs) caused by pathogenic *Staphylococcus aureus*. This can result in several complications and challenges including poor prognosis of the affected patients, delays in providing effective treatment or, in worst case scenario, inability to provide treatment by any means. Infections including UTIs caused by *Staphylococcus aureus* are becoming more difficult to treat because of increasing resistance to antibiotics [1]. One of the resistance/adaptation strategies of *S. aureus* like other bacteria is the formation of an organized community of aggregated cells, embedded in a hydrated matrix of Extracellular Polymeric Substances (EPS) called biofilms-which allows bacteria to survive in hostile environment including those orchestrated by

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antimicrobial onslaught [2]. For example, populations of *S. aureus* within biofilms exhibit differences in the expression of surface molecules, antibiotic resistance, nutrient utilization and virulence factors [3,4]. Antibiotic resistant *S. aureus* can cause various clinical conditions from superficial infections to serious life-threatening infections [5,6]. Some strains of *S. aureus* are methicillin-resistant (methicillin-resistant *S. aureus*, MRSA) and can easily be spread from patient to patient through the hands of hospital staff, thus causing nosocomial infections [7,8]. Hand hygiene therefore is critical to the prevention of such hospital-acquired infections including those caused by antibiotic resistant *S. aureus* and MRSA strains. And when such important infection control strategy (such as hand washing) is supplemented with proper antimicrobial susceptibility studies prior to antimicrobial therapy, the patient's prognosis tends to be good and curable. The importance of *S. aureus* as a persistent nosocomial and community acquired pathogen has become a global health concern-owing to the rate at which some strains of *Staphylococcus* are fast becoming resistant to some commonly used and available antibiotics. *S. aureus* has a remarkable capability of evolving different mechanisms of resistance to most antimicrobial agents [9,10,11]. This warrants the need for continuous profiling of their susceptibility patterns in order to effectively guide therapy and ensure better patients prognosis in the hospital environment. Resistance in microbial pathogens is an ever increasing global health and economic danger that has been brewing for so many years without appropriate detection, surveillance and reporting; and if nothing is done to assuage this health problem, some common and life threatening infections such as UTI may become difficult to treat. This study investigated the susceptibility profiles of some *Staphylococcus aureus* clinical isolates from UTI patients in a University Teaching Hospital in Abakaliki, Nigeria-as a panacea to raising awareness on the brewing problem of antibiotic resistance in this part of the world.

Materials and Methods

Bacterial Isolates

The bacterial isolates used in this study were non-duplicate clinical isolates of pathogenic *Staphylococcus aureus* (n=39) isolated from urine samples of UTI patients seeking medical care at a University Teaching Hospital in Abakaliki, Southeast Nigeria. The clinical isolates were recovered from the culture collection unit of the Microbiology Laboratory Unit of the University Teaching Hospital after obtaining ethical approval. In accordance with the Nigerian legislation and World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects and identifiable human material or data [12], written informed patient consent was not required for the use of the collected clinical isolates bacteriologically analyzed in this study.

Re-characterization of isolates

All the clinical isolates of *S. aureus* (n=39) analyzed in this study were re-characterized using standard microbiology techniques including Gram staining, culture, biochemical testing's and microscopy to re-authenticate and re-confirm the taxonomy of the isolates.

Antibiotics Susceptibility Testing (AST)

Antimicrobial susceptibility testing was attempted using some selected antibiotics used clinically for the treatment and management of infections caused by pathogenic *S. aureus*. The modified Kirby-Bauer disk diffusion technique was used for AST. AST was carried out using Mueller-Hinton agar plates (Oxoid, UK), and in conformity to the guidelines of Clinical Laboratory Standard Institute [13]. Single antibiotic disks (Oxoid, UK) comprising: clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), cefoxitin (30 µg), oxacillin (1 µg), bacitracin (10 µg), mupirocin (5 µg) and cloxacillin (30 µg) were used to determine the susceptibility patterns of the test *S. aureus* isolates. The ensuing Inhibition Zone Diameters (IZDs) from the respective *S. aureus* isolates was measured and interpreted as either susceptible, intermediate or resistant using standard antibiotic breakpoints as outlined by CLSI [13,14].

Determination of Multiple Antibiotic Resistance Index (MARI)

Multiple Antibiotic Resistance Index (MARI) was determined for each resistant *S. aureus* isolate to determine their respective levels of multiple antibiotic resistance nature. The multiple antibiotic resistance nature of the *S. aureus* isolates was determined by a mathematical formula: MARI = a/b, where 'a' represents the number of antibiotics to which the test *S. aureus* isolate showed resistance and 'b' represents the total number of antibiotics to which the test *S. aureus* isolate has been evaluated for [14].

Results

This present study evaluated the antimicrobial susceptibility profiles of pathogenic *Staphylococcus aureus* isolates (n=39) from the urine samples of Urinary Tract Infection (UTI) patients who received medical care and treatment at University Teaching Hospital in Abakaliki, southeast Nigeria. Table 1 shows the susceptibility and resistance profile of the *S. aureus* isolates tested in this study. Our study shows that the pathogenic isolates of *S. aureus* analyzed in this study were completely resistant to cloxacillin (100%), a first-line antibiotic used for the treatment of infections caused by *Staphylococcus* species. The *S. aureus* isolates also showed significant reduced susceptibility to bacitracin, oxacillin, mupirocin and cefoxitin-which are all used as either front-line or last-line antibiotic to treat and manage infections caused by Gram negative bacteria (Table 1).

The result of the multiple antibiotic resistance indexes of the *S. aureus* isolates showed varying levels of multidrug resistance profiles. While a handful of the *S. aureus* isolates showed multidrug resistance to all the antibiotics tested, others were resistant to at least 3 of the total antibiotics tested. Interestingly, only 18 isolates of the *S. aureus* were found to show multidrug resistance towards the tested

Table 1: Antimicrobial susceptibility profile of *S. aureus*.

Antibiotics (µg)	Resistance n(%)	Intermediate n(%)	Susceptible n(%)
Cefoxitin (30)	25(64.10)	0(0.00)	14(35.90)
Bacitracin (10)	36(92.31)	0(0.00)	3(7.69)
Erythromycin (15)	21(53.84)	14(35.90)	4(10.26)
Oxacillin (1)	35(89.74)	3(7.69)	1(2.57)
Clindamycin (2)	34(87.18)	2(5.13)	3(7.69)
Gentamicin (10)	15(38.46)	1(2.57)	23(58.97)
Mupirocin (5)	32(82.05)	7(17.95)	0(0.00)
Cloxacillin (5)	39(100.00)	0(0.00)	0(0.00)

Table 2: Multiple antibiotic resistance indexes of the *S. aureus* isolates.

Isolate No.	MARI	Antivbiotics
S3	0.8	FOX, B, E, OX, DA, MUP and OB
S4	0.7	FOX, B, OX, DA, CN, MUP and OB
S5	0.4	FOX, B, E, OX, DA, CN, MUP and OB
S6	1	B, E, OX, DA, CN, MUP and OB
S7	0.7	FOX, B, OX, DA, MUP and OB
S8	0.7	FOX, E, OX, DA, MUP and OB
S9	0.6	FOX, B, E, OX, DA, MUP and OB
S10	0.5	B, E, OX, DA, CN, MUP and OB
S11	0.7	FOX, B, OX, DA, MUP and OB
S12	0.5	FOX, B, E, OX, DA, MUP and OB
S24	0.8	FOX, B, OX, DA, CN, MUP and OB
S25	0.7	FOX, OX, DA, CN, MUP and OB
S26	0.8	FOX, B, E, OX, DA,MUP and OB
S30	0.7	FOX, B, OX, DA, MUP and OB
S33	0.3	FOX, B, E, OX, DA, MUP and OB
S34	0.4	FOX, B, OX, DA, MUP and OB
S36	0.7	FOX, B, E, OX, DA, MUP and OB
S39	1	FOX, B, E, OX, DA, CN, MUP and OB

Keywords: FOX=Cefoxitin; B=Bacitracin; E=Erythromycin; OX=Oxacillin; DA=Clindamycin; CN=Gentamicin; MUP=Mupirocin; OB=Cloxacillin.

antibiotics (Table 2). The remaining 21 isolates of *S. aureus* were not multidrug resistant in nature as mathematically determined by the MARI model used in this study.

Discussion

Staphylococcus aureus is an important member of the normal skin flora and other sites of the body of humans. They are also abundantly found in the nose and mouth of animals and humans. Pathogenic *S. aureus* are important bacterial pathogens that have the capacity to cause a range of infections and diseases in humans including skin infections, bacteremia and pneumonia. Infections caused by *S. aureus* are becoming more difficult to treat because of increasing resistance to some available first-line, second-line and even last-line antibiotics [1]. Thus, this study investigated the antimicrobial susceptibility profile and multiple antibiotics resistance index of 39 non-duplicate isolates of *S. aureus* from UTI patients in order to update on their susceptibility profiles and create awareness on the need for hospital laboratories to always be on the lookout for multidrug resistant bacteria in their routine practice. The *S. aureus* isolates showed varying rates of resistance and susceptibility to the antibiotics tested. Interestingly, the *S. aureus* isolates were more resistant to cloxacillin (100%), bacitracin (92.31%), oxacillin (89.74%), clindamycin (87.18) and mupirocin (82.05). Meanwhile, most of the isolates were susceptible to gentamicin (58.97%). The likely reasons for the growing levels of resistance in *S. aureus* as observable in this study may be attributable to blind treatment, irrational antibiotic usage and other resistance factors in the organism. As previously reported and in conformity to this study, researchers elsewhere also showed that clinical isolates of *S. aureus* are fast becoming resistant to some commonly available antibiotics [9,15,16]. In two separate studies conducted elsewhere, the increasing resistance of clinical isolates of *S. aureus* to some commonly used antibiotics particularly the beta lactam agents including clindamycin, oxacillin and methicillin as observed in our studies was reported amongst patients admitted in the hospital [2,17]. The problem of antibiotic resistance and multidrug resistance in species of *Staphylococcus* is mainly attributed to some combination of resistance (genetic) factors or genes that could be acquired vertically or horizontally. Among these resistance genes notable in pathogenic *S. aureus* is the *mecA* gene-which mediates methicillin resistance in species of *Staphylococci*. The presence of *mecA* gene [which encodes for Penicillin-Binding Protein (PBP), PBP-2a] and other genetic resistance factors in species of *Staphylococcus* gives the organism the undue advantage of being resistance to an array of antibiotics [14]. This phenomenon is bad for therapeutic measures directed at bacterial related infections caused by species of *Staphylococcus*. In this study, we evaluated the multidrug resistance nature of the pathogenic *S. aureus* used in this study-to try to find out if they show multidrug resistance to some first-line, second-line and last-line antibiotics used clinically for the treatment and management of infections caused by pathogenic *S. aureus*. The *S. aureus* isolates were found to be multidrug resistant in nature-as calculated from the Multiple Antibiotics Resistance Index (MARI) model. Out of the 39 clinical isolates of *S. aureus* analyzed in this study, only 18 isolates were found to be multiply resistant in nature to at least two or more of the tested antibiotics. This portends a serious health risk to the treatment and management of infections (e.g., UTI e.t.c) caused by pathogenic species of *Staphylococcus* -especially in this part of the world where Antimicrobial Susceptibility Testing (AST) in hospital laboratories does not go beyond the routine susceptibility studies to lookout of for other resistance phenotypes or genotypes. It has been reported that multiple antimicrobial resistant *S. aureus* are more common in clinical samples which arise as a result of antibiotics misuse and other genetic or environmental factors [14,16]. When proper AST is not carried out in the clinical and/or hospital laboratory, it will result in poor prognosis of the patients because antimicrobial therapy will not be properly guided to effectively tackle and eradicate the resistant microbe responsible for the disease condition of the individual.

Conclusions

In conclusion, our study has shown with some preliminary epidemiological data that there is occurrence of multidrug resistant *Staphylococcus aureus* from UTI patients. Since Multidrug Resistant (MDR) bacterial infections are usually difficult to treat, it is therefore important that hospital laboratories in Nigeria modify their routine AST to properly detect and report any case of MDR so that therapy can be properly guided in order to ensure good prognosis of the affected patients.

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