The Contribution of Curable Plasmid-Mediated Resistance in Isolates of Staphylococcus aureus at the University of Benin Teaching Hospital, Benin City, Nigeria

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Abstract

*S. aureus,* an important human pathogen and commensal that is responsible for infections ranging from minor to deep-seated life-threatening conditions. Multi-drug resistant *S. aureus* or MRSA is a major cause of hospital acquired infection (HAIs) or nosocomial infections with consequential reduction in treatment options and overtly increased cost of healthcare, morbidity and mortality. The study was conceived to determine the contribution of curable transmissible plasmids to the ever-increasing proportion of multi-drug resistant *S. aureus* at the University of Benin Teaching Hospital, Benin City. A total of 448 consecutive multi-drug resistant clinical isolates of *S. aureus* were collected, confirmed by SCT and TCT and resistance to commonly used antimicrobial agents. Each isolate was inoculated into Mueller-Hinton Broth containing 100 μg/mL acridine orange and incubated at 37°C for 24 h. Each broth culture was subsequently sub-cultured onto blood agar plates and incubated at 37°C for 24 h. Sensitivity tests were thereafter done on each sub-culture by the Kirby-Bauer disc diffusion method. SCT and TCT were re-tested on each sub-culture. Isolates with curable transmissible plasmids were 31/448 (6.9%) and there was complete reversion to sensitivity in all the cured strains to antimicrobial agents tested including ampicillin. The remaining isolates (93.1%) retained their resistance to all the antimicrobial agents. The isolates with curable plasmids (6.9%) also lost the coagulase activity of both types. Plasmid-mediated resistance in *S. aureus* remain an important route of multi-drug resistance, however this is thwarted by chromosomally-mediated resistance as the major mechanism of resistance in multi-drug resistance *S. aureus*. Additionally, the cure of drug resistance was also concomitantly associated with loss of the pathogenicity factor-coagulase in these isolates.

Keywords: *S. aureus*, multi-drug resistance, transmissible plasmids.

INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is a major human pathogen and commensal that is responsible for infections that range from minor to deep-seated life-threatening conditions 1-3. *S. aureus* colonizes between 30% and 50% of humans which increases the tendency to initiate infection 4. After the introduction of penicillin into clinical practice in the 1940s, the first case of methicillin-resistant strains of *S. aureus* (MRSA) was reported 5. There has been a steady increase in the number of methicillin-resistant strains of *S. aureus* worldwide due to antimicrobial agents’ selective pressure 6. Rates that range from 61.8 – 80% have been reported in Nigeria 7 and 61% in the United States 8. Resistance to commonly prescribed antimicrobial agents has been recognized as a contributory to morbidity and mortality in hospitalized patients 9-11. MRSA strains are some of the commonest microorganisms associated with hospital-acquired infection (HAIs), possessing improved capabilities for resistance to methicillin and other antimicrobial agents have been reported 12. Resistance of *S. aureus* has been largely attributed to chromosomal and extra-chromosomal genes acquisition 13. Infection with MRSA strains comes with increased costs, treatment failure and longer hospital bed-time 14-15. This study was conceived to determine the relative contribution of curable transmissible plasmids to the resistance of *S. aureus* isolates in Benin City.

MATERIALS AND METHODS

Consecutive isolates of *S. aureus* that were resistant to gentamycin, amoxycllin-davulanate, ciprofloxacin, sparfloxac in ceftazidime, ceftriaxone azithromycin, and ampicillin, were collected. Each isolate coagulase activity was confirmed by the SCT and TCT and resistance to the antimicrobial agents.

Slide Coagulase Test (SCT)

Emulsion of each isolate was done on two separate spots on clean grease-free slide with a loopful of normal saline. To one emulsion was added a loopful of citrated human plasma and mixed with the loop and to the other spot was added a loopful of normal saline. The slide was rock-mixed in a figure "8" fashion. The presence of clumping or granulation was
Tube Coagulase (TCT)

Each isolate that was SCT test negative was further tested by the TCT as well the SCT positive as confirmation of the SCT. Each isolate was inoculated into the bijou bottle containing 1.0 ml of sterile nutrient both of which 0.2 ml of citrated plasma was added. A local coagulase-positive strain of S. aureus was treated in a similar manner as control. The preparations were incubated at 37°C and examined for the presence of a coagulum in 3 h, 6 h, if no coagulum developed, the bottles were re-incubated and examined after 18 h using the control test to validate the result.

Sensitivity Test

A standard inoculum was prepared from each isolate adhering to the Clinical Laboratory Standard Institute guidelines (2021) to obtain 10^6 organisms/mL. A plate of Mueller Hinton agar (Oxoid CM 0337) previously dried at 37°C for 30 minutes was inoculated with the prepared inoculum using a sterile swab stick. Oxford strain of S. aureus (NCTC6571) was treated in the same manner as control. Antimicrobial agent discs were placed with a sterile forcep at a minimum distance of 25 mm apart. The plates were thereafter incubated at 37°C for 18 h. The sensitivity tests were read with reference to the control strain as sensitive or resistant.

Plasmid Curing

Each isolate was inoculated into a bijou bottle containing 2 ml Mueller-Horton broth (Oxoid CM 0405B) containing 100 µg/mL acridine orange and incubated at 37°C for 18 h. This was subsequently sub-cultured onto a blood agar plates (Oxoid CM 55) and incubated at 37°C for 18 h. Sensitivity tests were carried out on each sub-cultured growth with the same antimicrobial agent discs prior the curing process. The Oxford strain of S. aureus was also used as a sensitivity test control organism. The results were recorded as sensitive or resistant.

RESULTS

Isolates with curable plasmids were 31/448 (6.9%) from the curing process as presented in Table 1. All the isolates with curable resistance plasmid genes showed complete reversibility to sensitivity with all the antimicrobial agents tested including ampicillin. The cure of resistance plasmids in these multi-drug resistant S. aureus isolates also resulted in the loss of the coagulase activity of both types. All the other isolates that were not cured of resistance genes (93.1%) during the process remained resistant to all the antimicrobial agents and tested positive by both SCT and TCT.

Table 1: Tests on isolates after growth in the presence of 100 µg/mL acridine orange

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of Cases (%)</th>
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<tbody>
<tr>
<td>Slide coagulase</td>
<td>448(100%)</td>
</tr>
<tr>
<td>Tube coagulase</td>
<td>448(100%)</td>
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<tr>
<td>Sensitivity to Ampicillin (10 µg)</td>
<td>31(6.9%)</td>
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DISCUSSION

The study revealed that 6.9% of S. aureus isolates possess curable or transmissible plasmids that mediate resistance to antimicrobial agents in this study population. This, in turn, suggests that the resistance of S. aureus to antimicrobial agents is to a large extent chromosomally mediated and less to extra-chromosomal or plasmid genes. Chromosomal resistance often results from mechanisms that involve target site alteration to antimicrobial agents known to bind penicillin-binding proteins (PBP’s) such as the beta-lactams or through the use of specialized exporter proteins (efflux pumps) to pump out harmful substances from the cell interior. This has also been observed in many bacteria populations. However, this study further shows that the acquisition resistance in S. aureus strains through infection with a virus (bacteriophage) or through extra-chromosomal genetic elements such as transposons and integrons in bacterial populations makes less degree of contribution to resistance in S. aureus strains as other studies have similarly observed. S. aureus resistance to antimicrobial agents as well as other bacterial species appears to be an evolutionary demand for the survival of living systems in an ever-changing environmental conditions which is inevitable and unavoidable consequence of the continuous use of antimicrobial agents. The contribution of curable transmissible plasmids to the ever-rising tide of MRSA in hospitals is disproportionately low in comparison to the chromosomally-mediated mechanism of resistance. The loss of resistance after the curing process also resulted in the loss of coagulase activity which may suggest that the pathogenicity factor (coagulase) is closely linked to the resistance gene. Transmissible plasmids contribute much less to antimicrobial resistance in strains of S. aureus than chromosomally-mediated resistance. Resistance genes in these isolates appear to be co-inherited genes with the pathogenicity factor coagulase. It is therefore, expedient to redouble efforts to protect reserved valuable antimicrobial agents against unnecessary exposure. A renewed and sustained search for new antimicrobial agents must be given the priority it demands in attempts to win the battle against bacterial infections and curtail the development of resistance.

REFERENCES


