Antibiotic Resistance Profiles of *Staphylococcus aureus* Isolated from Fomites in Community Schools within Abeokuta Environs Leading to Detection of MRSA.

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**Abstract**

MRSA is known to show resistance to β-lactam class of antibiotics and is among the highest superbugs posing dangerous threats to humans which cause serious infectious diseases, with extended resistance to other antibiotics making treatment ineffective. An additional challenging feature of MRSA bacteria is its rapid dissemination to human and animal through different fomites. This study aimed at determining the prevalence and antimicrobial susceptibility pattern of MRSA isolated from toilets, offices and classroom door handles in community schools within Abeokuta; which might serve as a reservoir for dissemination to the general population of students and staffs within the school community. Fifteen community schools in Abeokuta environs were picked for sampling, in which 300 sample swabs were taken from the toilet; office and classroom door handles using sterile swabs stick moistened with buffered peptone water. Growth and Isolation of S. aureus were carried out using Mannitol salt agar, while antimicrobial susceptibility test was performed on the isolates to determine resistance profile to available antibiotics used while Multiple Antibiotic Resistance index (MARI) of MRSA was determined in addition to their haemolytic pattern on blood agar.

The occurrence rate of Staphylococcus spp isolated from fomites is 50.60 %, while the prevalence rate of MRSA is 2.99 % as determined by antimicrobial susceptibility test. High Multiple Antibiotics Resistance index greater than 0.4 was observed in all MRSA isolates while 3 out of nine isolates showed partial α-haemolytic reaction representing 40 % and the remaining 6 representing 60 % showed γ- haemolytic reaction. The detection of MRSA in this study emphasizes the need to set up surveillance programs to monitor the spread of MRSA and formulate preventive measures to prevent the spread of MRSA amongst students and staffs hence curbing its dissemination.
Keywords: MRSA, Community Schools, Antimicrobial susceptibility, fomites, Public Health, MARI

1. BACKGROUND OF THE STUDY

*Staphylococcus aureus* is a bacterium which is commonly found as commensals on part of the human body of healthy individuals (Plata et al., 2009). Methicillin-Resistant *Staphylococcus aureus*- with an acronym MRSA is *S. aureus* that is resistance to the beta (β)-lactam class of antibiotics. According to the Centres for Disease Control and Prevention (CDC, 2013), MRSA is among the among the highest superbugs posing dangerous threats to humans of antibiotic resistant pathogens; although MRSA causes serious infectious diseases, many antibiotics remain resistance to it hence making treatment ineffective.

As the numbers of severe invasive MRSA infections acquired in health-care settings continue to decline, the numbers of such infections caused by pathogenic bacteria of medical importance among the general public continue to rise and are transmitted through different contacts (fomites) within our community i.e. schools (Dirk, 2006; Akinrotoyé et al., 2018). Historically, *S. aureus* rapidly developed resistance to antibiotics as they were brought successively into clinical use, and doctors came to recognize resistance to each such new drug within months to years of its introduction. The first penicillin resistant *S. aureus* pathogens emerged by 1942 after its introduction in 1940, *Staphylococcus aureus* remains resistant to that original type of penicillin and to other members of this class of antibiotics till date (Otto, 2012).

*S. aureus* becomes MRSA after acquiring genes that encode resistance to the broader class of β-lactam antibiotics. The specific gene taken up by *S. aureus* to make it resistant to Methicillin is called *mecA*. This gene is carried together with a larger set of genes, known as *SCCmec* (Staphylococcal cassette chromosome), that is mobile and capable of inserting into the DNA of other *S. aureus* bacteria. MRSA strains can also carry resistance to antibiotics other than members of the β-lactam class. This multidrug resistance makes the infections caused by these *S. aureus* strains very difficult to treat. In addition to producing toxins and virulence factors, MRSA bacteria can generate biofilms, sticky layers and clumps of cells that enable bacteria within them to adhere to medical devices such as catheters and implants.
A biofilm protects the bacteria within it from the actions of antibiotics and of the human immune system (Otto, 2012). The protective strategies used by MRSA strains to evade the host immune system in combination with multidrug resistance all contribute to the virulence of MRSA. Patients with these infections can have high morbidity and mortality rates, and caring for them contributes to high health-care costs for these patients (due to treatment difficulties and longer hospital stays).

An additional challenging feature of MRSA bacteria is their hardiness. They can survive for prolonged periods on surfaces and objects such as sinks, toilets, door handles, floors, medical devices, bed linens, cleaning equipment, and clothing. Therefore, extensive contact control and disinfection procedures are needed to limit the spread of MRSA in health-care and other community settings e.g. community school, stadia, markets, Malls etc.

MRSA can be divided into 3 categories:

• Healthcare-associated (HA)-MRSA bacteria contracted in health-care settings
• Community-associated (CA)-MRSA bacteria contracted outside of health-care-related environments (more common in close-knit communities) e.g. public schools
• Livestock-associated (LA)-MRSA – MRSA in a livestock (most commonly pigs) reservoir transmitted from animals to infect people working with or in close contact with those animals. Animals can acquire both HA-MRSA and CA-MRSA strains (Smith et al., 2013).

This study aims to determine and know if MRSA is present and detected from swabs sample taken from fomites in the community schools within Abeokuta environs, occurrence and prevalence rate of MRSA in all of the randomly selected community schools. Probable occurrence of MRSA in various centre of learning within Abeokuta may pose a threat to the health of students and staffs in the community school, which might serve as a possible reservoir of MRSA (point of contact) to the general population within the community.

2. MATERIALS AND METHODS

Study site: Study site was Abeokuta environs. Abeokuta has three LGAs namely; Odeda Local Government Area, Abeokuta South Local Government and Abeokuta North Local Government Area. Arc GIS 9.3 software was used to display the map of the study areas and study site. GPS was used to georeference the study sites namely Abeokuta South LGA, Abeokuta North LGA and Odeda LGA, Arc GIS 9.3 software was used to display the map of the study sites using their GPS coordinates.
Plate I: Geographical coordinates of the study site collected through Arc GIS 9.3 software.

2.1 Study population: The population of interest included selected community/public secondary schools in Abeokuta, Nigeria. The Principal of the schools gave consent of their willingness to participate in the study.
Sample Size: \( (n) = \frac{Z^2 \times (p) \times (1-p)}{c^2} \) (Maccallum et al., 2001)

- \( Z = 1.96 \) (95%)
- \( p \) = Occurrence rate of 20.23% (0.2023) of MRSA among environmental samples from previous studies
- \( c \) = Confidence interval, 0.05. The minimum number of 296 environmental samples was calculated.

**Cross-sectional study design:** This was prepared by randomization in which the community/public schools of interest was picked randomly and sampled across Abeokuta environs.

**2.2. Approval from Principals**

The schools contacted was selected from a list of community/public schools in Abeokuta environs, Ogun State; Nigeria. The Principals of the various community schools were brief and were asked for voluntary participation; principals of the various community schools gave approval by signing a consent form given to them.

**2.3 Collection of sample swabs from fomites**

300 swab samples were taken from various door handles (fomites) such as class rooms, offices, staff toilets, student toilets in selected public community schools in Abeokuta, Nigeria. Fomite was swabbed with a swab stick moistened with buffered peptone water and kept at room temperature by placing them in an ice-pack cooler. The swab samples were labelled to aid identification and then transported to the Laboratory for further examination and culture.

**2.4 Culture and examination of sample swabs**

The sample swabs was shaken vigorously so as to displace the microorganisms into the buffered peptone water solution, after which it was plated into Mannitol salt agar media and plates incubated at 35°C for 24 h. The high concentration of sodium chloride in selective media of Mannitol salt agar does not allow the growth of any other bacteria species on the agar media except that of *Staphylococcus species*, which is indicated by a change in the colour of red-phenol to a golden or yellowish coloration (Collee et al., 1996). It was later sub-cultured on blood agar to determine their haemolysis parameter.
2.4.1 Biochemical Characterization of bacterial isolates

Bacteria isolated from the various fomites were morphologically examined to determine the shape, size, elevation, surface, margin, colour, odour, and pigmentation of the various colonies on the agar plate; Biochemical tests including catalase, coagulase, oxidase, citrate utilization, Voges-Proskauer, and methyl-red were also carried out on the isolates (Fawole and Oso, 1998; Cheesbrough, 2006) for further identification and compared with Bergeys Manual of Determinative Bacteriology. Coagulase test and Novobiocin was used to differentiate between *Staphylococcus aureus* and *S. saprophyticus*.

2.5 Testing for antimicrobial susceptibility pattern

Antibiotics acquired for the antimicrobial susceptibility test for the *S. aureus* include gentamicin (10 μg), Trimethoprim + sulfamethoxadole (30 μg), ofloxacin (5 μg), pefloxacin (10 μg), cotrimoxazole (25 μg), chloramphenicol (5 μg), ciprofloxacin (5 μg), erythromycin (5 μg), amoxycillin (10 μg), oxacillin (5 μg). 10 μL of 0.5 McFarland standards (10⁶ CFU/ml) of *Staphylococcus aureus* were inoculated and plated on Mueller Hinton agar plates after which the antibiotics was placed at different region of the agar plate with the aid of a forceps and incubated at 37º C for 24 h.

The agar plate was then observed for clearer zone around the disc, which is then determined with rule-meter calibrated in mm. Oxacillin disc (5 μg) was improvised and used to test for oxacillin resistance in *Staphylococcus aureus* isolated from various fomites, a diameter of zone ≤ 10 mm will be regarded as Methicillin resistance (CLSI, 2011; Deresinski, 2005) and later confirmed according to the National Committee for Clinical Laboratory Standard.

2.5.1 Multiple Antibiotic Resistance (MAR) Index Determination

The Multiple Antibiotic Resistances (MAR) Index was calculated for each isolate as shown in the equation below. MAR index is the number of antibiotic(s) to which the organism is resistant divided by the total number of antibiotics tested (Akinjogunla and Enabulele, 2010)

\[
\text{MARI} = \frac{\text{Number of Antibiotic (s) to which isolate was resistant}}{\text{Total number of antibiotics tested}}
\]

2.5.2 Pilot Study/ Feasibility Study

A control reference Methicillin Resistant *Staphylococcus aureus* (ATCC 33591) was acquired to conduct the pilot study which was used as the control for all other parameters.
2.6 Statistical Analysis

Data collected and gathered were analysed using Microsoft Excel (Windows 10).

2.7 Ethical approval

Ethical clearance for the study was obtained from both State Ministry for Health & Education; Ogun State and was cleared by the Ethical Review Committee from College of Biosciences (COLBIOS), FUNAAB.

3. RESULTS AND DISCUSSION

3.1 Sample Collection

Figure 1 shows a total of 300 swabs collected from fomites in randomly selected secondary schools in the three Abeokuta Local government areas which were distributed as follows: Abeokuta South LGA- office doors (27), classroom doors (33), toilet doors (23), toilet water closet handle (17), Abeokuta North LGA- office doors (27), classroom doors (39), Toilet doors (21), Toilet WC handle (15); Odeda LGA- office doors (28), classroom doors (35), Toilet (21), Toilet WC handle (16). Highest number of swabs was obtained from the classroom doors and lowest number of swabs from the toilet doors respectively from all the local government areas.
3.2 Prevalence of *Staphylococci* organisms

A total of 300 sample swabs collected from fomites in different community secondary schools were microbiologically evaluated and processed. 83 *Staphylococcal* organisms were isolated from these fomites comprising 3 species namely, *Staphylococcus aureus* (50.60 %), *Staphylococcus saprophyticus* (20.48 %) and *Micrococcus spp* (28.92 %).

Plate II: Growth of *Staphylococcus aureus* on Mannitol Salt agar
3.2.1  Antibiotic susceptibility test for S. aureus isolates from fomites

Antibiotic susceptibility test were carried out on all the 42 S. aureus isolated from the fomites which show the proportion of S.aureus isolates which are considered to be susceptible, intermediate or resistant to the various antibiotics as shown in Fig 3. All S. aureus tested were highly resistant to ampicillin (100 %), Streptomycin (100 %), Amoxicillin (100 %), but showed various level of resistance to Trimethoprim/Sulfamethoxazole (77.78 %), Cotrimoxazole (66.67 %), Erythromycin (33.33 %), oxacillin (21.43 %) and Chloramphenicol (44.44 %).
Plate III: Multi drug resistance in *S.aureus* isolated from fomites within school in Abeokuta

Figure 3: Antimicrobial susceptibility profile of *Staphylococcus aureus* isolates from fomites in selected secondary schools in Abeokuta Environs

### 3.2.2 MAR Index of MRSA isolate

Table 1 shows the Multiple Antibiotic Resistance (MAR) index of the nine (9) MRSA strains in which second isolate has the maximum MAR index of 0.8, followed by the third, fifth and ninth isolates with MAR index of 0.7. The sixth and seventh isolates have the lowest MAR index of 0.44 and 0.55 respectively.
Table 1: MAR Index of MRSA isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Resistance pattern</th>
<th>MAR Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GEN, COT, AMX, TSX, OXL, EMX</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>GEN, COT, OFL, AMX, CPX, OXL, TSX, PFX</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>GEN, COT, AMX, TSX, CMP, EMX, OXL</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>GEN, AMX, PFX, CMP, CPX, OXL</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>GEN, OFL, AMX, TSX, OXL, CMP</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>GEN, COT, AMX, OXL</td>
<td>0.44</td>
</tr>
<tr>
<td>7</td>
<td>COT, AMX, EMX, TSX, OXL</td>
<td>0.55</td>
</tr>
<tr>
<td>8</td>
<td>AMX, TSX, CMP, OFL, OXL, PFX</td>
<td>0.67</td>
</tr>
<tr>
<td>9</td>
<td>AMX, EMX, TSX, OFL, COT, OXL</td>
<td>0.67</td>
</tr>
</tbody>
</table>

KEY: GEN = GENTAMYCIN, COT = COTRIMOXAZOLE, OFL = OFLOXACIN, AMX = AMOXYCILLIN, CPX = CIPROFLOXACIN, CMP = CHLORAMPHENICOL, EMX = ERYTHROMYCIN, TSX = TRIMETHOPRIM+SULFAMETHOXADOLE, PFX = PEFLOXACIN, OXL = OXACILLIN, MAR = MULTIPLE ANTIBIOTIC RESISTANCE

3.3 MRSA growth on blood agar

Plate IV: Growth of MRSA on blood agar showing α-haemolysis and γ-haemolysis
Staphylococcus aureus were isolated from fomites such as classroom, office, toilet and toilet water closet door handles in selected secondary schools within Abeokuta environs (Abeokuta North LGA, Abeokuta South LGA and Odeda LGA). Methicillin Resistance Staphylococcus aureus (MRSA) is the leading cause of bacterial infections, causing a wide range of diseases such as bacteraemia, wound infection, septicaemia and pneumonia; but when it has not acquired the resistant gene (just the normal S. aureus), it is an important human friendly microorganisms which is often found as commensal on body surface but its transfer to other part of the human body may lead to a wide variety of infections (David and Daum, 2010). From this study, some strains of S. aureus isolated from the fomites were found to be resistant to oxacillin confirming them to be Methicillin Resistant S. aureus (Taiwo et al., 2004) which is of a major concern to the public health safety of the students and staffs within the community (secondary schools) in Abeokuta environs.

According to the antimicrobial susceptibility test, using the disk diffusion method; strains of S. aureus isolated shows high resistance to ampicillin (100%), Streptomycin (100%), Amoxicillin (100%), but moderate resistance to Trimethoprim (77.78%), Cotrimoxazole (66.67%), but low resistance to Erythromycin (33.33%), oxacillin (21.43 %) and Chloramphenicol (44.44%). Based on this resistance profile, S. aureus was shown to be Multi-drug resistant and the clinical importance of this is that these drugs should not constitute a first line of therapeutic treatment in any hospital setting around the environs (Albuquerque et al., 2007).

The high level of resistance to the β-lactam antibiotics (Amoxicillin and ampicillin) in this study confirm β-lactamase activity which was due to the presence of meca gene which encodes a modified Penicillin-binding protein which has low affinity for β-lactams (Hartman and Tomasz, 1984; Katayama et al., 2000; Li et al., 2011; García et al., 2011). The antimicrobial resistance profile also revealed that the levels of resistance to trimethoprim/sulfamethoxazole, ofloxacin, ciprofloxacin were remarkable among the S. aureus strains which support earlier report by (Fridkin et al., 2005; Nordmann and Naas, 2005; Polyzou et al., 2001). Since our study was cross-sectional, data on contacts between staffs and students were not collected limiting the investigation on possible transfer of MRSA from one sub-group to another within the school community.
A Prevalence of 50.60 % for *S. aureus* was recorded in this cross sectional study in which 42 isolates of *S. aureus* were confirmed; 2.99 % (9 isolates) were confirmed to be MRSA by both antimicrobial susceptibility test and PCR amplification. The prevalence rate recorded for MRSA in this study were much lower than one recorded for MRSA in a similar study carried out in Nairobi county, Kenya (Caroline et al., 2013) in which a prevalence rate of 15 % was found. The results obtained from this study indicate that the prevalence rate of MRSA varies from one geographical region to another, since it is clear that MRSA is a threat to the health of both students and staffs within the school community; it is therefore pertinent to put in place infection control measures which may be necessary to nip its rapid dissemination amongst the population (Ghebremedhin et al., 2009; Okon et al., 2011, Akinrotoye et al., 2018).

Lack of surveillance programs in Nigeria monitoring the prevalence of MRSA (Taiwo et al., 2004; WHO, 2014) in school community serves as hindrance to conclusive evidence whether there has been an increase or decrease in the prevalence rate of MRSA in the school community within Abeokuta environs.

4. SUMMARY AND CONCLUSION

4.1 Summary

This study has described the Antibiotic resistance profiles and Multiple Antibiotics Resistance Index (MAR) of MRSA strains obtained from schools within Abeokuta environs of Ogun State. This information is helpful in establishing effective infection control measures in health care settings and school in Ogun State, Nigeria. Methicillin Resistant *S. aureus* are present on fomites from selected secondary schools within Abeokuta environs hence preventive measures should be formulated in order to regularly curb its dissemination and monitor the spread of MRSA and other bacteria of public health importance.

4.2 Conclusion

Hygiene measures such as hand washing should be encouraged in schools across Abeokuta and other part of the country to prevent the spread of *Staphylococcus aureus* amongst students and staffs which is capable of developing resistance to various antibiotics.
5. REFERENCES


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