Fusidic Acid and Rifampicin Resistant Strains of Methicillin Resistant \textit{Staphylococcus aureus} (MRSA) from Northwestern Nigerian Hospitals

Abdulhadi Sale Kumurya*

Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University, Kano, Nigeria

Abstract

\textbf{Background:} Glycopeptides have been the last in the line of antibiotics used to treat serious MRSA infections. The emergence and spread of multi-resistant methicillin resistant \textit{Staphylococcus aureus} (MRSA) strains, especially those resistant to fusidic acid and rifampicin in Nigerian hospitals is of concern. Objectives: The main objectives of the study were to determine the resistance rates of fusidic acid and rifampicin among MRSA strains in Northwestern Nigerian hospitals. \textbf{Methods:} In this study PCR was performed for detection of \textit{mecA} gene from \textit{S. aureus} isolates. Antibiotic susceptibility testing using disc susceptibility testing and minimum inhibitory concentration (MIC) were performed on 423 MRSA isolates from 8 health institutions across Northwestern Nigeria. The isolates were obtained from clinical samples collected for duration of two years from February 2008 to April 2010. The resistance was confirmed by determination of MIC of fusidic acid and rifampicin. \textit{Staphylococcus aureus} ATCC 25923 was used as a reference control organism. \textbf{Results:} Of 432 MRSA isolates, 21(4.9\%) and 27(6.4\%) were found to be resistant to fusidic acid and rifampicin respectively. All the 21 and 27 isolates were confirmed to be resistant with MIC of >25mug/L and >45mug/L to fusidic acid and rifampicin respectively. \textbf{Conclusion:} The information gained from the epidemiology of fusidic acid and rifampicin-resistant isolates can help the hospitals infection control teams understand the epidemiology of these organisms in their institutions. Surveillance of these strains should be carried out to prevent further spread of the clone. Increase resistance to fusidic acid and rifampicin will further reduce the already limited treatment options for MRSA infections.

Keywords

Fusidic Acid, Rifampicin, MRSA, Resistance, Northwestern Nigeria

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1. Introduction

\textit{Staphylococcus aureus} particularly methicillin-resistant \textit{S. aureus} or MRSA infections pose serious clinical problems in hospitals as well as community (Yu \textit{et al.}, 2005). It is resistant to most of the antibiotic classes currently in use (Sakoulas and Moellering, 2008). Glycopeptides have been the last in the line of antibiotics used to treat serious MRSA infections. However, there have been reports about vancomycin resistant strains in recent years (Tiwari and Sen, 2006; Sievert \textit{et al.}, 2008). Fusidic acid has a high degree of activity against \textit{Staphylococcus aureus}, including methicillin resistant \textit{Staphylococcus aureus} (MRSA). Resistance to fusidic acid can be produced by growing \textit{Staphylococcus aureus} with increasing concentration of this antibiotic (O’Brien \textit{et al.}, 1998). The development of resistance during treatment with fusidic acid when this antibiotic is used alone is being reported increasingly (Ravenscroft \textit{et al.}, 2000; Chang \textit{et al.}, 2000). Rifampicin is used also as a potent...
antistaphylococcal agent but its use alone has resulted in rapid emergence of resistance staphylococci (Kucers and Bennet, 1987). In Nigeria, treatment of choice for serious MRSA infection is vancomycin. However, a combination of fusidic acid and rifampicin is used as an alternative oral regimen for the treatment of bacteraemia, musculoskeletal and cardiovascular infections caused by MRSA. Fusidic acid and rifampicin are used in combination to prevent the emergence of resistance which may occur if these antibiotics are used individually. These antibiotics provide an alternative or switch therapy to vancomycin in Nigeria. However, in Northwestern Nigerian hospitals, the resistance rate for fusidic acid and rifampicin to MRSA isolates is not known. It is important to know the resistance rates of MRSA to both these antibiotics. The main objectives of this study were to determine the resistance rates to fusidic acid and rifampicin among MRSA isolates in Northwestern Nigerian hospitals.

2. Materials and Methods

2.1. Bacterial Isolates

A total of 423 methicillin resistant *Staphylococcus aureus* (MRSA) isolates (from various clinical samples) were obtained from eight health institutions (Microbiology department) across Northwestern Nigeria. The hospitals were two teaching hospitals, three Federal Medical Centres, two Specialist Hospitals and one Infectious Diseases Hospital. The isolates were collected for duration of two years from February 2008 to April 2010. The quality control and rejection criteria of specimen were followed (Isenberg, 1998).

2.2. Identification of *Staphylococcus aureus* Isolates

All plates were examined for Staphylococcus by colonial morphology on nutrient agar (Cheesbrough, 2000). Bacterial culture was done in Mueller-Hinton broth at 370°C. Characters of these strains were determined by traditional biochemical tests such as catalase, coagulase, DNase test, and test for ß-heamolysin, Beta-lactamase production test, hydrolysis of gelatin and galactose and fermentation of mannitol, lactose, maltose and sucrose were performed on all the isolates. *Staphylococcus aureus* ATCC 25923 was used as a reference control organism.

2.3. Storage of the Isolates

Using sterile swab, the entire growth of an overnight pure culture was sub-cultured in 5ml of 16% v/v sterile glycerol broth and immediately stored in freezer [Micro bank (Diagnostic pro-lab)] at -80°C. After 24 hours the viability of the organism was checked by thawing the suspension at 35°C and inoculated on blood agar plates.

2.4. PCR Amplification

PCR assays were all directly performed from the bacterial suspension obtained after the rapid DNA extraction method described by Bignardi et al., 1996; Cavassini et al., 1999; Perez et al., 2001; Anna-Kaarina et al., 2009. An aliquot of 5 µl of this suspension was added to 95 µl of PCR mixture consisting of 1× reaction buffer [16 mM (NH4)2SO4,67 mMTris-HCl (pH 8.8)], a 0.5 mM concentration of each of the four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, anddTTP) (Inqaba Biotechnical Industries (Pty) Ltd., South Africa), 1.0µM of each primer, and mecA primer, and 1.25 U of the Dream Taq™ Green PCR Master Mix (2x) (Fermentas Life Sciences, supplied by Inqaba Biotechnical Industries (Pty) Ltd., South Africa) is a ready-to-use solution containing Dream Taq™ DNA polymerase, optimized Dream Taq™ Green buffer and 4mM MgCl2. For each sample, one reaction was performed with the pair of primers to identify *S. aureus* specific sequence gene and with the mecA pairs of primers to detect both resistance gene (mecA). In order to reduce the formation of nonspecific extension products, a hot-start PCR protocol was used; the tubes were placed in the thermal cycler when the denaturing temperature was reached. All PCR assays were carried out with a negative control containing all of the reagents without DNA template. DNA amplification was carried out in a Techne PCR system TC-5000 thermocycler (Bibby Scientific Ltd.) with the following thermal cycling profile: initial denaturation step at 94°C for 5 min was followed by1 cycle of amplification this was followed by denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s ending with a final extension step at 72°C for 5 min. After PCR amplification, 5 µl was removed and subjected to agarose gel electrophoresis (1.5% agarose, 1× Tris-borate-EDTA, 100V , 40min) to estimate the sizes of the amplification products by comparison with a 100-bpO’ GeneRuler™ 100 bp molecular size standard DNA Ladder, ready-to-use designed by Fermentas Life sciences (supplied by Inqaba Biotechnical Industries (Pty) Ltd., South Africa) The gel was stained with ethidium bromide, and the amplicons were visualized using a UV light box. This protocol, including the rapid DNA extraction method from a single colony and electrophoretic analysis of the amplified products on an agarose gel, was performed in less than 4h.

2.5. Disk Susceptibility Test

The susceptibility testing of isolates to these antibiotics was carried out by the disk diffusion method according to the National Committee for Clinical Laboratory Standards (now Clinical Laboratory Standards institute) guidelines (NCCLS,
performed triplicates. Isolates were considered to be added to molten Mueller–Hinton II agar (BBL, USA) to fusidic acid (Leo pharmaceutical products, Denmark) were 10^4 to 10^5 CFU/ml (Zhao following incubation for 24 h at 37°C for the colony count of lowest concentration of drug that inhibited bacterial growth 2565 μm/L to 15 μm/L. The MIC was defined as the 15 μm/L. Serial dilutions were also made for rifampicin (Sigma Alderich) to give concentrations ranging from 2565 μm/L to 15 μm/L. The MIC was defined as the lowest concentration of drug that inhibited bacterial growth following incubation for 24 h at 37°C for the colony count of 104 to 105 CFU/ml (Zhao et al., 2003). Each assay was performed triplicates. Isolates were considered to be resistant to fusidic acid if the MIC was >25 μm/L and resistant to rifampicin if the MIC was ≥45 μm/L (Toma and Barriault, 1995).

3. Results

The hospitals with MRSA isolates resistant to both these antibiotics were one Specialist Hospital, two Federal Medical Centres, one University Teaching Hospital (ABUTH), and the Infectious Diseases Hospital. No such resistance was observed in one Teaching Hospital, one Specialist Hospital Sokoto and one Federal Medical Centre.

Simultaneous testing of 423 methicillin resistant Staphylococcus aureus isolates to fusidic acid and rifampicin susceptibility by the disk susceptibility and serial dilutions (MIC) showed 21 (4.9%) and 27 (6.4%) isolates that were found to be resistant to fusidic acid and rifampicin respectively (Table 1).

Table 1. Fusidic acid and rifampicin susceptibility profile.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No of resistant isolates (%) by:</th>
<th>Disk Susceptibility (%)</th>
<th>Serial dilutions (MIC) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusidic acid</td>
<td>21 (4.9)</td>
<td>21 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>27 (6.4)</td>
<td>27 (6.4)</td>
<td></td>
</tr>
</tbody>
</table>

X^2=0.00, Df=1, P=1.000

4. Discussion

Besideshaving the advantage of oral administration, fusidic acid and rifampicin penetrates tissues better than glycopeptides. The appearance of resistance to either of these antibiotics will adversely affect affective oral treatment of MRSA infections. Rifampicin and fusidic acid should be used in combination when treating MRSA infections to prevent emergence of resistance to either of these antibiotics. No information was available on the usage of fusidic acid and rifampicin in the hospitals studied but the emergence of such resistance has been associated with monotherapy (O’Brien et al., 1998; Tiemersma et al., 2004).

The MRSA isolates that were resistant to fusidic acid observed in this study did not agree with data from Southwestern Nigeria which observed a full susceptibility of S. aureus to fusidic acid (Adebayo et al., 2006). It is also not comparable with data obtained from multi-center in South Africa which also observed a full susceptibility of MRSA to fusidic acid (Lin et al., 2004). However, this study agreed with data obtained from Malaysian hospitals (Norazah et al., 2002) which recorded 5% resistance of MRSA to fusidic acid. The MRSA isolates that were resistant to rifampicin observed in this study did not agree with data obtained from Southwestern Nigeria by Adebayo et al., (2006) which observed a susceptibility of MRSA to rifampicin. But it is comparable with data obtained from Malaysia hospitals (Norazah et al., 2002) which reported 5% resistance of MRSA to rifampicin. This report is not in agreement with the finding of Shopsin and Kreswirth (2003) in South Africa which reported the resistance of MRSA to rifampicin of 74%. This study observed a prevalence of rifampicin resistance (6.4%) among the Staphylococcus aureus isolates, suggesting that this trend may be increasing worldwide. Rifampicin resistance in S. aureus has been reported in Australia (Gottlieb and Mitchell, 1998), United Kingdom (Aukken et al., 2002), Malaysia (Norazah et al., 2002), Turkey (Tosum et al., 2005) and Poland (Matynia et al., 2005). Furthermore, a recent study on MRSA in eight African countries noted that the prevalence of rifampicin resistance was high with the exception to two countries (Morocco and Kenya) (Kesah et al., 2003). Resistance to rifampicin has been reported to be a common trend among S. aureus in South Africa (Lin et al., 2004) and clinical isolates of Mycobacterium tuberculosis (Lin et al., 2004). This could be attributed to the widespread use of this antimicrobial agent. Interestingly, a high level of rifampicin resistance has also been observed in environmental isolates of members of the family Enterobacteriaceae in Northern KZN (Lin et al., 2004). These facts indicate the severity of rifampicin resistance in...
both clinical and environmental bacteria in South Africa.

In a study by Schmitz et al., (2000), it was shown that the development of higher incidence rates of MRSA to fusidic acid and rifampicin was not the result of hyper mutability of target genes or a faster emergence of different mutations but rather the consequences of clonal spreads of multi resistant MRSA. Based having the advantage of oral administration, fusidic acid and rifampicin penetrate tissues better than glycopeptides. The appearance to either of these antibiotics will adversely affective oral treatment of MRSA infections. Fusidic acid and rifampicin should be used in combination when treating MRSA infections to prevent the emerging of resistance to either of these antibiotics. However, monotherapy with fusidic acid has been associated with another antistaphylococcal agent (beta – lactam, rifampicin or glycopeptides) to minimize the emergence of fusidic acid resistance strains (Howden and Grayson, 2006).

5. Conclusion

The information gained from the epidemiology of fusidic acid and rifampicin-resistant isolates can help the hospitals infection control teams understand the epidemiology of these organisms in their institutions. Surveillance of these strains should be carried out to prevent further spread of the clone. Increase resistance to fusidic acid and rifampicin will further reduce the already limited treatment options for MRSA infections.

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References


