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Fusidic Acid and Rifampicin Resistant Strains of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Northwestern Nigerian Hospitals

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Abstract

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 *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. *Methods:* In this study PCR was performed for detection of mecA gene from S. aureus isolates. Antibiotic susceptibility testing using disc susceptibility testing and minimum inhibitory concentration (MIC) were performed on 423MRSA 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. *Staphylococcus aureus* ATCC 25923 was used as a reference control organism. *Results:* 0f 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 >25mumg/L and >45mumg/L to fusidic acid and rifampicin respectively. *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

Staphylococcus aureus particularly methicillin-resistant *S. aureus* or MRSA infections pose serious clinical problemsin hospitals as well as community (Yu *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 *et al.,* 2008). Fusidic acid has a high degree of activity against *Staphylococcus aureus*, including methicillin resistant *Staphylococcus aureus* (MRSA). Resistance to fusidic acid can be produced by growing *Staphylococcus aureus* with increasing concentration of this antibiotic (O'Brien *et al.,* 1998). The development of resistance during treatment with fusidic acid when this antibiotic is used alone is being reported increasingly (Ravenscroft *et al.,* 2000; Chang *et al.,* 2000). Rifampicin is used also as a potent

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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 423methicillin 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 370C. 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 µlof 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 thefour deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, anddTTP) (Inqaba Biotechnical Industries (Pty) Ltd., South Africa), 1.0µM of eachprimer, 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 4mMMgCl2. 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 detectboth 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 PCRamplification,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,

2006). Bacterial suspension adjusted to 0.5 MacFarland was inoculated onto Mueller – Hinton agar. Disks of fusidic acid (10μg) and rifampicin (5μg) were used. Staphylococcus aureus (ATCC25923) was the control strain in every test run. Zone diameters for resistance to fusidic acid and rifampicin are as follows: ≤ 18mm – fusidic acid (Skov *et al.,* 2001) and ≤ 14mm – rifampicin (Kin *et al.,* 2004) using the current guidelines of the Clinical Laboratory Standard Institute (NCCLS, 2006) as described by Ghebremedhin *et al*., (2007).

2.6. Susceptibility Testing

MICs were determined in IS broth by broth microdilution method adopted from Andrews (2001), based on CLSI guidelines (Sakharkar *et al.,* 2009). Serial two – fold dilution to fusidic acid (leo pharmaceutical products, Denmark) were added to molten Mueller – Hinton II agar (BBL, USA) to make up concentration ranging from 2565mumg/L to 15mumg/L. Serial dilutions were also made for rifampicin (Sigma Alderich) to give concentrations ranging from 2565mumg/L to 15mumg/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 105CFU/ml (Zhao *et al.,* 2003). Each assay was performed intriplicates. Isolates were considered to be resistant to fusidic acid if the MIC was ›25 mumg/L and resistant to rifampicin if the MIC was ≥45 mumg/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.

	No of resistant isolates (%) by:	
Antibiotics	**Disk Susceptibility (%)**	**Serial dilutions (MIC) (%)**
Fusidic acid	21 (4.9)	21 (4.9)
Rifampicin	27 (6.4)	27 (6.4)

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 agreed 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 agreed 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 treat 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 effective 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

[1] Adebayo, S., Johnson, L. and Deboye, K. (2006). Antimicrobial Susceptibility Patterns of Staphylococcus aureus and Characterization of MRSA in Southwestern Nigeria. Wounds 18(4):77-84.

[2] Andrews JM (2001). Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48(suppl 1): 5-16.

[3] Anna-Kaarina, J., SannaLaakso, P., Anne sAittakorpi, M., Lindfors, L., Huopaniemi, H. and Minna, M. (2009). Rapid identification of bacterial pathogens using aPCR- and microarray-based assay. BMC Microbiology 9:161.

[4] Bignardi, G. E., Woodford, N., Chapman, A., Johnson, A. P. and Speller, D. C. E. (1996). Detection of the mec-A gene and phenotypic detection of resistance In Staphylococcus aureus isolates with border ine or low-level methicillin Resistance. Journal of Antimicrobial Chemotherapy 37: 53—63.

[5] Cavassini, M. A., Wenger, K., Jaton, D. S., Blane, and J. Bille. (1999). Evaluation of MRSA-Screen, a simple anti-PBP-2a slide latex agglutination kit, for rapid detection of methicillin resistance in Staphylococcus aureus. J. Clin. Microbiol. 37: 1591-1594.

[6] Chang SC, Hsieh SM, Chen ML, Sheng WH, Chen YC. Oral fusidic acid fails to eradicate methicillin-resistant Staphylococcus aureus colonization and results in emergence of fusidic acid-resistant strains. DiagnMicrobiol Infect Dis 2000. 36:131–6.

[7] Cheebrough, M. (2000). District Laboratory Practice in Tropical Countries 2. pp 180-242.

[8] Cambridge Uni. Press.Ghebremedhin, B., W. Konig, W. Witte, K.J. Kardy, P.M. Hawkey, and B. Konig, (2007).

[9] Subtyping of ST22MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. J. Med. Microbiol. 54:365-375.

[10] Howden BP, Grayson ML (2006). Dumb and dumber - The potential waste of a useful antistaphylococcal agent: Emerging fusidic acid resistance in Staphylococcus aureus. Clin. Infect. Dis. 42(3): 394-400.

[11] Isenberg, H. D. (1998). Agar screening test to detect oxacillin (methicillin)-resistantStaphylococcus Spp. In: Essential procedures for clinical microbiology.ASM Press WashintonDCpp 232-234.

[12] Kesah, C., Ben Redjeb, S., Odugbemi, T.O., Boye, C.S.B., Dosso, M.Y., Achola, J.O. et al. (2003). Prevalence of methicillin-resistant Staphylococcus aureus. In eight African hospitals and Malta. Clin Microbiol infect. 9: 153-156.

[13] Kucers A (1997). Fusidate sodium. In: Kucers A, Crowe SM, Grayson ML, HoyJF. The use of antibiotics. 5th ed. Oxford: Butterworth-Heinemann. 580–6.

[14] NCCLS (2006). National Committee for Clinical Laboratory Standards, 2006. Performance standards for antimicrobial susceptibility tests. Smith informational supplement, NCCLSdocumentM100-56. Wayne, PA.Norazah A., Lim V. K. E., Koh Y. T., Rohani M. Y., Zuridah H., Spender P.P et al. (2002).

[15] Molecular fingerprinting of fusidic acid- and ripamficin-resistant strains of methicillin-resistant Staphylococcus aureus (MRSA) from Malaysian hospitals. Journal of Medical Microbiology 51:1113-1116.

[16] O'Brien, F. G., Botterill, C. I., Endersby, T. G., Lim, R.L.G., Gruubb, W. B., Gustafson, J. E.(1998). Heterogenous expression of fusidic acid resistance in Staphylococcus aureus with plasmid or chromosomally encoded fusidic acid resistance genes. Pathology 30:299-303.

[17] Perez, R. E., Claverie, M. F., Villar, J. and Méndez, Á. (2001).Multiplex PCR for Simultaneous Identification of Staphylococcus aureus and Detection of Methicillin and Mupirocin Resistance. Journal of Clinical Microbiology 39(11): 4037-4041.

[18] Ravenscroft JC, Layton A, Barnham M. Observations on high levels of fusidic acid resistant Staphylococcus aureus in Harrogate, North Yorkshire, UK. ClinExpDermatol 2000; 25:327–30.

[19] Sakharkar MK, Jayaraman P, SoeWM, Chow VTK, Sing LC, Sakharkar KR (2009). In vitro combinations of antibiotics and phytochemicals against Pseudomonas aeruginosa. J. Microbiol. Immunol., 42(5): 364-370.

[20] Sakoulas G, Moellering RC (2008). Increasing Antibiotic Resistance among MethicillinResistant Staphylococcus aureus Strains. Clin. Infect. Dis. 46(Supplement 5): S360-S367.

[21] Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC (2008).Vancomycin-resistant Staphylococcus aureus in the United States, 2002-2006. Clin. Infect. Dis., 46(5): 668-674.

[22] Schmitz, F. J., Fluit, A. C., Hatner, D (2000). Development of resistance to ciprofloxacin, rifampicin and mupirocin in methicillin-susceptible and resistant Staphylococcus aureus isolates. Antimicrob Agents chemother 44:3229-3231.

[23] Shopsin, B. and Kreiswirth, B.N. (2003). Molecular epidemiology of Methicillin-resistant Staphylococcus aureus. Emerging Infections Disease 7:323-326.

[24] Tiemersma, E. W., Brozwaer, S.L., Lyytikainen, O., Degener, J.E., Schrijnemakers. P., BruinsmaN. et al. (2004). Methicillin-resistant Staphylococcus aureus in Europe. 1999-2002. EmergInfect Dis. 10: 1627-1634.

[25] Tiwari H, Sen M (2006). Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis., 6(1): 156.

[26] Toma, E and Barriault, D. (1995). Antimicrobial activity of fusidic acid and disk diffusion susceptibility testing criteria for gram-positive cocci. J. Clin. Microbiol. 33:1712-1715.

[27] Yu HH, Kim KJ, Cha JD, Kim HK, Lee YE, Choi NY, You YO (2005). Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant Staphylococcus aureus. J. Med. Food. 8(4): 454-461.

[28] Zhao X, Eisner W, Perl-Rosenthal N, Kreiswirth B, Drlica K (2003). Mutant Prevention Concentration of Garenoxacin (BMS-284756) for Ciprofloxacin-Susceptible or -Resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 47(3): 1023-1027.