Detection of Rifampicin Resistance Rate among Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates in Kano, Nigeria

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Abstract
Background: Rifampicin is used in the treatment of staphylococcal prosthesis-associated infections, in which bactericidal activity against surface-adhering, slow-growing and biofilm-producing microorganisms is essential.
Objectives: To determine the resistance rates of methicillin-resistant *Staphylococcus aureus* isolates to Rifampicin and interpretation of susceptibility tests to guide therapy.
Methods: The study investigated the susceptibility pattern of 42 non-duplicate methicillin-resistant *Staphylococcus* isolates from different human clinical specimens to antibiotics in Aminu Kano Teaching Hospital and Murtala Muhammad Specialist Hospital, Kano. All the isolates were tested for rifampicin resistance using disc diffusion method with 30µg rifampicin disc on Mueller Hinton agar. In addition, biotyping characterization of the isolates was carried out.
Results: More than half of the total number of the MRSA 22 (52.4%) was recovered from blood culture. The prevalence of rifampicin resistance among MRSA isolates was 33.3%.The susceptibility patterns of MRSA against antibiotics tested showed a susceptibility of 92.9%, 69%, 69%, 69%, 64.3%, 64.3%, 50% and 38.1% to Ciprofloxacin, Erythromycin, Clindamycin, Cloxacillin, Tetracycline, Gentamycin, Cotrimoxazole, and Amoxyclav respectively.
Conclusion: Rifampicin monotherapy is associated with the development of resistance among MRSA isolates. For this reason, rifampicin should be used in combination with other antibiotics in the treatment of MRSA infections. The result of the antibiotic susceptibility testing revealed that Ciprofloxacin is the first line drug for treatment of MRSA infections. Control of MRSA infection is essential, and it can be achieved by proper implementation of hospital control measures.
Keywords: MRSA, Rifampicin Resistance, *Staphylococcus aureus*

Introduction

Staphylococcus aureus is an important pathogen in human infections and is implicated in a wide variety of infections, from mild skin infections to more serious and invasive infections, including septicemia, pneumonia, endocarditis, deep-seated abscesses, and toxinoses including food poisoning and toxic shock syndrome. The discovery of antimicrobial agents has been a critical element of the therapeutic armamentarium of modern medicine, but the treatment of infections caused by *Staphylococcus aureus* is still a challenge for clinicians.

The treatment of infections due to *Staphylococcus aureus* was revolutionized by the introduction of Penicillin in1942. However it did not take long for penicillin resistance to emerge and by 1950, 80% of hospital acquired staphylococcal infections were untreatable by penicillin because of the production of enzyme beta-lactamase by the organisms which destroys penicillin.

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Almost 100% of hospital strains of *Staphylococcus aureus* are presently resistant to Penicillin. Soon enough, newer penicillin derivatives including methicillin, flucloxacillin and oxacillin were introduced which were resistant to staphylococcal beta-lactamase. These beta-lactamase resistant Penicillins remained the drugs of choice for treating staphylococcal infections until the late 20thcentury, when the organisms developed newer mechanisms for evading the antibacterial activity of these Penicillins, namely the altered Penicillin Binding Proteins (PBP) (Hussain *et al*., 2005).

Rifampicin is a broad-spectrum antibiotic that exerts its activity by interacting specifically with the b subunit of the bacterial RNA polymerase encoded by the rpoB gene. The chemical structure of rifampicin allows this drug to penetrate well into tissues and abscesses, which are poorly penetrated by most other anti-staphylococcal agents. Rifampicin is also used in the treatment of staphylococcal prosthesis-associated infections, in which bactericidal activity against surface-adhering, slow-growing and biofilm-producing microorganisms is essential. Rifampicin resistance is typically due to amino acid substitutions in three specific clusters of the rifampicin-binding site of the b subunit of the bacterial RNA polymerase (Villar*et al.,* 2011).

There has been a major increase in the number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Antibiotic pressure is known to select mutants that can survive adverse conditions. Rifampicin monotherapy in MRSA infections is definitely associated with the occurrence of resistance; hence, this agent should be used in combination therapy so that development of resistance may be prevented. In practice, rifampicin resistance may emerge despite the use of minocycline, fusidic acid, or even vancomycin in combination (Neogi *et al*., 2009). The main aim of this study is to determine the resistance rates of rifampicin among MRSA and study the antibiotics susceptibility pattern of the MRSA isolates in Kano, Nigeria.

MATERIALS AND METHODS

The study areas

The study was conducted in Aminu Kano Teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital Kano (MMSH). The two hospitals are situated in Kano, Kano State, Nigeria.

Sample size

In a study on rifampicin-resistant strains of methicillin-resistant *Staphylococcus aureus* in Spain, the prevalence was found to be 3.22 (Villar *et al*., 2011). The sample size of this study was determined using formula as follows:

$$n = \frac{Z^2pq}{D^2}$$

Where N = number of samples,
Z = standard normal deviate at 95% = I.96
p = prevalence, given 3.26, (0.0326)
q = 1-p, given (1-0.0326)
D = allowable error of 5%, (0.05)

$$n = \frac{(1.96)^2 \times 0.0326 \times (1-0.0326)}{(0.05)^2}$$

n = 41.46
n = 42.

Specimen collection

From May 2015 to October 2015, a total of 48 consecutive non - duplicated isolates of MRSA were collected from Medical Microbiology Laboratories of Aminu Kano Teaching Hospital and Murtala Muhammad Specialist Hospital, Kano. The isolates were collected from different clinical specimens and processed using standard bacteriological technique (Cheesbrough, 2000). The quality control and rejection criteria of specimen were followed (Isenberg, 1998).

Scope of the Study

The study dealt only with *S. aureus* isolated from clinical specimens obtained from the Medical Microbiology Laboratory of Aminu Kano Teaching Hospital and Murtala Muhammad Specialist Hospital.

Identification of *Staphylococcus aureus* isolates

All plates were examined for *Staphylococcus aureus* by colonial morphology on nutrient agar (Cheesbrough, 2010). Gram staining, catalase, coagulase, mannitol fermentation, DNAase tests and test for hemolysin production were performed on all the isolates. *Staphylococcus aureus* ATCC 25923 was used as a reference control organism.

Preparation of Turbidity Standard Equipment to 0.5 McFarland Scale

A 0.5 McFarland standard was prepared by mixing 0.05ml of aliquot of 0.048mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) and 0.18mol/L H_2S0_4 (1% v/v) with constant stirring to make a suspension. It was stored in a well-sealed container in a dark place at room temperature (20-28^{0}c).

Latex Agglutination Test

A drop of latex reagent was placed on a slide-card. Using an applicator stick colonies from the test organism was placed on the drop to make a suspension. It was mixed gently and hand-rocked for 20 seconds on the slide-card to agitate the combination. It was examined for latex reaction. Agglutination/visible clumping were observed (Norazah *et al*., 2002).

Screening for MRSA

Using sterile cotton swab, a bacterial suspension adjusted to 0.5 McFarland standard was inoculated onto Mueller Hinton agar. Filter paper disc containing 30µg cefoxitin was placed on the inoculated Mueller Hilton agar containing 4% NaCl. The plate was incubated at 35^{o}C for 24 hours. The diameter of zone of inhibition was measured and recorded.

Determination for rifampicin resistance

The methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were subjected to rifampicin (30µg) disc susceptibility testing following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2006). Bacterial suspension of MRSA adjusted to 0.5 McFarland standard was inoculated unto Mueller Hinton agar. Filter paper disc containing 30µg rifampicin was placed unto the inoculated Mueller Hinton agar. The plate was then incubated at 35^{o}C for 24 hours. The diameter of zone of inhibition was then measured and recorded.

Antibiotic susceptibility testing

The susceptibility testing of the isolates to different antibiotics was carried out by the disc diffusion method according to the National committee for Clinical Laboratory standards (Now Clinical Standards Institute) guidelines (NCCLS, 2006). The antibiotics (purchased from Oxoid Co., USA) used include Ciprofloxacin (10µg), Erythromycin (15µg), Gentamycin (10µg), Tetracycline (30µg), Clindamycin (2µg), Cotrimoxazole (25µg), Amoxyclav (30µg) and Cloxacillin (5µg). *Staphylococcus aureus* (ATCC 25923) was the control strain in every test run. The resistance rate to each antibiotic was calculated.

Statistical analysis

Statistical analysis was carried out using computer database software from the statistical package from social sciences (SPSS version 16.0) to generate frequency distribution and percentage prevalence scores of the various parameters. Descriptive analysis of the percentages of continuous variables was reported.

Results

Of the 42 MRSA isolates tested, 19 were obtained from male patients (45.0%) and 23 from female patients (55.0%) (Table 1) According to this study, the rifampicin-resistant isolates were found to be 14 (33.0%), while the rifampicin-sensitive were found to be 28 (67.0%). Of the 42 MRSA isolates obtained from the two study areas, 21were obtained from AKTH and also21were obtained from MMSH. Of the 14(33.0%) rifampicin-resistant MRSA isolates obtained, 6 (43.0%) were obtained from AKTH while 8 (57.0%) from MMSH (Table 2).

The age group 21-30 years has the highest percentage of the isolates (31.0%), followed by the age group 11-20 years (29.0%), then 51-60 years (12.0%), followed by 41-50 years (10.0%). This is followed by the age groups 0-10 and 31-40 with the same percentage of (7.1%) and then the age group 61-70 and 71-80 years with the lowest percentage of (2.0%) (Table 3).

The source of isolates with the highest percentage was blood cultures (52.0%), followed by sputum (26.0%). This is followed by high vaginal swab, wound swab, throat swab and ear swab with the same percentage of 2.0% (Table 4).

The result of the characterization of the isolates according to their haemolysis on blood agar, mannitol fermentation and production of DNAase showed that forty two MRSA isolates tested, (86.0%) were DNAase positive while (14.0%) were negative for DNAase, 75% were positive for mannitol fermentation while 25% were found to be negative, (81.0%) showed Beta hemolysis while (19.1%) showed no hemolysis (Table 5).

The susceptibility pattern of MRSA against antibiotics tested showed a susceptibility of (93.0%), (69.0%), (69.0%), (69.0%), (64.0%), (64.0%), (50.0%) and (38.0%) to Ciprofloxacin, Erythromycin, Clindamycin, Cloxacillin, Tetracycline, Gentamycin, Cotrimoxazole, and Amoxyclav respectively (Table 6).

Table 1: Distribution of MRSA isolates according to gender

GENDER	FREQUENCY	PERCENTAGE (%)
MALE	19	45
FEMALE	23	55
TOTAL	**42**	**100**

Table 2: Distribution of rifampicin-resistant MRSA isolates according to study area

HOSPITAL	FREQUENCY	PERCENTAGE (%)
AKTH	6	43
MMSH	8	7
TOTAL	**14**	**100**

Table 3: Distribution of MRSA isolates according to age group

AGE GROUP (YEARS)	FREQUENCY	PERCENTAGE (%)
0 -10	3	7
11-20	12	29
21-30	13	31
31-40	3	7
41-50	4	10
51-60	5	12
61-70	1	2
71-80	1	2
TOTAL	**42**	**100**

Table 4: Distribution of MRSA according to source of isolates

SOURCE	FREQUENCY	PERCENTAGE (%)
BLOOD CULTURES	22	52
SPUTUM	11	26
HIGH VAGINAL SWAB	2	5
WOUND SWAB	2	5
EYE SWAB	1	2
THROAT SWAB	2	9
EAR SWAB	2	5
TOTAL	**42**	**100**

Table 5: Biotyping characteristic pattern of MRSA isolates

TEST	POSITIVE (%)	NEGATIVE (%)
DNAase	85.7	14
Hemolysis	80.9	20
Mannitol fermentation	75.0	25

Table 6: Antibiotic susceptibility pattern of MRSA isolates (N=42)

ANTIBIOTICS	SUSCEPTIBLE (%)	RESISTANT (%)
Oxacillin	0	100
Erythromycin	69.0	31
Ciprofloxacin	92.9	7
Clindamycin	69.0	31
Amoxyclav	38.1	62
Tetracycline	64.3	36
Gentamycin	64.3	36
Cloxacillin	69.0	31
Cotrimoxazole	50.0	50

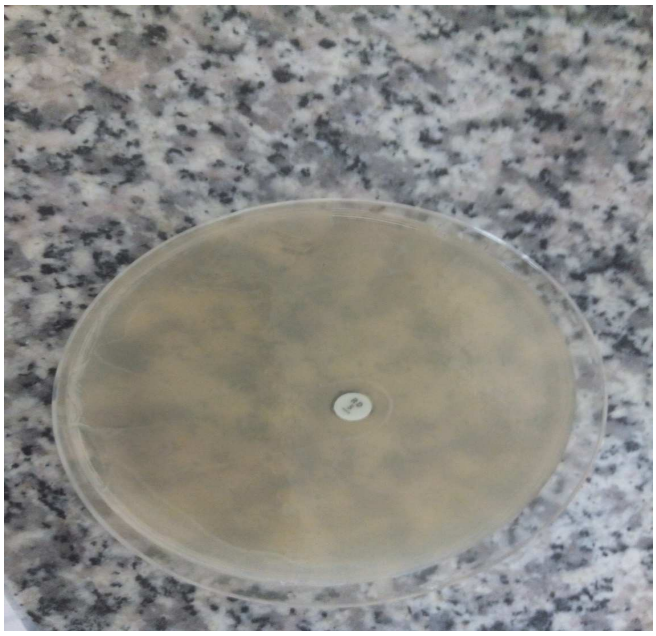

Figure 1: Rifampicinresistant *MRSAisolate on Muller Hilton Agar

*Methicillin Resistant *Staphylococcus aureus*

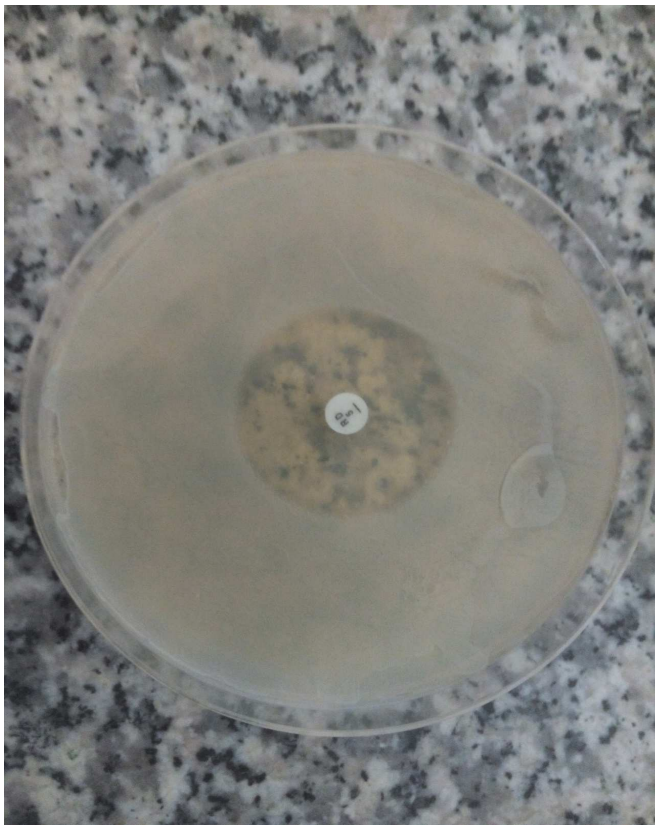

Figure 2: Rifampicin-sensitive *MRSA isolateon Muller Hilton Agar with ≥16mm in diameter zone of inhibition

Discussion

The high level resistance could be associated with earlier exposure of these drugs to isolates which may have enhanced development of resistance. There is high level of antibiotic abuse in this environment arising from self-medication which is often associated with inadequate dosage and failure to comply to treatment and availability of antibiotics to consumers across the counters with or without prescription (Monnet and Frimodt, 2001).

A number of factors drive the emergence and spread of antibiotic resistance, including antibiotic usage, infection control practices and the organism's genetics. Previous studies carried out in South Africa have reported large proportions of rifampicin-resistant MRSA isolates (39.7%-46.4%) (Rensburg *et al*., 2012), this has corroborate with the prevalence of rifampicin-resistance among MRSA isolates obtained from this study (33.3%). However, these results contradict the result of a rifampicin-resistance study conducted in San Sebastián, Spain which has a prevalence of 3.26% (Villar *et al*.,2011).It is likely that the frequent use of rifampicin to treat tuberculosis in this environment has driven the high prevalence of rifampicin resistance among local MRSA.

Due to the rapid development of resistance, rifampicin is not used as monotherapy, but it has been used in combined therapy for a wide range of staphylococcal infections, especially osteomyelitis and prosthetic device-related infections. Rifampicin resistance in *Staphylococcus aureus* is typically due to amino acid substitutions in the three known clusters (I, II and III) of the rifampicin-binding site of the RNA polymerase b subunit, and the genetic determinants for most rifampicin-resistant *S. aureus* isolates identified are point mutations that have been mapped in clusters I and II (Villar *et al*., 2011) .

According to this study, MRSA isolates obtained from blood cultures have the highest prevalence (52.0%) with the least obtained from eye swab (2.0%). The study conducted in Spain showed ulcers to be the source with the highest isolates (22.7%) (Villar *et al*., 2011). This is amply borne out by the findings of the present study, where 52.4% of MRSA isolates were from Blood Culture patient as compared to only 5.0% from wound swab patients. Its prevalence varies considerably from one region to another and even among the hospitals in the same city. Proximity to an infected or colonized patient increases the rate of acquisition of MRSA; the same is true of MRSA-colonized healthcare workers. Once strains of MRSA become established as endemic nosocomial pathogens they are difficult to eradicate.

Most of the MRSA isolates were isolated from female patients with a percentage of 55.0% with a male prevalence of 45.0%. This is in contrast to the study carried out in Spain where the isolates obtained from male patients were higher with a percentage of 77.3% (Villar *et al*., 2011).

Data from this study revealed that most of the isolates obtained were from blood cultures. As this organisms are multi-drug resistant, their high prevalence in septicemia suggests serious complications, thus control measures should be taken to prevent the infection. Rifampicin should not be used alone in MRSA infections treatment as the organisms tend to develop resistance to it. Another limitation of this study is the low number of the isolates used in the study, another study is recommended with higher number of Methicillin resistant *Staphylococcus aureus* isolates and to cover more region of the North western Nigeria.

Conclusion

Rifampicin monotherapy is associated with the development of resistance among MRSA isolates. For this reason, rifampicin should be used in combination with other antibiotics in the treatment of MRSA infections. The result of the antibiotic susceptibility testing revealed that Ciprofloxacin is the first line drug for treatment of MRSA infections. This is followed by Erythromycin and Clindamycin. These antibiotics should therefore be used in the treatment of MRSA infections in this region for routine clinical practice.

Acknwoledgement

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