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Full Length Research Paper

Prevalence and susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria

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Multi-resistant methicillin-resistant *Staphylococcus aureus* (MMRSA) has been commonly reported to be one of the commonest causes of nosocomial infections worldwide. Also, recent reports describe methicillin-resistant *S. aureus* (MRSA) carriage in persons in the community. The study investigated its prevalence in urine of healthy women and its susceptibility pattern to other antibiotics. Urine samples collected from healthy women volunteers in Zaria were cultured and screened for *S. aureus* using standard microbiological procedures. The isolates were then subjected to antibiotic susceptibility testing using disc diffusion technique. A total of 54 (36%) *S. aureus* isolates were isolated from 150 urine samples collected. The prevalence rate for married and single women was 31% and 46%, respectively. Of the *S. aureus* isolates, 37 (69%) were methicillin-resistant. The MRSA were highly resistant to ampicillin 100%, cephalexin 100%, clindamycin 92%, vancomycin 89% but had low resistance to pefloxacin 35%, ofloxacin 27% ciprofloxacin 27%, sparfloxacin 24% and gentamicin 16%. All the 37 (100%) MRSA isolates showed resistance to at least two antibiotics tested while 33 (89.2%) were multi-drug resistant.

Key words: Prevalence, methicillin-resistant *Staphylococcus aureus*, susceptibility, healthy women.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and it now represents a challenge for public health; as community-associated infections appear to be on the increase in both adults and children in various regions and countries (Layton et al., 1995). The overall incidence of MRSA isolation has gradually increased to reach levels of around 30% or more in some countries (Ayliffe, 1997). It was estimated that MRSA strains accounted for 84% of hospital-acquired *S. aureus* isolates and 45% of non-hospital acquired *S. aureus* isolates in Taiwan in 1998 (Ho et al., 1999).

S. aureus is an endogenous microorganism colonizing the nasal cavities, skin, gastrointestinal, anuses, and vaginal vaults of healthy women. Approximately 60% of women harbour this organism intermittently at one or more body sites (von Eiff et al., 2001). Studies have shown that 7-25% of women harboured toxin-producing *S. aureus* (Warner and Onderdonk, 2004). However, most persons with MRSA are not infected. MRSA may colonise mucosal and epithelial surfaces without causing any signs of inflammation or infection (Simor, 2001; Weems, 2001) but are at increased risk of becoming infected with these strains (Lowy, 1998).

Resistance in MRSA is related to a chromosomal *mecA* gene that specifies the production of an abnormal penicillin binding protein called PBP2a or PBP2[1]. Penicillin–binding proteins are membrane-bound enzymes, which targets for all β-lactam antibiotics. PBP2a has a decreased affinity for binding β-lactam antibiotics resulting in resistance not only to methicillin but also to all β-lactams including penicillins and

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cephalosporins (Weems 2001). The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non-β-lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin is common (Chambers, 1979; Chambers, 2001). Nevertheless, MRSA isolates susceptible to several non-β-lactam antibiotics have appeared in some European regions (Lelievre et al., 1999; Pournaras 2001).

This study reports the prevalence and susceptibility pattern of MRSA isolates among the healthy women in Zaria, Nigeria.

MATERIALS AND METHODS

Antibiotics and media

Antibiotics discs were used with their concentrations and sources as follows: ampicillin 10 µg (Medreich sterilab, India), cephalexin 30 µg (Fidson, India), ciprofloxacin (ciprotab®) 5 µg (Fidson, India), clindamycin (Dalacin C®) 2 µg (Pharmacia, Belgium), gentamicin (Hefogenta®) 10 µg (Wuham, china), methicillin 10 µg (Oxoid, UK), ofloxacin (Fluxor®) 5 µg (Pathoteq Lab, India), pefloxacin (Peflotab®) 5 µg (Fidson, India), sparfloxacin (Sparbact®) 5 µg (Pathoteq Lab. India) and vancomycin 30 µg (Dumex-Alpharma, S. Demark)

Mannitol salt agar, Mueller Hinton agar II, Nutrient agar and Nutrient broth Media used were from LAB M (International Diagnostics Group Plc, United Kingdom).

Sample Collection

First 'clean catch" urine sample were collected randomly from 150 healthy women who were classified into two groups: Married (pregnant women inclusive) and single of ages between 20-40 years over a period of two (2) months from Zaria community after an informed consent had been obtained from each woman. All the volunteers were not on any antibiotics at the point of sampling. Samples (100 from married and 50 from single) were collected into labelled sterile bottles, kept in an iced-bag and transported to the laboratory.

Bacteriology

Each urine sample was inoculated (in duplicates) into mannitol salt agar plates and incubated at 37°C for 24 h. The characteristic isolates were asceptically isolated and characterised using established microbiological methods, which include colonial morphology, Gram's, stain reaction and biochemical characteristics (Cheesbrough, 2002). Isolates that were Gram-positive cocci, catalase positive and coagulase positive were considered as *S. aureus* in this study.

Definition of community-associated isolates

For the purpose of this study, community-associated isolates were defined as isolates from the samples of the healthy women who were not on any antibiotic at the time of sampling and had not been admitted in the hospital in the last one year before the sampling.

Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern of the *S. aureus* isolates was determined using Kirby-Bauer-NCCLS modified disc diffusion technique (Cheesbrough, 2002). All the isolates were tested for sensitivity to ten (10) antibiotics. Standardised overnight culture of each isolate was used to flood the surface of Mueller Hinton agar (MHA) plates; excess drained off and allowed to dry while the lid was in plate. Standard antibiotic discs were asceptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 h. The plates (in duplicates for each isolates) were then incubated at 30°C for 24 h so as to favour the growth of methicillin resistant strains (BSAC 2002). The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolates were classified as "resistant", "intermediate" and "sensitive" based on the standard interpretative chart updated according to the current NCCLS standard (BSAC, 2002; NCCLS, 2002) and Fluka zone interpretative chart in accordance with WHO requirements (flukatec@eurnotes.sial.com).

Statistical analysis

Frequencies were obtained and percentages were calculated for study variables. Chi-square and two-tailed Fisher's exact test were used to calculate probabilities and determine significance. A p-value of less than or equal to 0.05 is considered to be statistically significant (P≤0.05).

Table 1. Distribution of *Staphylococcus aureus* and MRSA in married and single women in Zaria, Nigeria.

Source	Number Sampled	*S. aureus* No (%)	MRSA No (%)
Married	100	31 (31)	21 (68)
Single	50	23 (46)	16 (70)
Total	150	54 (36)	37 (69)

RESULTS AND DISCUSSION

A total of 54 (36%) *S. aureus* isolates were isolated from 150 urine samples screened. The prevalence rates from married and single women were 31% and 46%, respectively. Of the *S. aureus* isolated, 37 (69%) were methicillin-resistant (Table 1).

The result of antibiotic susceptibility of methicillin-resistant isolates to other antibiotics is shown in (Table 2). The results show high resistance to ampicillin (100%), cephalexin (100%), clindamycin (92%), vancomycin (89%) but low resistance to pefloxacin (35%), ofloxacin (27%), ciprofloxacin (27%), sparfloxacin (24%) and gentamicin (16%).

The prevalence of multi-drug resistance in the (MRSA) isolates is shown in Table 3. Multi-drug resistance was defined in this study as resistance to four or more of the antibiotics tested. Thus, 33 (89.2%) of the MRSA isolates showed multi-drug resistance to the antibiotics and none was fully susceptible to all the tested antibiotics.

Table 2. Resistance pattern of MRSA isolates from healthy women in Zaria, Nigeria.

Antibiotics	Resistant No (%)	Intermediate No (%)	Susceptible No (%)
Ampicillin (10 µg)	37 (100)	0 (0)	0 (0)
Cephalexin (30 µg)	37 (100)	0 (0)	0 (0)
Clindamycin (2 µg)	34 (92)	0 (0)	3 (8)
Ciprofloxacin (5 µg)	10 (27)	3 (8)	24 (65)
Gentamicin (10 µg)	6 (16)	0 (0)	31 (84)
Ofloxacin (5 µg)	10 (27)	1 (3)	26 (70)
Pefloxacin (5 µg)	13 (35)	1 (3)	23 (62)
Sparfloxacin (5 µg)	9 (24)	1 (3)	27 (73)
Vancomycin (30 µg)	33 (89)	0 (0)	4 (11)

Table 3. Prevalence of multiple-drug resistance among 37 MRSA isolates.

Parameter	Frequency of multi-drug resistance	
	Number	(%)
Fully sensitive	0	0
Resistant to 1 agent	0	0
Resistant to 2 agent	3	8
Resistant to 3 agent	1	3
Resistant to 4 agents	18	49
Resistant to 5 agents	4	11
Resistant to 6 agents	1	3
Resistant to 7 agents	0	0
Resistant to 8 agents	7	19
Resistant to 9 agent	3	8

New strains of community-associated methicillin-resistant *S. aureus* (CA-MRSA) that cause soft-tissue infections in healthy people have recently been detected worldwide (Vandenesch et al., 2003). Drug resistance in *S. aureus* including the emergence of MRSA in healthcare and community settings is an increasingly reported event that makes treating serious infection difficult.

The carrier rate of *S. aureus* in this study was 31% and 46% for urine of married and single women, respectively. This is in agreement with the report of Ehinmidu (2003). The difference in the colonization rate of *S. aureus* in the married and single women was not significant ($P>0.05$) indicating that marital status is not a notable factor in colonization and there is no activity or behaviour of any of the groups, which predisposes them to *S. aureus* infection. Also, the difference in MRSA colonization in the women groups was not significant ($P>0.05$). MRSA colonization may subsequently cause infections (Kluytmans et al., 1997).

In this result, MRSA isolated show total resistance to ampicillin and cephalexin, which support the findings, that MRSA strains are equally resistant to all β-lactam antibiotics (Weems, 2001; Gross-Schulman et al., 1998) which may be due to the presence of chromosomal mecA gene that specifies the production of an abnormal penicillin binding protein (PBP2a) which has low affinity for binding β-lactam antibiotics. A high resistance was observed to clindamycin (92%) and vancomycin (89%) in our study but this is contrary to the findings of earlier reports (Fridkin et al., 2005; Anupurba et al., 2003) that did show that community-based MRSA isolates had 87-100% susceptibility to clindamycin and vancomycin. Thus, these antibiotics have been used in the treatment of MRSA infections. However, our findings support the reports by Olayinka et al. (2005) who observed 57.7% vancomycin-resistant *S. aureus* (VRSA) and Lu et al. (2005) who found 92.9% CA-MRSA isolates resistance to clindamycin. These findings also support the recent alarming increasing emergence of vancomycin resistance of *S. aureus* worldwide (Fridkin, 2001).

There is a high level of MRSA susceptibility to gentamicin and the fluoroquinolones tested in this study. This may be due to the absence of resistance conferring-genes in these MRSA strains as reported by Polyzou et al., 2001). The observed high MRSA susceptibility to gentamicin and fluoroquinolones in this study support some previous reports (Fridkin et al., 2005; Nordmann and Nass, 2005). Thus, the existence of MRSA susceptible to these non β-lactam antibiotics may provide

an opportunity for the recommendation of these drugs for empirical treatment of community-associated MRSA strains and further reducing reliance on vancomycin.

The high level of multiple drug resistance shown by the MRSA isolates from healthy volunteers in this study is of great concern. All the MRSA isolates showed resistance to at least two antibiotics tested in this study, indicating the presence of strong selective pressure from antibiotics use in this community. This confirms the postulation that healthy members of the community are the highest reservoir of antimicrobial resistant bacteria (Lamikanra et al., 1996). The high prevalence rate of multi-drug resistant MRSA in the urine of healthy women investigated in the community demand serious concern. There is an urgent need to adopt basic principles of asepsis and high personal hygiene, for most staphylococcal infections are readily transmitted among susceptible populations by the individuals who have acquired them by hospitalization. Our study however involved only a small number of isolates, so we recommend a multi-centre study to be done to determine the true prevalence of MRSA in both community and hospital settings.

The central focus of this study is on women because of the inevitable vital role they play in human race as wives and mothers. Studies have shown that 60% of women harbour *S. aureus* (including the toxin producing ones) intermittently at one or more body sites (Daghistani et al., 2000, von Eiff et al., 2001; Warner and Onderdonk, 2004). Thus, understanding the prevalence of *S. aureus* among various women groups in our society is clinically important for the implication of measures to control the spread of *S. aureus.*

It can be concluded that the results from this study show the need to reassess policies on antibiotics use within and outside the hospital environments.

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