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ANTIBIOGRAM OF NASAL METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM ANTENATAL CLINIC ATTENDEES IN A TERTIARY HOSPITAL, SOUTH-SOUTH NIGERIA

C. A. Etok[*1], E. A. Ochang[2], V. Inyang[1], I. A. Onwuezobe[2], E. E. Asuquo[2]

[*1]*Department of Microbiology, University of Uyo, Nigeria*
[2]*Department of Medical Microbiology and Parasitology, University of Uyo Teaching Hospital, Uyo, Nigeria.*

Abstract
The antibiogram of nasal methicillin resistant *Staphylococcus aureus* (MRSA) from pregnant women attending University of Uyo Teaching Hospital was investigated using standard microbiological procedures. Out of 772 women, 180(23.3%) harboured nasal MRSA while 592 (76.7%) had MSSA (Methicillin Sensitive *Staphylococcus aureus*). The highest frequency (33.3%) occured at week 16 while the lowest occured at week 36 of the pregnancy period. Evaluation by logistic regression showed no risk factor involvement for MRSA. The patients were evaluated on their first visit (booking) therefore the MRSA were likely community-acquired. Antibiogram of isolates showed sensitivity mostly to clindamycin (80%), amoxacillin-clavulanic acid (76.7%), ceftriazone (69.4%) and resistance to co-trimoxazole (51.7%). The asymptomatic nasal colonisation of MRSA in pregnant women may therefore be a risk factor for serious systemic infection after delivery.
***Keywords*:** MRSA, antibiogram, anterior nares, antenatal attendees

1. Introduction

Most staphylococci are resistant to penicillin due to the production of β-lactamase. A positive β-lactamase test indicates resistance to pencillin, ampicillin, amoxillin, azlocillin, carbenicillin, mezlocillin, piperacillin and ticacillin[1]. Resistance to the antistaphylococcal, penicillinase-stable penicillins (methicillin, nafcillin and oxacillin) has been referred to as "methicillin resistance".

Most resistance to oxacillin in staphylococci is mediated by the mecA gene which directs the production of a supplemental penicillin-binding protein, PBP2a in the cell wall of resistant strains[2]. MRSA appeared in 1961 shortly after the introduction of methicillin in clinical practice and its prevalence in US hospitals approached 50% in 2004[3]. MRSA has been shown to be one of the most common causes of nosocomial infections accounting for 40 to 70% of *S. aureus* infections in intensive care unit[4]. It also causes infections in neonatal intensive care unit[5], among families as well as in pregnant and postpartum women[6]. Infection with MRSA is likely to be more severe and requires longer hospitalization[7].

The study of nasal carriage of MRSA is important to the community since carriage plays a key role in the epidemiology and pathogenesis of Community Associated MRSA (CAMRSA). CAMRSA having the PVL gene is associated with skin infections like furuncles and with necrotizing pneumonia[8].

The treatment options of MRSA are limited to few antibiotics like vancomycin, linezolid and teigecycline. Since there are reports of reduced susceptibility and resistance of *S. aureus* to vancomycin from different countries especially Japan and USA[9], the study was conducted to determine current prevalence of MRSA as well as antimicrobial susceptibility patterns of isolates from pregnant women. This is because the presence of MRSA in anterior nares of these women could pose a pregnancy-related threat to both mother and neonate.

2. Materials and Methods

2.1 Source of samples

Swab specimens were obtained from antenatal attendees at the University of Uyo Teaching Hospital, Uyo. The hospital is a tertiary facility with 7,250 beds and serves as a referral centre. All the women consented to participation in the study and permission from the hospital authority was obtained.

2.2 Collection of samples

A swab specimen from the anterior nares was obtained from each pregnant women involved in the study. Swabs were carefully inserted into each nostril so that the tip is entirely at the nasal osteum level (about 2.5 cm from the edge of the nares) and rolled gently 4 times.

2.3 Culture procedures and isolation

The swabs were immeditately plated on blood agar and on mannitol-salt agar and incubated for 24 hours at 35^{o}C. Mannitol fermenting colonies were subcultured and identified by morphology, microscopy and biochemical tests[1].

2.4 Screen test for MRSA

A suspension equivalent to MacFarland 0.5 was prepared from each isolate. A swab was dipped and surface-plated on Mueller-Hinton agar. Thirty (30)µg oxacillin disk was placed on the medium and the plates incubated at 35^{o}C for 24 hours. Plates with inhibition zones less than 12mm were considered methicillin resistant while those with zones greater than 13mm were susceptible.

2.5 Antibiotic susceptibility test

The Kirby-Bauer disk diffusion technique was adopted. Individual discs of the antibiotics were aseptically placed on Mueller-Hinton agar previously

Corresponding Author*: ekomedem@yahoo.com

seeded with 0.5 MacFarland suspension of each isolate. Plates were incubated at 37°C for 24 hours and zones of inhibition were recorded. All the MRSA isolates were used in the test.

2.6 Inducible Macrolide Lincomycin Streptogramin B (iMLS$_B$)

All isolates resistant to erythromycin but susceptible to clindamycin were subjected to iMLS$_B$ test. A 15µg erythromycin disc and 2µg clindamycin disc (Oxoid Ltd, Cambridge, UK) were placed 15-26mm apart on an already *S. aureus* seeded Mueller-Hinton agar plate. The plates were incubated for 18-24 hours at 35°C. Isolates that showed a flattening of the clindamycin zone of inhibition adjacent to the erythromycin disc (referred to as D-zone) were considered to exhibit inducible clindamycin resistance.

2.7 Data anaylsis

Data were analysed using SPSS software involving regression and correlation.

3. Results

Figure 1 represents the distribution of MRSA and Methicillin Sensitive *Staphylococcus aureus* (MSSA) among antenatal attendees. Exactly 23.33% of the isolates were MRSA while 76.67% were MSSA.

Figure 2 shows the frequency of occurence of MRSA with age. The highest frequency was obtained at age 31 years.

The frequency of occurence of MRSA at different gestational ages is shown on Figure 3. The highest frequency (33.3%) occured at week 16 while the lowest (3.3%) was at week 36 of the pregnancy period.

Table 1 shows the risk factors associated with MRSA colonisation in the attendees. Exactly 86.7% of the attendees were not previously admitted in the hospital, did not visit clinics and were not on any form of antimicrobials during the pregnancy period.

Table 2 shows the antibiotic susceptibility patterns of MRSA isolates. The isolates where mostly sensitive to clindamycin, amoxycillin-clavulanic acid and ceftriazone with susceptibilities of 80%, 76.7% and 69.4% respectively. The isolates were however resistant to cotrimozaxole.

Table 3 shows frequency of inducible macrolide streptogramin B. The MRSA isolates exhibited 31.1% resistance to erythromycin, 20% to clindamycin, 29.4% to ceftriazone, 35.6% to ciprofloxacin, 51.7% to sulfamethoxazole-trimetoprim and 22.2% to amoxillin-clavulanic acid.

4. Discussion

Methicillin resistant *S. aureus* has evolved as one of the most important causes of nosocomial infections worldwide. The data obtained from this study revealed that 23.33% antenatal women in the study area harboured MRSA in their anterior nares. However, 45% colonization by MRSA in health care workers was obtained in India[10], while 36.2% colonization by MRSA occured in 9 Provinces in Iran[11]. Also, it was reported that 47% of all *S. aureus* isolates from US intensive care units in 1998 were methicillin-resistant[12]. The prevalence of MRSA among clinical specimens has been found to be 23.9%[13] and in other reports, the prevalence ranged from 20% to 32.8% [14, 15]. The prevalence of MRSA in some countries is still low. In the Netherlands for example, it is as low as 1%[16]. Thus the prevalence differ from one geographical location to another. Different epidemiological factors such as geographical and health system capability in running infection control programme influence the variability of prevalence of MRSA[11].

There was negative correlation between gestatinal age (GA) and antimcrobial use (P=0.001) ie women at early GA are more likely to take antibiotics than those at a later GA age.

Although some reports show signicantly increased rate of MRSA in elderly people [17, 18], in this study, there was no significant difference between MRSA colonization and age. Apart from pregnancy, age is a risk factor because of its role in long term hospitalization, loss of immunity and longer antibiotic therapy[19].

From the study, the MRSA isolates were community acquired because 86.7% of the attendees were not previously admitted in the hospital and 80% had no clinic visit history, therefore, no risk factor for colonisation by MRSA was noted.

Identifying the incidence of MRSA infections within the community would imply the existence of significant reserviors of MRSA colonization within the community. It holds important implications for efforts to control the emergence of glycopeptide resistance among staphylococci. Recognising the emergence of CAMRSA might serve as a warning that MRSA control measures have failed[12].

The importance of a dose-effect association, supporting a causal relationship between MRSA and antimicrobial drug use has been demonstrated[20]. However, in the study, 96.7% of attendees were not exposed to antimicrobial use.

In this study, the isolates were susceptible to clindamycin, amoxycillin-clavulanic acid and ceftriazone but resistant to cotrimoxazole (51.7%). A 100% resistance of MRSA to penicillin, co-amoxyclav and ampicillin and more than 70% resistance to cefotaxinic, erythromycin, ceftriaxone and nalidixic acid has been reported[11].

5. Conclusion

The MRSA isolates were community acquired and therefore no risk factor for colonisation was observed. Accurate and early detection of these strains in hospitals and community is encouraged. Also, a systematic review of isolation policies in the hospitals management of MRSA coupled with increased compliance with hand hygiene recommendation could lead to a significant reduction in MRSA.

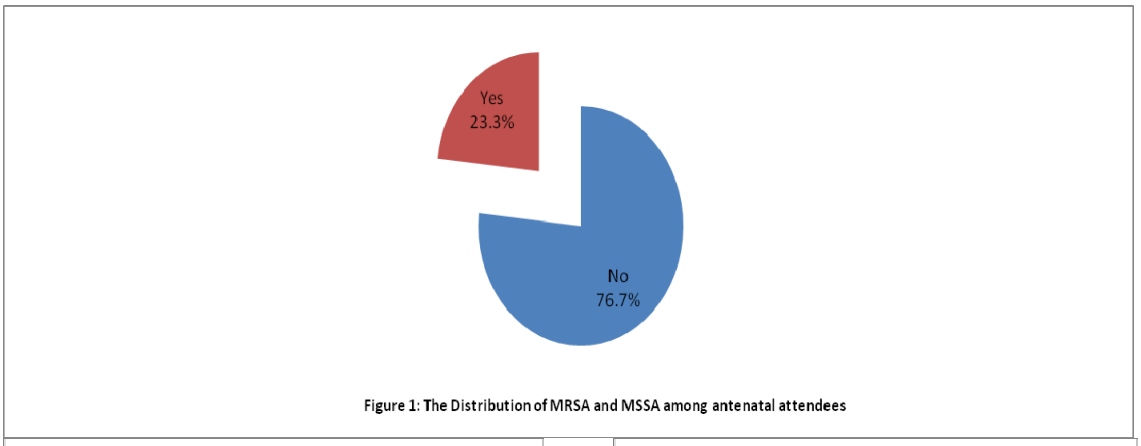

Figure 1: The Distribution of MRSA and MSSA among antenatal attendees

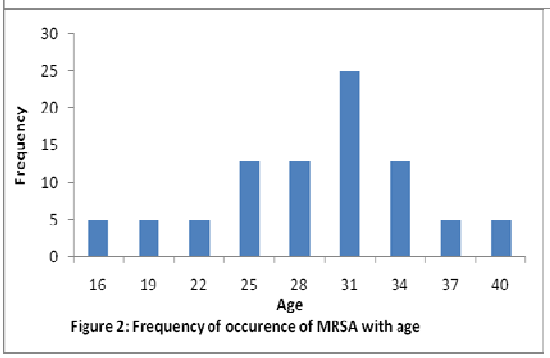

Figure 2: Frequency of occurence of MRSA with age

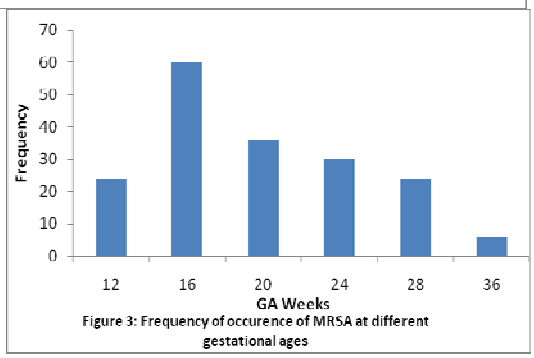

Figure 3: Frequency of occurence of MRSA at different gestational ages

Risk factor		Frequency	Percentage (%)
Previous Hospital Admissions	none	156	86.7
	Last 3 months	18	10.0
	Last 12 months	6	3.3
Clinic Visit	None	144	80
	Last 3 months	24	13.3
	Last 6 months	12	6.7
Antimicrobial use history	none	174	96.7
	Last 3 months	6	3.3

Table 2: Susceptibility pattern of MRSA isolates

Antibiotics	Frequency	Susceptibility (%)
Ciprofloxacin	114	63.3
Cotrimoxazole	84	46.7
Amoxycillin-clavulanic acid	138	76.7
Erythromycin	120	66.7
Clindamycin	144	80.0
Ceftriazone	125	69.4

Table 3: Frequency of Inducible Microlide streptogramin B

Antibiotics	Frequency (R)	Percentage (%)
Ciprofloxacin	64	35.6
Cotrimoxazole	93	51.7
Amoxycillin-clavulanic acid	53	29.4
Erythromycin	56	31.1
Clindamycin	36	20.0
Ceftriazone	53	29.4

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