Carriage and Resistance Pattern of Methicillin Resistant Staphylococcus aureus among Pregnant Women Attending an Ante- Natal Clinic in Uyo – Nigeria

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ABSTRACT

The study was designed to investigate the carriage and resistance pattern of Methicillin resistant *Staphylococcus aureus* among pregnant women attending ante-natal clinic at the university of Uyo medical centre in Nigeria. Seventy (70) urine samples collected from pregnant women were screened for *Staphylococcus aureus* using standard microbiological techniques. The isolates were then subjected to antibiotic susceptibility testing using the disc diffusion method during which resistance to 30µg cefoxitin was used as a yardstick for Methicillin resistance. Results obtained showed that 50 (71%) of the urine samples were positive for *Staphylococcus aureus* and 21 (42%) of them were resistant to 30µg cefoxitin confirming them phenotypic MRSA isolates. Results obtained further showed that the MRSA isolates were resistant to norfloxacin (100%), ampicillin/cloxacin (81%), chloramphenicol (62%) but showed low resistance to levofloxacin (19%) and streptomycin (19%). Results obtained further confirmed that 76% of the MRSA isolates were multi- drug resistant and may have been contracted from the environment where they reside before coming to hospital. It is concluded that methicillin resistant *Staphylococcus aureus* (MRSA) carriage is still high among pregnant women in Uyo-Nigeria and the implications are highlighted.

Key words: Carriage, Resistance, *Staphylococcus aureus,* Cefoxitin, Multi- drug resistance

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) are certain isolates of *Staphylococcus aureus* which have acquired genes encoding resistance to methicillin and all other penicillins. This resistance is conferred by the Mec A gene that encodes a penicillin binding protein PBP2A that has a decreased affinity for β-lactam antibiotics (Udobi et al, 2013). They cannot be controlled with the conventional β- lactams and have continued to pose a lot of problems in clinical settings (Gengroug et al, 2010). MRSA has remained the most recognized multi drug resistant pathogen globally and they have been found in increasing numbers in various infections where they continue to cause various therapeutic problems (Grundman et al, 2006). Generally, *Staphylococcus aureus* is both a commensal and a pathogenic organism. While the anterior nares are the main ecological niche of *Staphylococcus aureus*, so many other sites in man can also be colonized (Rachael and Franklin, 2008). Through these sites, the organisms can easily invade other areas when the immune system is compromised. The female genital tract is one part of the body that can be conveniently colonized by *Staphylococcus aureus* from where it can then be passed to the new born through the mother's birth canal.

The carriage rate of methicillin resistant *Staphylococcus aureus* in Nigeria is becoming worrisome. Even though it has been the trend to regularly investigate the antibiotic susceptibility pattern among pregnant women worldwide, not much data is available for Nigeria. This is a thing to worry about since most pregnant women who carry this organism may just remain carriers but are 'willing' to transfer the organisms to their new born.

This study was therefore designed to ascertain the carriage and resistance pattern of methicillin resistant Staphylococcus aureus among pregnant women attending ante-natal clinic at the University of Uyo medical centre in Akwa-Ibom state Nigeria.

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METHODOLOGY

Urine Collection

A total of seventy (70) urine samples were collected from pregnant women attending ante-natal clinic at the University of Uyo medical centre. A lot of these women are known to come from the adjoining local communities along Ikpa road. All the samples were collected in sterile urine bottles and taken immediately upon collection to the laboratory for examination.

Isolation and confirmation of Staphylococcus aureus

Mannitol salt agar prepared according to the manufacturer's instruction was used. Collected urine samples were inoculated unto mannitol salt agar and incubated for 24 hours at 37ºC. Where growth was observed, they were Gram stained and their morph ological characteristics observed. The catalase and coagulase tests as described by cheesebrough (2000) were also employed for further confirmation.

Identification of Methicillin Resistant Staphylococcus aureus (MRSA)

All confirmed *StaphIylococcus aureus* isolates were tested for their methicillin resistance ability. This was done by testing their sensitivity or otherwise to 30µg cefoxitin according to the recommendations of (CLSI). A suspension of the isolates was inoculated unto appropriate agar and inoculated at 37ºC. Inhibition zones were measured and interpreted using the CLSI (2008) interpretative criteria for methicillin resistance in *Staphylococcus aureus*.

Susceptibility Profile and Multiple Antibiotic Resistance (MAR) Index

The susceptibility profile of the confirmed *Staphylococcus*. *aureus* and MRSA isolates against a range of antibiotics commonly used in the hospital was studied. This was done using the Kirby Bauer disc diffusion method and the results obtained were interpreted using the CLSI (2008) interpretative criteria for *Staphylococcus aureus*. The Multiple Antibiotic Resistance (MAR) index which is a comparison of the number of antibiotics the organism is resistant to with the total number of antibiotics tested was thus determined for each of the confirmed MRSA isolates.

RESULTS

Specimen collection and Staphylococcus aureus identification

Fifty (50) out of the seventy (70) fresh urine samples aseptically collected from pregnant women attending University of Uyo medical centre were positive for *Staphylococcus aureus*

Antibiogram

Antibiogram results showed that fourty four (44) of the fifty (50) positive S. *aureus* isolates showed highest resistance (88.0%) to Ampicillin/Cloxacillin (20µg) while the least resistance of 10.0% was shown by 5 isolates to Streptomycin. (Table1).

Table1: Summary of the antibiogram for the resistance pattern of fifty (50) confirmed *Staphylococcus aureus.*

	Number of Isolates			
Antibiotic	Highly Susceptible	Moderately Susceptible	Resistant	Percentage resistant
Ciprofloxacin (10µg)	23	8	19	38.0
Norfloxacin (10µg)	2	5	43	86.0
Gentamycin (10µg)	16	16	18	36.0
Amoxicillin (20µg)	18	10	22	44.0
Streptomycin (30µg)	42	3	5	10.0
Rifampicin (20µg)	19	19	12	24.0
Erythromycin (30µg)	20	6	24	48.0
Chloramphenicol (30µg)	14	10	26	52.0
Ampicillin/Cloxacillin(20µg)	3	3	44	88.0
Levofloxacin (20µg)	38	6	6	12.0

Identification and antibiogram of Methicillin Resistance Staphylococcus aureus

Results obtained showed that out of fifty (50) isolated *Staphylococcus aureus* samples, twenty-one (42%) were resistant to cefoxitin (30µg) according to the interpretative scheme of CLSI (2008) and this confirmed them MRSA (Phenotypically). Antibiogram results showed that four (4) isolates (19%) out of these were resistant to Streptomycin while all the isolates (100%) were resistant to norfloxacin (Table 2).

Table 2: Summary of antibiogram of confirmed MRSA isolates

Antibiotic	Highly Susceptible	Moderately Susceptible	Resistant	Percentage resistant
Ciprofloxacin (10µg)	8	5	8	38.0
Norfloxacin (10µg)	0	0	21	100
Gentamycin (10µg)	4	5	12	57.1
Amoxicillin (20µg)	4	5	12	57.1
Streptomycin (30µg)	17	0	4	19.0
Rifampicin (20µg)	5	7	9	42.8
Erythromycin (30µg)	7	3	11	52.3
Chloramphenicol (30µg)	4	4	13	61.9
Ampicillin/Cloxacillin(20µg)	2	2	17	80.9
Levofloxacin (20µg)	14	3	4	19.0

Multiple Antibiotic Resistance (MAR) Index

Results of the multiple antibiotic resistance index showed the varying resistance pattern of the 21 isolates of confirmed Methicillin Resistant *Staphylococcus aureus* to ten (10) antibiotics surveyed. Out of the 21 isolates, sixteen (16) (76.1%) had Mar index greater than 0.3 showing that such high percentage were resistant to more than three (3) of the ten (10) antibiotics tested. (Table 3)

Table 3: Resistance pattern of 21 confirmed MRSA to commonly used antibiotics at the University of Uyo Medical centre.

Organism	Resistant Antibiotics	No of resistant antibiotics out of ten	Multiple Antibiotic Resistance (MAR) Index	Resistance Classification (Magiorakos et *al*, 2012)
A1	NB,AML,RD, E,CH,APX	6	0.6	MDR
A2	NB,CH,APX	3	0.3	MDR
A3	NB	1	0.1	------
A4	CPX,NB,CN,AML,S,RD,E,CH,APX,LEV	10	1.0	MDR
A6	CPX,NB,CN,AML,S,RD,E,CH,APX,LEV	10	1.0	MDR
A8	NB, CN,E	3	0.3	MDR
A10	CPX,NB,AML,CH,APX	6	0.6	MDR
A12	NB,APX	2	0.2	NIL
A17	NB, CN,APX	3	0.3	MDR
B2	NB	1	0.1	-------
B4	NB,CN,APX	3	0.3	MDR
B8	NB,CPX,CN,AML,E,CH,APX	7	0.7	MDR
B19	NB, AML,CH,APX	4	0.4	MDR
1C	NB,CN,AML,APX	4	0.4	MDR
2C	NB,CPX, CN,AML,RD, E, CH, APX	8	0.8	MDR
3C	CPX,NB,CN,AML,E,CH, APX, LEV	8	0.8	MDR
4C	NB, CN,AML,S,RD,E,CH,APX	8	0.8	MDR

10C	CPX,NB,CN,AML,S,RD,E,CH,APX	9	0.9	MDR
11C	NB,CN,AML, RD, E, APX	6	0.6	MDR
12C	NB,CN,RD,E,CH,APX,S	7	0.7	MDR
13C	CPX,NB,RD,CH,APX	5	0.5	MDR

DISCUSSION

With the development of resistance to a wide range of antibacterial agents being reported by scientists across the globe, the continuous monitoring of the carriage and antibiotic resistance pattern of the known problematic MRSA which has continued to present a great deal of therapeutic difficulties recently remains expedient. Methicillin resistant *Staphylococcus aureus* is an increasingly prevalent pathogen found in both community and hospitalized patients where they present a lot of therapeutic difficulties. Even though the detection of the MecA gene has been the gold standard for the confirmation of MRSA, the cefoxitin (30µg) disc diffusion test has been reported to be in concordance with the PCR for MecA gene and has thus been an acceptable alternative for the detection of MRSA (Swenson et al, 2005; Mimica et al, 2007; Anand *et al*, 2009; Uzun et al, 2013). In this study, the cefoxitin disc diffusion method was effectively used for the identification of MRSA. Though the anterior nares are known to be the main ecological niche of most S *aureus*, so many other sites in man can also be colonized. Through these sites, they can invade other areas especially when the immune system is compromised (Rachael and Franklin, 2008). Generally, the female genital tract is one part of the body that can be colonized by *Staphylococcus aureus* from where it can conveniently be passed to the new born through the mother's birth canal. For instance, it has been suggested that infants born to mothers who are colonized by *Staphylococcus aureus* are more likely to be colonized as neonates through early post natal acquisition (Jimenez *et al*, 2012).

Results obtained from this study showed that 50(71.49%) of the seventy (70) urine samples were positive for *Staphylococcus aureus* (Table 1). Results obtained further showed that 21 (42%) of the confirmed *Staphylococcus aureus* were resistant to 30µg cefoxitin and were therefore considered Methicillin resistant *Staphylococcus aureus* (CLSI, 2008). The high carriage of MRSA (42%) observed in the study has similarities to results obtained in other areas of Nigeria such as Jos by Ikeh et al, 2003 where a prevalence of 43% was reported from University of Jos Teaching hospital and that obtained by Taiwo *et al*, (2005) in Ilorin-Nigeria where a prevalence of 34% was reported from the university of Ilorin teaching hospital. If such a high carriage rate is still being reported, it confirms that MRSA continues to remain a menace in many areas of Nigeria. This high carriage may be associated with the low economic status of the women as the medical center is highly patronized by the local of the university (Ikpa road) which is a rural community. The results of the multiple antibiotic resistance index obtained in this study seem to confirm this. In it, sixteen (16) (76.1%) of the 21 isolates had MAR index greater than 0.3 suggesting that the resistant organisms were picked up from an environment like Ikpa road where these antibiotics are likely to be used and abused frequently.

The antibiogram confirmed the MRSA isolates multi-resistant with the organisms showing to be resistant to a wide range of the organisms tested (Table 3). Strains of MRSA are known to contain factors which enhance their virulence, enabling them to cause particular clinical syndrome or enhance their multi drug resistance ability. While only levofloxacin showed 66.7% susceptibility, the other floroquinolones like norfloxacin, ciprofloxacin was not of much use in the control of the organisms. Our previous published work (Udobi *et al*, 2013) had shown that ciprofloxacin showed better potentials in the control of MRSA than now. This may be among many other reasons because these floroquinolones are the older and cheaper members of the group which may be more prone to abuse. Whatever may be the reasons, these organisms are certainly gaining more grounds and scientists need to do more work in combating them. We are currently working on a possible combination therapy using quinolones and certain plant metabolic products which have been reported to potentiate the activity of certain antibiotics (Oluwatayo *et al*, 2017).

In conclusion results obtained from this work showed that MRSA continues to be a menace as the carriage rate obtained compares with that obtained in different parts of the country before now. The high MAR index obtained shows that the socio- economic and educational status of a community plays a significant

role in this problem too. The Government must therefore work hard concerning improving the economic and educational status of the people. This work further confirms that there are no longer any lines of separation between hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA) as both now show the same attributes initially believed to be the preserve of HA-MRSA.

REFERENCES

Anand, K. B; Agrawal, P; Rumar, S and Kapila, K (2005). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. *Indian Jour. Med. Microbiol.* 22(1) 27-29.

Cheesbrough, M. (2000). District laboratory practice manual in tropical countries part 2. Cambridge University press, Cambridge, 178-179.

Clinical Laboratory Standard Institute (CLSI) (2008). Performance standard for antimicrobial susceptibility testing, 15th International supplement, wayne.

Gengrong, Lin; Lois, M. Ednie and Peter, C. Appelbum (2010). Antistaphylococcal activity of ACHN-490 tested alone and in combination with other agents by time kill assay. *Antimicrob. Agents chemother*. 54(5): 2258–2261.

Grundman, H; Aires-de-souza, M; Boyce, J and Tiemersma, E. (2006). Emergence and resurgence of methicillin resistant *Staphylococcus aureus* as a public health threat, *Lancet* (368) 874-885

Ikeh E. C. (2003). Methicillin resistant *Staphylococcus aureus* at Jos University Teaching Hospital, *Afri. Journ Clin. Exper. Microbiol.* 4, (1), 52–55.

Jimenenz- Iruque, N; Tedeschi, S;Soye, E. J; Mekenna, B. O; Langdon, W; Wright, J. P; Alsentzer, A; Arnod, S; Seville, B. R; Wang, W; Thomsen, I and Creach, C. B (2012). Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Paediatrics,* 129 (5): 1252-9.

Mimica M. J; Berezin E. N; Carvalho R.L.B[I]; Mimica I.M; Mimica L.M.J; Sáfadi M.A.P; Schneider E and Caiaffa-Filho H.H. (2007). Detection of methicillin resistance in *Staphylococcus aureus* isolated from pediatric patients: is the cefoxitin disk diffusion test accurate enough. *Braz. J. Infec.t Dis.* 11 (4)

Oluwatayo, E. Abioye; David, A Akinpelu and Anthony I. Okoh. (2017). Synergistic effects of n-hexane fraction of *Parkia biglobosa* (Jacq) bark extract and selected antibiotics on bacterial isolates. Sustainability 9, 228; doi:103390/su9020228.

Rachael, J. Gordon and Franklin, D. Lowy (2008). Pathogenesis of methicillin resistant *Staphylococcus aureus* infection. *Clin. Infect. Dis*. 46(5) 350-359

Taiwo, S. S; Bamidele, M; Omonigbehin, E.A; Akinsinde, K.A; Smith S.I; Onile, B. A and Olowo, A, O. (2005). Molecular epidemiology of methicillin resistant *Staphylococcus aureus* in Ilorin, Nigeria. *West Afr. Journ. Med*. 24 (2) 100-106.

Udobi, C.E; Obajuluwa, A. F and Onaolapo, J. A. (2013). Prevalence and antibiotic resistance pattern of methicillin resistant *Staphylococcus aureus* infection from an orthopaedic hospital in Nigeria. *Biomed Res. Journ*. (2013) I D 860467.

Swenson J.M., Tenover F.C. (2005) Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* Spp. *J Clin Microbiol*;43:3818-23.

Uzun B, Karataş Şener AG, Güngör S, Afşar I, Yüksel Ergin O, Demirci M.(2013). [Comparison of Cefoxitin Disk Diffusion Test, Automated System and Chromogenic Medium for Detection of Methicillin Resistance in *Staphylococcus aureus* Isolates]. *Mikrobiyol Bul.* ; 47(1):11-18.