Correlation of Hemolysin Production and Multi-Drug resistant Phenotype among Methicillin-Resistant Staphylococci Species

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ABSTRACT

Methicillin-resistant Staphylococci are shown to cause various forms of infections in human with fatal consequences on the health and economy of the patient. This study is aimed at determining the prevalence and antibiotic resistance pattern of methicillin resistant Staphylococci in relation to hemolysin production. A total of 100 clinical samples were screened for Staphylococci spp by standard method. Isolates obtained were tested for methicillin-resistance and susceptibility to other antibiotics by standard procedure. A total of 30 Staphylococcus spp comprising 18 S. aureus and 12 Coagulase-Negative Staphylococci (CoNS) where obtained. Twenty-seven (90%) of the Staphylococci spp were methicillin resistant comprising 17(94.4%) MRSA and 10(83.3%) MRCoNS. Thirteen (13) resistance profiles were exhibited by MRSA out of which 9 were MDR, while 9 resistance profiles were shown by MRCoNS out of which 5 were MDR. There isa significant correlation in antibiotic resistant phenotype between MRSA and MRCoNS (P=0.01). The result also revealed that 5 and 12 MRSA were -hemolytic and -hemolytic respectively, whereas 2 and 8 MRCoNS were -hemolytic and -hemolytic respectively. The high prevalence rate of methicillin resistance coupled with high multi-drug resistance phenotype among Staphylococcus spp in the study area is alarming. There is therefore need for periodic or regular surveillance of MRSA and MRCoNS infections not only in the hospital settings but also in the communities.

Keywords: Methicillin, Resistance, Hemolysin, Staphylococci

INTRODUCTION

Staphylococci are Gram-positive, non-motile, non-spore forming, facultatively anaerobic, spherical bacteria with inherent ability to breakdown carbohydrates, producing peculiar characteristic colour (white to deep yellow) on culture media. Species are classified as coagulase positive (e.g. *Staphylococcusaureus)* and coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*and *Staphylococcus-saprophyticus*.). Fermentation of mannitol and deoxyribonuclease (DNase), catalase and coagulase enzymes is often used for their identification[1]. The ability of *Staphylococcus* spp to caused infection is usually associated with the presence of some virulence factors such as hemolysins, toxic-shock syndrome toxin, coagulase, enterotoxins, exfoliatins, Panton-Valentine leukocidin, protein A and capsular polysaccharide.[2]The high incidence of antibiotic resistance among *Staphylococci spp*in humans has been associated with increased cost of healthcare, coupled with a huge burden of disease among different populations. Among the antibioticresistant strains of *S. aureus,* increased attention has been devoted to the methicillin-resistant *S. aureus* (MRSA) due to its significance in clinical environments globally.[3] Resistance to this class of antibiotics is usually mediated by mecA gene which encodes for the production of penicillinbinding protein 2A (PBP-2A) mediating resistance to all penicillins including methicillin and resulting in reduced affinity for binding betalactam antibiotics.[4]Apart from resistance to methicillin or its derivatives, MRSA also exhibits multi-drug resistant (MDR) phenotype[4] and a low-level resistance to vancomycin;[5] this is a major call for concern. This is possible because the mecA gene complex is said to contain insertion sites for plasmids and transposons that facilitate the acquisition of resistance to other antibiotics.[6] Consequently, studies have shown that methicillin-resistant Staphylococci are often

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associated with infections which hardly respond to therapy.[7] They are also implicated in sepsis, endocarditis and neonatal meningitis.[8]

Risk factors for the acquisition of hospital-acquired methicillin-resistant Staphylococci include prolong usage or abuse of antibiotics, prolonged hospitalization, parenteralfeeding, nasal carriage of MRSA, direct or indirect contact with an infected individuals or materials, regular exposure to clinical specimens without adequate preventive measures, crowded and unhygienic living conditions, compromised immune system and underlying chronic illness amongst others.[9] A study showed that the economy and health loss dueto methicillin-resistant Staphylococci infection is far greater than the one caused by methicillin-sensitive *Staphylococcus spp.*[10] In Africa, the frequency of MRSA is low and changes from place to place.[11] Prevalence rates between 10-49% has been reported in some Africa countries.[9]

Studies have shown that the pathogenicity of Staphylococci spp is enhanced by a variety of virulence factors, one of which is hemolysin. Staphylococci spp is reported to produce different types of hemolysin which include , and - hemolysin.[12]

Gamma haemolysin cannot haemolyse human or rabbit red blood cells, unlike - and -haemolysins.[13] Surprisingly, some studies have associated hemolysin production with increased virulence and antibacterial resistance.[13, 14]

This study therefore reports the prevalence and susceptibility pattern of methicillin-resistant Staphylococci and their resistance pattern in relation to hemolysin production.

MATERIALS AND METHODS

Study area

Mubi metropolis comprises of two local government areas; Mubi North and Mubi South lies between latitudes 10° 05' and 10° 30'N of the equator and between longitude 13° 12'E and 13° 19'E of the Greenwich meridian. The area shares boundaries with Maiha, Hong, Michika Local Government Areas (LGA) in Nigeria and the Cameroun Republic to the south west and east respectively. Majorly, Mubi is made up of the following ethnic groups; Gude, Njanyi, Kilba, Fali, Higgi and Margi.[15]

Sample population

A total of 100 clinical samples were collected randomly from patients attending Mubi general hospital and New life medical clinic from May 2017 to August 2017. The clinical samples include urine (68), high vaginal swab (14), wound swab (7), sputum (1), semen (1), ear swab (1) and stool (8).

Ethical consideration

Verbal inform consent was obtained from both hospital managements and patients.

Identification of *S. aureus*

Standard procedure was used to identify Staphylococci isolates. This was based on their ability to grow and utilised Mannitol in Mannitol salt agar (MSA) medium, morphological characteristics, reaction to Gram staining, coagulase and catalase tests.[16]

Hemolysin production:

Hemolysin production was detected using blood agar. All bacterial isolates were grown on blood agar (Nutrient agar supplemented with sheep erythrocyte) and incubated at 37°C for 24 hours. The organisms were classified as either , or -hemolytic. Detectionof clear zone around the colonies was taken as -hemolysis (complete lysis of RBC). The presence of a halo (greenish colouration) around the bacteria growth was taken as -hemolysis (partial hemolysis), while -haemolysis was recorded when there was normal growth without changes in the culture medium (no hemolysis).

Antibiotics Susceptibility Testing

Susceptibility of the isolates to antibiotics was carried out by modified Kirby-Bauer disc diffusion method based on CLSI procedure and guidelines for reading zone of inhibition interpretative table.[17] The antibiotic discs used include; perfloxacin(10ìg), gentamycin (10 g), ampiclox (30 g), cefuroxime (20 g), amoxicillin (30 g), ceftriaxone (25 g),

ciprofloxacin (10 g), streptomycin (30 g), cotrimoxazole (30 g) and erythromycin (10 g).

Phenotypic Detection of Methicillin-Resistant *Staphylococcus* spp

Methicillin-resistant *Staphylococcus* spp isolated from clinical samples were detected phenotypically using 1 g oxacillin disc CT0159B (Oxoid, UK). Approximately 0.1ml of 0.5 McFarland standard *Staphylococcus* spp was inoculated onto Mueller-Hinton Agar (MHA) plates. Commercially available oxacillin disc was placed on the plate of Mueller-Hinton agar and incubated aerobically at 37°C for 24 hrs. Zone of inhibition ≤12mm was interpreted as methicillin-resistant, while inhibitory zone ≥13mm was interpreted as methicillin-sensitive.[12]

Statistical analyses:

Bivariate correlation was used to determine the association in antibiotic resistance between MRSA and MRCoNS and also between -hemolytic and -hemolytic MRCoNS. More so, Non-Parametric Mann-Whitney statistics was used to determine the level of significance in MDR phenotype between -hemolytic and -hemolytic methicillin-resistant Staphylococci spp. All statistical analyses were carried out using the SPSS 17.0 Windows based program. Significant difference and Non-significant difference was defined when p=0.05 and p>0.05 respectively.

RESULTS

The result showed that 30 staphylococcal spp were isolated from the 100 clinical samples screened; of these, 18(60%) were *Staphylococcus aureus* while 12(40%) were coagulase-negative staphylococci (CoNS). *S.aureus* was mostly isolated from urine (38.9%) followed by HVS (33.3%) and wound swab (27.8%). Whereas CoNS were isolated mostly from urine (75%) and HVS (25%) (Table 1).

Table 2 showed the prevalence of methicillin resistance among the isolated staphylococci spp. A total of 27(90%) Staphylococci spp were observed to be methicillin-resistant. This included17(94.4%) MRSA and 10(83.3%) MRCoNS. There is a significant correlation in antibiotic resistance phenotype between MRSA and MRCoNS (P=0.01).

The hemolysin production and multi-drug resistant (MDR) phenotype among methicillin-resistant staphylococci spp. is shown in Table 3. Thirteen (13) resistance profiles were exhibited by MRSA of which 9 are MDR. In the same vein, 9 resistance profiles were shown by MRCoNS out of which 5 are MDR. The result also revealed that 5 and 12 MRSA are -hemolytic and -hemolytic respectively. More so, 2 and 8 MRCoNS are -hemolytic and -hemolytic respectively. The MDR phenotype is significantly higher in -hemolytic than -hemolytic methicillin-resistant staphylococci spp. (P=0.001).

Antibiotic resistance pattern in -hemolytic MRCoNS is significantly higher than -haemolytic MRCoNS (P=0.030). However, the antibiotic resistance pattern in -haemolytic MRSA is significantly higher than that of -hemolytic MRSA (P=0.006).

Table 1: Frequency of Staphylococci spp from clinical Samples

SN	Specimen	Frequency	*S. aureus*(%)	CoNS (%)
1.	Urine	68	7(38.9)	9(75.0)
2.	High Vagina swab	14	6(33.3)	3(25.0)
3.	Wound swab	7	5(27.8)	-
4.	Sputum	1	-	-
5.	Semen	1	-	-
6.	Ear swab	1	-	-
7.	Stool	8	-	-
	Total	100	18(60)	12(40)

Legend: CoNS: coagulase negative staphylococci

Table 2: Prevalence of methicillin resistance among Staphylococci spp

Sex	*S.aureus*			CoNS		
	No. tested	MRSA (%)	MSSA (%)	No. Tested	MRCoNS (%)	MSCoNS (%)
Male	5	5(100)	0	3	3(100)	0
Female	13	12(92.3)	1(7.7)	9	7(78)	2
Total	18	17(94.4)	1(5.6)	12	10(83.3)	2

Table 3: Resistance profile of Methicillin Resistance Staphylococci spp based on hemolysin production

Organisms	No. of resistance profile	Resistance Profile	No. of isolates -hemolytic	-hemolytic	No. of antibiotics
MRSA	1	pef, cn, apx, cxm, am, cro, cip, s, sxt, e	2	2	10
	2	pef, cn, apx, cxm, am, cro, s, sxt, e	0	1	9
	3	pef, cn, apx, cxm, am, cro, cip, sxt, e	0	1	9
	4	pef, cn, apx, am, cro, cip, s, sxt, e	1	0	9
	5	pef, cn, apx, cxm, am, cro, sxt, e	1	1	8
	6	pef, apx, cxm, am, cro, sxt, e	0	1	7
	7	apx, cxm, am,sxt	1	0	4
	8	apx, cn, am, sxt	0	1	4
	9	am, sxt, e	0	1	3
	10	am, cip, s	0	1	3
	11	apx, am	0	1	2
	12	am, sxt	0	1	2
	13	Am	0	1	1
		Total	**5**	**12**	
MRCoNS	1	pef, cn, apx, cxm, am, cro, cip, s, sxt, e	0	2	10
	2	pef, cn, apx, cxm, am, cip, s, sxt, e	0	1	9
	3	pef, cn, apx, cxm, am, cro, cro, e	0	1	8
	4	pef, cn, apx, am, s, sxt, e	0	1	7
	5	apx, cxm, am, cro, s	0	1	5
	6	cn, am, cro	1	0	3
	7	cn, sxt, e	1	0	3
	8	cn, apx, am	0	1	3
	9	Am	0	1	1
		Total	2	8	

Legend: pef =perfloxacin, cn=gentamycin, apx=ampiclox, cxm=cefuroxime, am=amoxicillin, cro=ceftriaxone, cip=ciprofloxacin, s=streptomycin, sxt=cotrimoxazole, e=erythromycin

Table 4: Resistance pattern of Methicillin Resistance Staphylococci sp

Antibiotic	MRSA (%)[a] N= 17	MSSA N = 1	MRCoNS (%)[a] N= 10	MSCoNS N = 2
Perfloxacin	10(59)	-	5(50)	1(50)
Gentamycin	10(59)	-	8(80)	1(50)
Ampiclox	13(76)	-	7(70)	1(50)
Cefuroxime	9(53)	-	5(50)	-
Amoxicillin	17(100)	1(100)	9(90)	2(100)
Ceftriaxone	11(65)	-	5(50)	-
Ciprofloxacin	7(41)	-	3(30)	-
Streptomycin	7(41)	-	5(50)	-
Cotrimoxazole	14(82)	-	6(60)	-
Erythromycin	11(65)	-	6(60)	1(50)

[a]= correlation is significant (P=0.01)

Table 5: Resistance pattern of Methicillin Resistance Staphylococci sp based on Hemolysin production

Antibiotic	MRSA (%)		MRCoNS (%)	
	-hemolytic[a] (n=5)	-hemolytic[b] (n=12)	-hemolytic[c] (n=2)	-hemolytic[d] (n=8)
Perfloxacin	4(80)	6(50)	0	5(63)
Gentamycin	4(80)	6(50)	2(100)	6(75)
Ampiclox	5(100)	8(67)	0	7(88)
Cefuroxime	3(60)	6(50)	0	5(63)
Amoxicillin	5(100)	12(100)	1(50)	8(100)
Ceftriaxone	5(100)	6(50)	1(50)	4(50)
Ciprofloxacin	3(60)	4(33)	0	3(38)
Streptomycin	3(60)	4(33)	0	5(63)
Cotrimoxazole	5(100)	9(75)	1(50)	5(63)
Erythromycin	4(80)	7(58)	1(50)	5(63)

a and b (statistics is significant, (P=0.006), c and d (statistic is significant, P=0.030).

DISCUSSION

The finding that Staphylococci spp is mostly isolated from urine as shown in this study is in conformity with previous studies.[18] In contrast, others opined that wound swab harbours more Staphylococci spp than other clinical samples.[19,20]

Quite a number of recent studies have shown that *S.aureus* is the causative agent of many infections in Nigeria involving the bloodstream, ear, skin and lower respiratory tract including many other infections which are difficult to treat.[4,21] On the other hand, Coagulase-negative Staphylococci (CoNS) which were previously regarded as either contaminants[22] or normal flora are now recognized as a major cause of significant clinical infections. They are associated with infections in the immune compromised host, bacteremia, wound-related infections, intravascular catheter-related infections and a variety of post-operative infections.[23]

Data from previous studies have shown that methicillin resistance among *Staphylococcus* spp is on the increase and constitute health challenges not only in Africa but also in Europe, America and Asia.[24] Due to the ability of Staphylococci to mutate over time; methicillin-resistant *Staphylococcus* spp will continue to constitute nuisance both in the hospital and community settings. The high rate of resistance to methicillin (Oxacillin) by *S. aureus* and CoNS isolates from clinical samples in this study area with no previous reasonable report of MRSA and MRCoNS prevalence, is alarming but not unexpected because it has been reported that MRSA prevalence is ever increasing.[25] This is similar toearlier reports from Ota, Ogun state in which 94-100% *S. aureus* isolated were reported to be methicillin-resistant.[26] The high prevalence rate shown in this study is also comparable to report in Lagos where 85% of *S. aureus* were methicillin resistant and multi-drug resistant.[27] Another study in Benin-city demonstrated that 79% of *S. aureus* isolates were methicillin resistant.[19] Similarly, MRCoNS prevalence rates of 82.4 % and 83.3%[28] were reported in conformity with the findings of this study.

High MRCoNS prevalence rate comparable to this study were previously reported as 87%[23] and 90%.[29] Contrary to the findings of this study, several previous studies in Nigeria have reported a lower prevalence rate of MRSA. These include69% prevalence rate reported from Zaria,[6] 47.8% prevalence rate reported from Osogbo,[30] 38.5%,[31] 35.7%,[12] 33.3%,[24] 30.4%,[20] and 11%[32] prevalence rates reported from Enugu, Uyo, Jimeta-Yola, Ibadan and Benin-city respectively. Similarly, the low prevalence

rate of 33.3%[12] for MRCoNS was also reported in Uyo, Nigeria. It is evident in this study that the proportion of *S.aureus* resistant to methicillin (94.4%) were more than that of *CoNS (83.3%) as shown by their prevalence rate*. This is contrary to the previous finding from Iran[10] *which revealed that CoNS are more resistant to methicillin than S.aureus.* Although the antibiotic resistant pattern of MRSA in developing countries is not uniform but varies from one country to the other, various reports have indicated that MRSA exhibits extreme resistance to other antibiotics in addition to methicillin.[33] Previous studies have also shown that methicillin-resistant *S.aureus* (MRSA) of clinical origin exhibits multi-drug resistant (MDR) phenotype.[18,27] This is in agreement with the findings of this study which demonstrated high MDR phenotype among MRSA and MRCoNS. Relatively high MDR MRSA has also been reported in some African countries.[32]High MDR phenotype in this study indicates the presence of strong selective pressure from antibiotics use in this community. Also, therapy associated with MDR strains are usually problematic and incurred huge financial drain on the hospital resources.[34] MDR was considered when an organism was non-susceptible to at least one agent in three or more antimicrobial categories or class.[35] The study further showed that MRSA and MRCoNS have high resistance to amoxicillin, ampiclox, ceftriaxone and cefuroxime which are -lactam antibiotics. This is comparable to an earlier report from Ogun State,[27] Delta State[36] and Ibadan[20] where it was reported that MRSA were also resistant to all antibiotics of -lactam class.[6] This may be due to the *mec*A gene, by a unique mobile genetic element, staphylococcal cassette chromosome *mec* (SCC*mec*) integrated into the *S.aureus* chromosome.[37]This observation can be correlated to commonly used and unauthorized prescription of these antibiotics. Also, exposure of isolates to these drugs enhances the development of high level of resistance.

The study demonstrated that both MRSA and MRCoNs are also resistant to classes of antibiotics other than the -lactam antibiotics which includes; perfloxacin, gentamycin, erythromycin, cotrimoxazole and streptomycin in varying proportions ranging from 30%-82%. This is commensurable to previous reports on MRSA and MRCoNS.[26] Contrary to the findings, a previous study in Zaria,[6] Delta[36] and Ibadan Nigeria[20] demonstrated a higher level of MRSA susceptibility to gentamycin and the fluoroquinolones. Thus, the existence of MRSA resistant to classes of antibiotics other than â-lactam antibiotics, in addition to penicillin and cephalosporin may limit the chances for recommending these drugs for therapy in the study area.

The pathogenicity of both *S. aureus* and Coagulase negative *Staphylococci* comes from their produced stock of virulence factors that enhance host's tissues invasion, their spread within the tissues and inhibition of phagocyte engulfment.[38] One of such virulence factors is hemolysin. Detection of and -hemolysin among MRSA and MRCoNS as shown in this study was reported previously[12] and detection of and -hemolysin among *S.aureus* and CoNS has been reported by various authors.[39] But -hemolysin was also reported among both MRSA and MRCoNS[12]unlike this study. Another study revealed that CoNS did not produce -hemolysin[40]which was contrary to the findings of this study. Most of the methicillin-resistant staphylococcal spp are rather -hemolytic (non-hemolytic) than -hemolytic contrary to previous report.[12] In agreement with findings of this study, a number of other studies have associated hemolysin production to antibacterial resistance.[12,13] In this study, lack of hemolysin (-hemolysis) contributed immensely to the antibiotic resistance pattern of MRCoNS, while production of hemolysin (-hemolysis) contributed immensely to the antibiotic resistance pattern of MRSA. This is in agreement with the report of Martinez-Martinez *et al*.[13] with respect to MRCoNS. According to them, antibiotic resistance (especially quinolones) was more on non-hemolytic *E.coli* than haemolytic *E.coli*. Contrary to the finding of this study however, a study from Edo state Nigeria showed that

there was no significant association between haemolysin productions and resistance to the antibacterial agents used in their study.[40] The reasons for this discrepancy are rather obscure but might not be unconnected with the sample size of each genera and the type of genera used. In the studies of Martinez-Martinez *et al.*[13] and Drews *et al.*[14] only one genera of *Escherichia* with a large number of strains was used while various genera of bacteria were used in the study of Egbe and Enabulele.[40] None of the studies used methicillin-resistant *Staphylococcus* spp as employed in this study.

CONCLUSION

The widespread dissemination of MDR phenotype among MRSA and MRCoNs may increase morbidity and mortality and complicate diagnosis. They may also decrease the effectiveness of drugs, increased hospital stay including the cost of chemotherapy and also limit the chances of recommending these drugs for empirical treatment. Therefore, this study underscores the need for periodic surveillance of MRSA infections especially in areas or among people that are at risk. There should be reasonable dispensing of antibiotics based on swift and dependable laboratory test. By and large, there is need to develop and enforce programs, policies and strategies that will underscore the menace of irrational or misuse of antibiotics in both hospital and community settings.

CONFLICT OF INTEREST

Authors have declared that there is no conflict of interest

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