Methicillin Resistant Staphylococcus Aureus And Coagulase Negative Staphylococci Recovered From Clinical Specimens In A Tertiary Hospital In Benin City, Nigeria

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Abstract

Background: Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCoNS) are prevalent worldwide, and are considered the most important cause of nosocomial and community-acquired infections, resulting in increased morbidity and mortality. This study was conducted to determine the prevalence of MRSA and MRCoNS from clinical specimens. **Materials and methods:** A total of 689 consecutive non-repetitive clinical isolates of staphylococci consisting of 566 Staphylococcus aureus and 123 coagulase negative staphylococci were recovered from clinical specimens using standard microbiological techniques. Methicillin resistant was determined by resistance to 30µg cefoxitin discs. Antibiotic susceptibility tests were performed on all isolates using the British Society for Antimicrobial Chemotherapy Method. **Results:** The prevalence of MRCoNS (67.48%) was significantly higher than that of MRSA (56.54%) and statistically significant ($p<0.05$). MRSA were recovered more from umbilical cord (100.00%) and catheter tips (85.71%) than other clinical specimens, while the prevalence of MRCoNS from various clinical specimens did not differ significantly ($P=0.3991$). There was no significant difference ($P > 0.05$) in the susceptibility profiles of MRSA and methicillin-susceptible Staphylococcus aureus (MSSA), as well as MRCoNS and methicillin-susceptible coagulase negative staphylococci (MSCoNS) recovered from in- and out-patients. However, there was statistical significance in the susceptibility of MRSA and MSSA recovered from in-patients to ofloxacin, gentamicin and ceftriaxone ($p<0.05$). Generally, there was no significant difference ($P> 0.05$) in the susceptibility profiles of MRSA and MRCoNS recovered from in- and out-patients. **Conclusion:** The prevalence (58.49%) of methicillin-resistant staphylococci was observed in this study and prudent use of antibiotics to stem the tide of antibacterial resistance is advocated.

Keywords: Coagulase negative, Methicillin resistance, Staphylococci, Clinical specimens, Benin

Introduction

Staphylococcus aureus is a major pathogen both within hospitals and in the community (1). This organism is associated with a variety of clinical infections and variable antibiotic resistant pattern (2). Coagulase negative staphylococci (CoNS) have been identified as the aetiologic agent in various infections and are among the microorganisms most frequently isolated in nosocomial infections (3). Isolates from hospitals and other healthcare settings are often resistant to penicillinase–resistant penicillins, oxacillin or methicillin, and this indicates cross–resistance to all classes of β–lactam antibiotics (4).

Methicillin–resistant staphylococci are a group of staphylococci that are resistant to methicillin – a penicillinase–resistant penicillin (5). Methicillin–resistant Staphylococcus aureus (MRSA) and methicillin–resistance CoNS (MRCoNS) are prevalent worldwide, and are

considered the most important cause of the nosocomial and community–acquired infections, resulting in increased morbidity and mortality (6,7,8,9). MRSA are resistant to different classes of antimicrobial agents, thereby, limiting therapeutic options. Surveillance of MRSA provides relevant information on the extent of the MRSA epidemic, identifies priorities for infection control and the need for adjustments in antimicrobial drug policy (10,11). Therefore, the knowledge about the prevalence of MRSA, MRCoNS and their antibiotic susceptibility pattern has become fundamental in the selection of appropriate treatment (12). There is paucity of data on MRSA and MRCoNS in Benin City, Nigeria. Against this background the study aims to determine the prevalence of MRSA and MRCoNS among clinical isolates of staphylococci. The effect of age and gender of the host on this prevalence will be determined as well as the susceptibility profiles of all recovered staphylococci isolates.

MATERIALS AND METHODS

Study area

The study was conducted in the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Nigeria. The hospital is a tertiary hospital with referral status. All isolates used were recovered from clinical specimens processed in the hospital laboratory.

Bacterial isolates

A total of 689 consecutive non–repetitive clinical isolates of staphylococci consisting of 566 strains of Staphylococcus aureus and 123 CoNS were recovered and used for this study. All isolates were identified using standard techniques (13). An isolate was identified as S. aureus if it was a Gram–positive cocci, catalase positive and coagulase positive. Similar criteria were used for CoNS except that they were coagulase negative.

Detection of methicillin-resistance

Methicillin resistance in both S. aureus and CoNS was indicated by resistance to 30µg cefoxitin discs (Abtek U.K) using the British Society for Antimicrobial Chemotherapy (BSAC) method (14). Briefly, test organisms were emulsified in sterile water and the turbidity matched with 0.5 McFarland standards. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. A 30µg cefoxitin disc was place at the centre of the plates and the plates were incubated at 350C overnight. An isolate was deemed methicillin resistant if the inhibition zone diameter is ≤21mm for both S. aureus and CoNS.

Disc susceptibility testing

Disc susceptibility tests were performed using the British Society for Antimicrobial Chemotherapy (BSAC) method (14).

Data analysis

The data obtained were analyzed with Chi-square (X2) test or fisher's exact test as appropriate and odd ratio analysis using the statistical software INSTAT® (Graph Pad Software, Inc., La Jolla, USA)Results

The prevalence of methicillin resistance was significantly higher in CoNS (67.48%) compared with S. aureus (56.54%) (P=0.0331, Table 1). All the three S. aureus isolates from umbilical cord swabs were MRSA (100%), followed by 85.71% of S. aureus strains recovered from catheter tips while the prevalence of MRSA among S. aureus isolates recovered from pus was the least (16.67%) compared to isolates recovered from other specimens. There was significant association between the prevalence of MRSA among S. aureus recovered from different clinical specimens (p=0.0225). Among CoNS, the prevalence of MRCoNS did not differ significantly (p>0.05) among strains recovered from various clinical specimens (Table 2).

Gender, age and source of isolates (in-patient and out patient) did not significantly affect the prevalence of MRSA and MRCoNS (P > 0.05) (Table 3).

The susceptibility profiles of MRSA, methicillin-susceptible S. aureus (MSSA), MRCoNS and methicillin-susceptible CoNS (MSCoNS) recovered from in– and out– patients are shown in Table 4. There was no significant difference (P > 0.05) in the susceptibility profiles of MRSA and MSSA, and MRCoNS and MSCoNS recovered from in– and out– patients, with the exception of

the susceptibility of MRSA and MSSA recovered from in-patients to ofloxacin, gentamicin and ceftriaxone as well as susceptibility of MRCoNS and MSCoNS recovered from in-patients ($p<0.05$).

Table 5 shows the comparison the susceptibility profiles of MRSA recovered from in– and out–patients; there was no significant difference in the values observed ($P > 0.05$). A similar picture was observed for MRCoNS with the exception that no MRCoNS recovered from in–patients were susceptible to erythromycin compared to the 44.26% recovered from out–patients that were susceptible to erythromycin ($P= 0.0004$).

Table 1: Prevalence of Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant Coagulase negative staphylococci

Organism	No. tested	No. positive for Methicillin Resistance (%)	OR	95% CI	P value
Staphylococcus aureus	562	320(56.94)			
Coagulase negative staphylococci	120	83(67.48)	1.569	1.039,2.371	0.0403

OR = Odd ratio; CI = Confidence interval

Table 2: Distribution of MRSA and MRCoNS from different clinical specimens

Specimens	*Staphylococcus aureus*		Coagulase negative staphylococci	
	No. tested	No. positive for MR (%)	No. tested	No. positive for M (%)
Urine	93	53(56.99)	22	15(68.18)
Seminal fluid	24	13(54.17)	5	4(80.00)
Blood	14	5(35.71)	1	0(0.00)
Sputum	7	5(71.43)	-	-
Wound	144	84(58.33)	39	24(61.53)
HVS/endo-cervical swab	148	82(55.41)	24	10(41.67)
Eye swabs	13	3(23.08)	4	0(0.00)
Ear swabs	46	30(65.22)	11	7(63.64)
Urethral swabs	23	8(34.78)	6	0(0.00)
Oral swabs	5	1(20.00)	-	-
Skin swabs	10	3(30.00)	-	-
Nasal swabs	2	1 (50.00)	3	2(66.67)
Umbilical swabs	3	3(100.00)	-	-
Pus	6	1(16.67)	-	-
Catheter tip	14	12 (85.71)	2	2(100.00)
Aspirates	14	5 (35.71	2	2 (100.0.)

MR = Methicillin–resistant; *Staphylococcus aureus* ($P = 0.045$); Coagulase negative staphylococci ($P = 0.1485$).

Table 3: Effect of age, gender and source of isolates on the prevalence of MRSA and MRCoNS

Characteristics	No. tested	No. positive for MR (%)	OR	95% CI	P value
Staphylococcus aureus					
Gender					
Male	244	140(57.38)	1.032	0.737,1.446	0.9223
Female	318	180(56.60)			
Age (years)					0.0700
≤ 1 – 10	72	36(50.00)			
11 – 20	67	44(65.57)			
21 – 30	108	64(59.26)			
31 – 40	101	57(56.44)			
41 – 50	51	20(39.22)			
51 – 60	25	11(44.00)			
≥ 61	142	83(58.45)			
Source of isolates					
In–patients	159	94(59.12)	1.091	0.752,1.583	0.7163
Out–patients	407	232(57.00)			
Coagulase negative staphylococci					
Gender					
Male	60	44(73.33)	1.692	0.787,3.639	0.2461
Female	63	39(61.90)			
Age (years)					0.0615
≤ 1 – 10	16	12(75.00)			
11 – 20	8	6(75.00)			
21 – 30	25	18(72.00)			
31 – 40	28	22(78.57)			
41 – 50	12	8(66.67)			
51 – 60	7	7(100.00)			
≥ 61	23	10(43.48)			
Source of isolates					
In–patients	38	20(52.63)	0.490	0.223,10.75	0.1117
Out–patients	85	59(69.41)			

MR = Methicillin resistance; OR = odd ratio; CI = confidence interval.

Table 4: Comparison of the susceptibility profiles of MRSA and MSSA, and MRCoNS and MSCoNS recovered from in-patients and out-patients

Antibacterial agents (µg/disc)	In-patients			Out–patients		
	MR(n= 94)	MS(n=65)	P value	MR(n=232)	MS(n=175)	P value
Staphylococcus aureus						
Amoxicillin–clavulanate (30)	4(4.3)	6(9.2)	0.3481	12(5.2)	10(5.7)	0.9857
Ceftriaxone (30)	11(11.7)	17(26.2)	0.0323	43(18.5)	29(16.3)	0.7020
Ceftazidime (30)	0(0.0)	2(3.1)	0.3233	8(3.5)	2(1.1)	0.2444
Cefuroxime (30)	1(1.0)	1(1.5)	0.7918	4(1.7)	5(2.7)	0.6679
Cloxacillin (5)	7(7.5)	5(7.7)	0.9541	12(5.2)	13(7.4)	0.4654
Gentamicin (10)	36(38.3)	37(56.9)	0.0312	95(41.0)	89(50.9)	0.0590
Ofloxacin (5)	45(47.9)	44(67.7)	0.0207	110(47.4)	94(53.7)	0.2467
Erythromycin (5)	45(47.9)	31(47.7)	0.9822	119(51.3)	94(53.7)	0.7010
Coagulase negative staphylococ	**n=21**	**n=17**		**n=60**	**n=25**	
Amoxicillin-clavulanate(30)	0(0.0)	0(0.0)	ND	9(15.0)	3(12.0)	0.7463
Ceftriaxone(30)	3(14.3)	3(17.7)	1.0000	4(6.7)	9(36.0)	0.0016
Ceftazidime(30)	1(4.8)	0(0.0)	1.0000	3(5.0)	0(0.0)	0.6336
Cefuroxime(30)	0(0.0)	0(0.0)	ND	1(1.7)	0(0.0)	0.5211
Cloxacillin(5)	0(0.0)	0(0.0)	ND	3(5.0)	2(8.0)	0.9560
Gentamicin(10)	2(9.5)	6(35.3)	0.1066	13(21.7)	8(32.0)	0.4266
Ofloxacin(5)	3(14.3)	6(35.3)	0.2493	23(38.3)	11(44.0)	0.7420
Erythromycin(5)	0(0.0)	5(29.4)	0123	27(45.0)	8(32.0)	0.4269

Figures in parenthesis are percentages; n = Number tested; MS = Methicillin susceptible; MR = Methicillin resistant; ND =Not done

Table 5: Comparison of susceptibility profiles of MRSA and MRCoNS from in-patients and out-patients

Antibacterial agents (µg/disc)	MRSA		P value
	In–patients n=94	Out–patients n= 232	
Amoxicillin–clavulanate (30)	4(4.26)	12(5.17)	0.9488
Ceftriaxone (30)	11(11.70)	43(18.53)	0.1807
Ceftazidime (30)	0(0.00)	8(3.45)	0.1534
Cefuroxime (30)	1(1.00)	4(1.72)	0.6603
Cloxacillin (5)	7(7.45)	12(5.17)	0.5940
Gentamicin (10)	36(38.30)	95(40.95)	0.7509
Ofloxacin (5)	45(47.87)	110(47.41)	0.9401
Erythromycin (5)	45(47.87)	119(51.29)	0.6619
	MRCoNS n=21	n=60	
Amoxicillin-clavulanate (30)	0(0.00)	9(15.00)	0.1391
Ceftriaxone (30)	3(14.29)	4(6.67)	0.5364
Ceftazidime (30)	1(4.76)	3(5.00)	0.9654
Cefuroxime (30)	0(0.00)	1(1.67)	0.5516
Cloxacillin (5)	0(0.00)	3(5.00)	0.7092
Gentamicin (10)	2(9.52)	13(21.67)	0.3646
Ofloxacin (5)	3(14.29)	23(38.33)	0.0784
Erythromycin (5)	0(0.00)	27(45.00)	0.0005

MRSA = Methicillin – resistant *Staphylococcus aureus;* MRCoNS = Methicillin- resistant coagulase negative staphylococci; n = number tested; figures in parenthesis are percentages.

Discussion

Methicillin–resistant *Staphylococcus aureus* (MRSA) and methicillin–resistant coagulase negative staphylococci (MRCoNS) are prevalent worldwide and are important cause of nosocomial and community-acquired infections (6,9). Knowledge about the prevalence and susceptibility patterns of MRSA and MRCoNS is fundamental in the treatment of infections caused by these organisms (12).

The prevalence of MRSA and MRCoNS observed in this study were 56.94% and 67.48% respectively. The higher prevalence (67.48%) observed from MRCoNS agrees with previous reports (15,16), but differs from previous findings (12); where the prevalence of MRSA (60.40%) was higher than that of MRCoNS (43.80%). It is important to note that the prevalence of MRSA and MRCoNS reported by Mir and Srikanth (15) was 32.22% and 40%, respectively, while Raakhee and Sreenivasa (16) was 31.31% and 42.62% respectively, and were lower than that observed in this study. This may be due to differences in geographical location. Indeed, the frequency of MRSA has been suggested to vary in relation to geographical location and site of sample collection (9). In this study, methicillin resistance was significantly more prevalent and/or associated with CoNS than *S. aureus*. The reason for this is unclear.

The prevalence of MRSA differ significantly (P=0.0225) among strains of *S. aureus* recovered from different clinical specimens with isolates recovered from umbilical cord and catheter tips harbouring most MRSA strains. All *S. aureus* strains were recovered from umbilical cord were MRSA while 85.71% of *S. aureus* strains recovered from catheter tips were MRSA. Microorganisms on catheter tips and other indwelling devices readily form biofilms which results in increase resistance to antimicrobial agents (17). This may explain the high prevalence of MRSA recovered from catheter tips. Other authors have reported pus (12,15) and wounds (18) specimens as harbouring most MRSA. It is important to note that the specimen types from which *S. aureus* were recovered in this study were more than those examined by the above authors. Specimen type did not significantly affect the prevalence of MRCoNS (P =0.3991).

Age, gender and source of isolates (in–patients and out-patients) did not significantly (P > 0.05) affect the prevalence of MRSA and MRCoNS in this study. In relation to age and gender of patients, a similar finding has previously been reported (18). This non–significant difference in the prevalence of MRSA and MRCONS may reflect the unregulated use of antibiotics in Nigeria (19).

Methicillin–resistant staphylococci have been reported to be more resistant to antimicrobial agents when compared with methicillin-susceptible staphylococci (15,20). Among in-patients, with the exception of ceftriaxone, gentamicin and ofloxacin in which MRSA strains were significantly (P < 0.05) less susceptible than MSSA, the susceptibility profiles between MRSA and MSSA did not differ significantly (P > 0.05). Indiscriminate use of antibiotics, prescription of antibiotics without laboratory guidance and poor economic status has been reported as risk factors for multi-drug resistant MRSA (15). There was no significant difference in the susceptibility profiles of MRSA and MSSA from out-patients (P > 0.05). In Nigeria, over the counter sales of antibiotics without prescriptions are rife (20, 21). The findings in this study may be explained due to fact that the highest volumes of antibiotics are being prescribed and consumed in ambulatory care (22).

Susceptibility profiles of MRCoNS and MSCoNS recovered from in-patients showed that MSCoNS were significantly more susceptible to erythromycin only (P =0.0087). One may surmise that erythromycin is used more in hospital settings than in community setting. Prior antibiotic use is a risk factor for the development of methicillin resistance (15). A similar picture was seen among CoNS isolates recovered from out-patients; whereby, MSCoNS were significantly more susceptible to ceftriaxone (P=0.0012) instead of erythromycin as seen in isolates recovered from in-patients.

There was no significant difference in the susceptibility profiles of MRSA from in-patients and out-patients (P > 0.05). This is a reflection of the indiscriminate use of antibiotics in our environment. There is no antibiotic policy or where they exist, they are not adhered to. This coupled with over the counter sales of antibiotics

without prescription and prescription without laboratory guidance may explain this finding. The result with strains of MRCoNS recovered from in-patients and out-patients were similar with the exception that 44.26% of MRCoNS only from out-patients were significantly susceptible to erythromycin with none of the isolates from in-patients been susceptible (P=0.0004).

In conclusion, the prevalence of MRSA and MRCoNS (56.54% and 67.48%, respectively) were relatively high with CoNS being twice more likely to be methicillin resistant than *S. aureus*. With the exception of few antibiotics, there was no significant difference in susceptibility to antibiotics between methicillin–resistant and methicillin–susceptible *S. aureus* and CoNS. Prudent use of antibiotics is advocated to stem the tide of antibacterial resistance.

Conflict of Interest

The authors declare no conflict of interest**.**

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