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Frequency of Methicillin-resistant *Staphylococcus aureus* isolates from Clinical Specimens in Gondar University Hospital, Northwest Ethiopia

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Abstract: The development of resistance to multiple antibiotics and control of disease transmission by MRSA isolates in hospitals/communities have been recognized as the major challenges as the bacterial population that expresses the resistance phenotype varies according to the environmental conditions. This study was conducted to determine the magnitude of MRSA strain and to investigate the *in vitro* antimicrobial susceptibility pattern and β-lactamase production of strains isolated from clinical specimens. Total of 1,295 clinical specimens including: pus, wound swab and discharge and body fluids were collected from patients presenting with infection. The presence of *Staphylococcus aureus* was detected using conventional microbiological methods. Isolated *S. aureus* were further subjected to MRSA screening and subsequently the antibiotic susceptibility test was performed. The data were entered and analyzed using SPSS. Of the total 279 *S. aureus* isolates during the study period (21.5%), 49 (17.6%) were found to be MRSA. Most of *MRSA* were isolated from wound swab and discharge and from inpatient. All MRSA strains recorded susceptibility to vancomycin, flucloxacillin, cefadroxil and cefoxitin, which was followed by 95.9% to clindamycin. In contrast, all strains of MRSA were found to be resistance to penicillin and 78.7% of them were found to be multidrug resistant. Both β-lactamase productions were detected in all *S. auresus* irrespective of methicillin-resistant. According to this study, vancomycin, flucloxacillin, cefadroxil and cefoxitin seems to be most effective antimicrobial agents which shows 100% sensitivity even with multi-drug resistance.

Keywords: Clinical specimens, frequency, gondar ethiopia, MRSA, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is a common cause of community and hospital acquired infections. It is associated with infections in all age groups, including surgical wounds, skin abscess, osteomylitis, septicemia, food poisoning and toxic shock syndrome (Jawetz *et al*., 2007). Over the past 50 years, treatment of these infections has become more difficult because of the emergence and spread of resistance to various antibiotics (Jun *et al*., 2004). The most notable example of this phenomenon was the emergence of methicillin resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the use of methicillin (Qureshi *et al*., 2004; Cookson *et al*., 2003). Methicillin resistance in staphylococci is mediated by the *mecA* gene, which encodes for the Penicillin-Binding Protein 2A (PBP2A) resulting in reduced affinity for the beta-lactam antibiotics including the penicillinase-resistant penicillin and thus it become a major hospital pathogen in human medicine (Rohrer *et al*., 2003). Since, the emergences of MRSA there have been many reports of MRSA causing various infections throughout the world. MRSA is of concern not only because of its resistance to methicillin but also many of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptides antibiotics such as vancomycin (Mehta *et al*., 1998). Indeed, low level resistance even to vancomycin is emerging at present (Assadullah *et al*., 2003). The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital are the possible predisposing factors for the emergence of MRSA (Anupurba *et al*., 2003).

The magnitude of MRSA infections has been increased in the past decades worldwide. For example it

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has increased from 2.0% in 1974 to 22.0% in 1995 and to 63.0% in 2004 in USA (CDC, HA-MRSA 2010). Similarly, it has increased from 29% to 42.5% in India (Metha *et al*., 1996; Chandrashekhar and Basappa, 2012). Although studies in Africa are limited, previous reports showed high rate of MRSA infection (Hayanga *et al*., 1997; Omari *et al*., 1997; Urassa *et al*., 1999). In Ethiopia, similar to other African countries little is known on the magnitude of MRSA. However, previous studies in the country have shown the importance of *S. aureus* in diseases and the emergency of multiple drug resistance strains with 19% MRSA (Yohannes *et al*., 1999; Beyene and Abdissu, 2000; Aseffa and Yohannes, 1996; Moges *et al*., 2002a, b). The development of resistance to multiple antibiotics and control of disease transmission by MRSA isolates in hospitals and/ or in communities have been recognized as the major challenges as the bacterial population that expresses the resistance phenotype varies according to the environmental conditions (McDonald, 1997; Qureshi *et al*., 2004). And since methicillin disc is used as a representative of the group of penicillinase resistant penicillin including oxacillin, cloxacillin, naficillin, flucloxacillin and dicloxacin isolation of MRSA is useful for in clinical practice to appropriately select antibiotics. Therefore, it is imperative to know the magnitude of MRSA and their current antimicrobial profile in areas where drug susceptibility testing is lacking. In the present study, we determined the magnitude of MRSA strain in different clinical specimens and define the in vitro antimicrobial susceptibility pattern and β-lactamase production of strains isolated from patients at Gondar University hospital, North West Ethiopia.

MATERIALS AND METHODS

Source of specimens: A total of 1,295 clinical specimens including: Pus, wound swab and discharge and body fluids were collected for *Staphylococcus aureus* screening from in and out patients of University of Gondar Teaching Hospital. These specimens were collected from September 2009 to June 2010 and analyzed in Microbiology Laboratory of Gondar University Teaching Hospital. The specimens were collected as part of routine clinical management of patients both from in-patient and out-patient with various clinical profiles. Specimens were collected following standard procedures for specimen collection (Cheesbrough, 2002).

Isolation and identification of clinical specimens: All the specimens were aseptically handled and processed. The morphotypes were done for all the specimens based on the Gram staining method to determine the likely organism present. Subsequently, the clinical specimens were inoculated on to 10% sheep blood agar, mannitol salt agar, MacConkey agar and nutrient agar and incubated at 37ºC for 24 hours. Initial screening and identification of *S. aureus* were done according to standard laboratory protocols where colonial morphology, Gram's stain reaction and biochemical characteristics were used for identification of *S. aureus*. *Staphylococcus aureus* ATTC 25923 of known coagulase production was included as control strain. Isolates that were gram-positive cocci, catalase positive, coagulase positive and mannitol fermentation were considered as *S. aureus* in this study.

Antimicrobial susceptibility test: Drug susceptibility test for each *S. aureus* isolates were carried out following the standard agar disc diffusion methods. Briefly, 3-4 morphologically identical colonies of *S. aureus* were picked up by sterile inoculating loop and suspended in about 3ml of nutrient broth with sterile normal saline to a turbidity that matches to 0.5 McFarland standard (approximately contains 10^7 to 10^8 CFU/ml). A sterile absorbent cotton swab was immersed into the bacterial suspension and rolled on the wall of the tubeand then inoculated on Muller Hinton agar (Oxoid Ltd; Basingstoke, Hampshire, England) uniformly in aseptic condition. The plates were then incubated at 35°C for 24 h so as to favor the growth of methicillin-resistant strains. Susceptibility testing was done for methicillin (5 μg) and others antimicrobials including chloramphenicol (30 μg), penicillin G (30 μg), ampicillin (30 μg), amoxicillin (30 μg), tetracycline (30 μg), gentamicin (10 μg), erythromycin (15 μg), co-trimoxazole (25 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), clindamycin (30 μg), flucloxacillin (5 μg), cefadroxil (30 μg), cefoxitin (30 μg) and vancomycin (10 μg). The antimicrobial susceptibility tests were interpreted based on the recommendation of the Clinical and Laboratory Standards Institute (CLSI). A zone of inhibition of less than 10 mm or any discernible growth within the zone of inhibition was taken as indicative of methicillin resistance (screening out MRSA strains) and for the others antimicrobials the diameter of 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 CLSI standard (NCCLS, 2002). Reference strains of *S. aureus* (ATCC 25923) was tested regularly and methicillin-resistant and methicillin-sensitive strain were included as controls according to the CLSI.

Detection of β-lactamase production: β-lactamase production was determined by acidimetric filter paper test. Briefly, benzylpenicillin was dissolved in phosphate buffer (pH8) and then bromocresol purple indicator solution was add and mixed. The mixture then placed in a strip of Whatman filter paper (number 1)

and kept in the bottom of a Petri dish. After the paper is fully saturated a few drops of buffered crystalline penicillin bromocresol purple solution was added. Using a sterile wire loop, few colonies from the medium plate were transferred to the filter paper. If the color of the paper changes in 60 min incubation period, the bacteria were considered as beta-lactamase positive.

Data analysis: All data were registered in laboratory logbook during the study period. Then the data were entered and analyzed using SPSS statistical software package (version 16) and a p-value <0.05 was considered statistically significant.

Ethical consideration: Ethical approval was obtained from Ethical Review Committee of the University of Gondar.

RESULTS

Bacteriological isolates: A total number of 279 *S. aureus* were isolated. The highest percentage of these isolates were collected from pus specimens and the least number of isolates were recovered from eye discharge (Table 1). The isolation rates of methicillin resistance were found to be 17.6% (49/279). The prevalence of MRSA was significantly different among various clinical specimens (p<0.05) and was found that 24.2% of these isolates were from wound swab and discharges, followed by pus (14.6) and 0% from eye discharge. The number of MRSA isolates were significantly different between in-patient and outpatient (X^2 = 7.81; 95% CI = 1.286, 4.504; p = 0.005) (Table 2).

Antimicrobial susceptibility test: All the 49 MRSA isolates were resistant to penicillin, 93.9% to ampicillin, amoxicillin, chloramphenicol and co-trimoxazole each, 40.8% to tetracycline, 36.7% to erythromycin and 24.5% to gentamicin. However, all MRSA strains were found to be susceptible to vancomycin, flucloxacillin, cefadroxil and cefoxitin, which was followed by 95.9% to clindamycin. In contrast, 83.9% of methicillin sensitive *S. aureus (*MSSA*)* were resistant to penicillin and ampicillin, 56.5% resistance to amoxicillin, 39.6% resistance to tetracycline, 13.0% resistance to chloramphenicol and co-trimoxazole, 9.6% resistance to erythromycin, 1.3% resistance to gentamicin as compared with MRSA (Table 3).

Table 1: Number and percentage distribution of MRSA from different clinical specimens at university of Gondar teaching hospital, Gondar, Ethiopia from September, 2009 to June, 2010

Clinical specimens	Total specimens (N = 1295)	*S. aureus* (n = 279)	%	MRSA (n = 49)	%
Wound swab and discharge	320	120	37.5	29	24.2
Pus	210	89	42.4	13	14.6
Body fluid	115	11	9.6	1	9.1
Genital swabs	85	13	15.3	1	7.7
Ear discharge	200	30	15.0	3	10.0
Eye discharge	150	2	1.3	0	0.0
Urine	215	14	6.5	2	14.3

N = Total number of specimens processed, n= individual number of isolates

Table 2: Distribution of S. *aureus* and MRSA based on source of specimens at university of Gondar teaching hospital, Gondar, Ethiopia from September, 2009 to June, 2010

Specimen source	Total specimens (N =1259)	*S. aureus* n (%)	MRSA n (%)	X^2	*P*-value	95%CI
In-patient	660	110 (16.7)	28 (25.6)	7.81	0.005	1.286, 4.504
Out-patient	635	169 (26.6)	21 (12.4)			

N = total number of specimens processed, n = individual number of isolate, X^2 = chi-square

Table 3: Antimicrobial susceptibility pattern of MRSA and MSSA isolated at University of Gondar teaching hospital, Gondar, Ethiopia from September, 2009 to June, 2010

	MRSA(n = 49)			MSSA (n = 230)			Total (N = 279)
	No of isolates that were			No of isolates that were			No of resistance
Antimicrobials	S	R	Resistance rate (%)	S	R	Resistance rate (%)	rate (%)
Penicillin G	0	49	100.0	37	193	83.9	242(86.7)
Ampicillin	3	46	93.9	43	187	81.3	233(83.5)
Amoxicillin	3	46	93.9	100	130	56.5	176(63.1)
Chloramphenicol	3	46	93.9	200	30	13.0	76(27.2)
Co-trimoxazole	3	46	93.9	200	30	13.0	76(27.2)
Erythromycin	31	18	36.7	208	22	9.6	40(14.3)
Gentamicin	37	12	24.5	227	3	1.3	15(5.4)
Tetracycline	29	20	40.8	139	91	39.6	111(39.8)
Ciprofloxacin	45	4	8.2	230	0	0.0	4(1.4)
Ceftriaxone	46	3	6.1	230	0	0.0	3(1.1)
Vancomycin	49	0	0.0	230	0	0.0	0(0.0)
Clindamycin	47	2	4.1	230	0	0.0	2(0.7)
Flucloxacillin	49	0	0.0	230	0	0.0	0(0.0)
Cefadroxil	49	0	0.0	230	0	0.0	0(0.0)
Cefoxitin	49	0	0.0	230	0	0.0	0(0.0)

N = Total number of *S.aureus* isolates, n = individual number of isolates

Table 4: Multi-drug resistance and β-lactamsese production from *Staphylococcus aureus* isolates at university of Gondar teaching hospital, Gondar, Ethiopia from September, 2009 to June, 2010

	β-lactamase (N = 230)		
S. aureus isolates	Producing (n = %)	Non- producing (n = %)	MDR (n = %)
MRSA	49 (100.0)	0 (0.0)	39 (79.6)
MSSA	181 (78.7)	49 (21.3)	92 (40)
Total	230 (82.4)	49 (17.6)	131 (47%)

N = total number of β-lactamase, n = individual number, MDR = multi-drug resistance

Detection of multi-drug resistance and β-lactamsese production: All the 49 MRSA isolates was found to be of β-lactamase producing strains. Of which 79.6% (39/49) showed multidrug resistance. Among the 230 MSSA isolates, 78.7% were beta-lactamase positive (Table 4). Further analysis indicated significant rate of multi-drug resistance ($X^2 = 25$; 95% CI = 0.081, 0.359; p = 0.0001) and β-lactamase ($X^2 = 12.4$; 95%CI=1.183, 1.350; p = 0.001) production observed among MRSA compared to MSSA.

DISCUSSION

The data in the present study shows a frequent isolation of MRSA in inpatients and is comparable with previous studies from African (Bouchillon *et al*., 2004; Zinn *et al*., 2004) and Asian countries (Sanjana *et al*., 2010; Saikia *et al*., 2009; Rajaduraipandi *et al*., 2006). We have observed that the resistant rate to different antibiotics among MRSA strains was higher than those sensitive to methicillin and this phenomena was reported elsewhere (Tahnkiwale *et al*., 2002). In this study all the strains showed susceptibility to vancomycin, flucloxacillin, cefadroxil and cefoxitin and most of them were susceptible to clindamycin and ceftriaxone. This report was in line with the studies conducted in Nepal (Rajaduraipandi *et al*., 2006), Trinidad & Tobago (Patrick *et al*., 2006), Addis Ababa (Yohannes *et al*., 1999) and in middle Tennessee (Kilic *et al*., 2006). All MRSA isolates were significantly less sensitive to antibiotics as compared with MSSA isolates. As expected, all the strains were resistant to penicillin and most of them were resistant to ampicillin which was not observed in case of MSSA. This report was similar with the studies conducted in Trinidad and Tobago (Patrick *et al*., 2006) and Nepal (Zinn *et al*., 2004). Lower penicillin resistance of 80% also was observed in India (Sanjana *et al*., 2010). The present study also showed that relatively high antibiotic resistance profile of MRSA to the commonly used antibiotics like, amoxicillin, chloramphenicol and co-trimoxazole (93.9% each) and 40.8% of tetracycline. In one study on spectrum of antimicrobial resistance among MRSA, ciprofloxacin resistance was as high as 90% and Qureshi had reported the same as 98.9 % (Qureshi *et al*., 2004; Pulimood *et al*., 1996). In contrast, we have 8.2% of the strains resistant to ciprofloxacin. These discrepancies may result from the low number of methicillin-resistant isolates collected for our study, may be due to overuse of the drugs since ciprofloxacin prescribed in our study as final option and finally may be due to misuse of drugs. However, Pulimood had observed only 8% resistance of MRSA to gentamicin (Pulimood *et al*., 1996) as against 24.5% in our study. Gentamicin resistance is on the rise since 1996. An increase of gentamicin resistance from 0% before 1996 to 80% after 1996 has been reported (Price *et al*., 1998). Qureshi had reported a gentamicin resistance of 97.8% (Qureshi *et al*., 2004), which is higher compared to our study. Many of MRSA (79.6%) strains were multidrug resistant (resistance of two and more antibiotics) compared to MSSA (40%). Higher percentages of multidrug resistant were also reported from different studies (Saikia *et al*., 2009; Majumder *et al*., 2001). Similarly we observed all MRSA strains were produce β-lactamase which help the bacteria to develop multiple drug resistance pattern for β-lactam antibiotics including penicillin and its derivatives as compared to that of MSSA strains which was more sensitive for these drugs. Identical finding was also reported from other similar studies (Paradisi *et al*., 2001; Olowe *et al*., 2007).

In conclusion, the emergence of multi-drugs resistance in MRSA is worrisome in the present study and more cases were identified among inpatients. A regular surveillance of hospital associated infection including monitoring antibiotic sensitivity pattern is mandatory to controlling the spread in the hospital and strict drug policy are of importance or else the threat will increase. According to this study, vancomycin, flucloxacillin, cefadroxil and cefoxitin seems to be most effective antimicrobial agents in our area which shows 100% sensitivity even with multi drug resistance. These drugs remains the first choice of treatment for MRSA and to preserve their value, their use should be limited to those cases where there are clearly needed. Further detection and molecular characterization of the gene (*mec A*), phage typing and analyses of the plasmids of MRSA is necessary.

Declaration of competing interests: The author (s) declares that they have no competing interests.

Authors' contributions: BA, AM, FM, AK &YS designed the study, carried out the testing, performed the statistical analysis and interpretation of data and drafted the manuscript. BA participated in statistical analysis. YS, CU and FB conceived the study, participated in antibiotic testing and in the preparation of the settings. All authors read and approved the final manuscript.

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