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Antimicrobial Resistance Profile of Staphylococcus aureus from Clinical Samples of Patients Visiting Sushma Koirala Memorial Hospital

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Abstract

Staphylococcus aureus is commonly isolated pathogen from clinical specimen with increasing trend of antimicrobial resistance. The aim of the study was to isolate S. aureus from the pus and wound swabs and determine their susceptibility pattern. This study was carried out in Sushma Koirala Memorial Hospital (SKM), Kathmandu, from June 15, 2013 to May 30, 2014, following the standard microbiological protocol.

Out of 285 samples (pus and wound swabs), S. aureus was isolated from 65 (22.80%), of which 38 (58.46%) from male and 27 (41.53 %) from female. Fourteen (21.53%) isolates were identified as MRSA and remaining 51 (78.47%) were Methicillin-Susceptible (MSSA). Among MSSA isolates, all isolates were 100 % susceptible to Amikacin, Tetracycline and Vancomycin. The susceptibility to Ciprofloxacin, Erythromycin and Penicillin was 52.94%, 68.63%, and 10.20% respectively. Among MRSA isolates, no resistance was seen to Amikacin, Chloramphenicol, Tetracycline, and Vancomycin but only 66.67 % of isolates were susceptible to Clindamycin. To remaining all antibiotics tested, Cefotaxime, Cephalexin, Ciprofloxacin, Erythromycin, Gentamicin and Penicillin, the susceptibility was lower than the 37%. In this study, all the MRSA isolates were multidrug resistant (MDR), whereas 25.49 % of MSSA isolates were MDR. Six (9.2 %) of the total isolates were obtained as Penicillinase producing S. aureus by penicillin disk zone-edge method.

Keywords: ***S.aureus, Penicillinase, MRSA, MDR.***

Introduction

S. aureus is a potentially pathogenic gram positive bacterium that causes a broad spectrum of diseases; minor infections of the skin to post-operative wound infections, bacteriemia, meningitis, endocarditis and infections associated with foreign bodies and necrotizing pneumonia, with substantial mortality and morbidity. *S.aureus* can adapt rapidly to the selective pressure of antibiotics, and this has resulted in the emergence and spread of Methicillin-Resistant *S. aureus* (MRSA).[1,2] However, Methicillin-Resistant *S. aureus* (MRSA) and Vancomycin-Resistant *S.aureus* (VRSA) have become threat level of concern worldwide. MRSA may reach to the "threat level of urgent" if its infection rates increase or becomes more resistant to other antibiotic agents.[3]

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S. aureus was extra ordinarily adaptable pathogen and uniformly sensitive to Penicillin in the beginning, but soon the resistant strains began to emerge after the extensive miss use of the drugs. Methicillin-Resistant *S. aureus*(MRSA) is, any strain of *S. aureus* that has developed resistant to beta lactam antibiotics, due to the horizontal transfer of resistance genes from outsides and chromosomal mutation.[4] However, due to horizontal transfer of resistance genes from outside and chromosomal mutation, it has become notorious pathogen in both hospital settings as well as community settings.[5]

Materials and Methods

Study site and population

The present study was conducted in Sushma Koirala Memorial (SKM) Hospital, Kathmandu from June 15, 2013 to May 30, 2014. A total of 285 clinical samples (wound and pus swabs) received for culture in the laboratory from outpatients and inpatients which met the inclusion criteria were included in the study.

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Isolation, Identification & AST (Antibiotic Susceptibility Test) of *S. aureus*

Consecutive clinical samples collected by authorized technician were immediately cultured on nutrient agar plate and incubated at 37°C for 24 hours. Next day gram staining and biochemical tests; Catalase test, Oxidase test, Coagulase test and O/F test were performed to identify the isolates to be *S. aureus* confirm. Antibiotic susceptibility of the isolates was examined on Muller Hinton Agar (MHA) plate by Modified Kirby-Bauer disc diffusion method following clinical laboratory standard institute protocol with commercial antibiotics disks (Hi-Media).[6]

Detection of MRSA and MSSA

Screening for Methicillin Resistance was performed by Cefoxitin (30mcg) disc diffusion method and interpreted according to CLSI guidelines. Isolates which gave zone of inhibition (ZOI) ≥ 22 mm were identified as Methicillin-Susceptible (MSSA) and isolates with ZOI ≤ 21 mm identified as Methicillin-Resistant (MRSA).[6]

Detection for Beta lactamase production

In all isolates, irrespective of their susceptibility or resistance to penicillin, test for β lactamase production was performed in MHA Plate.[6,7] For this assay Penicillin 10U was placed over the inoculum in MHA plate and incubated at 35±2°C for 16-18 hours. Sharp zone edge ("cliff") was regarded as β-lactamase positive whereas fuzzy zone edge ("beach") was regarded as β-lactamase negative.

Quality Control

Quality of each test was performed by using standard protocol *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were respectively used as positive and negative control.

Results

Table 1 shows out of 285 pus and wound swabs processed, *S. aureus* were isolated from 65 samples: thirty six (31.03%) were from Out-patients whereas the remaining 29 (17.16%) were from in-patients; it was statistically significant (p value<0.05).

Table 1: Patient-wise distribution

Study groups	Growth (%)	No growth (%)	Total	P-value
Out-patients	36 (31.03)	80 (68.97)	116	
In-patients	29 (17.16)	140 (82.84)	169	0.006
Total	65	220	285	

Table 2: Antibiotic Susceptibility Pattern of MSSA and MRSA isolates

ANTIBIOTICNUMBER OF S. AUREUS ISOLATES				
	MSSA (n=51)		MRSA (n=14)	
	S (%)	R (%)	S (%)	R (%)
FOX	51 (100)	0 (0)	0 (0)	14 (100)
AMK	51 (100)	0 (0)	14 (100)	0 (0)
CTX	45 (88.23)	6 (11.77)	2 (14.29)	12 (85.71)
CEP	32 (62.75)	19 (37.25)	3 (21.43)	11 (78.57)
CHL	45 (88.23)	6 (11.77)	14 (100)	0 (0)
CIP	27 (52.94)	24 (47.06)	3 (21.43)	11 (78.57)
CLI	41 (80.39)	10 (19.61)	10 (66.67)	4 (28.57)
ERY	35 (68.63)	16 (31.37)	0 (0)	14 (100)
GEN	48 (94.12)	3 (5.88)	5 (37.71)	9 (64.29)
PEN	5 (9.80)	46 (90.20)	0 (0)	14 (100)
TCY	51 (100)	0 (0)	14 (100)	0 (0)
VAN	5151 (100)	0 (0)	14 (100)	0 (0)

S= Susceptible, R= Resistant.

In table 2 shows among all the isolated 14 (21.5%) cefoxitin-resistant (MRSA) and were susceptible to the Amikacin, Tetracycline, and Vancomycin. The high level of susceptibility to Cefotaxime, Chloramphenicol and Gentamicin was 88.23%, 88.23% and 94.12% respectively. The susceptibility to Ciprofloxacin and Penicillin was 52.94% and 11.76% respectively. To remaining all antibiotics tested, Cefotaxime, Cephalexin, Ciprofloxacin, Erythromycin, Gentamicin and Penicillin the susceptibility was below 38%. All the 14 MRSA isolates were resistant to Penicillin and Erytrhomycin.

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Table 3: Antibiotic resistant profile of *S. aureus*

Resistance profile	Number of Classes Resistance	MDR	Number of Isolates
PEN*	1		11
PEN CIP	2		5
PEN CHL	2		4
PEN CEP	2		4
PEN CEP CIP	3		3
PEN CHL ERY	3		2
PEN ERY CLI	3		2
PEN CTX CEP FOX CIP ERY	4	MDR	4
PEN CTX CEP FOX ERY CLI	4	MDR	6
PEN CEP CIP ERY	4	MDR	5
PEN FOX TCY CIP ERY	5	MDR	5
PEN CTX CEP FOX GEN CIP ERY	5	MDR	3
PEN CTX CEP FOX CEN ERY CLI	5	MDR	2
PEN CEP TCY ERY CLI	5	MDR	2
Isolates not resistant to any antibiotics			7

** Penicillin not included defining MDR because S. aureus has intrinsic resistance to it.*

Table 3 shows that seven (10.76%) isolates were susceptible to all antibiotics tested, whereas 11 (16.92%) isolates were found to be only resistant to Penicillin. There were 13 (20%) such isolates which were resistant to Penicillin and other antibiotic. Seven (10.77 %) isolates were resistant to Penicillin and other two antibiotics from different classes. And remaining 29 (44.61 %) isolates were classified as MDR. MDR were resistant to antibiotics of three or more different classes, not counting Penicillin.[8]

Table 4: Comparison of AST of beta-lactamase producing (n=59) and non-producing *S. aureus* (n=6)

Antibiotic	NUMBER OF S. AUREUS ISOLATES			
	β-lactamase producer(n=59		β-lactamase non-producer(n=6)	
	S (%)	R (%)	S (%)	R (%)
PEN	0 (0)	59 (100)	6 (100)	0 (0)
AMK	59 (100)	0 (0)	6 (100)	0 (0)
CTX	43 (72.88)	16 (27.22)	6 (100)	0 (0)
FOX	47 (79.66)	12 (21.34)	6 (100)	0 (0)
CEP	32 (54.23)	27 (45.77)	6 (100)	0 (0)
CHL	55 (93.22)	4 (6.88)	6 (100)	0 (0)
CIP	21 (35.59)	38 (64.41)	6 (100)	0 (0)
CLI	59 (100)	0 (0)	6 (100)	0 (0)
ERY	30 (50.84)	29 (49.16)	6 (100)	0 (0)
GEN	49 (83.05)	10 (16.95)	6 (100)	0 (0)
TCY	59 (100)	0 (0)	6 (100)	0 (0)
VAN	59 (100)	0 (0)	6 (100)	0 (0)

S= Susceptible, R= Resistant

Table 4 shows that the comparison of AST of beta-lactamase producing and non-producing *S. aureus* isolates. In beta lactamase producing test, out of 65 total isolates, 59 isolates gave zone of inhibition below 29 mm also gave a sharp edge, i.e. they were beta-lactamase producing strains.[6] The remaining six isolates gave a hazy (beech) edge and they were non beta-lactamase producing strains, susceptible to penicillin, i.e. ZOI ≥29 mm.

Discussion

In this study, outpatients were significantly infected with *S. aureus* than inpatients (p-value <0.05). The rate of isolation was more common in out patents (31.03%) of age group 21-

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30 (29.2%). Similar infection rates among the out patients have been noted by previous researcher.[9] This high frequency of isolation from out patients may be due to the trend of unhealthy medicine practice by the pharmacist with-out susceptibility test.

The AST results revealed the (21.5%) MRSA isolates among the 65 *S. aureus* isolates which was similar to the recent studys[10,11,12] All the *S. aureus* were 100 % susceptible to the Amikacin, Tetracycline and Vancomicin, followed by Gentamicin (94.12%), Chloramphenicol (88.23%) and Cefotaxime (88.23%). Among the MRSA isolates high level of resistance was observed against Cefotaxime (85.71%) followed by Cephalexin (78.57%), Ciprofloxacin (78.57%) and Gentamicin (64.29%). None of the MRSA isolates were susceptible to Penicillin and Erythromycin whereas 9.80% and 68.63% MSSA isolates were susceptible to Penicillin and Erythromycin respectively. Selective pressure from the use of antimicrobial agents is the major determinant for the emergence of the resistance. A decreased susceptibility of *S. aureus* to commonly used antibiotics has been noted by previous researchers.[12,13]

In this study, 44.61% of the total isolates were found to be MDR. This data suggest the high prevalence of MDR isolates in the clinical settings. The previous researcher has been noted similar prevalence of MDR.[13] Proper use of antibiotics and the proper infection control strategies is the most to reduce the prevalence of MDR strains. However, increased MDR scenario has been noted 73.38% in the western Nepal by previous researcher.[14]

In this study 90.76% isolates were found to be β-lactamase producer (resistant to penicillin with zone of inhibition ≤28 mm).This result agree with 91.94% *S. aureus* isolates resistant to Penicillin and had also compared the Penicillin resistance with three different beta-lactamase detection results.[15] This high level of Penicillin resistance in *S. aureus* in both studies is in concordance with the reported 90 % in US.[16] However, recent research showed 71.82% isolates were β-lactamase producer.[17]

Conclusion

The incidence of MRSA in *S. aureus* was high with worse multi drugs resistance. The high load of MDR was screened among MRSA and MSSA Penicillinase producing *S. aureus*. The high load of MDR organism provokes the necessities of strictly performing susceptibility testing before starting antibiotic therapy, or there may be chance of clinical failure.

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Refferences

[1] Greenwood D, Slack RCB and Peutherer JF (2002).Medical Microbiology 16th Edition, Churchill Livingstone, New York.

[2] Collier L, Balows A. and Sussman M (1998).Topley and Wilson's Microbiology and Microbial Infections. 3: 231-48.

[3] Centre for Disease Control and Prevention (CDC) (2013). Antibiotic Resistance Threats in the U S. 85-86.

[4] Turnidge JD, Kotsanas D (2009) *S. aureus* bacteraemia: a major cause of mortality in Australia and New Zealand. Med J. 191: 368-73.

[5] Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, Coombs GW, Pearman JW, Tenover FC, Kapi M, Tiensasitorn C, Ito T and Hiramatsu K (2002). Dissemination of new methicillin-resistant *S. aureus*clones in the community.J ClinMicrobiol.40:4289-4294.

[6] Clinical and Laboratory Standards Institute (2013). Performance Standards for Antimicrobial Disk Susceptibility Testing; Twentieth Informal Supplement M100-S21. 31 (1), CLSI, Villanova, PA

[7] Kasse M, Lenga S, Friedrich S, Szabados F, Sakinc T, Kleine B and Gatermann SG (2008). Comparison of phenotypic methods for penicillinase detection in *S. aureus*. ClinMicrobiol Infect. 14**:** 616-618.

[8] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT and Monnet DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. ClinMicrobiol Infect.18: 268-281.

[9] Sapkota K, Basnyat S.R, Shrestha C.D, Shrestha J.S, Dumre S.P and Adhikari N. (2010).Prevalance of MthicillinresiatantS*.aureus* (MRSA) in tertiary referral hospital in Nepal.

[10] Baral R, Khanal B and Acharya A (2011). Antimicrobial susceptibility patterns of clinical isolates of *S.aureus* in Eastern Nepal. HealthRenaissance**.9:**78-82.

[11] Shrestha B (rev 2013). Comparative prevalence of MRSA in two Nepalese tertiary care hospitals.

[12] Bhatt AS, Manzo VE, PedamalluCS, Duke F, Cai D, Bienfang DC, Padera RF, Meyerson M, Docken WP (2014) .In search of a candidate pathogen forgiant cell arteritis: sequencing-based characterization of the giant cell arteritis microbiome. 66 (7):1939-44

DOI: 10.23958/ijirms/vol02-i08/20
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[13] Tiwari HK, Das AK, Sapkota D, Sivarajan K and Pahwa VK (2009). Methicillin resistant *S. aureus*: Prevalence and antibiogram in a tertiary care hospital in western Nepal. J Infect DevCtries. 3:681-4.

[14] Bhatta, Dharm R., Cavaco, Lina,Nath, Gopal; Gaur, Abhishek; Gokhale, Shishir; Bhatta, Dwij Raj (2015).Threat of multidrug resistant Staphylococcus aureus in Western Nepal.Asian Pacific Journal of Tropical Disease. 5:8. 617-621.

[15] Shrestha B, Pokhrel BM and Mohapatra TM (2009). Antibiotic susceptibility pattern of nosocomial isolates of *S. aureus* in a tertiary care hospital, Nepal. J Nepal Med Asso.48**:** 234-248.

[16] Centre for Disease Control and Prevention (CDC) (2005). Laboratory diagnosis of: oxacillin/methicillin-resistant *S. aureus.*

[17] Adhikari R. Pant N.D, Neupane S, Neupane M, Bhattarai R, Chaudharai, Lekhak B (2015).Detection of methicillin resistant Staphylococcus aureus and determination of minimum inhibitory concentration of vancomycin for Staphylococcus aureus isolated from pus/wound swab samples of the patients attending a tertiary care hospital in Kathmandu, Nepal.

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