Methicillin Resistant *Staphylococcus aureus* Isolated from Wound Infections

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ABSTRACT

Objectives: The aim of this study was to determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) and assess antibiotic resistance pattern of the isolates from wound infections.

Methods: A total of 706 wound specimens including pus and wound swab were processed in the laboratory of B and B Hospital, Lalitpur from May to October 2014. The specimens were cultured on blood agar and mannitol salt agar plates and incubated at 37°C for 24 hours. Antibiotic susceptibility test was performed by modified Kirby-Bauer disc diffusion method. Strains resistant to cefoxitin (30mcg) with inhibition zone ≤ 21mm were identified as MRSA.

Results: Out of 366 bacterial isolates, 90 (24.6%) were *S. aureus* and among them 16.7% were MRSA and 54.4% multi-drug resistant (MDR). All isolates were sensitive to vancomycin and most of the isolates were sensitive to cefoxitin (83.3%). High rate of resistance was observed towards penicillin (98.9%) and ampicillin (86.7%). All MRSA isolates and 52.9% of methicillin sensitive *S. aureus* (MSSA) were MDR.

Conclusion: MRSA incidence is increasing in the population, and therapeutic measures are few and accompanied by diverse side effects. It is noteworthy to state that vancomycin is still the first line drug although vancomycin-resistant strains have been reported.

Key words: Wound infection, antimicrobial resistance, MRSA, MDR

INTRODUCTION

Wound is a breach in the skin, which can lead to infections with the presence of replicating microorganisms with the discharge of pus (Dulon et al. 2011). *Staphylococcus aureus* has been recognized as an important cause of disease around the world ranging from relatively mild infections of the skin and soft tissue to life-threatening sepsis. The emergence of strains resistant to methicillin and other antimicrobial agents has become a major concern, especially in the hospital environment (Spagnolo et al. 2014).

Methicillin resistance is mediated by PBP-2a, a penicillin binding protein encoded by the *mecA* gene that is located on a mobile genetic element called a Staphylococcal cassette chromosome (Mahasenan et al. 2017). The relative ease of transfer of this genetic element explains the growing resistance to β-lactam antibiotics such as penicillin and its chemical derivatives as well as cephalosporins. MRSA is now endemic in both community and hospital environments (Sit et al. 2017).

MRSA strains have spread among hospitals and disseminated worldwide. The development of resistance to multiple antibiotics and control of disease transmissions by MRSA isolates in hospitals have been recognized as a major challenge (Chen and Huang 2014). The recent studies conducted in different parts of Nepal reported the rate of MRSA to be 21.1% (Khanal et al. 2018), 35.5% (Adhikari et al. 2017) and 43.6% (Raut et al. 2017). The knowledge on prevalence of MRSA and their current antimicrobial profile has become

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necessary in the selection of appropriate empirical treatment of these infections. This study aimed to determine the prevalence of MRSA in wound infections among the patients attending B and B hospital and their susceptibility pattern towards various antimicrobial agents.

MATERIALS AND METHODS

Study site and population

The research work was conducted at laboratory of Department of Microbiology, B and B Hospital, Gwarko, Lalitpur from May to October 2014. During this period, a total of 706 aspirated pus and wound swabs from male and female patients of age group 1-87 years was collected, properly labelled and transferred to laboratory for further processing.

Isolation and identification of *S. aureus*

The specimens were directly inoculated on blood agar and mannitol salt agar plates and incubated at 37°C for 24 hours. The isolates were identified by morphological appearance of the colonies, microscopic findings; biochemical properties like catalase production test and coagulase production test by slide and tube methods. The colonies with golden yellow pigmentation on mannitol salt adgar and cream colored hemolytic or non-hemolytic on blood agar; Gram-positive cocci in grape-like cluster in Gram staining and catalase and coagulase tests positive were identified as *S. aureus* (Forbes et al. 2007).

Antibiotic susceptibility testing

Antibiotic susceptibility tests of all *S. aureus* isolates towards various antibiotics were performed by modified Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI 2018). In this study the antibiotics used were amoxicillin (AMX/10mcg), cefoxitin (CX/30mcg), ciprofloxacin (CIP/5mcg), cotrimoxazole (COT/25mcg), erythromycin (E/15mcg), gentamicin (GEN/10mcg), ofloxacin (OF/5mcg), penicillin (P/10mcg) and vancomycin (VA/30 mcg). Screening for methicillin resistance was performed by cefoxitin disc diffusion method and interpreted according to CLSI (2018). Isolates with diameter of zone of inhibition (ZOI) ≥ 22mm were identified as MSSA and isolates with ZOI ≤ 21mm identified as MRSA. Isolates resistant to three or more classes of antibiotics were considered MDR (Nair et al. 2013).

RESULTS

A total of 706 wound specimens included 39 aspirated pus and 667 wound swabs in which bacterial growth was observed in 366 wound specimens (Table 1).

Table 1: Bacterial growth pattern in wound samples

Specimens	Bacterial growth N (%)	No growth N (%)	Total sample
Wound swab	352 (52.8)	315 (47.2)	667 (94.5)
Aspirated pus	14 (35.9)	25 (64.1)	39 (5.5)
Total	**366 (51.8)**	**340 (48.2)**	**706 (100)**

Among 366 isolated different organisms, 90 (24.6%) were identified as *S. aureus*. *Klebsiella* species was found to be most predominent Gram negative bacteria constituting 89 (24.3%). Other most frequently isolated organisms were *E. coli* (22.1%) and *Enterococcus* spp (11%) (Table 2).

Table 2: Different bacterial isolates from wound infection

Organisms	Number of isolates	%
Staphylococcus aureus	90	24.6
Klebsiella spp	89	24.3
E. coli	81	22.1
Enterococcus spp	40	11
Proteus spp	35	9.6
Pseudomonas spp	21	5.7
Citrobacter spp	6	1.6
Acinetobacter spp	4	1.1
Total	**366**	**100**

Out of 706 patients under study, 470 (66.6%) were inpatients and 236 (33.4%) were outpatients. The culture positive cases in inpatients were 216 (59%) and 150 (41%) in outpatients (Table 3).

Table 3: Case wise distribution of patients

Patient type	Specimen N (%)	Culture positive N (%)
Inpatients	470 (66.6)	216 (59)
Outpatients	236 (33.4)	150 (41)
Total	**706**	**366**

The higher numbers of isolates of *S. aureus* were recovered from the age group 31-40 years (20%) followed by age group 21-30 years (17.8%). Least isolates were from age group 71-80 years (10%) and none were isolated from 80 years and above (Table 4).

Table 4: *S. aureus* isolates from different age groups of patients

Age group (years)	Total	*S. aureus*	
		N	%
<10	41	6	6.7
11 - 20	72	11	12.2
21 - 30	85	16	17.8
31 - 40	196	18	20
41 - 50	174	13	14.4
51 - 60	35	12	13.3
61 - 70	60	5	5.6
71 - 80	33	9	10
80 above	10	0	0
Total	**706**	**90**	**100**

Out of 366 culture positive cases, a total of 90 *S. aureus* were isolated. Among them, 61 isolates (67.8%) were from male patients and 29 (32.2%) were from female patients. The distribution of *S. aureus* was higher in males than in females and the result was statistically significant ($p<0.05$) (Table 5).

Table 5: Distribution of *S. aureus* according to the gender of patients

Gender	*S. aureus*		*P*-value
	N	%	
Male	61	67.8	
Female	29	32.2	**0.005**
Total	**90**	100	

S. aureus isolated were tested with different antibiotics by using modified Kirby-Bauer disc diffusion method. Antibiotic susceptibility pattern of *S. aureus* isolates showed that the high proportion of isolates were resistant to penicillin (n=89, 98.9%) and amoxicillin (n=78, 86.7%). All the isolates of *S. aureus* were susceptible to vancomycin and most of the isolates were susceptible to cefoxitin (n=75, 83.3%) and gentamicin (n=48, 53.3%). The prevalence of MRSA was found to be 16.7% as shown by resistance with cefoxitin (Table 6).

Table 6: Antibiotic susceptibility pattern of *S. aureus* (N = 90)

Antibiotics	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Amoxicillin	10 (11.1)	2 (2.2)	78 (86.7)
Penicillin	1 (1.1)	0	89 (98.9)
Gentamicin	48 (53.3)	8 (8.9)	34 (37.8)
Cotrimoxazole	41 (45.6)	27 (30)	22 (24.4)
Cefoxitin	75 (83.3)	0	15 (16.7)
Erythromycin	12 (13.3)	29 (32.2)	49 (54.4)
Vancomycin	90 (100)	0	0
Ofloxacin	30 (33.3)	9 (10)	51 (56.7)
Ciprofloxacin	30 (33.3)	7 (7.8)	53 (58.9)

Among 90 *S. aureus* isolates, 49 (54.4%) were found to be MDR. All 15 MRSA isolates and 34 (45.3%) MSSA were MDR (Table 7).

Table 7: MDR pattern of *S. aureus*

Drug resistance	MRSA N (%)	MSSA N (%)	Total *S. aureus* N (%)
MDR	15 (30.6)	34 (69.4)	49 (54.4%)
Non-MDR	0	41 (100)	41 (45.6%)
Total	**15 (16.7)**	**75 (83.3)**	**90 (100%)**

DISCUSSION

MRSA has emerged as a serious public health problem globally. Because of the ability of Staphylococci to acquire antimicrobial resistance over time, MRSA has been and will continue to be a problem in the future. Today, most of the MRSA are multi-drug resistant thus causing a clinical problem as antibiotic treatment becomes useless. As such, this study was undertaken to determine the prevalence of *S. aureus* and MRSA, along with their antibiotic susceptibility patterns.

Out of 706 specimens, 366 were culture positive cases and *S. aureus* (24.6%) was found to be predominant bacteria causing wound infection. Pandey et al. (2012) and Hussain et al. (2005) reported similar results with bacterial growth of 26.1% and 20% respectively. This suggested that *S. aureus* is the constantly isolated pathogen in hospital settings and regular intervention is required for the control of infection caused by this organism.

The present study showed that male (67.8%) had a higher infection rate of wounds than females, which was statistically significant ($p<0.05$) and similar result was found in a study carried out by Mama et al. (2014). Some other studies showed statistically insignificant results in the distribution of *S. aureus* between males and females (Adhikari et al. 2017; Khanal et al. 2018). The number of wound specimens was highest in 31-40 years of age group with higher incidence of *S. aureus* infection (20%). This might be explained by the fact that this group of population is mainly involved in occupations such as farming, construction works, transportation and industry works where the likely exposure to trauma is common.

Among 470 samples from inpatients, 216 culture positive results were observed and 150 positive cases were observed from 236 samples of outpatients. The prevalence of *S. aureus* was higher in outpatients (33.3%) as compared to inpatients (18.5%). The result was not in the agreement with the study done by Bhatta et al. (2014) who have reported higher prevalence of *S. aureus* in hospital setting accounting 54% as compared to outpatients (46%).

S. aureus isolated in this study in overall showed higher rate of sensitivity towards cefoxitin (83.3%) followed by gentamicin (53.3%) and cotrimoxazole (45.6%) whereas higher rate of resistance was observed towards penicillin (98.9%) and amoxicillin (86.7%) followed by ciprofloxacin (58.9%) and erythromycin (54.4%). In this study, most of *S. aureus* isolates were resistant towards β-lactam antibiotics making them the least effective drugs. The high resistance to penicillin and total susceptibility to vancomycin is commonly noted for *S. aureus* isolated at different hospitals worldwide (Adhikari et al. 2017). Bacterial

resistance to β-lactam antibiotics is primarily due to the production of β-lactamase that opens its β-lactam ring rendering them to deactivate and also its penicillin binding protein (PBP2a) (Richmond 2000). None of the isolates were resistant to vancomycin as this antibiotic has unique mode of action to bacteria. It acts by inhibiting the second stage of cell wall synthesis of various susceptible bacteria altering bacterial cell wall membrane permeability and RNA synthesis (Rijal et al. 2008). Knowledge about MRSA and carrier status needs to be raised among the health staffs of the hospital and control measures need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment (Holmes et al. 2005).

Among 90 isolates of *S. aureus* 49 isolates (54.4%) were found to be MDR. Similar studies by Banjara (2002), Rajbhandari et al. (2003) and Surucuoqlu et al. (2005) reported 40%, 54.9% and 31% MDR *S. aureus* respectively from wound sample. The overuse of antibiotics clearly drives the evolution of resistance. In bacteria, antibiotic resistance occurs due to horizontal gene transfer among different species of bacteria and spontaneously through mutation. Antibiotics remove drug-sensitive competitors, leaving resistant bacteria behind to reproduce as a result of natural selection. Despite warnings regarding overuse, antibiotics are overprescribed worldwide (Read and Woods 2014).

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives (Golkar et al. 2014). The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Spellberg and Gilbert 2014).

CONCLUSION

S. aureus was the most common bacteria causing wound infection and the prevalence of MRSA and MDR *S. aureus* was 16.7% and 54.4% respectively. Most of the isolates were sensitive towards and gentamicin and resistant towards penicillin and amoxicillin. Continuous surveillance on antimicrobial susceptibility of *S. aureus* is essential for the detection of emerging trends and the development of appropriate therapeutic strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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