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Original Article

Antibiogram of Bacteria Isolated From Wound Exudates

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ABSTRACT

The objective of this study was to isolate, identify and determine the antibiotic susceptibility pattern of aerobic causative infectious agents of wound infection from pus specimen. Determination of prevalence of methicillin-resistance among S. aureus (MRSA) and their Minimum Inhibitory Concentration (MIC) to vancomycin was the other objective.

The study was carried out in KIST Medical College and Teaching Hospital, Lalitpur, Nepal form November 2012 to June 2013. Pus sample collected aseptically were processed in the microbiology laboratory. The culture of the specimen, identification of the isolates and their antibiotic susceptibility testing were done as per the standard guidelines.Results: In a total of 149 culture positive specimens, 83(55.7%) were S. aureus, a leading cause of wound infection followed by (23.4%) belonging to the members of Enterobacteriaceae family. Coagulase Negative Staphylococci accounts for 14(9.4%), 8(5.4%); Acinetobacter spp., 5(3.4%); Pseudomonas aeruginosa and 4(2.7%); Enterococcus spp. In vitro susceptibility testing of all of these isolates showed that Imipenem was the most efficient drug for the Gram-negative isolates. Vancomycin was reported to be the most sensitive drug for Gram-positive isolates with 100% susceptibility rate. Among 83 S. aureus, the prevalence of MRSA was 27(32.5%). Detection of inducible clindamycin resistance in S. aureus showed that 10(12%) were iMLSB phenotypes. Similarly, β-lactamase production in S. aureus was detected in 53(62%) isolates. All of the MRSA strains were sensitive to vancomycin while subjected to determine Minimum Inhibitory Concentrations.

S. aureus is one of the major pathogen that causes wound infections. Prevalence of drug resistant superbugs like MRSA is increasing which is a major concern and thus, antibiotic susceptibility testing is crucial in empirical drug therapy. MIC determination is more sensitive than disc diffusion.

Introduction

Wounds have been with humankind from the prehistoric beginning . They have been regarded as the most common nosocomial infections and are probably the major complications of surgery and trauma . They remain as a major public health problem, despite the progress made on improving surgical techniques and antibiotic prophylaxis application . Probably the first step in bacterial wound infection is the attachment of bacteria to the surface of wounded skin . Bacterial contamination, wound colonization, critical colonization and wound infection are the series of events in a bacterial wound infection . Wound colonization is most frequently poly-microbial involving numerous micro-organisms that are potentially pathogenic; any wound is at some risk of becoming infected . In developing countries, wound infections are recognized as a prominent route of bacterial infections . Aerobic and anaerobic, gram-negative and gram-positive organisms, whose origins are the endogenous oral, gastrointestinal and skin flora may be present in such infections, where they exist synergically .

Antibiotics are always necessary in a bacterial infection, but their abuse and misuse in order to prevent bacterial infections contribute to increased bacterial drug resistance . Antimicrobial resistance is a serious problem that not only threatens the continued effectiveness of antimicrobials but also risks global health security . Following the ubiquitous use of antibiotics, multidrug-resistant nosocomial pathogens such as methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Acinetobacter baumannii and enteric bacteria such as extended spectrum β-lactamase (ESBL) producing E. coli and Klebsiella spp. have emerged as the predominant pathogens in complex wound related soft tissue infection . The knowledge of the causative agents of wound infections will be therefore helpful in the selection of empiric antimicrobial therapy .

S. aureus is a potentially pathogenic bacterium which is an important cause of skin and soft tissue infections (SSTIs) and other life threatening disease ranging from endovascular infection to sepsis . Infections due to methicillin-resistant S. aureus (MRSA), have been a common cause of severe, hospital acquired infections since 1960s and its emergence in the community has added another serious concern to the epidemiology of S. aureus infections .

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After the emergence of MRSA in 1960s, the glycopeptides, particularly vancomycin, became the mainstay of therapy for serious MRSA infections . It continues to be used as a first-line antimicrobial agent for the treatment of infection with MRSA then .

The clinical use of vancomycin is, however, threatened by increasing reports of a higher likelihood of mortality or treatment failure among patients infected with MRSA presenting with a vancomycin MIC at the high end of susceptible range .

MATERIALS AND METHODS:

Sample collection: Pus specimen of 229 patients of all age group was collected for the purpose of study from November 2012 to June 2013. Pus specimens collected from the patients and then transported to the laboratory were processed aseptically.

Sample processing: Pus specimen were inoculated onto MacConkey Agar (Himedia) and Blood Agar (HImedia) and incubated at 370C for 24-48 hours. The growth of organism was observed. Further biochemical tests were performed to identify the isolates. Macroscopic and microscopic observation of the specimen was also performed. Macroscopic observation included observation of the color, odour, consistency and presence of RBCs, granules, etc. Microscopic observation of the specimen included Gram-staining and its observation under microscope.

Antibiotic susceptibility testing: All the isolates were subjected to in-vitro susceptibility testing by Modified Kirby-Bauer disc diffusion method. The S. aureus isolates were screened for methicillin resistance by cefoxitin disc diffusion. The Methicillin resistant S. aureus (MRSA) were further subjected to determine minimum inhibitory concentrations (MIC) of vancomycin by agar dilution method.

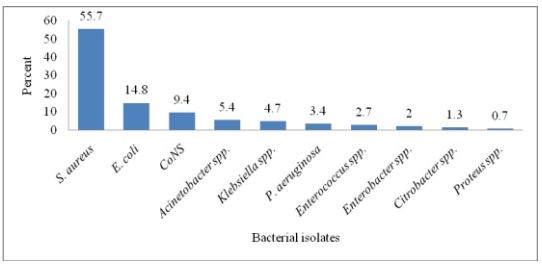
Quality control: Control strain of S. aureus (ATCC 25923) and E. coli (ATCC 25922) were used wherever applicable for quality control throughout the study.

Data analysis: Analysis of the data was carried out using software, namely; Statistical Package for Social Sciences (SPSS) version 16 and WHONET 5.6.

RESULTS:

Altogether 229 pus and wound swabs were collected from both male and female patients of all age groups and processed. Of total specimen processed; 80(34.9%) showed no growth of any organism while remaining 149(65.1%) were culture positive. Among the culture positive specimens; 83(55.7%) were S. aureus, 14(9.4%) were coagulase-negative staphylococci (CoNS), 22(14.8%) were E. coli, 7(4.7%) were Klebsiella spp., 1(0.7%) was Proteus spp., 5(3.4%) were *P. aeruginosa*, 4(2.7%) were Enterococcus spp., 3(2.0%) were Enterobacter spp., 2(1.3%) were Citrobacter spp. and 8(5.4%) were Acinetobacter spp. This showed that Gram positive S. aureus is the most common causative agent of wound infection followed by Gram Negative E. coli (Chart 1).

Chart 1: Bacteriological profile of wound exudate



Age wise distribution of the pathogens causing wound infections showed that adult (age ≥ 19 years) were more susceptible to wound infection than the pediatric patients (age ≤ 18 years). The rate of wound infection in adults was 69.1% while it was 30.9% in pediatric patients.

Similarly, 57.7% of the causative organisms were isolated from in-patients while 42.3% were isolated from out-patients. On the basis of gender, 53.7% of the isolates were isolated from the male patients while the remaining 46.3% from the female patients.

In a total of 83 S. aureus isolates, 56(67.5%) were methicillin sensitive (MSSA) and the remaining 27(32.5%) were methicillin resistant (MRSA) strains (Chart 2).

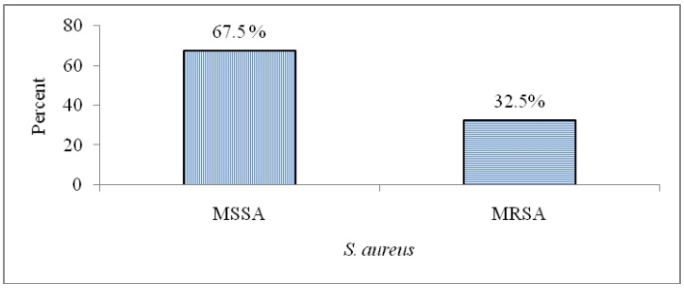


Chart 2: Distribution of MRSA and MSSA among S. aureus isolates

Out of 83 S. aureus isolates, 42(50.6%) were isolated from in-patients and the remaining 41(49.4%) were isolated from out-patients. Such a distribution of S. aureus among in-patients and out-patients is statistically insignificant (p value 0.531 > 0.05) (Table 1).

Table 1: Distribution of *S. aureus* on the basis of type of patients

S. aureus	Type of patient		p-value
	Out-patients	In-patients	
MRSA	12	15	
MSSA	29	27	0.531

Among 83 S. aureus, gender wise distribution of the isolates showed that 41(49.4%) were from female patients and 42(50.6%) were from male patients. However, there was no statistical significance of such distribution pattern (p value; 0.118 > 0.05).

Similarly, on the basis of age of the patient, the rate of S. aureus infection was found to be higher in the adults (61.4%) than in the pediatric patients (38.6%). Such distribution pattern of S. aureus on the basis of age-group was statistically significant (p value; 0.027 < 0.05) (Table 3).

Table 3: Distribution of S. aureus on the basis of age

S. aureus	Age-group		P-value
	Pediatric	Adult	
MRSA	15	12	
MSSA	17	39	0.027

Antibiotic susceptibility pattern of S. aureus isolates showed that none of the isolates were resistant to vancomycin and chloramphenicol. However, all the isolates showed resistance towards penicillin. Among all the S. aureus each of 1MRSA and MSSA showed resistance to amikacin. Similarly, 7(25.9%) MRSA and only 2(3.6%) MSSA were resistant to gentamicin. Antibacterial activity of tetracycline showed that 4(14.8%) of MRSA were resistant to it while only 1(1.8%) MSSA was resistant. 50(83.9%) of MSSA isolates were resistant to oxacillin. But, S. aureus resistant to oxacillin was confirmed by using cefoxitin, which showed all the MSSA isolates were sensitive to it (Table 4).

Similarly, antibiotic susceptibility profile showed that all the Gram-negative isolates belonging to the family Enterobacteriaceae were susceptible to imipenem. All the isolates of P. aeruginosa were sensitive to imipenem, while 75% of Acinetobacter spp. were sensitive to imipenem. Most of the strains of belonging to both Gram-positive and Gram-negative bacteria were multi-drug resistant.

Table 4: Antibiotic resistance profile of S. aureus (MRSA,N= 27 and MSSA, N=56)

Antibiotics (Concⁿ.)	Antibiotic resistance profile		Total
	MRSA n(%)	MSSA n(%)	
CX (30)	27 (100)	0 (0.0)	27(32.5%)
P (10U)	27 (100)	56 (100)	83(100%)
OX (1)	27 (100)	50 (83.9)	77(92.7%)
CD (2)	13 (48.1)	6 (10.7)	19(22.8%)
E (15)	22 (81.5)	19 (33.9)	41(49.3%)
CIP (5)	12 (44.4)	23 (41.4)	35(42.1%)
OF (5)	15 (55.6)	22 (39.3)	37(44.5%)
TE(30)	4 (14.8)	1 (1.8)	5(6.1%)
AK (30)	1 (3.7)	1 (1.8)	2(2.4%)
GEN (10)	7 (25.9)	2 (3.6)	9(10.8%)
C (30)	0 (0.0)	0 (0.0)	0(0%)
VA (30)	0 (0.0)	0 (0.0)	0(0%)

In this study, detection of β- lactamase production by S. aureus was based on disc diffusion test. As per the test so performed, 53(64.0%) of all S. aureus isolate produced β- lactamase. Such a distribution of β- lactamase production by the S. aureus was found to be statistically significant (p-value; 0.011 < 0.05) (Table 5).

Table 5: β -Lactamase production in S. aureus

S. aureus	β- Lactamase Production		P-value
	Negative	Positive	
MRSA	15	12	
MSSA	15	41	0.011

Similarly, out of 83 S. aureus isolates, 10(12%) showed D-zone test positive. Among them 4(4.8%) were MSSA and 6(7.2%) were MRSA. Such a distribution of clindamycin resistance in S. aureus was statistically significant (p-value 0.048 < 0.05) (Table 6).

Table 6: Inducible Clindamycin Resistance in S. aureus using D-test

S. aureus	D-Zone test		P-value
	Negative	Positive	
MRSA	21	6	
MSSA	52	4	0.048

Finally, Minimum Inhibitory Concentration (MIC) of vancomycin was performed by agar dilution method. MIC was carried out only among 27 MRSA isolates. Some of the suspected MRSA strains (with diameter of zone of inhibition to vancomycin disc = 6mm) and other susceptible MRSA strains were subjected to MIC. MIC of vancomycin all the strains was below 2 µg/ml i.e. all the strains were susceptible to vancomycin.

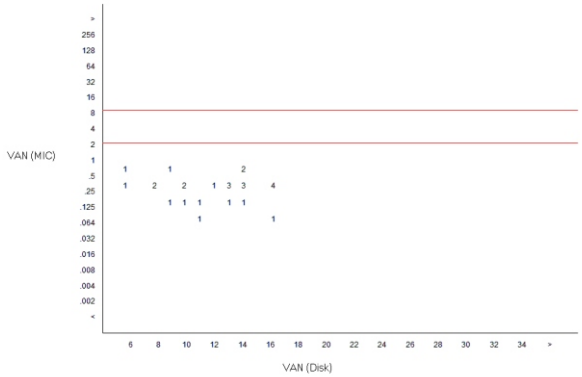


Chart 3: Scatter-plot of Vancomycin (Disc diffusion test v/s MIC)

DISCUSSION:

This study was carried out in KIST Medical College and Teaching Hospital with an objective to study the etiological agents of wound infection. In total, 229 wound exudates (pus) were received from both in-patients and out-patients and processed during the study period in order to study the bacteriological profile of wound exudate. Microbiological profile of wound infection or skin and soft tissue infection shows that S. aureus is the predominant causative agent. As in this study, S. aureus was the leading cause of wound infection in other similar studies performed by Banjara et al., in 2002 . Bhatta and Lakhey (2010), Egbe et al., (2011) and Suwal et al., (2012), where the rate of

infection due to S. aureus was 24.9%, 50%, 21.5% and 24.3% respectively . However, different studies showed that P. aeruginosa was the leading cause of burn wound infections. In 1997, Mousa conducted a study to assess the rate of burn wound infection by aerobic bacteria and found that 19.1% of the wound infection was caused by P. aeruginosa. Similar study on burn wound infection by Nasser et al. (2002) showed P. aeruginosa (21.6%) as the most common isolate .

Determination of the prevalence of MRSA among the isolated S. aureus was the other objective. The prevalence of MRSA was 27/83(32.5%). Several studies have been carried out in different parts of Nepal in order to assess the prevalence of MRSA. The prevalence of MRSA was 68% among skin infection cases in a hospital in Chitwan, Nepal . Similar study by Sanjana et al. (2010) to assess the prevalence of MRSA in a teaching hospital found it to be 39.6% . Similar study carried out in BPKIHS, Eastern Nepal showed a prevalence of MRSA as 26% . Overall prevalence of MRSA in clinical samples of hospitals located in Kathmandu Valley, Nepal was 62% .

Antibiotic susceptibility testing (AST) of all the isolates was performed by modified Kirby-Bauer disc diffusion method. It was reported that imipenem was the most effective drug for the Gram-negative bacteria. Similarly, vancomycin was the most effective dug for Gram-positive bacteria followed by chloramphenicol. And, minimum inhibitory concentration determination of vancomycin to MRSA strains of S. aureus showed that all of them were sensitive to it.

Penicillin is rarely considered for treatment of staphylococcal infection but it might be considered for infections requiring lengthy therapy (e.g., endocarditis, osteomyelitis) if penicillin were known to be sensitive. However, some staphylococci that tests "Sensitive" to penicillin by MIC or disc diffusion may possess a β-lactamase (BL) that may cause the patient to fail penicillin therapy . In this study, S. aureus resistance to penicillin by the production of β-lactamase was detected by penicillin disc zone edge test as recommended by CLSI –– . Out of 83, 53(64%) of the S. aureus isolates were found to produce β-lactamase.

Clindamycin (a lincosamide) is an attractive option for infections due to MRSA . However, therapy for MRSA infections is complicated by the possibility of inducible macrolide, lincosamide and streptogramin B (iMLSB) resistance . Clindamycin resistance in S. aureus may be of two types, namely; inducible clindamycin resistance (ICR), designated as iMLSB and the other is constitutive resistance designated as cMLSB. Thus, from 19 strains that were resistant to clindamycin, 10 strains were iMLSB and the remaining 9 strains were cMLSB. From the 10 iMLSB phenotypes, 4 were MSSA while 6 were MRSA. Such a distribution of iMLSB strains among MRSA and MSSA is statistically significant (p value 0.048< 0.05).

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Antibiotics (Concentration)	Antibiotic susceptibility pattern	
	Susceptible n(%)	Non-susceptibile n(%)
Ampicillin (10mcg)	7(20)	28(80)
Cefipime (30mcg)	16(45.8)	19(54.2)
Ceftazidime (30mcg)	8(22.8)	27(77.2)
Chloramphenicol (30mcg)	21(60)	14(40)
Ciprofloxacin (5mcg)	12(34.3)	23(65.7)
Ofloxacin (5mcg)	16(45.8)	19(54.2)
Gentamicin (10mcg)	22(62.8)	13(37.2)
Amikacin (30mcg)	23(65.7)	12(34.3)
Imipenem (10mcg	35(100)	0(0)
Tetracycline (30mcg)	14(40)	21(60)

Antimicrobial susceptibility pattern for *Pseudomonas aeruginosa* (n=5)

Antibiotics (Concentration)	Antibiotic susceptibility pattern	
	Susceptible n(%)	Non-susceptibile n(%)
Cefipime (30mcg)	2(40)	3(60)
Ceftazidime (30mcg)	1(20)	4(80)
Ciprofloxacin (5mcg)	5(100)	0(0)
Ofloxacin (5mcg)	4(80)	1(20)
Gentamicin (10mcg)	3(60)	2(40)
Amikacin (30mcg)	2(40)	3(60)
Imipenem (10mcg	5(100)	0(0)

Antimicrobial susceptibility testing for *Acinetobacter* spp. (n=8)

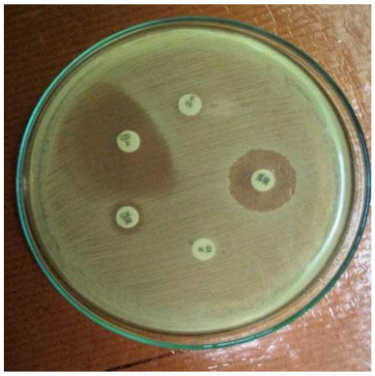
Antibiotics (Concentration)	Antibiotic susceptibility pattern	
	Susceptible n(%)	Non-susceptibile n(%)
Cefipime (30mcg)	2(25)	6(75)
Ceftazidime (30mcg)	0(0)	8(100)
Ciprofloxacin (5mcg)	3(37.5)	5(62.5)
Ofloxacin (5mcg)	1(12.5)	7(87.5)
Gentamicin (10mcg)	2(25)	6(75)
Amikacin (30mcg)	3(37.5)	5(62.5)
Imipenem (10mcg	6(75)	2(25)

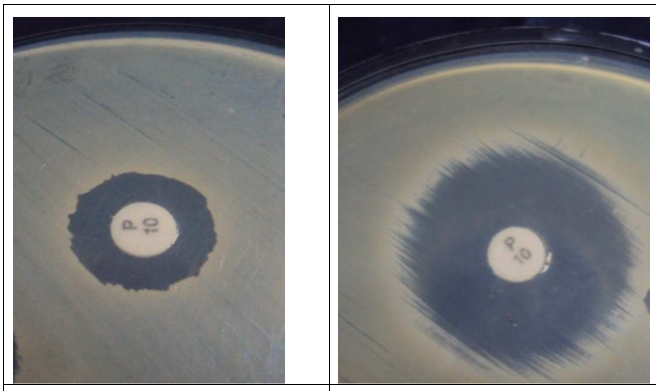
Antibiotics (Concentration)	Antibiotic susceptibility pattern	
	Susceptible n(%)	Non-susceptibile n(%)
Penicillin (10units)	0(0)	14(100)
Ciprofloxacin (5mcg)	9(64.2)	5(35.8)
Ofloxacin (5mcg)	11(78.5)	2187.5)
Gentamicin (10mcg)	9(64.2)	5(35.8)
Amikacin (30mcg)	11(78.5)	3(21.5)
Chloramphenicol (30mcg)	12(85.7)	2(14.3)
Tetracycline (30mcg)	12(85.7)	2(14.3)
Vancomycin (30mcg)	14(100)	0(0)

Antimicrobial susceptible pattern of *Enterococcus* spp. (n=4)

Antibiotics (Concentration)	Antibiotic susceptibility pattern	
	Susceptible n(%)	Non-susceptibile n(%)
Penicillin (10units)	1(25)	3(75)
Ampicillin (10mcg)	1(25)	3(75)
Ciprofloxacin (5mcg)	2(50)	2(50)
Erythromycin (15mcg)	1(25)	3(75)
Chloramphenicol (30mcg)	4(100)	0(0)
Tetracycline (30mcg)	2(50)	2(50)
Vancomycin (30mcg)	4(100)	0(0)

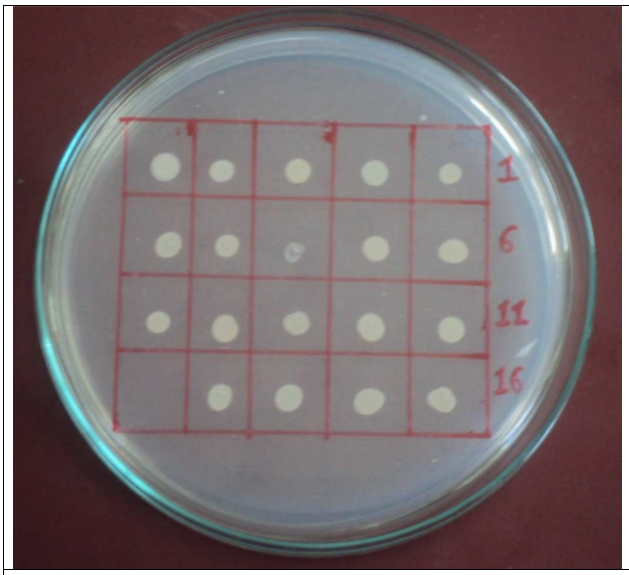
Photograph 1: Antibiotic Susceptibility pattern of S. aureus (MRSA and Inducible clindamycin resistant strain) on Muller Hinton Agar (Isolate No. T- 1026)





A positive test (Isolate No. T-988)	A negative test (Isolate No. T- 999)

Photograph 2: Penicillin disc zone-edge test for the detection of β-lactamase production by *S. aureus*



Photograph 3: Minimum Inhibitory concentration of vancomycin to MRSA strain of *S. aureus* by agar dilution method (concentration of vancomycin= 0.25 µg/ml)

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