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Antibiogram of bacterial species causing skin wound infections

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Abstract

Wounds occur when the integrity of any tissues is compromised. Infection causes significant increase in costs, morbidity, and potential mortality. This study was conducted during the period from July, 2015 to January, 2016 with the aims of identifying the etiological agents causing skin wound infections, and their antibiotic susceptibility profile among patients visiting International Friendship Children's Hospital (IFCH), Maharajgunj, Kathmandu, Nepal. Specimens were processed by conventional culture technique and antibiogram of isolates were done by modified Kirby-Bauer disc-diffusion method. Out of 219 skin pus samples, 132 (60.3%) were reported to be bacterial culture positive. Eight different bacterial species were identified as; *S. aureus* 75 (56.8%), Coagulase negative *S. aureus* (CONS) 20 (15.2%), *Escherichia coli* 13 (9.8%), *Citrobacter* spp. 7 (5.3%), *Pseudomonas aeruginosa* 5 (3.7%), *Klebsiella* spp. 5 (3.7%), *Proteus* spp. 5 (3.7%) and *Enterobacter* spp. 2 (1.5%), all were isolated from culture positive specimens. Antibiotic susceptibility test (AST) of all Gram-negative isolates showed that Colistin and Imepenum were the most effective antibacterial drugs. Out of total 75 *S. aureus* isolates, all were reported to be susceptible to Vancomycin, whereas, 23 (30.7%) were resistant to methicillin. This study reported that *S. aureus* strains were the predominant isolates. Prevalence of multi-drug resistant strains of *S. aureus* is increasing. Current results demonstrated that antibiotic resistance in Gram-positive and Gram-negative bacteria is increasing in alarming trends that lead to failure of treatment.

Key words: Wound infection, *S. aureus*, AST, Disc-diffusion, MRSA

1. Introduction

Healthy skin is the first line of defence and barrier against microbial invasion (Landis, 2008). Wound is a type of injury in which the skin is turned, cut or punctured (open wound) or where blunt force trauma causes a contusion (closed wound) (Bhatta and Lakhey, 2007). It may be caused as a result of a fall, a surgical procedure, an infectious disease or an underlying pathological condition. Certain parasites such as Hookworm larvae and bacteria e.g. *Treponema pallidum*, can penetrate the intact skin. However, certain primary skin infections like impetigo are caused by *Streptococcus pyogens* or *S. aureus*, both gain access through abrasions as minor trauma of skin is part of our daily life.

Patients with diminished immunity are highly susceptible and at high risk of developing a wound infection (Heinzelmann *et al*., 2002). There are several factors including; age, obesity, malnutrition, endocrine and metabolic disorders that influence development of wound infection. Previously, Baquero, (1997) reported that virulence factors, quantity and antibiotic resistance of bacteria may also lead to contamination that result in wound infection.

Wound can be infected by variety of microbes such as bacteria, fungi and parasites (Bowler *et al*., 2001). The most common Gram-positive bacteria that cause wound infection are the haemolytic *Streptococcus* and *S. aureus*, while the Gram-negative bacteria are mainly rod shaped *P. aeruginosa*. Infection caused by *P. aeruginosa* is particularly challenging because of its resistance to most antibacterial drugs. The facultative anaerobes include *Enterobacter* spp., *E. coli*, *Klebsiella* spp. and *Proteus* spp. Meanwhile, the fungal strains are mainly unicellular *Candida* spp. and mold fungi such as *Aspergillus* spp.

Microorganisms responsible for wound infection depend mainly on the surgical site, and misuse of antimicrobials within the hospital. *S. aureus* remains a significant cause of mortality and morbidity in tropical countries (Rasoul *et al*., 2010). Surgical site infections (SSI) caused by *S. aureus* are harmful to patients and more costly for society (Schmidt *et al*., 2015). Moreover, Weiner *et al*., (2016) added that *S. aureus* is the most frequently reported microbe that causes SSI after clean surgery.

The misuses of antibiotics together with the duration of time over which they were available, led to major problems of antibiotic resistant microorganisms contributing to morbidity and then mortality. Following the ubiquitous use of antibiotics, multi drug resistant (MDR) nosocominal pathogens such as MRSA, *P. 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 causing complex wound related soft tissue infections (Alavi *et al*., 2010). Recently, Parvez, (2018) added that MRSA is no longer limited to hospitals, but occurs among otherwise healthy communities as well. Accordingly, knowledge of the causal agents of wound infections will be therefore helpful for the selection of suitable antibiotic therapy.

The present study was carried out with the aims of exploring the bacteriological etiologic agents of wound infections, and the AST of the pathogens.

2. Materials and Methods

This study was conducted over a period of 6 months, from July, 2015 to January, 2016 in the microbiological laboratory of IFCH. A total of 219 pus samples from skin were collected for culture and AST assay from patients of age below 20 years. The collected samples were processed following the standard laboratory techniques.

Samples were collected from wounds using a sterile cotton swab. These swabs were then streaked onto Nutrient agar (NA), MacConkey agar (MA) and Blood agar (BA) plates, and then incubated at 37ºC for 24 h. After incubation, bacteria recovered from positive cultures were identified according to standard microbiological criteria such as; colonies morphology, Gram stain and biochemical assays carried out according to Bergey's Manual of Determinative Bacteriology, (2000).

The AST of all isolates was performed by modified Kirby Bauer's disc diffusion method on Muller Hilton Agar medium (Bauer *et al*., 1966), using antibiotics as per Clinical Laboratory and Standard Institute (CLSI) guidelines. All the culture media and biochemicals used were from Hi-media Company (India). Control strains of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used wherever applicable for quality control throughout this study.

Statistical analysis

Analysis of data was carried out using software SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) and Chi square test was applied. P-value <0.05 was considered statistically significant.

3. Results

Out of 219 skin pus samples, 132 (60.3%) were bacterial culture positive, while the rest of samples 87 (39.7%) showed no growth of bacteria on each of NA, MA and BA isolation media (Fig. 1). These samples were collected from all hospital departments, 63.1% of the causative bacteria were isolated from In-patient department (IPD), and 56.2% were from Out-patient department (OPD).

On the basis of gender, 59.8% of isolates were recovered from male patients, while the remaining 40.2% were from female patients. Age wise distribution of the pathogens causing wound infections showed that high infection rate was found in age group of 1-5 years. From the 132 cultures positive specimens, 8 different bacterial species were identified biochemically. The predominant bacteria were *S. aureus* (56.8%), CONS (15.2%), followed by *E. coli* (9.8%). The remaining isolates were *P. aeruginosa*, *Citrobacter* spp., *Proteus* spp., *Klebsiella* spp. and *Enterobacter* spp. (Fig. 2).

The most effective antibiotics for the Gram-negative bacterial isolates were Imepenum and Colistin showing 100% sensitivity, followed by Amikacin (86.5%), Azithromycin (72.9%) and chloramphenicol (72.9%), respectively. On the contrary, Cotrimoxazole (29.7%) and Amoxycillin (5.4%) were recorded as the least effective antibacterial drugs (Table 1).

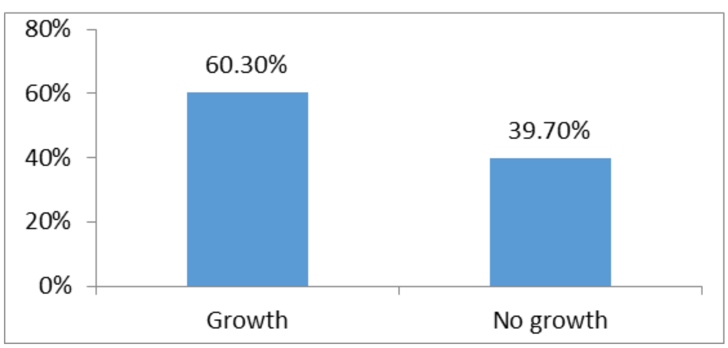

Fig. 1: Culture positivity of 219 samples of processed pus specimens

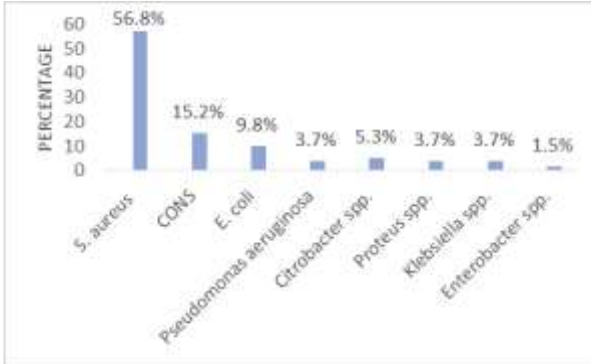

Fig. 2: Bacterial isolates recovered from 132 wound pus samples, CONS; Coagulase negative *S. aureus*

Table 1: Antibiotic susceptibility test (AST) of Gram-negative bacterial isolates

Antibiotics Used	Sensitive isolates		Resistant isolates		Total
	Number	Percentage (%)	Number	Percentage (%)	
Amikacin	32	86.5	5	13.5	37
Azithromycin	27	72.9	10	27.1	37
Ceftazidime	10	27.0	27	73.0	37
Cotrimoxazole	11	29.7	26	70.3	37
Gentamicin	21	56.8	16	43.2	37
Cefotaxime	10	27.0	27	73	37
Amoxycillin	2	5.4	35	94.6	37
Chloramphenicol	27	72.9	10	27.1	37
Colistin	37	100	0	0.0	37
Meropenem	21	56.8	16	43.2	37
Imepenum	37	100	0	0.0	37
Piperacillin/Taz	19	51.4	18	48.6	37

-Results of AST were obtained from 37 isolates of Gram-negative bacteria

Out of 75 *S. aureus* isolates, 52 (69.30%) were methicillin sensitive (MSSA), whereas, 23 (30.70%) were MRSA. Among these *S. aureus* isolates, 43 (57.3%) were recovered from OPD, 18 (24%) were from General ward, however, 14 (18.6) were isolated from Intensive care unit (ICU) (Table 2). This distribution demonstrated that higher percentage of MRSA was isolated from out patients. However there was no statistical significance of such distribution pattern. (P- value; 0.79 > 0.05).

The rate of *S. aureus* infection was found to be higher in the age group of 1-5 years (45.3%), however, the age group below 1 was least affected (Table 3). There was no statistical significance of such distribution pattern (P-value; 0.220 > 0.05).

Similarly on the basis of gender of patients, *S. aureus* were isolated from female patients composed of 9 (17.3%) MSSA and 10 (43.5%) MRSA. Meanwhile, isolates of male patients composed of 43 (82.7%) MSSA and 13 (56.5%) MRSA. Accordingly, the rate of infection due to MRSA was higher in males than in females (Table 4). Such distribution of *S. aureus* on the basis of gender of patients was statistically significant (P-value; 0.016 < 0.05). Antibiotic susceptibility pattern of *S. aureus* isolates showed that none of them were resistant to Vancomycin. However, all isolates showed resistance towards Penicillin. Among all *S. aureus* isolates, 4 (17.4%) MRSA and 1 (1.9%) MSSA showed resistance to Amikacin. Similarly, only 1 (4.3%) MRSA and 3 (5.7%) MSSA showed resistance to Chloramphenicol. Also 7 (30.4%) MRSA and 2 (3.8%) MSAA were resistant to Tetracycline. This was followed by Gentamicin antibiotic, where 8 (34.7%) MRSA and 6 (11.5%) MSSA were resistant to it (Table 5).

Table 2: Department wise distribution of *S. aureus* isolates

Department	MRSA	MSSA	Total	P-value
OPD	12 (52.2)	31 (59.6)	43 (57.3)	
General ward	9 (39.1)	9 (17.3)	18 (24)	
ICU	2 (8.7)	12 (23.1)	14 (18.6)	0.79
Total	23 (30.7)	52 (69.3)	75 (100)	

-Results were obtained from 75 isolates of *S. aureus*, P-value <0.05 was considered statistically significant

Table 3: Age wise distribution of *S. aureus* isolates

Age Group	**MRSA**	**MSSA**	**Total**	**P-value**
Below 1	3 (13.0)	3 (5.8)	6 (8)	
1-5	10 (43.5)	24 (46.2)	34 (45.3)	
5-10	4 (17.4)	13 (25)	17 (22.7)	
10-15	5 (21.7)	4 (7.6)	9 (12)	0.220
15-20	1 (4.3)	8 (15.4)	9 (12)	
Total	23 (100)	52 (100)	75 (100)	

-Results were obtained from 75 isolates of *S. aureus*, P-value <0.05 was considered statistically significant

Table 4: Gender wise distribution of *S. aureus* isolates

Gender	**MRSA**	**MSSA**	**Total**	**P-value**
Female	10 (43.5)	9 (17.3)	19 (25.3)	
Male	13 (56.5)	43 (82.7)	56 (74.7)	0.016
Total	23 (1000)	52 (100)	75 (100)	

-Results were obtained from 75 isolates of *S. aureus*, P-value <0.05 was considered statistically significant

Table 5: Antibiotic susceptibility pattern of *S. aureus* isolates

Antibiotics	Antibiotic sensitivity pattern				Total R (%)
	MRSA		MSSA		
	R {n, (%)}	S {n, (%)}	R {n, (%)}	S {n, (%)}	
Amikacin	4 (17.4)	19 (82.6)	1 (1.9)	51 (98.1)	5 (6.7)
Chloramphenicol	1 (4.3)	22 (95.6)	3 (5.7)	49 (94.2)	4 (5.3)
Ciprofloxacin	18 (78.3)	5 (21.7)	24 (46.2)	28 (53.8)	52 (69.3)
Cotrimoxazole	19 (82.6)	4 (17.4)	31 (59.6)	21 (40.3)	50 (66.7)
Cefoxitin	23 (100)	0 (0.0)	0 (0.0)	52 (100)	23 (30.7)
Erythromycin	16 (69.5)	7 (30.4)	27 (51.9)	25 (48.1)	43 (5.3)
Gentamicin	8 (34.7)	15 (65.2)	6 (11.5)	46 (88.5)	14 (18.7)
Penicillin	23 (100)	0 (0.0)	52 (100)	0 (0.0)	75 (100)
Ofloxacin	17 (73.9)	5 (21.7)	20 (38.5)	32 (61.5)	49 (65.3)
Oxacillin	23 (100)	0 (0.0)	30 (57.6)	22 (42.3)	52 (69.3)
Tetracycline	7 (30.4)	16 (69.5)	2 (3.8)	50 (96.5)	9 (12)
Vancomycin	0 (0.0)	23 (100)	0 (0.0)	52 (100)	0 (0.0)

-Results were obtained from 75 isolates of *S. aureus*

4. Discussion

This study was carried out in IFCH with an objective to study the bacterial etiological agents of wound infections. Among the total pus samples processed 132 (60.3%) were culture positive, whereas the rest 87 (39.7%) showed no growth of bacteria. Current results agreed with those of Yakha, (2014) and Arjun, (2015) in Nepal, who reported 65.1% samples with bacterial growth and 44.8% without growth, respectively. Overall 130 (59.4%) patients were from IPD and remaining 89 (40.6%) from OPD. The rate of wound infection was higher in IPD (63.1%) than in OPD (56.2%). Similar study was carried out by Yakha, (2014) who found that prevalence of wound infection was higher in inpatients (54.9%) than in out-patients (52.63%). The bacterial growth was found to be higher in male patients (59.8%) than in female patients (40.2%) in accordance with Yakha, (2014). The relative higher percentage of male patients might be due to active involvement of male children with knives, pencils, sharp instrument, and fighting each other's. Both Gram-positive and Gram-negative aerobic bacteria were isolated from the pus specimens. *S. aureus* was the predominant cause of wound infection which accounted for (56.8%) of all isolates, followed by CONS (15.2%) and *E. coli* (9.8%). Similar study performed by Arjun, (2015) reported that *S. aureus* (55.7%) was the predominant species followed by CONS (31%), moreover, Dryden, (2009) in UK previously pointed that *S. aureus* was present in 45% of skin and soft tissue infections.

AST of all the isolates demonstrated that Imepenum and Colistin were the most effective antibacterial drugs for Gram-negative bacteria. Whereas, Vancomycin and Chloramphenicol antibiotics were highly effective against the Gram-positive bacteria.

One of the objectives of this study was to determine the prevalence of MRSA among isolated *S. aureus*. Generally, MRSA is associated with skin and soft-tissue infections, endovascular infections, pneumonia, septic arthritis's, endocarditis and osteomyelitis (Yasmin *et al*., 2016). Currently, the prevalence of MRSA was 23/75 (30.70%). These results are in accordance with a study carried by Rijal *et al*., (2008) among the school children of Pokhara, Nepal, where the prevalence rate was 56.1%. Moreover, prevalence was 18.1% in another study of Thapa *et al*., (2008) performed in Birendra Sainik Hospital, Nepal. In MRSA, the most effective antibiotics were Vancomycin 100.0% (23/23) followed by Chloramphenicol 95.6% (22/23), and Amikacin 82.6% (19/23), respectively. Antibacterial drug resistance of MRSA was highest with Penicillin and Oxacillin (100.0%). These findings are in accordance with those of Sanjana *et al*., (2010). Current results are attributed to fact that MRSA strains are often resistant to all standard β-lactams, macrolides and aminoglycosides antibacterials (Fang *et al*., 2016). Our study confirmed that all MRSA isolates were significantly less sensitive to antibiotics compared with MSSA ones.

Conclusion

Gram-positive *S. aureus* was found to be more predominant in skin wound infections compared with Gram-negative bacteria in IFCH, Maharajgunj, Kathmandu, Nepal. In addition, our results demonstrated high prevalence of MRSA among patient's pus samples, thus prescription and sale of antibiotics without laboratory guidance should be discouraged.

Conflict of interests

The authors declare no conflict of interests.

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