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Research Article

Bacteriological Profile and Antibiogram of Infected Wound from Patients Visiting in Tertiary Care Children's Hospital, Kathmandu, Nepal

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Abstract

Introduction and Objective: Wound infection is the most common nosocomial health issue especially among patients undergoing surgery. This study was conducted in the Microbiology Laboratory of Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal from June 2016 to December 2016. The objectives of this study are to determine the bacteriological profile of wound infections and antimicrobial sensitivity pattern of the isolates from the wound at Kanti Children's Hospital, Kathmandu, Nepal.

Results: Among 300 pus samples, 188 (62.7%) were reported as positive bacterial growth. Among total positive growth, the highest bacterial isolates were reported from the male population (67.0%), in-patients' population (54.2%), and pus-aspirated samples (94.7%). The bacteriological profile showed *Staphylococcus aureus* (62.2%) was predominant bacteria followed by *Escherichia coli* (10.1%). The most sensitive antibiotics against all the isolates were vancomycin, amikacin, polymyxin B, and imipenem. All isolates were highly resistant to Amoxycillin and cotrimoxazole. Among total *S. aureus*, 35.9% isolates were shown to be methicillin-resistant *S. aureus*. The highest (57.9%) multi-drug resistance was reported from the in-patient samples.

Conclusion: This study concluded that a broad range of bacteria is associated with wound infection which should be treated following routine antibiotic sensitivity testing.

Keywords: *Pus; Wound Infection; MRSA; MDR; Bacteriological Profile; Antibiotic Susceptibility*

Introduction

Wound infection has become a serious threat in the medical field, and it is one of the most common hospital-acquired infection and accounts for 70-80% morbidity rate [1]. The presence of foreign materials even with low bacterial inoculums can increase the risk of serious infection in the skin and subcutaneous tissues [2]. Furthermore, the emergence of antimicrobial resistant organisms due to misuse of antibiotics has created challenges in the recovery of wound infections contributing to the high morbidity and mortality rate [3].

Globally, the wound infection has been regarded as the most common nosocomial infection, especially in patients undergoing surgery [1,2,4,5]. In Nepal, wound infection is a major threat among postoperative patients [6]. In addition, failure of treatment of wound infections has been reported due to antimicrobial resistant bacteria [6]. Therefore, the adequate data of bacteriological isolates involved in wound infection and their antibiotic susceptibility pattern is predominantly required for effective treatment of wound infections. The present study aims to determine the bacteriological profile of wound infection and antibiotic sensitivity pattern of the bacterial strains isolated from the patients visiting Kanti Children's Hospital, Nepal. The findings of this study could be valuable for the selection of appropriate antibiotics and designing prevention measures of wound infections.

Materials and Methods

This study was conducted in the Microbiology Laboratory of Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal from June 2016 to December 2016.

Sample size: A total of 300 pus samples were enrolled in this research which was collected from infected wounds of indoor and outdoor patients.

Methodology: The pus swab and pus aspirate (excluding drained pus) were collected from the wounds which were suspected as infected by the presence of purulent material following standard aseptic techniques. The purulent material was aspirated with a sterile syringe. In cases where aspiration was not possible, sterile cotton swabs were used. The samples were delivered quickly to the microbiological laboratory and inoculated on the nutrient agar (NA; HiMedia, M0010), MacConkey agar (MA; HiMedia, M081), and blood agar (BA; HiMedia, M073). The NA and MA plates were incubated aerobically and BA plates were incubated in micro-aerophilic condition using carbon dioxide enriched candle jar at 37°C for 24 hrs. After incubation, subculturing was performed on NA and incubated at 37°C for 24 hrs from the plates which were reported as positive for bacterial growth. The pure isolates were selected and identified by following the standard microbiological techniques, which involved studies of colony characteristics, Gram-staining reaction [7]. The biochemical studies included during identification of bacterial isolates were oxidative-fermentative tests, catalase, oxidase, coagulase, SIM, MRVP, citrate utilization, TSI, urease tests, and bacitracin and optochin sensitivity tests [8].

The antibiotic sensitivity test for pure identified isolates was performed by Kirby-Bauer sensitivity testing method according to the guidelines given by Clinical and Laboratory Standards Institute [9]. The antibiotics used for sensitivity tests were cloxacillin (5 mcg), amoxicillin(10 mcg), cotrimoxazole (25 mcg), chloramphenicol (30 mcg), amikacin (30 mcg), gentamicin (10 mcg), vancomycin (30 mcg), cefoxitin (30 mcg), imipenem (10 mcg), polymyxin B (5 mcg), cefotaxime (30 mcg), ciprofloxacin (5 mcg), cefepime(5 mcg), and ceftazidime (5 mcg). All the antibiotics discs used in this study were purchased from Himedia, India. The reference strains used for quality control during biochemical and antibiotics sensitivity tests were *E. coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC25923.

The Chi-square and P-value were calculated based on data entered in Microsoft Excel worksheet and considered significant only when it was less than or equal to 0.05.

Ethical approval and consent to participate: Ethical approval (Dispatch Number: 313) was obtained from Institutional Review Committee (IRC) of Kanti Children's Hospital, Kathmandu, Nepal before conducting the research. Informed written consent was obtained from patients of each participant involved in this study.

Results

Out of 300 pus samples, 188 (62.7%) samples were reported as positive for bacterial growth and 112 (37.3%) samples showed negative for bacterial growth. Among total positive cases, the highest prevalence of bacterial growth was observed in the male population (67.0%), in-patients population (54.2%), and pus-aspirated samples (94.7%). There was no significant relationship between gender and bacterial growth positivity ($p > 0.05$). In regard to patient wards and types of sample, statistical analysis revealed that both categories of samples were significantly associated with bacterial culture positivity ($p < 0.05$) (Table 1). Out of 188 positive bacterial cultures, the bacteriological profile reported 66.0% were Gram-positive isolates and 34.0% were Gram-negative isolates. Among Gram-positive isolates, *S. aureus* account to be more prominent (62.2%) causative agent of wound infection followed by Gram-negative isolates, *E. coli* (10.1%). The least bacterial strain reported was *Proteus mirabilis* (0.5%). The other Gram-negative isolates observed were *Klebsiella* spp., *Acinetobacter* spp., *Proteus vulgaris*, *Citrobacter* spp., *Pseudomonas aeruginosa* and *Enterobacter* spp. (Table 2).

S. No.	Sample distribution	Bacterial growth (positive)	Bacterial growth (negative)	p-value
1	**Sex**			> 0.05
	Male	126	82	
	Female	62	30	
2	**Patient wards**			< 0.05
	In-patient	102	78	
	Out-patient	86	34	
3	**Types of sample**			< 0.05
	Pus-aspirated	178	83	
	Pus-swabbed	10	29	

Table 1: *Sample distribution pattern and bacterial growth positivity among various wound infected samples.*

Bacterial Isolates	In-patients n (%)	Out-patients n (%)
Gram-positive bacteria		
Staphylococcus aureus	46 (53.5)	71 (69.6)
CoNS	2 (2.3)	5 (4.9)
Gram-negative bacteria		
E. coli	8 (9.3)	11 (10.8)
Klebsiella spp.	6 (7.0)	2 (2)
Acinetobacter spp.	5 (5.8)	5 (4.9)
Proteus vulgaris	2 (2.3)	3 (2.9)
Proteus mirabilis	0 (0)	1 (1)
Citrobacter spp.	7 (8.1)	1 (1)
Pseudomonas aeruginosa	6 (7.0)	1 (1)
Enterobacter spp.	4 (4.7)	2 (2)

Table 2: *Bacteriological profile of wound infections distributed among In-patients and Out-patients samples.*

The antibiotic sensitivity patterns showed that all the isolates of *S. aureus* were sensitive to vancomycin (100.0%) followed by gentamicin (95.7%) and amikacin (94.9%). The least sensitive antibiotic against *S. aureus* was amoxicillin (23.9%) followed by cloxacillin (44.4%). Amikacin was most effective against all the isolates of CoNS followed by gentamicin (85.7%). Amoxycillin and cotrimoxazole were less effective against CoNS. All the strains of *E. coli* were sensitive to polymyxin B whereas amoxicillin was found to be less (10.5%) sensitive. For *Acinetobacter* spp., imipenem antibiotic was reported to be the highest (80.0%) sensitive among all the tested drugs. Amoxycillin, cefepime, ceftazidime, cefotaxime, and polymyxin B were completely resistant towards all the strains of *Acinetobacter* species. The isolates of *Citrobacter* spp. showed high sensitive to amikacin (87.9%) but resistant to antibiotic such as cotrimoxazole, ciprofloxacin and cefepime. Both amikacin and imipenem were equally (85.7%) effective against the isolates of *Pseudomonas* species but amoxicillin, cotrimoxazole, and cefepime were not effective. For *Klebsiella* spp., the most effective antibiotic was polymyxin B (75%) followed by imipenem (62.5%). All the isolates of *Klebsiella* spp. were resistant to amoxicillin and ciprofloxacin (Table 3A).

Out of total *S. aureus* isolates, 75 (64.1%) were methicillin-sensitive *S. aureus* (MSSA) and 42 (35.9%) were methicillin-resistant *S. aureus* (MRSA). The distribution of MRSA was observed to be high among out-patient samples compared to in-patient samples. The distribution of MRSA was not significantly associated with the in-patient and out-patient samples ($p > 0.05$) (Table 3B). Among 38 isolates from in-patient samples, 22 (57.9%) were reported as multi-drug resistant (MDR) strains. Out of 26 isolates from out-patient samples, 11 (42.3%) were considered as MDR strains. The distribution of MDR was not significantly related to the in-patient and out-patient samples (P > 0.5) (Table 3C).

A. Antibiotic susceptibility pattern among different bacterial isolates							
Antibiotics	***S. aureus* n (%)**	**CoNS n (%)**	***E. coli* n (%)**	***Acinetobacter* spp. n (%)**	***Citrobacter* spp. n (%)**	***Pseudomonas* spp. n (%)**	***Klebsiella* spp. n (%)**
Amoxycillin	28 (23.9)	1 (14.2)	2 (10.5)	0	NT	0	0
Cotrimoxazole	88 (75.2)	2 (28.5)	3 (15.7)	1 (10.0)	0	0	1 (12.5)
Ciprofloxacin	93 (79.4)	3 (42.8)	2 (10.5)	3 (30.0)	0	5 (71.4)	0
Imipenem	NT	NT	16 (84.2)	8 (80.0)	6 (75.0)	6 (85.7)	5 (62.5)
Amikacin	111 (94.87)	7 (100.0)	15 (78.9)	7 (70.0)	7 (87.9)	6 (85.7)	4 (50.0)
Cefepime	NT	NT	4 (21.0)	0	0	0	NT
Ceftazidime	NT	NT	5 (26.3)	0	1 (12.5)	2 (28.5)	1 (12.5)
Cefotaxime	NT	NT	8 (42.1)	0	NT	NT	NT
Polymyxin B	NT	NT	19 (100.0)	0	NT	NT	6 (75.0)
Gentamicin	112 (95.7)	6 (85.7)	NT	2 (20.0)	NT	5 (71.4)	2 (25.)
Chloramphenicol	97 (82.9)	5 (71.4)	NT	NT	NT	NT	2 (25.0)
Cloxacillin	52 (44.4)	4 (57.1)	NT	NT	NT	NT	NT
Cefoxitin	67 (57.2)	NT	NT	NT	NT	NT	NT
Vancomycin	117 (100.0)	NT	NT	NT	NT	NT	NT

B. Distribution of MRSA among In-patient and Out-patient samples			
Samples	**MRSA**	**MSSA**	**p-value**
In-patient	19	27	> 0.05
Out-patient	23	48	

C. Distribution of MDR among In-patient and Out-patient samples			
Patient	**MDR**	**Non-MDR**	**p-value**
In-patient	22	16	> 0.05
Out-patient	11	15	

Table 3: *Antibiotic sensitivity profile of bacterial isolates from infected wounds.*

Discussion

Wound infection is a major health challenge especially among patients who are under post-operative care. In Nepal, several studies have reported frequent cases of wound infections from patients visiting hospitals and considered a serious health issue [10,11]. There is a wide range of etiological agents involved in wound infections. In addition, the emergence of antimicrobial resistance creates difficulty during the treatment of wound infections [1,3]. Therefore, the studies on bacteriological profile and determining antibiotic susceptibility pattern are utmost necessary to treat the wound infection effectively.

In this study, the high frequency of samples showed a wide range of bacterial growth and a significant number of samples did not show any bacterial growth. The culture negativity is due to the reason of history of antibiotic medication by the patients from whom the samples were collected and difficulty of fastidious organisms to survive during laboratory processes. The higher rate of bacterial growth positivity was reported from in-patients samples compared to out-patients samples. This finding agrees with previous studies [10,12]. Patients who are prolonged hospitalized are more prone to be infected with nosocomial pathogens and therefore higher incidence of wound infections were resulted from in-patients samples [13,14].

The bacteriological profile showed Gram-positive bacteria were predominant isolates compared to Gram-negative isolates. *S. aureus* was reported to be the highest followed by *E. coli*. Other bacterial isolates detected in this study are presented in table 2. These bacteria are major causative agents of wound infections and have been frequently reported by previous studies [15-17]. *S. aureus* is ubiquitous in nature and is normal flora of the human body. It is also an opportunistic pathogen and can infect the human body under favorable conditions [18]. *E. coli* is commonly associated with abdominal wounds and abscess [19]. It is more common in wounds after surgery of urinary tract [19]. This study reported *Acinetobacter* spp. as second highest Gram-negative isolates. *Acinetobacter* spp. possesses the ability of long-term survival on the intimate surfaces and is commonly isolated from hospital environments [20].

The antibiotic sensitivity profile revealed that vancomycin, gentamicin, and amikacin were the most effective antibiotics among all the Gram-positive isolates, whereas polymyxin B, chloramphenicol, and imipenem showed the highest sensitivity against all the Gram-negative isolates. Vancomycin and amikacin were detected as the most effective drug against all the strains of *S. aureus* and CoNS, respectively. On the other hand, amoxicillin appeared to be less effective against *S. aureus*, CoNS, and *E. coli*. Polymyxin B can be used for effective treatment of *E. coli* infected wound. Imipenem was observed effective against most of the isolates of *Acinetobacter* spp. but was resistant to most of the tested antibiotics (Table 3). For *Citrobacter* spp. and *Pseudomonas* spp., amikacin showed the highest sensitivity. However, cotrimoxazole, ciprofloxacin, and cefepime were found to be resistant towards *Citrobacter* species. Amoxycillin, cotrimoxazole and cefepime were completely ineffective against *Pseudomonas* strains. The effective antibiotic for *Klebsiella* isolates was imipenem, whereas drugs such as amoxicillin and ciprofloxacin reported as resistant antibiotics in significant number of cases. The antibiotic susceptibility profile of bacterial isolates of this study can be useful for the selection of antimicrobial therapy and can be implemented to control nosocomial pathogens in the hospitals. The resistance reported for amoxicillin, cotrimoxazole, and cefepime is possibly due to mutations in regulatory genes, the emergence of resistant genes, and misuse and frequent use of antibiotics [6,22,23].

This study found a high prevalence of MRSA strains from wound infected samples. The prevalence of MRSA from the wound has been frequently reported by previous studies [24,25]. This indicates that the prevalence of MRSA is gradually increasing in similar hospitals settings. Therefore, it is important to evaluate the presence of MRSA colonization among wound infected patients and administration of appropriate antimicrobial drugs is essential for controlling MRSA. The highest number of MDR was found among in-patients samples which revealed a high number of MDR pathogens prevail in the hospitals. The detection of MDR pathogens indicates a major threat to human life [10,12]. Thus, a combinatorial therapy and appropriate antimicrobial policy are essential to control these pathogenic strains of wound infections.

Conclusion

This study found *S. aureus* as predominant Gram-positive strains and *E. coli* as predominant Gram-negative strains from the wound samples. The antibiotic susceptibility pattern showed highest susceptible to vancomycin, amikacin, polymyxin B, and imipenem and the most resistivity was found with amoxicillin and cotrimoxazole antibiotics.

Limitations

This study determines the bacteriological profile of wound infections based on morphological, biochemical, and physiological characteristics. Further study should focus on the molecular identification of bacterial isolates and determine resistant genes from those isolates.

Consent for Publication

Not applicable.

Availability of Data and Material

All data obtained during this study are available within the article.

Competing Interests

The authors declare that they have no competing interests.

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Author's Contribution

BP, MSSH, and PKM conceived the concept and design of this study and performed experimental work. BP, RHC, DKC and AS analyzed the data and prepared the final draft of the manuscript. All authors read and approved the final manuscript.

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