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Research Article

Bacteriological Profile and Antibiotic Susceptibility Pattern of Wound Infection in Children

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Abstract

The growth of organism in wound is very common which sometimes leads severe infection. For proper treatment, it is very necessary to understand bacteriological profile of the infection. In recent studies antibiotic resistance pattern is also elevating. So, proper choice of antibiotic is important for effective treatment of the wound infection. In this study, 223 pus samples were examined from In-patients and Out-patients in Kanti Children's Hospital (KCH). Among 223 pus samples, 175(78.47%) samples were pus swabs and 48(21.52%) were pus aspirates. Out of all the samples analyzed, 128(57.4%) samples were growth positive. Among 128 growth positive samples, 117(91.4%) samples showed monomicrobial isolate and 11 (8.6%) samples showed polymicrobial isolates. *Staphylococcus aureus* (26.52%) was found to be the most predominant isolates, followed by Coagulase negative Staphylococci (17.94%); *Escherichia coli* (17.18%); *Pseudomonas aeruginosa* (12.5%); *Klebsiella* spp. (12.5%); *Acinetobacter* spp. (4.68%); *Proteus* spp. (3.12%); *Enterobacter* spp. (2.34%); *Streptococcus* pyogenes (1.56%); *Streptococcus* pneumoniae (0.78%); and *Citrobacter* spp. (0.78%). The most effective antibiotic against Gram positive bacteria was found to be Amikacin with susceptibility (78.33%) followed by Gentamicin (75%) and Cloxacillin (65%). The least effective drugs were Cephalexin (45%). Similarly among Gram negative isolates, Amikacin was found to be the most effective drug with susceptibility (51.5%) followed by Gentamicin (45.5%). The outcome of this study contributes for identification of common causative bacteria involved in wound infection and findings of antibiotic susceptibility pattern lead the stakeholder for appropriate selection of antibiotics during treatment of wound infection.

Keywords: *Staphylococcus aureus; Escherichia coli; Pseudomonas aeruginosa; Klebsiella; Wound Infection*

Introduction

Wound infection is defined as the presence and growth of microorganism in wound [1]. Infection of wound results in discharge of pus formed [2]. Pus is an exudates, typically white yellowish fluid that forms by the process of necrosis [1]. Development of wound infection depends on the interplay of a many factors; the breaking of the host protective layer, the skin, and muscles. This disturbance in the protective layer induces many cell types into the wound to initiate host response [2]. Wound infection is one of the common hospital acquired infection [3]. A number of bacteria may potentially cause wound infection which includes Gram positive bacteria involving *Staphylococcus aureus, Enteococcus faecalis* and other β-hemolytic Streptococci. Gram negative bacteria involving aerobic rods, *Pseudomonas aeruginosa*, and facultative rods, including *Escherichia coli, Enterobacter* spp., *Klebsiella* spp., *Proteus* spp. etc. and some anaerobes like: *Clostridium* spp., *Bacteroides* spp. etc are involved in wound infection [4]. The emergence of resistant strains is becoming a threat and has increased morbidity and mortality associated with wound infection. Multi drug resistant (MDR) organism is defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antibiotics. For being MDR, the organism must be resistance to ≥ 3 classes of antibiotics. Many nosocomial isolates resistance to ≥ 3 classes of antibiotics are called as MDR organism [5]. This study is intended to determine the common bacterial profile from the wound infection and focuses on antibiotic susceptibility pattern of isolated strains from the wound infection.

Materials and Methods

The study was conducted at Microbiology Laboratory of Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal from March 2015 to September 2015.

Sample size: The total of 223 samples (175 pus swabs and 48 pus aspiration) were collected from patient with different types of wounds like boils, lesions, trauma, burns, surgical, and systemic ulcers from different wards.

Culture of the Specimens: The inoculation of the collected specimens was done on Blood agar (BA) and MacConkey Agar (MA). Blood agar plates were incubated in microaerophilic condition using carbon dioxide enriched candle jar. Other plates were incubated at 370C for 24 hours aerobically. If no growth was observed, it reports as no growth. In case of bacterial growth, single colony was sub-cultured in the nutrient agar (NA) and incubated at 370C for 24 hours.

Identification of Isolates: Gram-staining reaction was performed for all the isolates. The gram-reaction positive bacteria were further tested for catalase, oxidase, coagulase, oxidative/fermentative, bacitracin, and optochin sensitivity test. For the identification of gram-reaction negative bacteria, colony morphology, staining reactions and various biochemical properties were studied.

Antibiotic sensitivity test: The antimicrobial susceptibility testing of the isolates towards various antimicrobial discs was done by modified Kirby-Bauer disk diffusion methods as recommended by Clinical and Laboratory Standards Institute using Mueller Hinton agar (MHA). The antibiotics disc used were amikacin, ciprofloxacin, ceftriaxone, meropenem, amoxicillin, piperacillin/tazobactam, gentamicin, cefixime, cotrimoxazole, cloxacillin and cephalexin.

Ethical Consideration: Verbal informed consent was taken from the patients attending the hospital suspected for wound infection and the study was approved by Kanti Children's Hospital, Maharajgunj, Nepal.

Results

The distribution pattern of pus samples enrolled in this study is shown in Table 1.

Types of sample	No	%
Pus swab	175	78.47
Pus aspirate	48	21.53
Total	223	100

***Table 1:** Distribution Pattern of Pus Sample in Total Patients.*

Out of total samples processed, 128 (57.4%) samples showed growth and 95 (42.6%) samples did not detect any growth. Among 128 growth positive cases, 107 (83.6%) samples were from pus swab and 21 (16.4%) were from aspirate. Among 95 negative cases, 68 (71.57%) samples were from swab and 27 (28.43%) were from aspirate (Table 2).

Types of sample	Growth		No growth		Total
	No	%	No	%	
Pus swab	107	61.11%	68	38.89%	175
Pus aspirate	21	43.75%	27	56.25%	48
Total	128	57.40%	95	42.60%	223

***Table 2:** Growth pattern in Total Sample.*

Monomicrobal isolates was found 100 (85.5%) in pus swab and 17 (14.5%) pus aspirate. The Monomicrobal isolate was high in both types of samples (Table 3).

Type of samples	Monomicrobal isolate		Polymicrobial isolate		Total no
	No	%	No	%	
Pus swab	100	85.5%	7	63.6%	107
Pus aspirate	17	14.5%	4	26.4%	21
Total	117	91.4%	11	8.6%	128

Table 3: *Growth pattern of Monomicrobal and Polymicrobial Isolates in Pus Samples.*

High number of pus sample (94) were collected from patients of age group 1 - 3 years and the least i.e. 10 samples were obtained from patients of age group 10 - 12 years. Among 128 positive cases, the highest positive cases 47 (36.7%) was found in the patients of age 1 - 3 years followed by 27 (21.0%) in age group of below 1 years; whereas the least bacterial growth of 6(4.0%) was found in patients of age group 10-12 years (Table 4).

Age (year)	Male		Female		Total Case	Total Positive Case (%)
	Total	Positive	Total	Positive		
Below 1	30	18	13	9	43	27 (21.0%)
1 - 3	71	35	23	12	94	47 (36.7%)
4 - 6	25	13	7	4	32	17 (13.2%)
7 - 9	26	17	7	5	33	22 (17.1%)
10 - 12	8	5	2	1	10	6 (4.0%)
13 - 15	11	9	0	0	11	9 (7.0%)
Total	171	97	52	31	223	128 (100%)

Table 4: *Age and Gender wise Distribution of Growth positive sample.*

Among 128 isolates, Gram negative bacteria were predominant constituting 68 (53.2%) of the total isolates and Gram positive bacteria constituted 60 (46.8%) isolates. *Staphylococcus aureus* was the most predominant species with 34 (26.52%) of total isolated. Among Gram negative isolates, *E. coli* was found to be the predominant species with 22 (17.18%) isolates (Table 5 and Figure 1).

	No. of Isolates (%)	Percentage of Total Isolates (%)
Gram positive organisms		
Staphylococcus aureus	34 (56.68%)	26.52%
CONS	23 (38.33%)	17.94%
Streptococcus pyogenes	2 (3.33%)	1.56%
Streptococcus pneumonia	1 (1.68%)	0.78%
Gram negative organisms		
Escherichia coli	22 (32.3%)	17.18%
Pseudomonas aeruginosa	16 (23.5%)	12.5%
Klebsiella spp.	16 (23.5%)	12.5%

Proteus spp.	4 (6%)	3.12%
Enterobacter spp.	3 (4.4%)	2.34%
Citrobacter spp.	1 (1.5%)	0.78%
Acinetobacter spp.	6 (8.8%)	4.68%
Total	68 (100%)	53.2%

Table 5: *Distribution of Bacterial Isolates.*

Figure 1: *Colonies of E. coli in MacConkey Agar.*

Antibiotic susceptibility pattern of the isolates showed amikacin was effective for both Gram-negative and Gram-positive strain. Gentamicin was alternative and cephalexin was found to be weak in inhibiting Gram-positive bacterial growth. For Gram-negative isolates amoxacilin showed highest resistant pattern (Table 6 and Figure 2).

Antibiotics	***Klebsiella* Spp.**			***Acineto-bacter* Spp.**			***Pseudomonas* Spp.**			***Escherichia Coli***			**Coagulase negative *Staphylococcus***			***Staphylococcus aureus***		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Amikacin	8	2	6	0	0	6	2	3	11	18	0	4	16	1	6	28	3	5
Ciprofloxacin	3	1	12	0	0	6	2	0	14	10	0	12	11	1	11	20	1	13
Ceftriaxone	5	2	9	0	0	6	7	0	9	14	0	8	15	2	6	17	6	11
Meropenem	13	0	3	0	0	6	8	0	8	13	3	6	-	-	-	-	-	-
Amoxicillin	3	0	13	0	0	6	0	0	16	5	0	17	15	0	8	11	3	20
Piperacillin/ tazobactam	13	0	3	1	0	5	13	0	3	14	2	6	-	-	-	-	-	-
Gentamicin	7	6	9	0	0	6	3	0	13	16	3	3	14	2	7	29	0	5
Cefixime	5	1	10	0	0	6	2	0	14	8	0	14	-	-	-	-	-	-

Cotrimoxazole	-	-	-	-	-	-	-	-	-	-	-	-	12	1	10	16	1	17
Cloxacillin	-	-	-	-	-	-	-	-	-	-	-	-	15	0	8	17	1	16
Cephalexin	-	-	-	-	-	-	-	-	-	-	-	-	10	0	13	15	15	18

Table 6: *Antibiotic Sensitivity Pattern of bacteria isolated from wound infection.*

Abbreviation: S, sensitivity; I, intermediate; R, resistance.

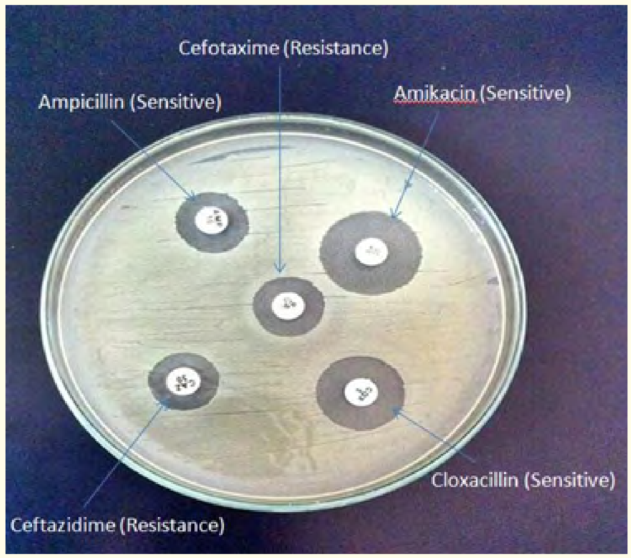

Figure 2: *Antibiotic Susceptibility Test for Staphylococcus. The susceptibility patterns were determined based on Clinical & Laboratory Standards Institute Guideliness and Zone size interpretation chart of Himedia.*

Discussion

Out of 223 Samples, 175 (78.47%) were pus swabs and 48 (21.53%) were aspirated pus (Table 1). The sample size was similar with many other studies related to wound infection [6,7]. In this study, monomicrobial and polymicrobial isolates were accounted to be 91.4%, and 8.6%, respectively (Table 2 and Table 3). Similar study carried out by Shrestha 2009, showed that monomicrobial and polymicrobial cases were 82.87% and 17.13%, respectively [6]. Similarly, a study carried out by Shrestha 1997, showed that monomicrobial isolates were seen in 56% and polymicrobial isolates in 44% cases [8]. In contrast, Brook & Finegold found 24% of pure culture growth and 72% of mixed culture growth from cutaneous abscesses children [9]. Mixed infections are common in wounds and abscess. Presence of single type of organism in infection assumed to be the only invading organism. If pyogenic organisms are found in mixed infection, they are considered to be primary cause of infection and rest as secondary invaders [10].

Of the total 128 isolates, Gram negative isolates 68 (53.2%) were the predominant pathogens followed by Gram positive 60 (46.8%) (Table 5). Among the Gram positive, *Staphylococcus aureus* 34 (56.68%) was the most prevalent and followed by Cogulase negative staphylococci (CoNS) 23 (28.33%). Among Gram negative isolates *Escherichia coli* account to be the most predominant 22 (32.3%) followed by *Pseudomonas aeruginosa* and *Klebsiella* spp., equally 16 (23.5%). Higher prevalence of *Staphylococcus aureus* seen in this study resembled with other studies [6,7,13]. Gram positive bacteria were predominant organism in traumatic wounds in children. However, higher incidence of gram negative bacteria in a study of gastrostomy site wound infection in children was also reported [11]. *Staphylococcus aureus* are also present as a normal flora in our body that may act as opportunistic pathogens under favorable condition. *Staphylococcus aureus* are robust, can resist high salt concentration, and produce various virulence factors such as coagulase, staphylokinase, leucocidins, exfolitians etc. thus, they are well studied in case of wound infection.

In this study, 68 cases were growth positive in surgical wound. The most predominant organisms were *S. aureus* 20 (29.41%) followed by *E. coli* 14 (20.5%), *Klebsiella* spp. 9 (13.23%), *P. aeruginosa* 8 (11.76%). However in a study done by Brook on microbiology of gastrostomy site wound infection in children, the most predominant isolates were *E. coli* 16 (28.07%), *Enterococcus* spp. 14 (24.56%) and *S. aureus* 6 (10.53%) [12]. In Lorsone study in nosocomial infection after cardiac surgery in infants and children found *Klebsiella* spp. (22%) to be the predominant organism followed by *Enterobacter* spp. (17%), *S. aureus* (16%) and *P. aeruginosa* (16%) [14]. Similarly in a study carried by Bhattacharya and Kosloske, the predominant bacterial isolates were *S. aureus, E. coli* and alpha haemolytic *Streptococcus* [15].

Among the growth positive cases, second highest number of organisms was isolated from 49 (38.3%) burn wound in which 23 (46.94%) were Gram positive and 26 (53.06%) were Gram negative. Gram negative bacteria outnumbered Gram positive one. Although burn wound surface are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms. The type and amount of microorganisms colonizing the burn wound influence the frequency of invasive burn wound infections and the clinical characteristics of such infections. Similarly reports showed the higher prevalence of Gram negative bacteria. Zhang and Zhao in their study on changing trends of bacteria and their sensitivity pattern in burn wound revealed the higher occurrence of Gram negative bacteria in burn wound [16]. Therefore, these studies support the fact that incidence of burn wound infection by Gram negative were greater than Gram positive bacteria. However, Shrestha 1997, reported higher incidence of Gram positive over Gram negative bacteria in a similar study [8].

Among the growth positive cases in burn wound, the most predominant organisms were CoNS 12 (24.5%) followed by *S. aureus* 10 (20.4%) and *E. coli* 8 (16.33%). But study carried by Nepal (2007), where the most predominant organism was *S. aureus* 42 (30%) followed by *P. aeruginosa* 34 (24.29%) and *E. coli* 23 (16.43%). Similarly, study carried out by Zhang and Zhao on changing trends of bacteria and their sensitivity pattern in burn wound revealed *S. aureus* to be the commonest isolate followed by *P. aeruginosa* and *E. coli* [16]. In other study showed (5.10%) of E. coli from burn wounds, 14.1%. The other organisms isolated from burn wound were *P. aeruginosa* 6 (12.24%), *Acinetobacter* spp. 5 (10.2%), *Klebsiella* pp. 3(6.12%), *Proteus* spp. and *Enterobacter* spp. 2(4.08%) and single isolate of *Streptococcus pyogenes* [17,18].

Out of 11 growth positive cases of other types of wounds (except surgical and burn wound), the most predominant isolates were *S. aureus* and *Klebsiella* spp. followed by *E. coli* and *Klebsiella* spp. and one isolates of CoNS. Brook and Frazier conducted a study on aerobic and anaerobic bacteriology of wounds and cutaneous abscess and found *S. aureus* (65%) to be the predominant organism followed by group A *Streptococci* (17.5%) and *E. coli* (17.3%) [19]. Similarly, in a study carried out by Brook and Finegold in cutaneous abscess of children, *S. aureus* (45.17%) was found to be the most common bacteria followed by non-haemolytic streptococci (14.7%), β-haemolytic *streptococci* (8.12%), *Enterobacter* spp. (5.07%) and *E. coli* (4.06%) [9].

In the present study, after *Escherichia coli* the dominant organisms were *Pseudomonas aeruginosa* and *Klebsiella* spp. equally (23.5%) of a total Gram negative isolates and (12.5%) of total isolates. Similar study carried out by Shrestha showed *Pseudomonas aeruginosa* (12.15%), *Klebsiella pneumoniae* (4.42%) [6]. The study carried out by Karki showed *Pseudomonas aeruginosa* (23.6%), *Klebsiella* spp (5.9%) [13], which is totally different in this study. Pseudomonas aeruginosa is most commonly associated with infected burn wound and other hospital acquired infection. In this study, (81.25%) of *Pseudomonas aeruginosa* were sensitive to piperacilline/tazobactam, and totally non effective drug was Amoxicillin and followed by cefixime, ciprofloxacin (87.5%). Whereas *Klebsiella* spp. were most susceptible to meropenem and piperacilline/tazobactam (81.25%) but least susceptible to amoxicillin (81.25%) followed by ciprofloxacin. Similarly, in a study carried out by Estahabanati, found amikacin to be the most effective antibiotic for *P. aeruginosa* [20]. In a study carried out by Brown and Izundu in Jamaica, reduced resistance of *P. aeruginosa* to ciprofloxacin has been reported (19.6%) [21]. In our study, chloramphenicol was 95.24% resistant to *P. aeruginosa*. In this study, all the 6 isolates of *Acinetobacter* spp. was 100% resistant to the used antibiotics except one was sensitive to Piperacillin/tazobactam.

Conclusion

The growth of organism was found to be higher in pus swab than pus aspiration. The growth was higher in In-patient than Out-patient. Male children's were found to be more susceptible to wound infection than female. Higher proportion of Gram negative isolated from In-patients was found to be statistically significant. Most predominant organism was *Staphylococcus aureus* followed by CoNS, *E. coli*. Gram negative isolates were the most predominant isolates. Amikacin and gentamicin were found to be the most effective antibiotic for Gram positive isolates and cephalexin was found least effective one. Most of Gram negative isolates were found to be susceptible to amikacin whereas amoxicillin was found to be least effective one.

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