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RESEARCH ARTICLE OPEN ACCESS

Determination of Bacterial Profile and Spectrum of Antimicrobial Drug Resistance in Pediatric Wound Infections

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Abstract

Pediatric wound infections lead to the prolonged morbidity of the child and financial burden for the parents. The present study aimed to determine the causative pathogens and the spectrum of their drug resistance to reduce the fatality in pediatric wound infections. The study conducted prospectively on wound specimens collected from a pediatric facility of Lahore, Pakistan. The specimens were processed on routine culture media, and the conventional biochemicals, analytical profile index (API) 20 E, and 20 NE identified the organisms. The spectrum of drug resistance observed against several classes of antibiotics. Of 960 cases, 695 (72.4%) were culture positive and 265 (27.6%) culture-negative cases. The pediatric cases of < 1 year of age had significant (p = 0.01) association with negative cultures. Illiteracy and primary education of the parents were significantly (p < 0.01 and 0.01, respectively) associated with pediatric wound infections. The distribution of the bacterial pathogens demonstrated 216 (27.6%) Gram-positive and 568 (72.4%) Gram-negative bacteria. Gram-positive isolates were resistant to most of the penicillins, co-trimoxazole, and cephalosporins, while none of these was resistant to linezolid and vancomycin. The majority of Gram-negative bacteria were resistant to cephalosporins and aminoglycosides, while lesser resistance observed against carbapenems, piperacillin-tazobactam, and cefoperazone-sulbactam. The wound infections could be polymicrobial with the expanded spectrum of drug resistance to most of the penicillin, cephalosporins, aminoglycosides, and fluoroquinolones. These infections lead to serious pediatric morbidity if not treated meticulously.

Keywords: Bacterial profile, wound infections, antibiotic resistance, pediatric wounds.

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INTRODUCTION

Human skin is a first protective obstacle that inhibits microbial infections by creating an effectual barrier, leaving the tissue underneath intact[1]. Most of the bacterial species have a weak potential for infections because of the intact human skin[2]. However, an open entrance for bacterial infections is provided by any breach of the skin surface, whether traumas, surgeries, accidents, or burns. Child injuries are a common public health concern and usually occur when the child has a burn injury, falls from the height, or a road accident. These multiple injuries lead to the formation of wounds that bring the patients into health care facilities[3].

Trauma may happen accidentally or deliberately in cases of elective surgeries or intravenous medical devices, which could lead to nosocomial wound infections[4]. Wound infection is triggered by weak skin integrity, type of organism, virulence, nature of the operation, antibiotics, and mechanism of host defense. The bacterial pathogens, chronic inflammation, and unbalanced cellular defense mechanisms characterize infected wounds[5]. An inflammatory process due to the enormous attraction of activated granulocytes that release inflammatory mediators is responsible for auxiliary damage to the human tissues leads to the chronicity of the wounds[6]. Predominantly, bacterial species are responsible for wound infections though fungi, protozoa, and viruses can also cause infections in the injured sites[7]. *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus*, other *Streptococci*, and *Enterococci* are common bacterial pathogens associated with wound infection[8]. The bacterial wound infection can be polymicrobial with the co-existence of multiple pathogenic bacteria in the same wound[9].

Wound infections are a paramount concern for healthcare professionals not only because of the patients' morbidity but also enhanced stress and cost-effectiveness of the healthcare system. Patient injuries vary between environments and range from acute operating wounds, traumatic injuries such as accidents, burn injuries, and chronic wounds, like diabetic foot, leg, and pressure sores[10]. Different saprophytic commensals of the skin, which can vary in quantities, contaminate the wounds. Effective wound management, especially chronic wounds, can ameliorate the health of the population, decrease morbidity, and improve the quality of life[11].

Children have a weak immune system, which makes them susceptible to severe infections that need appropriate treatment[12]. The chances of the indiscriminate use of antibacterials are immense if regional antibiotic sensitivity data is not available, which leads to the vicious circle of bacterial drug resistance. Trauma is the most common reason behind all injuries, and the unsuccessful treatment of these wounds may lead to fatal sepsis. Nearly 95% of injury-related pediatric mortalities occur in low and middle-income countries[13]. The study aimed to determine the aerobic and facultatively anaerobic pathogens responsible for pediatric wound infections and the spectrum of their antimicrobial drug resistance to reduce the fatality in children.

MATERIALS AND METHODS

Study methods and patients

The study conducted prospectively, and specimens from the cases of pediatric wound infections were collected from January 2016 to March 2017 from a pediatric facility of Lahore, Pakistan. A total number of 960 wound specimens (swabs, pus, drainage, and tissues) were obtained from the infected sites of the patients with traumatic injuries, burns, and post-operative wounds. The specimens were collected from in-patients and out-patients units with the convenient sampling method. The patients' demographic factors were also observed at the time of sample collection.

Collection of specimens

A pre-cleaned wound with sterile normal saline used to obtains the specimens aseptically. Amies transport media swabs were used to collect the wound specimens, and draining pus was collected directly in sterile syringes. The samples were carefully monitored not to be contaminated with skin microbiota and transported immediately for culture.

Isolation of wound pathogens

Blood, MacConkey's, and Chocolate agar plates were used to culture the samples and incubated at 37°C for up to two days with daily monitoring before discarding the cultures. The

culture plates of Blood and MacConkey were kept under aerobic conditions while Chocolate cultures were provided with 5 - 8% CO_2. The study included only cases with positive cultures, while the cases of negative cultures were excluded. The bacterial identification completed by bacterial morphology, growth characteristics, biochemical reactions (catalase, coagulase, oxidase, indole, urease, motility), API 20 E, and 20 NE (bioMerieux). The fermentation of glucose, sucrose, and other sugars also helped in the identification of the pathogens. The well-defined individual colonies were selected for further processing of the cultures[14].

Drug resistance testing

The spectrum of Gram-positive and Gram-negative bacterial drug resistance observed against various classes of antibiotics such as aminoglycosides, cephalosporins, macrolides, fluoroquinolones, carbapenems, glycopeptide, penicillins, and their combinations. The commercially available antibiotic discs (Oxoid) were applied in bacterial cultures streaked on Mueller Hinton agar (Blood agar for *S. pyogenes*) by disc diffusion procedure. The spectrum of drug resistance observed against individual cases with a subsequent overnight incubation at 37°C[15,16].

Statistics

SPSS 23 used to apply statistics, and regression analysis was done to calculate odd ratio (OR) with 95% CI and p-value < 0.05 considered as significant.

RESULTS

Characteristics of enrolled cases

Out of 960 enrolled cases, 695 (72.4%) were culture positive and 265 (27.6%) culture-negative patients. A total number of 542 (78%) cases yielded positive cultures in males and 153 (22%) in females without any significant (p = 0.10) gender association. The greater number of the cases belonged to the 1 – 5 years (23; 33.1%) and 6 – 10 years (270; 38.8%) of age. There was a significant association (p = 0.01) of negative cultures with patients < 1 year of age. The literacy status of the parents of the pediatric patients showed a significant association (< 0.01) of illiterate parents with 6.56 OR of having culture positive results. The OR is decreased to 1.75 in people with primary education but remained significant (p = 0.01) statistically (Table 1).

Distribution of bacterial pathogens

Of the 695 positive cultures, polymicrobial wound infections were observed in 89 (12.8%) cases. The distribution of the total number of 784 bacterial pathogens in pediatric wound infections demonstrated 216 Gram-positive (27.6%) and 568 Gram-negative bacteria (72.4%). Of these, 245 (31.1%) were *Pseudomonas aeruginosa*,

Table 1. Demographic factors of enrolled cases of pediatric wound infections (n = 960)

Characteristic	Culture-Positive n (%)	Culture-Negative n (%)	P-value	Odd Ratio (95% CI)
Overall positive and negative cultures				
Result	695 (72.4%)	265 (27.6%)	-	-
Gender				
Male	542 (78%)	220 (83%)	0.10	0.72 (0.50-1.04)
Female	153 (22%)	45 (17%)		
Age				
< 1 year	49 (7.1%)	31 (11.7%)	0.01	0.49 (0.28-0.8)
1 - 5 years	230 (33.1%)	90 (34%)	0.30	0.80 (0.53-1.21)
6 - 10 years	270 (38.8%)	99 (37.4%)	0.46	0.85 (0.57-1.28)
10 - 15 years	146 (21%)	45 (17%)	-	-
Parents' Literacy Status				
Illiterate	270 (38.8%)	33 (12.5%)	< 0.01	6.56 (3.97-10.85)
Primary education	173 (24.9%)	78 (29.4%)	0.01	1.75 (1.13-2.72)
Higher Secondary School Certificate	180 (25.9%)	97 (36.6%)	0.06	1.50 (0.97-2.28)
Graduation or more	72 (10.4%)	57 (21.5%)	-	-

187 (23.9%) *Staphylococcus aureus*, 87 (11.1%) *Escherichia coli*, 66 (8.4%) *Klebsiella pneumoniae*, 57 (7.3%) *Enterobacter cloacae* and 44 (5.6%) *Proteus* species. Few other Gram-positive and Gram-negative pathogens isolated from the positive cultures (Table 2).

Spectrum bacterial drug resistance

The spectrum of drug resistance was monitored against various groups of antibiotics against all of the isolated pathogens. The frequently isolated Gram-positive pathogen was *S. aureus*, and 174 (93%) isolates were resistant to ampicillin and penicillin, 118 (93%) co-trimoxazole, 75 (40.1%) cefixime and cefotaxime, 72 (38.5%) ceftazidime and cefuroxime. There were 31 (16.6%) isolates resistant to teicoplanin, and none of them was resistant to linezolid and vancomycin (Table 3). The most commonly isolated Gram-negative strains of *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. cloacae*, and *Proteus* species were resistant to most of the cephalosporins and aminoglycosides, while lesser resistance observed against carbapenems, piperacillin-tazobactam, and cefoperazone-sulbactam. The fluoroquinolones showed variable results against different pathogens (Table 4).

Table 2. Distribution of pathogens recovered from pediatric wound infections (n = 784)

Bacterial pathogens		Frequency	Percentages
Gram-positive bacteria n = 216 (27.6%)	*Staphylococcus aureus*	187	23.9
	Streptococcus pyogenes	15	1.9
	Enterococcus spp.	14	1.8
Gram-negative bacteria n = 568 (72.4%)	*Pseudomonas aeruginosa*	245	31.3
	Escherichia coli	87	11.1
	Klebsiella pneumoniae	66	8.4
	Enterobacter cloacae	57	7.3
	Proteus spp.	44	5.6
	Acinetobacter baumannii	28	3.6
	Citrobacter freundii	26	3.3
	Stenotrophomonas maltophilia	9	1.0
	Serratia marcescens	6	0.8

Table 3. Spectrum of drug resistance in Gram-positive bacterial pathogens (n = 216)

Antibiotic	*Staphylococcus aureus* n = 187	*Streptococcus pyogenes* n = 15	*Enterococcus* spp. n = 14
Amikacin	40 (21.4%)	3 (20%)	3 (21.4%)
Ampicillin	174 (93%)	1 (6.7%)	2 (14.3%)
Cefixime	75 (40.1%)	0 (0%)	2 (14.3%)
Cefotaxime	75 (40.1%)	0 (0%)	1 (7.1%)
Ceftazidime	72 (38.5%)	0 (0%)	2 (14.3%)
Ceftriaxone	70 (37.4%)	2 (13.3%)	1 (7.1%)
Cefuroxime	72 (38.5%)	2 (13.3%)	2 (14.3%)
Ciprofloxacin	65 (34.8%)	6 (40%)	2 (14.3%)
Co-amoxiclav	70 (37.4%)	2 (13.3%)	3 (21.4%)
Co-trimoxazole	118 (63.1%)	4 (26.7%)	3 (21.4%)
Erythromycin	62 (33.2%)	1 (6.7%)	2 (14.3%)
Gentamicin	48 (25.7%)	3 (20%)	3 (21.4%)
Imipenem	70 (37.4%)	0 (0%)	0 (0%)
Linezolid	0 (0%)	0 (0%)	0 (0%)
Penicillin	174 (93%)	0 (0%)	2 (14.3%)
Teicoplanin	31 (16.6%)	2 (13.3%)	3 (21.4%)
Vancomycin	0 (0%)	0 (0%)	2 (14.3%)

Table 4. Spectrum of drug resistance in Gram-negative bacterial pathogens (n = 568)

Antibiotic	*P. aeruginosa* n = 245	*E. coli* n = 87	*K. pneumoniae* n = 66	*E. cloacae* n = 57	*Proteus* n = 44	*A. baumannii* n = 28	*C. freundii* n = 26	*S. maltophilia* n = 9	*S. marcescens* n = 6
Amikacin	142 (58%)	26 (29.9%)	27 (40.9%)	22 (38.6%)	14 (31.8%)	22 (78.6%)	15 (57.7%)	5 (55.6%)	1 (16.7%)
Amoxicillin-Clavulanate	220 (89.8%)	53 (60.9%)	58 (87.9%)	50 (87.7%)	29 (65.9%)	25 (89.3%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Cefepime	193 (78.8%)	35 (40.2%)	58 (87.9%)	49 (86%)	24 (54.5%)	26 (92.9%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Cefixime	236 (96.3%)	41 (47.1%)	61 (92.4%)	50 (87.7%)	25 (56.8%)	28 (100%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Cefoperazone-Sulbactam	39 (15.9%)	19 (21.8%)	22 (33.3%)	16 (28.1%)	10 (22.7%)	15 (53.6%)	10 (38.5%)	1 (11.1%)	0 (0%)
Cefotaxime	225 (91.8%)	41 (47.1%)	61 (92.4%)	50 (87.7%)	27 (61.4%)	28 (100%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Ceftazidime	172 (70.2%)	38 (43.7%)	61 (92.4%)	50 (87.7%)	27 (61.4%)	28 (100%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Ceftriaxone	215 (87.8%)	41 (47.1%)	61 (92.4%)	50 (87.7%)	25 (56.8%)	28 (100%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Cefuroxime	235 (95.9%)	41 (47.1%)	61 (92.4%)	50 (87.7%)	25 (56.8%)	28 (100%)	22 (84.6%)	8 (88.9%)	1 (16.7%)
Ciprofloxacin	98 (40%)	61 (70.1%)	54 (81.8%)	22 (38.6%)	25 (56.8%)	26 (92.9%)	22 (84.6%)	4 (44.4%)	1 (16.7%)
Gentamicin	159 (64.9%)	36 (41.4%)	34 (51.5%)	24 (42.1%)	17 (38.6%)	24 (85.7%)	19 (73.1%)	6 (66.7%)	2 (33.3%)
Imipenem	47 (19.2%)	14 (16.1%)	27 (40.9%)	11 (19.3%)	6 (13.6%)	10 (35.7%)	9 (34.6%)	1 (11.1%)	0 (0%)
Levofloxacin	52 (21.2%)	36 (41.4%)	40 (60.6%)	17 (29.8%)	20 (45.5%)	20 (71.4%)	16 (61.5%)	3 (33.3%)	0 (0%)
Meropenem	69 (28.2%)	15 (17.2%)	30 (45.5%)	11 (19.3%)	5 (11.4%)	11 (39.3%)	10 (38.5%)	1 (11.1%)	0 (0%)
Moxifloxacin	95 (38.8%)	58 (66.7%)	53 (80.3%)	22 (38.6%)	23 (52.3%)	26 (92.9%)	9 (34.6%)	4 (44.4%)	1 (16.7%)
Piperacillin-Tazobactam	42 (17.1%)	18 (20.7%)	21 (31.8%)	14 (24.6%)	8 (18.2%)	12 (42.9%)	11 (42.3%)	1 (11.1%)	0 (0%)
Trimethoprim-Sulfamethoxazole	130 (53.1%)	47 (54%)	43 (65.2%)	23 (40.4%)	9 (20.5%)	27 (96.4%)	13 (50%)	7 (77.8%)	1 (16.7%)

DISCUSSION

The pathogens in wound infections play a significant role in chronicity development and slow wound healing. It is the most common postoperatively complicated infection that used to occur throughout all ages and causes significant patient morbidity and raises treatment costs. The extensive abuse of antibiotics has resulted in elevated selection pressures and compromised treatment options resulting in a worse situation.

The current study reports 72.4% culture-positive and 27.6% culture-negative wound samples with higher (78%) positivity in male children. A prior study reported comparatively low positivity (58.5%), while the nearly similar rate of culture positivity (70.5%) has been reported in Ethiopia with a higher incidence of wound infections in male (64.2%) patients[17,18]. A Pakistani study conducted in a hospital of Peshawar reported 59% of wound infections in males and 41% in females[19]. Another study reported similar findings of a higher incidence of wound infections in male (59.1%) patients than female (40.9%) patients[20]. The culture-negativity was significantly associated with children < 1 year of age in the current study, which is quite the opposite of the findings of a tertiary care hospital in Nepal, which reported a higher rate of wound infections in children under the age of 1 year[12]. The incidence of wound infections can vary in different age groups in different geographical areas. Younger children are more vulnerable to infections due to a weak immune system and hygienic conditions[21,22].

Detrimental virulence of commonly isolated bacterial strains of *S. aureus* and *P. aeruginosa* have been proven a significant reason for the delay in the healing process of the wounds[11]. The high prevalence of *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* infections in this study could be due to colonization of nosocomial pathogens with surgical instruments or invasive devices which helps the bacteria to breach the natural skin barrier. The higher incidence of *S. aureus* (54.1%), *K. pneumoniae* (20.8%) and *E. coli* (8.3%) are reported in an Indian study[23]. An Ethiopian study reported 28.7% *S. aureus*, 12.5% *Klebsiella*, 11.8% coagulase-negative *Staphylococci*, 11% *Citrobacter*, 9.6% *Enterobacter*, 5.9% each of the *P. aeruginosa* and *E. coli* and 4.4% *Proteus* which is contrary to the finding of the present study[20]. The results of a Nigerian study also reported predominantly *S. aureus* (38%) in wound infections followed by *P. aeruginosa* (18.7%), *Klebsiella* (17%), which differ in frequencies reported in the current study[24]. The pathogens reported in different studies are similar but differ in the frequency of their distribution. Most of the wounds are colonized with a single microbial growth, but some cases of wound infection may have polymicrobial growth. A polymicrobial etiology was observed in cases of pediatric wound infections in this study, which is closer to the polymicrobial growth reported by an Ethiopian study[20].

The selection of appropriate antibiotics is required in cases of evident wound infections keeping in view the concern of growing drug resistance[25]. The present study reports the spectrum of drug resistance against various classes of antibiotics, which are commonly used in the treatment of wound infections. Gram-positive isolates exhibited no or little resistance against linezolid, vancomycin, and teicoplanin in this study, whereas most of these strains were resistant to the antimicrobials belong to penicillins, cephalosporins, and co-trimoxazole. The antibiotic resistance to penicillin (59%), tetracycline (57%), ampicillin (55%), and co-trimoxazole (35%) was observed in *S. aureus* infected wounds[26]. Synergistic effects of beta-lactamase inhibitors (tazobactam and sulbactam) in combination with penicillins found to be effective against *S. aureus* infections isolated from wound and other sources[27]. A higher rate of *S. aureus* resistance in wound infections have been observed against amoxicillin (82%), ofloxacin (80%), sparfloxacin (78%), ciprofloxacin (71%), levofloxacin (46%) and gentamicin (36%). A single case of VRE was observed, while none of the *S. aureus* or *S. pyogenes* found to be resistant to penicillin[20]. A variable number of VRE have been reported in other studies[28,29]. *Staphylococci* resistant to methicillin and clindamycin have been reported in postoperative wound infections in Sudan[30].

Gram-negative isolates of *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. cloacae,* and *Proteus* species were resistant to most of the cephalosporins and aminoglycosides whereas found to be less resistant to carbapenems, piperacillin-tazobactam, and cefoperazone-sulbactam.

Multi-drug resistance has been reported against the eighteen different antibiotics in *E. coli* and *P. aeruginosa*. The pathogens showed expanded resistance to cephalosporins, fluoroquinolones, aminoglycosides, and monobactams in wound infections[20]. *E. coli* strains have been found resistant to co-trimoxazole (63%), ampicillin, and tetracycline (87% each) in a different study[26]. Massive self-medication and indiscriminately advised antibacterial drugs without scientific investigations could result in multi-drug resistant Gram-negative pathogens, particularly against cephalosporins, aminoglycosides, and fluoroquinolones in the wound as well as in other infections[20,31,32]. The production of AmpC, extended-spectrum beta-lactamases, and imipenemases among the Gram-negative isolates explicit the resistance against expanded spectrum cephalosporins[33-35].

CONCLUSION

The variable spectrum of antibacterial resistance tends to be broader in wound infections leaving fewer therapeutic options. The study reports a high occurrence of Gram-positive and Gram-negative single and polymicrobial pathogens in wound cultures, which emphasizes the need for efficient implementation of modern infection control strategies. The spectrum of drug resistance monitored against several antibacterial drugs left linezolid and vancomycin as empirical drugs in Gram-positive wound infections, and the use of carbapenems, piperacillin-tazobactam, and cefoperazone-sulbactam in Gram-negative wound infections. Continuous monitoring is indispensable in order to contribute to effective wound infection treatment and to prevent drug-resistant pathogens from evolving rationally. A meticulous antibacterial therapy in wound infection could protect the patients from painful procedures of debridement or amputation.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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