

Microbial Profile in Paediatric Patients with Wound Infection and Their Antibiotic Susceptibility Pattern

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Abstract

Wound infection is one of the major health problems that frequently occur among patients reporting or admitted in the hospital. This observational study was conducted in the Department of Microbiology at Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh, between November 2023 and May 2024, to observe the microbial profile in paediatric patients with wound infection and their antibiotic susceptibility pattern. A total of 372 wound swabs were collected and analyzed by using culture and antibiotic sensitivity tests. From total wound swab samples, culture positive samples were 281(75.54%). Among those, 52(18.5%) were Gram-positive and 229(81.5%) were Gram negative. *Pseudomonas spp* was the most commonly 83(36.24%) isolated organisms, followed by *Escherichia coli* 48(20.96%), *Acinetobacter spp* 33(14.41%), *Klebsiella spp* 24(10.48%) among Gram-negative bacteria. On the other hand, *Staphylococcus spp* 23(44%) was the most prevalent, followed by *Staphylococcus aerues* 18(35%) among Gram-positive isolates. 94% of the isolated *Staphylococcus aerues* were methicillin resistant *Staphylococcus aerues* (MRSA). *Staphylococcus spp* showed higher resistant pattern against azithromycin (91%), erythromycin (91%), linezolid (78%), ciprofloxacin (69%), moxifloxacin (65%), levofloxacin (52%) and gentamycin (52%). Most of the Gram-negative bacteria showed higher resistant against ampicillin followed by third and fourth generation cephalosporins, fluroquinolones. Periodic surveillance of microbial profile and appropriate antibiotic selection are necessary to prevent multidrug resistant bacteria in hospital patients. Moreover, infection prevention campaigns must be strengthened among hospital wards and premises.

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Introduction

Wound infection is one of the most common hospital acquired infection and is an important cause of morbidity and mortality worldwide.¹ A wound is the disruption in the continuity of soft parts of the body structures.² Wound infection occurs due to invasion and proliferation by one or more species of microorganisms resulting in pus formation.³ Bacterial infection causes serious complication in wound which may lead to fatal sepsis.⁴ Chronic wounds can be colonized on the surface by a wide range of organisms. Common bacterial pathogens associated with wound infection include *Staphylococcus aerues*,

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus spp*, *Streptococcus spp*, *Enterococcus spp*.⁵ These organisms exhibit natural resistance to many antibiotics and antiseptics in which they may survive for long periods and may even multiply in the presence of minimal nutrients and have the ability to colonize in traumatized skin.^{6,7}

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Use of antimicrobial agents cause a 'selective pressure' on microbial population.⁸ As a result of indiscriminate use of antimicrobial agents, significant changes occur in microbial genetic ecology.⁹ During last few decades, multidrug resistant bacterial strain such as *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, Methicillin resistant *Staphylococcus aureus* (MRSA) are increasingly associated with infections under hospital settings.^{10,11} Thus, wound infection caused by drug resistance pathogen is commonly reported from developing world.^{12,13} This development of resistance is worrisome with a resultant increase in morbidity, mortality and cost not only to patients and their relatives but including hospital management. This study was designed to evaluate the diversity of pathogenic bacteria found in wound infection and their antibiotic sensitivity pattern among paediatric patients in a tertiary level hospital.

Methods

This observational study was conducted in the Department of Microbiology at Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh, between November 2023 and May 2024. All wound swabs samples from both inpatient and outpatient departments of the hospital sent to the microbiology laboratory for culture and sensitivity tests. Data regarding the identity of the patient, referring departments, type of the specimen and sensitivity reports were collected from the records of the laboratory. Samples were inoculated in blood agar and MacConkey's agar media and incubated aerobically at 37°C for 72 hours. The inoculated plates were examined for bacterial growth and organisms were identified by colony morphology,

hemolytic criteria, pigment production, Gram staining and different biochemical tests like catalase test, coagulase test, oxidase test, reaction in TSI agar, MIU, Simmon's citrate agar media and bile esculin agar media. Antimicrobial susceptibility pattern of isolated organisms were done following Kirby-Bauer disk diffusion method.¹⁴ Sensitivity was done using commercially available antibiotic discs (Oxford, UK); amikacin(30µg), ampicillin (10µg), chloramphenicol (30µg), sulphamethoxazole/trimethoprim (25µg), meropenem (10µg), ciprofloxacin (5µg), cefixime (5µg), ceftriaxone (30µg), Cefepime (30µg), azithromycin (15µg), ceftazidime (30µg), gentamycin (10µg), Imipenem (10µg), levofloxacin (5µg), linezolid (30µg), doxycycline (5µg), penicillin (10µg), moxifloxacin (5µg), netilmicin (30µg), erythromycin (15µg), vancomycin (30µg). Interpretations as 'Sensitive' or 'Resistant' was done on the basis of diameters of zones of inhibition of bacterial growth as recommended by the disc manufacturer. Zone of inhibition was measured according to CLSI guideline.¹⁵ Collected data were classified according to characteristics and 'Microsoft Excel' software were used for analysis. The study was approved by the Ethical Review Committee of Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh.

Results

A total 372 samples collected from paediatric patients were sent for bacterial culture tests. 291(78.22%) samples were from out-patient department (OPD) patients and 81(21.78%) were in-patient department (IPD). Culture positive samples were 281 (75.54%). Among them

52 (18.5%) were Gram positive and 229(81.5%) were Gram negative (Table-I). Among Gram-positive organisms, *Staphylococcus spp* 23(44%) was the most prevalent, followed by *Staphylococcus aerues* 18(35%) and *Enterococcus spp* 11(21%). (Table-II). Among Gram-negative organisms, *Pseudomonas spp* was the most common isolated bacteria 83(36.24%), followed by *Escherechia coli* 48(20.96%), *Acinetobacter spp* 33(14.41%), *Klebsiella spp* 24(10.48%) and *Enterobacter spp* 15(6.55%), and *Proteus mirabilis* 14(6.11%) (Table-III). All the bacterial isolates were tested for antimicrobial susceptibility. 94% of the isolated *Staphylococcus aerues* were methicillin resistant *Staphylococcus aerues* (MRSA). *Staphylococcus spp* showed higher resistant pattern against azithromycin (91%), erythromycin (91%), linezolid (78%), ciprofloxacin (69%), moxifloxacin (65%), levofloxacin (52%) and gentamycin (52%) (Table-IV). Most of the Gram-negative bacteria showed higher resistant against ampicillin followed by third and fourth generation cephalosporins, fluroquinolones (Table-V).

Table-I: Culture results in clinically diagnosed wound infection cases (N=372)

Culture Results	Frequency	Percentage
Positive	281	75.54
Negative	91	24.46

Table-II: Gram positive organisms' profile (N=52)

Organisms	Frequency	Percentage
<i>Staphylococcus spp</i>	23	44
<i>Staphylococcus aerues</i>	18	35
<i>Enterococcus spp.</i>	11	21

Table-III: Gram negative organisms' profile (N=229)

Organisms	Frequency	Percentage
<i>Pseudomonas spp</i>	83	36.24
<i>Escherechia coli</i>	48	20.96
<i>Acinetobacter spp</i>	33	14.41
<i>Klebsiella spp</i>	24	10.48
<i>Enterobacter spp</i>	15	6.55
<i>Proteus mirabilis</i>	14	6.11
<i>Morganella morganii</i>	4	1.74
<i>Citrobacter spp</i>	4	1.74
<i>Serratia mercescens</i>	2	0.87
<i>Hafnia alvei</i>	2	0.87

Table-IV: Antibiotic resistance pattern of isolated Gram-positive bacteria.

Antibiotic	<i>Staphylococcus spp</i> (23)	<i>Staphylococcus aerues</i> (18)	<i>Enterococcus spp</i> (11)
Penicillin	21(91%)	17(94%)	2(18%)
Ampicillin	**	**	2(18%)
Oxacillin	21(91%)	17 (94%)	**
Linezolid	18(78%)	3(16%)	1(9%)
Moxifloxacin	15(65%)	5(27%)	**
Ciprofloxacin	16(69%)	0(0%)	4(36%)
Levofloxacin	12(52%)	10 (55%)	5(45%)
Cotrimazole	10(43%)	0(0%)	**
Azithromycin	21(91%)	16(88%)	**
Chloramphenicol	3(13%)	0(0%)	1(9%)
Doxycycline	0(0%)	0 (0%)	1(9%)
Erythromycin	21(91%)	15(83%)	11(100%)
Gentamycin	12(52%)	1(5%)	**
Vancomycin	**	**	3(27%)

** = Susceptibility not done.

Table-V: Antibiotic resistance pattern of isolated Gram-negative bacteria

Antibiotic	<i>Pseudomonas</i> spp (83)	<i>Escherichia coli</i> (48)	<i>Acinetobacter</i> spp (33)	<i>Klebsiella</i> spp (24)	<i>Enterobacter</i> spp (15)	<i>Proteus mirabilis</i> (14)	<i>Morganella morganii</i> (4)	<i>Citrobacter</i> spp (4)	<i>Serratia mercescens</i> (2)	<i>Hafnia alvei</i> (2)
Amikacin	34 (40%)	28 (58%)	28 (84%)	20 (83%)	6 (40%)	13 (92%)	0 (0%)	2 (50%)	1 (50%)	2 (100%)
Ampicillin	**	45 (93%)	**	23 (95%)	14 (93%)	13 (92%)	4 (100%)	2 (50%)	1 (50%)	2 (100%)
Ceftriaxone	**	40 (83%)	33 (100%)	21 (87%)	9 (60%)	8 (57%)	2 (50%)	2 (50%)	1 (50%)	2 (100%)
Cefixime	**	44 (91%)	33 (100%)	21 (87%)	9 (60%)	8 (57%)	2 (50%)	2 (50%)	1 (50%)	2 (100%)
Ceftazidime	64 (77%)	41 (85%)	32 (96%)	21 (87%)	9 (60%)	8 (57%)	2 (50%)	2 (50%)	1 (50%)	2 (100%)
Cefepime	8 (9.63%)	38 (79%)	30 (90%)	21 (87%)	9 (60%)	8 (57%)	2 (50%)	2 (50%)	1 (50%)	2 (100%)
Chloramphenicol	**	10 (20%)	**	9 (37%)	3 (20%)	4 (28%)	-	-	1 (50%)	1 (50%)
Ciprofloxacin	54 (65%)	36 (75%)	21 (63%)	21 (87%)	10 (66%)	8 (57%)	4 (100%)	2 (50%)	1 (50%)	2 (100%)
Levofloxacin	55 (66%)	36 (75%)	24 (72%)	15 (62%)	6 (40%)	8 (57%)	4 (100%)	2 (50%)	-	2 (100%)
Doxycycline	**	1 (2%)	1 (3.03%)	2 (8.33%)	-	4 (28%)	1 (25%)	-	1 (50%)	-
Gentamicin	**	24 (50%)	27 (81%)	17 (70%)	8 (53%)	8 (57%)	2 (50%)	2 (50%)	1 (50%)	2 (100%)
Imipenem	32 (38%)	25 (52%)	29 (87%)	16 (66%)	3 (20%)	2 (14%)	2 (50%)	1 (25%)	-	-
Meropenem	31 (37%)	25(52 %)	30 (90%)	17 (70%)	3 (20%)	-	1 (25%)	1 (25%)	-	-
Netilmicin	36 (43%)	21(43 %)	**	18 (75%)	3 (20%)	7 (50%)	0 (0%)	2 (50%)	1 (50%)	2 (100%)
Cotrimoxazole	**	32(66 %)	27 (81%)	20 (83%)	5 (33%)	8 (57%)	2 (50%)	2 (50%)	1 (50%)	2 (100%)

** = Susceptibility not done.

Discussion

Infections of the wound can prolong hospitalization and increase mortality rates by 70-80%.⁴ Clinical management of such infections are based on 2 essential factors, antibiotic therapy & wound care.¹⁶ In spite of proper application of the basic principles of wound care a number of patients develop infections, needing proper identification of the organisms for appropriate management.¹⁷ In this study, 75.54% was culture

positive which was almost same 79% to a study done in another private diagnostic centre in Dhaka.¹⁸ Culture negative was 24.46%, suggestive possibility of anaerobic organisms. Anaerobic culture was not done in this study. Gram-negative bacteria were more prevalent (81.5%) than Gram-positive bacteria (52%), supporting the findings of earlier research in Bangladesh and other countries.^{18,19}

Among Gram negative bacteria *Pseudomonas* spp was the most commonly isolated organism in this study followed by *Escherichia coli*, *Acinetobacter* spp, *Klebsiella* spp, *Enterobacter* spp, *Proteus*. *Pseudomonas* spp was found to be the most common Gram negative bacteria in a study in Bangladesh and India.^{20,21} The frequency of *Pseudomonas* spp as the causative agent of wound infection was 43.8% in Bangladesh 2021, which was higher than that of our study (36.24%).²⁰

In contrast to the present findings, *E coli*, *Acinetobacter* spp, *Klebsiella* spp were reported as predominant Gram negative bacterial pathogen.^{18,22,23} This result may be explained by the fact that most of these microbial isolates are part of skin and gut normal flora; hence, they are easily spread when there are breaks or cuts in the skin or soft tissue. Another possible explanation for this is that these isolates frequently found in health care environment as a contaminate.^{22,24}

Among Gram positive bacteria *Staphylococcus* spp was the most commonly isolated organism in this study followed by *Staphylococcus aureus*, *Enterococcus*. Similar findings were found in a study which was done in Brazil.²

The antibiotic susceptibility data in this study showed that some common antibiotics have very limited usefulness for treatment of wound infection. In this study, *Staphylococcus* spp shown highest resistant to penicillin (91%), followed by oxacillin (91%), azithromycin (91%), erythromycin (91%), linezolid (78%), ciprofloxacin (69%), moxifloxacin (65%), levofloxacin (52%) and gentamycin (52%). All most same resistant patterns are also found in *Staphylococcus aerues*

except linezolid, ciprofloxacin, moxifloxacin, and gentamycin in this study. Ciprofloxacin showed 100% sensitivity followed by gentamicin 95%, linezolid 94%, moxifloxacin 73% sensitive in *Staphylococcus aerues*. 100% sensitive to linezolid followed by gentamicin 88.88% were observed, which are almost similar to the findings of previous studies.^{9,26,27} About 94% of the isolated *Staphylococcus aerues* were methicillin resistant *Staphylococcus aerues* (MRSA). In Bangladesh the rate of MRSA infection ranges from 32% to 63% in different studies.²⁸ The rate of MRSA detection is quite higher than other studies, it may be due to lack of awareness, overuse of antimicrobial medication, increase in the infections due to lack of sanitation and hygiene.

Highest resistance by Gram negative bacilli was noted against ampicillin followed by third and fourth generation cephalosporins, fluoroquinolones in this study. This pattern of resistance has been shown by several studies.^{29,30} Fourth generation cephalosporins showed (57-100%) resistant in *Escherichia coli*, *Acinetobacter* spp, *Klebsiella* spp, *Enterobacter* spp, *Proteus* spp, *hafnia alvei*. This finding is different from other studies. The observed disparity in bacterial susceptibility profile could be related to the variation in the level of irrational antibiotic use. Carbapenems were very effective antibiotics showing 62-100% sensitivity against Gram negative bacilli except *Acinetobacter*, *klebsiella*, *E. coli* in this study, which is comparable to the findings of several other studies.³¹⁻³³ It is matter of great concern in treatment of infection because it is a reserve drug and being used for those who are resistant to most other antibiotics. There was variation in the antibiotic sensitivity rate of various organisms

isolated in the present study when compared to different past studies. Increasing MRSA, resistance to fourth generation cephalosporin and carbapenems as a matter of great concern.

Conclusion

There are limited treatment options available for resistant bacteria. Therefore, periodic surveillance of microbial profile and appropriate antibiotic application remain a significant priority in controlling the development and spread multidrug resistant organisms. Besides, infection prevention campaigns must be strengthened among hospital wards and premises.

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