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Original Article

Pattern of Antimicrobial Resistance amongst Pathogens Isolated from Children's Blood at a Private Diagnostic Clinic in Sylhet District of Bangladesh

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Abstract

The incidence of antibiotic-resistant sepsis in children, particularly multidrug-resistant (MDR) sepsis, is increasing day by day. The aim of this study is to describe the pattern of antimicrobial resistance amongst pathogens isolated from blood sepsis of children. This descriptive type of cross-sectional study was carried out in the Microbiology Section of the Popular Diagnostic Centre, Sylhet from April to October 2021. Data were collected from the data record software at centre. All the blood culture positive reports of children of age 0-120 months were collected and antibiotic sensitivity tests were done to identify different organisms. Patients with incomplete data were excluded from this study. A total of 83 patient reports were appraised, and data were analyzed by Statistical Package for Social Sciences (SPSS) software version 22. According to the reports majority of the children (59%) were male and male-female ratio was 1.4:1. Mean age of the children was 16.54±26.4 months (Mean±SD). About one third (33%) of children were within 1 month, 60 % were between 31 to 60 days, and 7.2% within more than 60 days. Most frequent (72.3%) organisms were gram positive, among them nearly half (49.4%) of the organisms were Staphylococcus aureus. More than one fourth (27.7%) of the organisms were gram negative, among them E. Coli was common organism and found in 15.7% of the cultures specimen of children. Here we found Azithromycin, Ceftazidime, Ceftriaxone and Colistin were resistant in most of the gram-positive cases and in gram-negative cases most of the antibiotic were resistant, where Ceftazidime was resistant in all cases. Nearly two-third (64%) of the organisms were multidrug resistant (MDR), 6% were extensively drug-resistant (XDR) and none were pan-drug resistant. About 61% Staphylococcus spp, 77% of E. coli, 43% of Klebsiella were MDR. The majority of sepsis in children are MDR, with Staphylococcus spp. and E. coli being the most common organism. Anti-microbial resistance surveillance and farther large scale studies are now crucial to revise the National Antibiotic Guideline.

Keywords: *Multidrug-resistant (MDR), sepsis, children, Bangladesh.*

INTRODUCTION

Sepsis is the leading cause of sickness, mortality, and healthcare utilization among children around the world. Globally, there are an estimated 22 cases of childhood sepsis per 100,000 person-years and 2,202 cases of neonatal sepsis per 100,000 live births, for a total of 1.2 million cases of childhood sepsis every year.[1] Sepsis is defined as a systemic inflammatory response syndrome (SIRS) triggered by bloodstream infections.[2,3] SIRS in children is defined by at least two of the following criteria, one of which is an abnormal temperature or leukocyte count: Temperatures of more than 38.5 °C or less than 36 °C, tachycardia (defined as a mean heart rate more than two standard deviations above the normal for age) or bradycardia for infants younger than 1 year (defined as a mean heart rate less than the tenth percentile for age), tachypnea (defined as a mean respiratory rate more than two standard deviations above the normal for age), increased or decreased leukocyte count for age, or Over 10% of Neutrophils are Premature.[4] There is a large variety of pathogens that can cause sepsis in children, and this range varies not only by age but also by underlying condition and geographic location.[5] Neonatal sepsis is typically caused by Group B streptococci (GBS),

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Escherichia coli (E. coli), and Listeria monocytogenes. In children younger than 5 years old, H. influenzae type b (Hib), Neisseria meningitidis, and Streptococcus pneumoniae are likely causes of bacterial illness. 6 Frequent hospitalizations of children may increase their exposure to MRSA and vancomycin-resistant enterococci infections. Pseudomonas aeruginosa, along with alpha-haemolytic streptococci and fungi, can cause sepsis in immunocompromised and chronically ill children.[6,7] In both adults and children with sepsis, MDR-GN bacteria are becoming increasingly resistant to antibiotics. MDR pathogens (resistance to at least three distinct classes of antibiotics) are increasingly reported, while pan-resistant strains (resistance to all listed medicines) have already emerged.[8,9] The situation is even worse in low- and middle-income nations than in the rich world.[10] Southeast Asia is regarded as having the highest AMR risk among all WHO regions.[11]

The abuse and misuse of antibiotics, the dissemination of successful clones owing to globalization, and poor hospital hygiene, which allows the development of resistant clones, are the primary causes of antibiotic resistance. Concurrently, there is a dearth of new antimicrobial drugs with the ability to tackle resistant microbes.[12] Antibiotic-resistant microorganisms are becoming increasingly prevalent worldwide. To comprehend the gravity of the threat posed by AMR, the World Health Organization (WHO) estimates that infections caused by multidrug-resistant (MDR) bacteria result in 700,000 fatalities annually across all age groups, including almost 200,000 infants.[13] The treatment of children with sepsis caused by MDR pathogens presents numerous difficulties for clinicians. The absence of data is the most significant issue. Due to the paucity of evidence-based pediatric trials, the majority of data about the efficacy of treatments for sepsis caused by multidrug-resistant (MDR) pathogens in children are drawn from adult studies. Appropriate research can somewhat fill in existing gaps.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in the Microbiology Section of the Popular Diagnostic Centre, Sylhet from April to October 2021. Data were collected from the blood culture positive reports and antibiotic sensitivity tests (for various species) reports of children aged 0-120 months obtained from record software of the microbiology section at the Popular Diagnostic Centre, Sylhet, Bangladesh. Blood samples were directly inoculated into FAN blood culture bottles using aseptic precautions. The BACT/Alert machine incubated bottles for up to 5 days. On MacConkey (MC) agar, chocolate agar and blood agar (5% sheep blood) plates, positive culture samples were placed directly. Pathogenic bacteria were discovered using normal bacteriological techniques. Patients with insufficient data were ruled out from this study. Age, sex differences, and antibiotic sensitivity of the organism were also examined. Based on in-vitro antibiotic susceptibility tests, multidrug resistance was defined as the inability to respond to at least one antimicrobial from three or more classes. Extensively drug-resistant (XDR) organisms are characterized as those with susceptibility to only one or two classes of antimicrobials and resistance to all other kinds. Resistance to all types of antibiotics is considered as pan-drug resistance. A total of 83 patient data were assessed, with the data entered into Microsoft Excel and analyzed using version 22 of the Statistical Package for the Social Sciences (SPSS) program. The purpose of the study was identified the most prevalent pathogenic organisms responsible for bloodstream infections (BSI) and MDR pathogens in children in Sylhet, Bangladesh.

RESULTS

Table I shows the distribution of the reported children by age; among 83 children with a positive blood culture, 49 (59%) males and 34 (41% females), for a male-to-female ratio of 1.40:1. The range of ages was from 6 days to 108 months, with a mean age of the children was 16.54±26.4 days (Mean±SD). Here, 32.5% of children were younger than 30 days, 60% were between 12 to 60 days, and 7.2% were older than 60 days.

Table- I: Distribution of patients by age

Patient Age Categories	No. of Cases (%)
≤1 month	27(32.5%)
> 1 month -<60 months	50 (60.3%)
≥ 60 months	6(7.2%)

Table II shows the distribution of the isolated pathogens from the children blood culture reports. Here, Staphylococcus aureus, detected in 41 (49.4%) of the blood cultures (54% from boys and other 46% from girls), followed by Coagulase-negative Staphylococcus in 18 (21.7) and Streptococcus pneumoniae in 1 (1.2%). Gram negative was detected in 23 (27.7%) with E. Coli was found in 13 (15.7%) of the cultures, followed by Klebsiella spp. in 33 (6%) and Pseudomonas aeruginosa in 1 (1.2%).

Table- II: Distribution of the pathogens according to type and Sex

Isolated Organism	Frequency n (%)	Boys (49)	Girls (34)
Gram positive	60 (72.3)	32	28
Gram negative	23 (27.7)	17	6
Staphylococcus Aureus	41 (49.4)	22(53.7)	19(46.3)
Coagulase negative Staph	18(21.7)	9 (50)	9 (50)
Streptoccus Pneumoniae	1(1.2)	1(100)	0
E. Coli	13 (15.7)	9 (69.2)	4(30.8)
Klebsiella spp	7 (8.4)	5 (71.4)	2 (28.6)
Pseudomonus Aeruginosa	3 (3.6)	3(100)	0

Table III displays the patterns of resistance exhibited by the gram-positive pathogens in the study samples. Here, Amoxyclav, Amikacin, Vancomycin, and Doxicyclin were sensitive antibiotics in higher frequency, whereas Azithromycin, Ceftazidime, Ceftriaxone, and Colistin were the resistance.

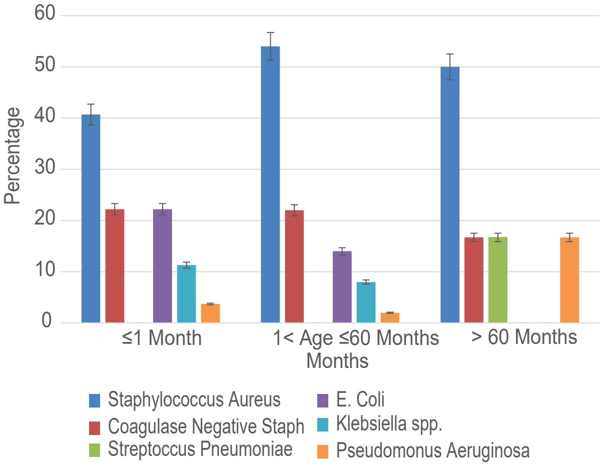

Figure- 1: *Distribution of the pathogen according to age*

Figure 1 displays the distribution of the pathogen by age. Among gram-positive bacteria, total cases were 27 in age group ≤1 month, where staphylococcus aureus were detected in 11 (40.7), Coagulase negative Staph. in 6 (22.2%), E. Coli in 6 (22.2%) children.

Table- III: Resistance patterns of the gram positive pathogens.

Antibiotic	Staphylococcus Aureus			Coagulase –ve Staphylococcus			S. pneumonae		
	R	S	I	R	S	I	R	S	I
Amoxyclav	2.4	**97.6**	00	16.7	**66.7**	16.7	00	**100**	00
Amikacin	4.9	**87.8**	7.3	11.1	**77.8**	11.1	00	**100**	00
Gentamicin	24.4	73.2	2.4	38.9	61.1	00	00	100	00
Vancomycin	7.3	**92.7**	00	11.1	**88.9**	00	00	**100**	00
Ciprofloxacin	36.6	63.4	00	22.2	72.2	5.6	00	100	00
Levofloxacin	4.9	80.5	14.6	27.8	66.7	5.6	00	100	00
Azithromycin	**80.5**	14.6	4.9	72.2	27.8	00	100	00	00
Cefaclor	41.5	56.1	2.4	61.1	27.8	11.1	100	00	00
Cefixime	92.7	7.3	00	88.9	11.1	00	100	00	00
Doxicyclin	2.4	**92.7**	4.9	5.6	**88.9**	5.6	00	**100**	00
Cefuroxime	31.7	63.4	4.9	44.4	50	5.6	100	00	00
Ceftazidime	**85.4**	12.2	2.4	77.8	16.7	5.6	100	00	00
Ceftriaxone	**56.1**	43.9	00	**72.2**	16.7	11.1	**100**	00	00
Imipenem	14.6	82.9	2.4	38.9	61.1	00	100	00	00
Meropenem	17.1	80.5	2.4	27.8	50	22.2	100	00	00
Colistin	**53.7**	46.3	00	**66.7**	33.3	00	**100**	00	00
Linezolid	4.9	95.1	00	16.7	83.3	00	00	100	00
Tazobactum+ Pipercilin	14.6	85.4	00	38.9	61.1	00	00	100	00

Table IV shows the resistance patterns of the gram-negative pathogens. Here, antibiotics were resistant in higher frequency, and Ceftazidime was resistant in every case.

Table- IV: Resistance patterns of the gram negative pathogens.

	Klebsiella			E.Coli			Pseudomonus		
Antibiotic	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**
Amoxyclav	71.4	14.3	14.3	41.7	58.3	00	00	100	00
Amikacin	57.1	28.6	14.3	46.2	46.2	7.7	66.7	33.3	00
Gentamicin	71.4	28.6	00	83.3	16.7	00	66.7	33.3	00
Vancomycin	57.1	42.9	00	75	25	00	100	00	00
Ciprofloxacin	57.1	42.9	00	23.1	76.9	00	00	100	00
Levofloxacin	57.1	42.9	00	23.1	76.9	00	00	100	00
Azithromycin	71.4	14.3	14.3	61.5	23.1	15.4	100	00	00
Cefaclor	85.7	00	14.3	83.3	16.7	00	100	00	00
Cefixime	85.7	00	14.3	100	00	00	66.7	33.3	00
Doxicyclin	14.3	85.7	00	25	75	00	00	100	00
Cefuroxime	85.7	14.3	00	92.3	7.7	00	100	00	00
Ceftazidime	100	00	00	100	00	00	100	00	00
Ceftriaxone	71.4	14.3	14.3	84.6	15.4	00	100	00	00
Imipenem	57.1	28.6	14.3	30.8	61.5	7.7	33.3	66.7	00
Meropenem	71.4	28.6	00	23.1	76.9	00	33.3	66.7	00
Colistin	14.3	85.7	00	50	50	00	100	00	00
Linezolid	85.7	14.3	00	83.3	16.7	00	100	00	00
Tazobactum+ Pipercilin	57.1	28.6	14.3	30.8	69.2	00	33.3	66.7	00

Table V demonstrates the distribution of AMR according to age; here, 61% of registances of organism in children younger than 5 years were MDR, while 100% of cases in children older than 5 years were MDR.

Table- V: Frequency AMR according to age

Patient Age Categories	MDR (%)	XDR	Pan-drug resistance
≤1 month (27)	18 (66.7)	2 (7.4)	00
> 1 month -<60 months (50)	29 (58)	3(6)	00
≥ 60 months (6)	6(100)	00	00

Table VI shows the extent of antibiotic resistance; here, 64% of the organisms were MDR, 6% were XDR, and none of the species were pan-drug resistant. 61% of Staphylococcus spp, 77% of E. coli, and 43% of Klebsiella were multidrug-resistant.

Table- VI: Type of Antibiotic resistance (ABR)

Antibiotic resistance (ABR)	Frequency n (%) n=83	Staph. Aureus n=41	Coagulase –ve Staphylococcus n=18	Strepto. Pneumoniae n-1	E.Coli n=13	Klebsiella n=7	Pseudomonas n=3
MDR	53(63.9)	25 (61)	12 (66.7)	1(100)	10 (76.9)	3 (42.9)	2 (66.7)
XDR	5 (6)	0	0	0	1 (7.7)	3 (42.9)	1(33.3)
Pan-drug resistance	0	0	0	0	0	0	

DISCUSSION

Antibiotic resistance is a global challenge, although impoverished countries are more at risk due to unsanitary environments and inadequate healthcare infrastructure. This is the first study conducted in Sylhet to examine antibiotic resistance in blood culture-positive septicemia in children. Staphylococcus aureus (40.7%), followed by Coagulase-negative Staphylococcus (22.2%), E. coli (22.2%), Klebsiella spp (11.3%), and Pseudomonas aeruginosa (3%), was the most prevalent organism in our study involving children less than one month. The majority of previous research has found that gram-negative organisms are the more prevalent. Shirin et al. discovered that gram negative bacteria comprised 77.4% of the neonate group, with Klebsiella pneumonae being the most prevalent (41.7%), while gram positive bacteria comprised 11.9%, with Staphylococcus Aureus and Streptococcus being equally represented (5.95% each).[15] According to Rafi et al, Escherichia coli was the most commonly recovered gram-negative bacterium from blood samples of suspected newborns with sepsis (40.7%), followed by Klebsiella Pneumoniae (18%). The most prevalent gram-positive organisms were Staphylococcus Aureus (27.5%) and Staphylococcus Saprophyticus (8.8%).[16] The most prevalent organisms in the study population were gram-positive (72,3%),with Staphylococcus aureus (49.4%) being the most prevalent, followed by Coagulase-negative Staphylococcus (21.7%) and Streptococcus pneumoniae (1.2%). E. coli (15.7%) was the most prevalent Gram-negative bacterium (27.7%), followed by Klebsiella spp. (3.6%) and Pseudomonas Aeruginosa (1.2%). This organism frequency differs from other studies. Chisti et al., research at ICDDR'B revealed that Gram-negative pathogens predominated, accounting for 83 (77%) of positive cultures. These were Pseudomonas (26.5%), Escherichia coli (20%), Salmonella enterica (17%), and Klebsiella Pneumoniae (13%). Pathogens that were Gram-positive included Pneumococcus (8%) and Staphylococcus Aureus (7%).[17] Ahmed et al., reported that S. Typhi was the most frequently isolated blood-borne bacterial pathogen, accounting for 36.9% of all blood-borne bacterial pathogens. Other commonly isolated organisms included coagulase-negative Staphylococcus species (21.5%), Pseudomonas species (12.5%), S. Paratyphi A, B (8.9%), and Acinetobacter species (5.1%). Pseudomonas species S. Paratyphi A, B, and Serratia species were prevalent in the over five-year-old age group. In contrast, non-typhoidal Salmonella species and S. pneumoniae were common among children younger than five years old.[18] Amoxyclav, Amikacin, Vancomycin, and Doxicyclin were the most sensitive antibiotics against gram-positive organisms, while Azithromycin, Ceftazidime, Ceftriaxone, and Colistin were resistant in the majority of instances. In gram-negative organisms, the majority of antibiotics were resistant in the majority of cases, and Ceftazidime was resistant in every case. According to a study by Shirin et al., the majority of isolated gram-negative bacteria were resistant to ampicillin, gentamicin, and ceftazidime, although gram-positive bacteria preserved 20-80% susceptibility. Amikacin, netilmicin, ciprofloxacin, and levofloxacin were less effective against Klebsiella than Acinetobacter. Approximately 45 to 65 percent of gram-negative bacteria exhibited resistance to imipenem and meropenem, but gram-positive bacteria exhibited less resistance. Klebsiella and Acinetobacter were resistant to piperacillin similarly to the carbapenem group, whereas gram-positive bacteria were completely sensitive to piperacillin.[15] Bacteria were 100% sensitive to imipenem, according to Islam et al. (86% for meropenem, 83% for ceftazidime, and 75% for ciprofloxacin).[19] Resistance to all commonly used empiric antibiotics, by Chisti et al (ampicillin, gentamicin, ciprofloxacin, and ceftriaxone).[17]This study found, None organism were found 100% sensitive. About 64% of the organisms were MDR, 6% XDR and non were pan-drug resistant. About 61% Staphylococcus spp, 77% of E. coli, 43% of Klebsiella, 68% of Pseudomonas and 100% of Strepto. Pneumoniae were MDR. Around 60% cases of organism in under 5 years children, were MDR and 100% cases of 5 years and above were MDR. Ahmed et al., found an overall increase in the presence of Gram-positive bacteria was observed, but most significantly we observed the percentage of MDR Gram-positive bacteria to double over the study period from 2004 to 2014. Overall, Gram positive bacteria were more resistant to most of the commonly used antibiotics than Gram-negative bacteria, but the MDR level was high in both groups. At 2014, about 74% gram-negative and 35% were gram-positive. Acinetobacter species (65.8%), Pseudomonas species (21.2%), Gram-Negative (74.1%), Escherichia coli (72.2%), Klebsiella species (81.4%), Enterobacter species (70.6%), Gram-Positive (35.4%), Staphylococcus Aureus (63.3%), Streptococcus pneumoniae) (15.4%), Streptococcus species (30.4%).[18] On the basis of a systematic review by Murray et al., the six leading pathogens responsible for AMR-related deaths (Escherichia coli, followed by Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa) were responsible for 929000 (660,000–1270,000) deaths.[20] Our research discovered a nearly identical pathogen in Sylhet, Bangladesh.

CONCLUSIONS

Anti-microbial resistance surveillance is an essential tool for antibiotic guidelines and successful treatment outcome. This study found that gram-positive bacteria, specifically Staphylococcus spp. and E. coli, are the most common organisms causing sepsis in children. All gram-positive and gram-negative microbes identified exhibited a high level of antibiotic resistance. About 64% of the organisms were MDR, while 6% were XDR. This is an important observation and requires further studies. The significance of the study is the observation of XDR, which may have a future impact for policy makers in revising the national antibiotic guideline for the management of patients.

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