Original Article

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Antimicrobial Resistance Pattern of Bacterial Isolates in Urine of the Children Suffering from UTI

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ABSTRACT

Background : *Use of antibiotics are important in the treatment of Urinary Tract Infections (UTIs). However, the inappropriate use of antibiotics contributing to the development of antimicrobial resistance. Urine culture is the most common and important laboratory test to isolate the microbial agents, their susceptibility pattern and subsequently helps in the selection of appropriate antimicrobial to treat the infection. To isolate and identify the bacterial agents in urine of children suffering from suspected urinary tract infection and determine their antimicrobial resistance pattern this research was performed.*

Materials and methods: *This retrospective study was conducted in the Department of Microbiology, IAHS, Chattogram, Bangladesh from March 2019 and April 2020. Total 2100 urine specimens were collected from the children attended in IAHS Inpatients and Outpatients Department of pediatrics at BBMH with suspected urinary symptoms. Bacterial culture and antimicrobial susceptibility testing were done in all urine specimens in BBMH Microbiology laboratory. The isolated samples were classified according to their age, sex, microorganisms and antibiotic susceptibility. Patients were divided into different age groups which are 0-2, 2-6, 6-12 and 12-17 years.*

Results: *Out of total 2100 urine samples, 720 samples showed bacterial growth on culture. That revealed the prevalence of UTI among child about 34%. The most common isolated bacterial agents was Escherichia coli (68.01%), Klebsiellapneumoniae (19.86%), Klebsiellaoxytoca (3.34%), Enterobacter cloacae (2.23%), and Pseudomonas aeruginosa (1.95%). In the 0-2 years age group was significantly high in this group ($p<0.05$) in terms of infectious agents diversity and antibiotic resistance. Isolated bacterial agents shows resistance to ampicillin (70.2%), amoxicillin- clavulanate (49.0%), cefixime (38.2%), and trimethoprim/sulfamethoxazole (37.1%). The frequency of UTIs were found more in girls (56.6%) than that of boys among 0-2 year age group.*

Conclusion: *In conclusion, ampicillin, cephalosporins, amoxicillin-clavulanate, and TMP/SMX should not be empirically commenced. In order to avert a rapid resistance development that is closely associated with widespread and inappropriate antibiotic use, the use of random antibiotics should be avoided and particularly the 3rd generation cephalosporins should be used more selectively. Although our data reflect only the results of patients admitted to a single hospital, classification of pathogens and determination of resistance rates against oral and parenteral antibiotics make this study valuable as it assesses the resistance patterns in the patients.*

Key words: AMR; Children; UTIs.

Introduction

Urinary Tract Infection (UTI) is the infection of the kidneys, ureters, bladder and urethra.[1] UTI is one of the most common bacterial infections of childhood. Among febrile infants, unwell children in general practice and older children with urinary symptoms, 6%–8% will have a UTI.[2,3] In 2000, according to the Urologic Diseases in America Project, UTIs accounted for more than 8 million office and 1.7 million emergency room visits, leading to around 350 000 hospitalizations.[3] Inaddition,12-30% of these children were also infected with recurrent fever infections.[4]

Flora alterations, urinary tract anomalies, and immature immune system are among the causes of frequent infections in this age group.[4,5] Renal damage induced by recurrent fever infection causes long-term complications including hypertension, renal dysfunction and chronic renal failure in later period.[6,7] Thus, detection of risk factors, development of appropriate diagnostic methods, and therefore a rapid and reliable treatment of UTI are of great importance to reduce the morbidity rate and related complications. The epidemiology of UTI varies depending on age and

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gender. Approximately 5% of girls and 2% of boys are infected with an UTI at least once.[8] The global prevalence in children under two is 7%.[9] In Infants, it is very difficult to identify the UTI symptoms. Clinicians should diagnose early to initiate appropriate treatment and prevent complications including hypertension and chronic kidney disease.[10]Microbial etiology of UTIs has been regarded as well established, with E. coli being the causative pathogen in 50–80% of cases, other Enterobacteriaceae (Klebsiella, Proteus, Enterobacter) together with Enterococci, Streptococci, Staphylococci, and Pseudomonas spp. account for most of the remaining positive urine cultures.[11,12] Empiric antibiotic treatment is therefore commonly adopted. However, due to significant local differences in frequency of urinary agents, the emergence of new pathogens, and changes of antimicrobial resistance, periodic evaluation of pathogens epidemiology is recommended, in order to revise treatment advices.[13] Majority of UTI agents are Escherichia coli, Klebsiella and Proteus species, although these agents differ depending on age and gender as well as the catheterization and hospitalization. Moreover, Enterococcus faecalis and Pseudomonas species showing high antibiotic resistance are also found in patients who were hospitalized or have anatomical anomalies.[14] Antibiotic resistance of these microorganisms varies depending on their location. In recent years, antibiotic resistance has been an increasingly important problem worldwide in the first stage of treatment.[15] Antibiotics used for the treatment include trimethoprim/sulfamethoxazole (TMP/SMX), cephalosporins, and amoxicillin.[16] Regarding the childhood UTI, E.coli, which is a common infection agent, indicates increasing resistance against beta lactam antibiotic and TMP/SMX. Wide spread antibiotic use increases the antibiotic resistance and therefore, triggers the invention of new antibiotics to be used in the treatment of infections. This situation increases the economic burden in the healthcare sector and leads to an additional increase in antibiotic resistance.

Materials and methods

This retrospective study was conducted in the Department of Microbiology, IAHS, Chattogram, Bangladesh from March 2019 to April 2020. The study protocol was reviewed and approved by Institutional Review Board (IRB) of IAHS. Total 2100 urine specimens were collected from the children attended in IAHS inpatients and outpatients department of BBMH with suspected urinary symptoms. The isolated samples were classified according to their age, sex, microorganisms and antibiotic susceptibility. Patients were divided into different age groups which are 0-2, 2-6, 6-12 and 12-17 years.

Inclusion criteria were children 2 months to 17 years of age presenting with symptoms of UTI with suspected UTI source in infants, a positive urine culture of organism according to standard guidelines. Patients who had received antibiotics before urine culture or samples with antibiotics resistant were excluded from the study.

Study specimens were cultured on Mac Conkey agar plates and blood agar plates by calibrated loop method. Clean catch midstream urine samples were collected into a wide mouthed sterile container. Then urine inoculated on Mac Conkey's and blood agar media using calibrated platinum loop following standard bacteriological technique and incubated at 37^0C for 24 hours. After 24 hours, the plate was examined for bacterial pathogen. Pure bacterial colony counting 100,000 or more was considered as significant and was subjected to identification based on colony characters and biochemical tests. In this study, culture was considered positive when the culture of a single microorganism found at a concentration of $>10^5$ colony forming units (CFU)/ml. Commercially available discs (supplied by hi media, india) were used to find the sensitivity of the organism on Mueller Hinton agar media after overnight incubation at 37^0C.

Antimicrobial resistance : susceptibility pattern of isolated bacteria from urine samples were tested against 19 different antimicrobial agents. These antimicrobial agents were, namely, ertapenem, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, ampicillin, amoxicillin/ clavulanate, norfloxacin, piperacillin/ tazobactam, cefixime, ceftazidime, ceftriaxone, cefepime, nitrofurantoin, TMP/SMX, fosfomycin, colistin and aztreonam.

Statistical Analysis

Data analysis was carried out using SPSS software (Version19.0, SPSS Inc. Chicago, IL). Descriptive values were specified as number (n), percentage (%), and median. The normal distribution of variables was evaluated using visual (Histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Pearson Chi-square test was used to compare the categorical variables. Continuous variables were compared using the Mann-Whitney U test because they did not match the normal distribution. Homogeneity of the groups was evaluated by the Levene's test and Tamhane's posthoc test. In this study, p values below 0.05 were considered as statistically significant.

Results

Out of total 2100 urine samples, 720 samples showed bacterial growth on culture. That revealed the prevalence of UTI among child about 34%. Urinary

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pathogens obtained from children in the age groups of 0-2 (n= 428), 2-6 (n= 154), 6-12(n=116), and 12-17 (n= 22) were examined. In total, 16 different UTI agents have been identified from these urine specimens (Table I). The most frequently isolated microorganism in UTI. Samples were Escherichia coli (68.01%) followed by Klebsiella, Enterobacter, Pseudomonas and Proteus species. Particularly in recent years, the incidence of high resistant gram-negative bacteria in complicated urinary tract infections has increased .In this study, among 19 different antimicrobial agents, the highest resistance rates were against ampicillin, amoxicillin/clavulanate, cephalosporin group, TMP/SMX and aztreonam, respectively. The lowest resistance rates were observed against imipenem, amikacin, and meropenem, respectively (Fig-1).The incidence of UTI varies according to age and gender. In childhood, UTI is one of the most common infection us diseases, especially in children under two years. The incidence of UTI in girls increases after the first age and the onset of toilet training. According to national and international studies, the majority of children with UTI are girls. The distribution of UTI agents according to age groups and gender status is presented in (Table II). Regarding age groups of patients, 428 (59.5%) patients are in 0-2 age, 153 (21.3%) in 2-6, 116 (16.1%) in 6-12, 22 (3.1%) in 12-17 years age. In this study, the low rate of resistance against carbapenem group antibiotics was identified in Escherichia coli and Klebsiella species. Carbapenem resistance was found to be quite low in accordance with other studies in the literature and there has been no significant increase in the resistance compared to previous studies. In 0-2 years age group, there were high levels of resistance against all of the tested antibiotics in this study (Fig-2). Resistance against these antibiotics was found to be mainly in Escherichia coli and Klebsiella strains. There is also a high degree of resistance against cephalosporins, which are frequently used in the treatment of UTI. This study found that there were resistances against cefixime, ceftriaxone and ceftazidime antibiotics which are among the 3rd generation cephalosporins. There was resistance development against cefepime that is among the 4th generation cephalosporins. The development of resistance against cefixime (38.2%), ceftriaxone (34.2%), ceftazidime (32.3%) and cefixime (29.6%) antibiotics over time can also be associated with the frequent use of these antibiotics. A previous study reported that resistance values of E. coli and Klebsiella spp. against cefixime, a common oral antibiotic, were 31.3% and 38.2%, respectively. The resistances against ampicillin (96.9%) and nitrofurantoin (76.4%) were also found to be relatively high in Klebsiella species which is the second most common agent isolated in this study (Table III).The highest resistance was against gentamicin (78.8%), whereas the lowest resistance was against imipenem (45.5%). Among the age groups, the lowest antibiotic resistance levels were found in 12-17 years age group. In this group, the highest resistance was against meropenem(12.5%), whereas the lowest resistance was against ertapenem (1.7%). Antibiotic resistance values in the four different age groups (0-2, 2-6, 6-12, and 12-17) indicated a normal distribution except for 12-17 years age group (p=0.44). But it was also evaluated as in a normal distribution. According to statistical analysis results, antibiotic resistance was significantly higher in 0-2 age group (group-1) significantly lower than other age groups ($p<0.05$) (Table-IV).

Table I Descriptive information about the bacterial strains isolated from UTI agent samples

Infective agents	Number	Percentage (%)
Escherichia coli	489	68.01
Klebsiella pneumoniae	143	19.86
Klebsiella oxytoca	24	3.34
Enterobacter cloacae	16	2.23
Pseudomonas aeruginosa	14	1.95
Proteus vulgaris	7	0.97
Providenciarettgeri	7	0.97
Enterobacter aerogenes	5	0.70
Proteus mirabilis	5	0.70
Morganella morganii	3	0.42
Citrobacter freundii	2	0.28
Cedecealapagei	1	0.14
Citrobacter koseri	1	0.14
Citrobacter werkmanii	1	0.14
Serratia fonticola	1	0.14
Serratiamarcescens	1	0.14
Total	720	100

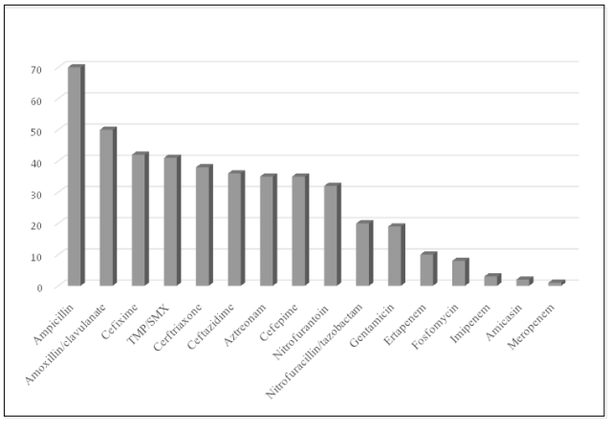

Figure 1 Antimicrobial resistance distribution of urinary tract infection agents isolated

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Table II Distribution of UTI agents according to age and gender groups in children

Organisms	Age (years)				Gender	
	0-2	2-6	6-12	12-17	Female	Male
E. coli	259	120	98	12	408	81
Klebsiella spp.	130	13	17	7	105	62
Enterobacterspp.	18	2	0	1	9	12
P. aeruginosa	7	4	2	1	9	5
Proteus spp.	5	7	0	0	9	3
M. morganii	3	0	0	0	1	2
Citrobacter spp.	2	1	0	1	3	1
P. rettgeri	2	5	0	0	6	1
Serratia spp.	2	0	0	0	1	1
C. lapagei	0	1	0	0	1	0
Total cases	428	153	117	22	552	168

Table III Antimicrobial resistance distributions according to urinary tract infection factors isolated from children (n=720)

Antibiotic agents	E, coli (n=489)	Klebsiella spp. (n=167)	Enterobacter spp. (n=21)	P. aeruginosa (n=14)	Proteus spp. (n=12)	M. morganii (n=3)	Citrobacter spp. (n=4)	P. rettgeri (n=7)	Serratia spp. (n=2)	C. lapagei (n=1)	Total
Ampicillin	301	160	15	13	8	3	1	2	1	0	504(70.2)
Amoxicillin/clavulanate	221	85	18	13	2	3	3	6	1	0	352 (49.0)
Trimethoprim /sulfamethoxazole	182	72	0	10	2	1	0	0	0	0	267(37.1)
Cefixime	158	81	11	14	4	2	1	2	1	1	275(38.2)
Ceftazidime	147	78	5	0	0	0	0	0	1	1	232(32.3)
Ceftriaxone	140	75	8	14	5	1	0	1	1	1	246(34.2)
Cefepime	135	71	4	0	1	0	0	0	1	1	213(29.6)
Aztreonam	135	73	6	2	0	0	0	0	1	1	218(30.3)
Norfloxacin	102	43	0	9	0	1	0	0	0	1	156(21.7)
Ciprofloxacin	72	21	0	0	0	1	0	0	0	1	95(13.2)
Gentamicin	43	50	1	0	4	0	0	0	1	0	99(13.8)
Piperacillin/tazobactam	43	64	5	1	0	0	0	0	0	0	113(15.7)
Ertapenem	12	23	6	14	1	0	0	2	1	1	60(8.3)
Meropenem	4	3	0	1	0	0	0	0	0	0	8 (1.1)
Nitrofurantoin	4	126	17	14	12	3	2	7	2	1	188(26.1)
Imipenem	2	4	0	1	4	0	0	0	0	0	11(1.5)
Amicasin	2	5	0	0	0	0	0	0	1	1	9(1.3)
Fosfomycin	2	17	3	13	2	3	0	2	1	0	43(6)
Colistin	0	1	0	0	12	3	0	7	2	1	26(3.6)

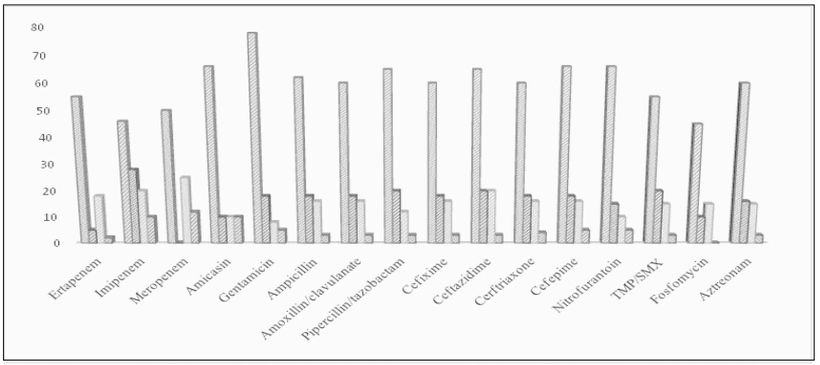

Figure 2 Comparison of antimicrobial resistance of urinary tract infection agents isolated from children with age groups (Group 1: 0-2, Group 2: 2-6, Group 3: 6-12 and Group 4: 12-17) (n=720)

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Table IV Statistical analysis of antibiotic resistance levels in four different age groups (Group 1: 0-2, Group 2: 2-6, Group 3: 6-12 and Group 4: 12-17).

Age group		Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval Lower Bound	Upper Bound
1.00	2.00	70.52632*	19.90999	.011	12.7880	128.2646
	3.00	73.31579*	19.75320	.008	15.8618	130.7698
	4.00	93.84211*	19.10285	.001	37.4724	150.2118
2.00	1.00	-70.52632*	19.90999	.011	-128.2646	-12.7880
	3.00	2.78947	7.67403	1.000	-18.5854	24.1644
	4.00	23.31579*	5.79835	.004	6.3200	40.3116
3.00	1.00	-73.31579*	19.75320	.008	-130.7698	-15.8618
	2.00	-2.78947	7.67403	1.000	-24.1644	18.5854
	4.00	20.52632*	5.23472	.005	5.2072	35.8454
4.00	1.00	-93.84211*	19.10285	.001	-150.2118	-37.4724
	2.00	-23.31579*	5.79835	.004	-40.3116	-6.3200
	3.00	-20.52632*	5.23472	.005	-35.8454	-5.2072

Note: * Symbol indicates that the mean difference is significant at 0.05 levels.

Discussion

The most frequently isolated micro organism in UTI agent samples was Escherichiacoli(68.01%); followed by Klebsiella, Enterobacter, Pseudomonas and Proteus species. Particularly in recent years, the incidence of high resistant gram-negative bacteria in complicated urinary tract infections has increased. In accordance with our study, the most common UTI agents isolated from children in previous years were reported as Escherichia coli, followed by Klebsiella, Proteus species, Enterococcus faecalis and Pseudomonas species.[11] Escherichia coli is known as the most common bacteria (80%) in children with UTI.[14] Klebsiellaspp., Proteus spp., Enterobacter spp., Serratia spp., Pseudomonas spp. and Proteus mirabilis are among the other most common infection agents.[15] In accordance with other studies in the literature, this study found that 76.6% of the patients were girls, whereas only 23.4% were boys. In similar studies,61.5% and 51% of patients were girls in 0-17 and 0-15 age groups, respectively.[16,17] Thus,the ratio of girls with UTI was found to be higher than that of boys. Similarly, 77.7% of patients (n=525) were girls and 59.1% (n=399) were under the age of two.[18] Therefore, the findings of this study are consistent with previous studies regarding both age and gender groups inchildren. In our study, the most common observed agent was E. coli (56.8%) across all age groups. However, the reproductive frequency in E.coli decreased as the age advanced in children. The situation was similar for not only in Klebsiella, but Enterobacter sp. and P. aeroginosa. However, contrary to our study, Topal etal found that the reproductive frequency in E. coli increased as the age advanced.[17]In children with a risk of increased antibiotic resistance, broad-spectrum antibiotics are used. Although amoxicillin, amoxicillin-clavulanate, cephalosporins, nitrofurantoin, and TMP-SMX are the most frequently prescribed drugs in the UTI treatment in Bangladesh. The lowest resistance rates were observed against imipenem, amikacin, and meropenem, respectively. Resistance against these antibiotics was found to be mainly in E. coli and Klebsiella strains. Low resistance against amikacin and imipenem antibiotics was also reported and amikacin resistance was found to be quite low in E.coli and Klebsiella strains, in accordance with this study.[17] Moreover, imipenem is noted as a broad-spectrum antibiotic that is less frequently used and it can be preferred in cases with resistance against other antibiotics. Our study indicated that ampicillin (70.2%), amoxicillin-clavulanate (49.0%), and TMX/SMX (37.1%) resistance were quite high in E. coli strains. Low resistance against amikacin and imipenem antibiotics was also reported and amikacin resistance was found to be quite low in E. coli and Klebsiella strains, in accordance with our study.[17] Aminoglycoside group antibiotics are one of the preferable antibiotics owing to low resistance in the treatment of UTI as supported by the findings of literature. Moreover, imipenem is noted as a broad-spectrum antibiotic that is less frequently used and it can be preferred in cases with resistance against other antibiotics. Our study indicated that ampicillin (70.2%), amoxicillin-clavulanate (49.0%) and TMX/SMX (37.4%) resistance were quite high in E. coli strains. Also, there was a resistance development for TMP/SMX (37.1%) and therefore, this antibiotic could not be used in the first stage treatment. Similarly, it is also reported that resistance values against ampicillin and TMP-SMX were high.[19] In Turkey, resistance values of Escherichia coli against TMP-SMX and ceftriaxone were reported to be 34.7%, 17%, respectively.[20] In another study, resistance rates of ampicillin and TMP-SMX in Escherichia coli were 81.5% and 67%, respectively.[21] Moreover, in a previous study in Turkey, ampicillin resistance values were56.4%, 78.3% and 42.3% in Escherichia coli, Klebsiella species and Proteus species, respectively.[21] Consequently,a high rate of ampicillin resistance detected in ths study was consistent with findings of previous literature. A possible reason behind these high values is that these agents are frequently used in the empirical treatment of UTI. There is also a high degree of resistance against cephalosporins, which are frequently used in the treatment of UTI. Our study

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found that there were resistances against cefixime, ceftriaxone and ceftazidime antibiotics which are among the 3rd generation cephalosporins, whereas there was resistance development against cefepime that is among the 4th generation cephalosporins. The development of resistance against cefixime (38.2%), ceftriaxone (34.2%), ceftazidime (32.3%) and cefepime (29.6%) antibiotics over time can also be associated with the frequent use of these antibiotics. A previous study reported that resistance values of Escherichia coli and Klebsiella spp. against cefixime, acommonoral antibiotic, were 31.3% and 38.2%, respectively. The highest levels of cefepime resistance were in Acinetobacter (53.3%), Klebsiella spp. (36.9%), and Escherichia coli (26.8%). Ceftazidime resistance values were 30.2% in E. coli, 39.4% in Klebsiellaspp.15.1% in Pseudomonas, 40.5% in Enterobacter and 47.8% in Acinetobacter. However, dissimilar to other agents, the resistance against nitrofurantoin in Klebsiella species was quite note- worthy in our study. In addition, aztreonam resistance values were found to be 27.8% and 44.2% in E. coli and Klebsiella species, respectively. The highest rates of ceftriaxone resistance were found in Enterobacter (47.1%), Klebsiella spp. (41.6%) and Escherichia coli (31.5%)[14]. Regarding age groups of patients, 428 (59.5%) patients are in 0-2 age, 154 (21.3%) in 2-6, 116 (16.1%) in 6-12,22 (3.1%) in 12-17 age. In a similar study, 1021 (23.1%) patients were under one year of age, 1547 (34.9%) were 1-5, 1018 (22.9%) were 6-10, and 835 (19.1%) were over 10 years old[16]. Moreover, another similar study found that 0-2 age group patients constitute a percentage of 56.6% (n=125), followed by 3-7 (26.2%, n=58) and 8-15 age groups (17.2%, n= 38).[17] Therefore, UTI is observed more often in 0-2 age group compared to other age groups as supported by this study and several other studies. A study in Greece, the sensitivity to the 3rd generation cephalosporins (Cefotaxime, ceftriaxone, and ceftazidime) in Escherichia coli was found to be between 95.6% and 97.4%. In our study, the cephalosporin resistance was found to be slightly higher compared with the literature. Given the presence of increasing resistance, especially the 3rd generation cephalosporinsshould be used moreselectively. Carbapenems are the most preferred group for parenteral treatment of UTI caused by microorganisms which are resistant to many antibiotics. It was reported that carbapenem resistance was the highest in E. coli strains (18.5%) whereas the carbapenem resistance was reported as 1.2% in a different study.[21,22] In the study of Konca et al imipenem and ertapenem resistance values were found to be 4.1% and 4.6% in all gram-negative bacteria, respectively.[23] In another study, meropenem resistance was 1.3%, imipenem resistance was1.5%, and ertapenem resistance was1.7% in E. coli.[19, 24] In our study, the low rate of resistance against carbapenem group antibiotics was identified in Escherichia coli and Klebsiella species. Carbapenem resistance was found to be quite low in accordance with other studies in the literature and there has been no significant increase in the resistance compared to previous studies. In 0-2 age group, there were high levels of resistance against all of the tested antibiotics. The highest resistance was against gentamicin (78.8%), whereas the lowest resistance was against imipenem (45.5%). Among the age groups, the lowest antibiotic resistance levels were found in 12-17 age group. In this group, the highest resistance was against meropenem (12.5%), whereas the lowest resistance was against ertapenem(1.7%). Antibiotic resistance values in the four different age groups (0-2, 2-6, 6-12, and 12-17) indicated a normal distribution except for 12-17 age group ($p=0.44$) but it was also evaluated as in a normal distribution. According to statistical analysis results antibiotic resistance was significantly higher in 0-2 age group (group-1), significantly lower than other age groups ($p<0.05$). Therefore, these resistance levels create a severe threat particularly for children not only in Bangladesh but also in the world.

Limitations

- The isolates were from one specific hospital, this did not make the findings generalized.
- Non availability as well as high cost of the reagents limited to this type of investigation.
- Time constrain-the study was done in a limited time period.

Conclusion

In conclusion, ampicillin, cephalosporins, amoxicillin-clavulanate, and TMP/SMX.should not be empirically commenced. In order to avert a rapid resistance development that is closely associated with widespread and inappropriate antibiotic use, the use of random antibiotics should be avoided and particularly the 3rd generation cephalosporins should be used more selectively. Although our data reflect only the results of patients admitted to a single hospital, classification of pathogens and determination of resistance rates against oral and parenteral antibiotics make our study valuable as it assesses the resistance patterns in patients.

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Disclosure

All the authors declared no competing interest.

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