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Original Article

Microbiology Section

Study of Aetiology and Anti-biogram of Uropathogens in Children-A Retrospective Analysis

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ABSTRACT

Objectives: Urinary Tract Infections (UTIs) are the most common serious bacterial infections which are seen during infancy. The aim of the present study was to evaluate aetiology, and antimicrobial resistance patterns among infants and children who approached our hospital for treatment of UTIs.

Methods: In this observational study which was carried out from 2007 to 2010, 1575 urine samples which were collected from children with suspected UTIs were studied. Demographic characteristics, aetiological agents and antimicrobial resistance were evaluated.

Results: UTIs were more common in the 0-1 year age group, among males. Among females, UTIs were commonly seen after 2 years of life. The most common isolated pathogen was *Escherichia coli spp* (45.12%), followed by *Klebsiella spp* (18.17%) and *Enterococcus spp* (9.23%). Isolated pathogens were highly resistant to ampicillin, co-trimoxazole, and norfloxacin (82%–98%) and highly sensitive to gentamicin (83%),amikacin (76.5%), and nitrofurantoin (71.5%).

Conclusion: The most common pathogen which caused UTIs in children was *E. coli spp*.

The isolated pathogens were highly resistant to commonly used antibiotics, ampicillin and co-trimoxazole, while they were highly sensitive to gentamicin, amikacin and nitrofurantoin. So, these antibiotics may be used as alternative drug therapies for the treatment of UTIs.

Keywords: Urinary tract infection, Antibiotic resistance, Sensitivity, *Escherichia coli*

INTRODUCTION

UTIs are the most common serious bacterial infections which are seen during infancy [1]. Prevalence of UTIs is 1-3% in girls and it is 1% in boys [2]. Paediatric UTI are associated with high morbidity and long term complications like renal scarring, hypertension and chronic renal failure [3]. The diagnosis of UTIs is difficult in the neonatal period, because the signs and symptoms are non-specific in this age group. Renal calculi, obstructive uropathy (posterior urethral valves), vesicourethral reflux and voiding disorders can lead to urinary stasis and they may predispose to the development of recurrent UTIs and their complications [4].

Common bacterial pathogens include gram negative enteric bacilli, especially *Escherichia coli* and *Klebsiella species* and gram positive organisms like group *B Streptococci*, *Enterococcus species* and *Staphylococcus aureus* [5]. Resistance of uropathogens to antibiotics is increasing [1]. A prompt diagnosis and management of UTIs can reduce the incidence of morbidity and life threatening bacteraemia. Recent studies which have been done on paediatric UTIs in India are limited. Therapy for these children requires urine culture and appropriate anti-microbial sensitivity testing. So, the aim of the present study was to analyze the aetiology and antimicrobial resistance patterns of uropathogens in all paediatric patients who approached our hospital with UTIs.

MATERIALS AND METHODS

The present retrospective study was carried out in the Department of Microbiology for a period of 3 years, from January 2007 to December 2010. A total number of 1575 urine samples from children who were suspected of having UTIs were received from among both inpatients as well as outpatients who approached our hospital, Smt. Kashibai Navale Medical College and General Hospital, Narhe, Pune, India. Samples were midstream urine specimens, catheterized urine samples and supra pubic aspirates. Screening of urine for significant bacteriuria was done by gram staining the samples. Urine culture was done by a semi-quantitative technique. Urine (0.001ml) was cultured by using a calibrated bacteriological loop on blood agar and Mac Conkey's agar. Recommendations of Kass [6] were followed, for distinguishing active UTIs from contaminations. Presence of 10^5 bacteria/ml of urine indicated an active urinary infection and so, this level was called significant bacteriuria. After an overnight incubation at 37°C, number of colonies which were obtained were counted and they were multiplied by 1000, to obtain the colony forming units (cfu)/ml. For suprapubic samples, any numbers of colonies were considered as significant [7].

Isolates were identified by gram staining, motility tests and routine biochemical reactions. Antibiotic sensitivity was performed by using Kirby Bauer disc diffusion method by following the Clinical Laboratory Standards Institute (CLSI) guidelines [8]. All *Enterobacteriaceae* and *Acinetobacter spp*. were tested against the first line agents: amikacin (30µg), ampicillin (10µg), cefotaxime (30µg), trimethoprim-sulphamethoxazole(1.25-23.75µg), gentamicin (10µg), nitrofurantoin (300µg), norfloxacin (10µg) and doxycycline (30µg). For gram positive cocci, penicillin (1U), cefazolin (30µg), ciprofloxacin (5µg), nitrofurantoin (300µg), norfloxacin (10µg), doxycycline (30µg), vancomycin (30µg), teicoplanin (30µg) were used. Pseudomonas aeruginosa was tested against amikacin (30µg), gentamicin (10µg), ceftazidime, (30µg), carbenicillin (100µg) piperacillin (100µg) and ciprofloxacin (5µg). Second line antibiotics were tested only for organisms who were resistant to all 1st line antimicrobials or if it was specifically requested for by the attending physician. These included; imipenem (10µg), cefepime (30µg), ofloxacin (5µg) and piperacillin-tazobactam (100/10µg). Extended spectrum β lactamase (ESBL) production in *Escherichia coli* and *Klebsiella pneumoniae* was tested as per CLSI guidelines [8]. For all *Enterobacteriaceae*, *Acinetobacter spp*. and *Pseudomonas isolates*, *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853 were used as controls.

Age groups (months/years)	Sex (No., %)		Total (No., %)
	Males	Females	
0-1	101 (25.89)	46 (11.79)	147 (37.7)
2-5	58 (14.87)	72 (18.46)	130 (33.3)
6-12	40 (10.25)	73 (18.71)	113 (29)
Total	199 (51.03)	191 (48.97)	390 (100)

[Table/Fig-1]: Age distribution in males and females with UTI

Organisms	0-1	2-5	6-12	Total (%)
Escherichia coli	74	54	48	176(45.12)
Klebsiella species	40	17	16	73(18.71)
Enterococcus species	15	12	09	36(9.23)
Pseudomonas species	02	06	10	18(04.61)
Citrobacter species	03	12	03	18(04.61)
Acinetobacter species	02	06	09	17(04.35)
Streptococcus species	01	05	10	16(04.10)
Proteus species	03	07	04	14(03.58)
Staphylococcus aureus	01	07	03	11(02.82)
Candida species	06	03	01	10(02.56)
Salmonella typhi	00	01	00	01(0.25)
Total	147	130	113	390

[Table/Fig-2]: Organisms isolated in different age groups

RESULTS

Out of the 1575 clinical specimens which were tested, 390 were culture positive (24.76%), 444 showed insignificant growth of organisms, i.e., due to contamination of urine samples and 741 were culture negative.

Overall, infection rate was higher in 0-1 year age group among males [Table/Fig-1]. Among females, the infection rate was higher in the age group of 6-12 years.

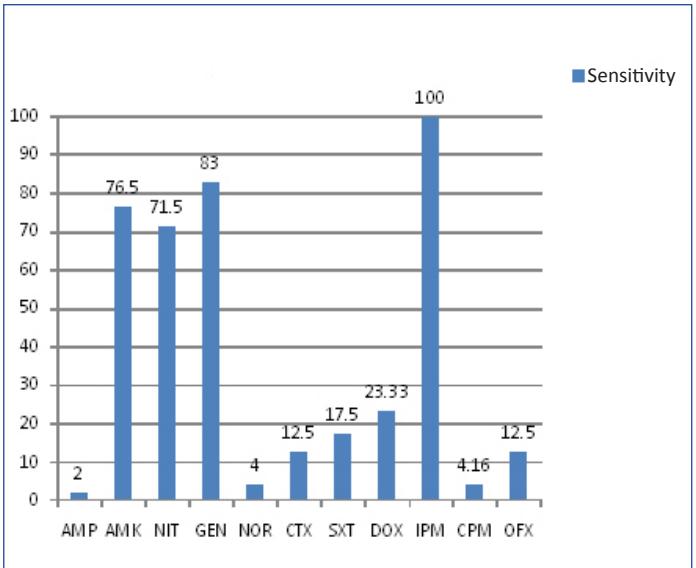
Escherichia coli (45.12%) was the commonest uropathogen which was isolated in all age groups, followed by *Klebsiella species* (18.71%) and *Enterococcus species* (9.23%) [Table/Fig-2].

Antibiotic susceptibility pattern among gram negative bacilli was as shown in [Table/Fig-3]. Among the first line antibiotics, higher sensitivity was observed against gentamicin, amikacin and nitrofurantoin. Maximum resistance was seen against ampicillin and norfloxacin. Among the second line antibiotics, all strains were sensitive to impenem. ESBL positivity was 6% in *Escherichia coli* and *Klebsiella spp*. Among *Enterococci*, resistance was not seen with vancomycin and teicoplanin (100% sensitivity) and a higher susceptibility was observed with nitrofurantoin (63.77%) [Table/Fig-4].

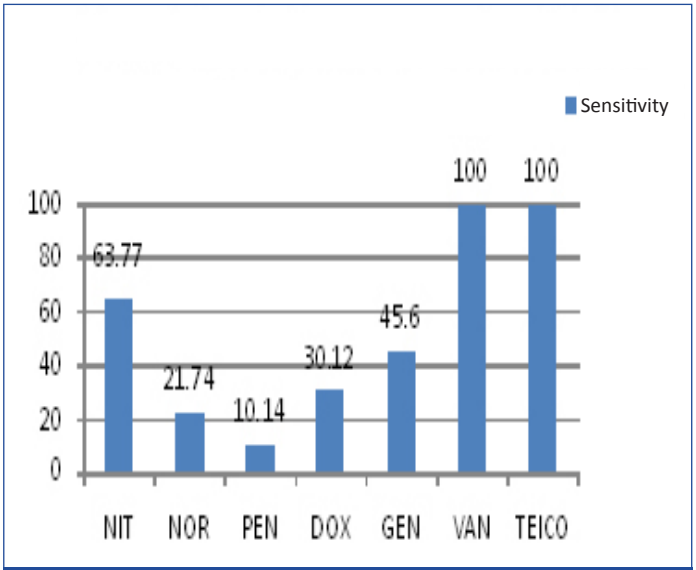
DISCUSSION

UTIs are one of the most common bacterial diseases in infants and children. Our hospital also caters to a significant number of children who present with UTIs, which could be estimated from 24.76% culture positivity which was seen in our study.

Frequency of UTIs in children varies according to age and sex. According to previous reports, [2,5,7] incidence of UTIs was highest in the first year of life. In the present study also, maximum infection (37.7%) was observed in the first year of life. A female preponderance with a male to female ratio of 1:10 has been reported in patients with UTIs, who were aged beyond 1-2 years [5,9]. In our study also, females were affected more after the first year of life.



[Table/Fig-3]: Sensitivity pattern of antibiotics in gram negative bacteria
[AMP= Ampicillin, AMK= Amikacin, NIT= Nitrofurantoin, GEN= Gentamicin, NOR= Norfloxacin, CTX= Cefotaxime, SXT= Co-trimoxazole, DOX= Doxycycline, IMP=Imipenem, CPM= Cefepime, OFX= Ofloxacin]



[Table/Fig-4]: Sensitivity Pattern of antibiotics in *Enterococcus spp*.
[NIT= Nitrofurantoin, NOR= Norfloxacin, PEN= Penicillin, DOX= Doxycycline, GEN=Gentamicin, VAN=Vancomycin, TEICO= Teicoplanin]

Gram negative organisms are the most commonly isolated organisms, with *Escherichia coli* (*E. coli*) accounting for 70 to 90% of infections [10]. In our study, *Escherichia coli* (45.12%), followed by *Klebsiella species* (18.71%) and *Enterococcus species* (9.23%), were isolated from most of the samples. Similar results were observed in other studies also [7,11,12].

There is a trend towards increasing resistance to the commonly used antimicrobial agents for the treatment of paediatric UTIs. In our study, antibiotic susceptibility which was seen against the gram negative uropathogens showed high resistance to ampicillin (98%), norfloxacin (96%) and co-trimoxazole (82.5%). They showed low resistance to gentamicin (17%), amikacin (23.5%) and nitrofurantoin (28.5%). These findings signified that ampicillin and co-trimoxazole which were commonly used for empirical therapy were now showing resistance in our region. This may be due to selection pressure of antibiotics. The studies from various regions, which were done during the past few years, also showed similar resistance patterns among uropathogens which caused paediatric UTIs [1,10-17].

Among gram positive organisms, *Enterococcus spp* (9.23%) were predominant isolates. All *Enterococcus spp*. strains were sensitive to vancomycin and teicoplanin (100%). A higher susceptibility was

seen against nitrofurantoin (63.77%). These results were consistent with those of Rudy M et al., [16] and Miskeen PA et al., [18] studies.

Thus, results of our study indicated that the presence of *Escherichia coli* as the aetiological agent and as a major uropathogen in children had not changed. But antibiotic susceptibility pattern had markedly changed. Drugs which were frequently used in the treatment of UTIs were now showing considerable resistance. This may have happened due to selection pressure of antibiotics. To avoid this situation, surveillance of antibiotic resistance patterns should be done periodically and an antibiotic policy should be made accordingly, for a better patient management.

CONCLUSION

This study concludes that *E.coli* is the predominant aetiological agent of UTIs which are seen in children. Ampicillin and co-trimoxazole which are commonly given as empirical treatment are now showing increased resistance. Due to low resistance to nitrofurantoin, amikacin and gentamicin, these may be considered as alternative drug therapies. Thus, trend of antibiotic susceptibility pattern should be detected periodically, to select the appropriate regimen for the treatment of UTIs.

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