Urinary Tract Infection among Children - A Study at a Tertiary Pediatric Care Hospital in Hyderabad, India

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A B S T R A C T

Urinary tract infection (UTI) is considered as the most common bacterial infectious disease seen among the pediatric patients. Pediatric urinary tract infections are associated with high morbidity and long term complications like renal scaring, hypertension and chronic renal failure The epidemiology of UTI during childhood varies by age, gender and other factors (Bickerton and Ducket, 1985). Most commonly bacteria causes of UTI in children are members of Enterobacteriaceae, particularly uropathogenic strains of E. coli and Klebsiella spp. which are the primary causative pathogens of UTI in the different part of the world (Gautam *et al.,* 2013). Susceptibility patterns of the bacterial isolates vary with geographic region and act as a reference for guiding the empirical therapy. The aetiology of paediatric UTI and the antibiotic susceptibility of urinary pathogens in both the community and hospitals have been changing, and drug resistance has become a major problem (Taneja *et al.,* 2010). With this background, the present study was conducted to observe the profile of the paediatric UTI, to find out the bacterial pathogens involved and their antimicrobial susceptibility pattern at a tertiary paediatric care centre in Hyderabad, Telangana. This retrospective study was undertaken for a period of one year in the department of Microbiology, Institute of child health, Niloufer hospital a tertiary care paediatric hospital in Hyderabad Telangana. A total of 262 cases aged between 2months to 14 years clinically suspected of UTI, were studied over a period of One Year. The rapid diagnostic test using urocolor 10 strips (Standard Diagnostics Inc.) was performed on all the samples of urine and was correlated with conventional culture. Urine cultures were carried out on blood agar, Mac conkey, CLED agar and Hichrome UTI agar plates and the isolates were identified by Gram staining and conventional biochemical methods. Antimicrobial susceptibility testing was performed by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI). Of the 262 children with suspected UTI included in this study, 100(38.16%) were found to be culture positive. Although urine culture is the standard criteria for diagnosis of UTI, it may take as long as 48 hours for culture to be positive. Hence the rapid diagnostic test using urocolor 10 strips (Standard Diagnostics Inc.) was performed on all the samples of urine and was correlated with conventional culture. Of the total 262 cases, 128 cases showed hematuria, 125 showed Leucocyte Esterase and 93 showed Nitrite test positive on the strip. Out of these the cultures were positive in 78 cases of hematuria, 97 cases of Leucocyte Esterase and 89 cases of Nitrite respectively The sensitivity of dipstick taken individually for hematuria, Leucocyte Esterase and Nitrite test were 60.93%, 77.6% and 95.69 % respectively. When all these parameters were taken combined the sensitivity was 78.07% and when taken for Leucocyte Esterase and Nitrite test was 86.65%. This confirms to the dipstick sensitivity of 85-90 %. In our study 87 samples showed growth with a single organism, while the total number of mixed infections were 13 (11%) out of which E.Coli + Enterococci were 4, Klebsiella + Enterococci 4, pseudomonas + candida 2 and E.Coli + CONS 3; An analyses of the results of present study reveals that amongst the various etiological agents isolated, Escherichia coli (55%) was found to be the most predominant organism in paediatric UTI,followed by klebsiella pneumoniae (26%)) The third in order of frequency was Enterococci which accounted for 10%.The other organisms isolated include Proteus mirabilis 2%, Pseudomonas aeriginosa 2%, Enterobacter 2%, Citrobacter 1% The present study reveals that most of the gram negative organism (GNB) were sensitive to Imipenem (95%), Amino glycosides 75%, Amoxyclav 60%, Piperacillin + Tazobactum 70% and 3rd generation cephalosporins 55%. The most resistant organism was pseudomonas which was sensitive only to Imipenam and piperacillin + Tazobactum. Pediatric urine culture isolates are becoming increasingly resistant to commonly used antibiotics. Finally, we suggest that empirical antibiotic selection should be based on knowledge of the local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal guidelines.

Introduction

UTI is one of the most common debilitating infections of childhood. Pediatric urinary infections are associated with high morbidity and long term complications like renal scaring, hypertension and chronic renal failure (Chon *et al.,* 2001). It is estimated that approximately 7% of children between 2 months to 2 years of age with undifferentiated febrile illness and 8% of children between 2-19 years of age presenting with possible urinary symptoms suffer from UTI. The reported incidence of UTI is 7% among girls and 2% among boys during the first 6 years of life (Edlin *et al.,* 2013). The epidemiology of UTI during childhood varies by age, gender and other factors (Bickerton and Ducket, 1985). The incidence is highest in the first yearr of life for all children (1%) but decreases substantially among boys after infancy. Under the age of 1 yr, the incidence of UTI in boys is higher than in girls; In the first year of life, especially in the first three months, UTI is seen more commonly in boys (3.7%) than in girls (2%). Thereafter, the UTI has been reported to be much more likely in girls (3%) than boys (1.1%) (Grabe *et al.,* 2013). Majority of UTI in children result due to ascending infection, although hematogeneous spread may be more common in the first year of life. Individual differences in susceptibility to UTI may be due to host factors such as production of urethral and cervical IgA antibodies as well as other factors that influence bacterial adherence to the introitus and the urethral epithelium. (Roberts, 1996; James-Ellison *et al.,* 1997). Children are at risk of developing UTI due to certain anatomic, physiologic factors and Congenital anomalies of the urinary tract among which vesicoureteric reflux (VUR) is the most common, leading to repeated infections and other complications like chronic pyelonephritis and eventual renal scarring which are components of reflux nephropathy, and other congenital anamolies like posterior urethral valve, ureteric duplex, etc., are also well known causes of UTI in children (Aggarwal and Verrier Jones, 1989)

In children most often urinary tract infection (UTI) manifests as fever of unknown origin. The clinical presentation of UTI in infants and young children can vary from occult and undiagnosed fever to gastrointestinal manifestations as well as upper and lower urinary tract symptoms, whereas in older children symptoms referring to the urinary tract may be observed (Grabe *et al.,* 2013). Hence, it is important to diagnose this condition at the appropriate time as it is a preventable cause of renal damage.

UTI is defined by the presence of organisms in the urinary tract, which is usually sterile. In the 1950's Kass studied adult women and established a threshold of $\geq 10^5$ cfu /ml in a voided specimen as the standard to define positive urine culture. Hobermann *et al.,* noted that high proportion (65%) of cultures with colony counts 10,000 to 49,000 grew mixed or Gram positive organisms suggestive of contamination. For boys and girls 10^4 and 10^5 cfu per ml of bacterial growth of a single type was taken as threshold (significant bacteriuria) respectively (Neelan Taneja *et al.,* 2010)

Bacteria are common causes of UTI in children with Escherichia coli being the most commonly isolated pathogen. The gold standard for diagnosis of UTI is growth of pathogenic bacteria in a urine culture Although urine culture is a gold standard for diagnosis of UTI, results are not available for 24-48 hrs. rapid technique to predict UTI include urine dipstick tests for lucocits sterase and nitrites and various forms of urinalysis including standard microscopy with a haemocytometer and Gram stain of uncentrifuged urine for organisms (Joseph, 2005).

Susceptibility patterns of the bacterial isolates vary with geographic region and act as a reference for guiding the empirical therapy. The aetiology of paediatric UTI and the antibiotic susceptibility of urinary pathogens in both the community and hospitals have been changing, and drug resistance has become a major problem (Taneja *et al.,* 2010). In recent years, widespread use of antibiotics has been resulted in an increasing incidence of antibiotic resistance among the urinary tract pathogens all over the world. Worldwide, emerging of antibiotic resistance is increasing among the urinary pathogens. (Kahlmeter, 2003; Runehagen and Kahlmeter, 2002) The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen. (Ashkenizi *et al.,* 1991; Marray, 1990; Alon *et al.,* 1987) Urinary tract infection (UTI) is the fourth most common reason for prescribing antibiotics, accounting for approximately 8% of all antibacterial prescriptions [Bickerton and Ducket, 1985]

With this background the present study was undertaken to determine the microbiological profile of UTI to find out the bacterial pathogens involved and their antimicrobial susceptibility pattern by traditional culture methods and also evaluation by rapid tests in early detection of pediatric UTI in children attending a tertiary care center - Institute of child health, Niloufer hospital, Hyderabad.

Materials and Methods

This cross-sectional study comprimising of 262 clinically suspected cases of UTI from the paediatric medicine and PICU and the paediatric surgical wards was undertaken in the department of Microbiology in collaboration with the departments of Pediatrics, at the institute of child health, Niloufer hospital a tertiary care centre for children in Hyderabad.

Inclusion Criteria

Children (2m -14years) attending Niloufer hospital suspected to have UTI with symptoms like burning micturition, dysuria, frequency, suprapubic pain, fever with or without chills, haematuria, flank pain

Exclusion criteria

Neonates below 2 month of age,

Informed written consent were obtained from their guardians/parents. Background information was collected from the guardian regarding the demographic details of the patient, previous incidence of UTI, family history of UTI/VUR, drug intake, previous interventions, and any other complaints.

Sample collection

The clean-catch mid-stream urine samples collected under strict aseptic conditions from patients were processed

Method of processing

Urine was processed soon after collection in order to minimize the multiplication of any contaminating organisms

The appereance and colour of urine was observed and noted as clear or turbid or blood tinged

Direct wet mount was done for presence of pus cells, apart from pus cells, urine was screened for epithelial cells, RBC, crystals, casts, bacteria, yeast cells which are of diagnostic significance.

Gram Stain of uncentrifuged urine sample was done for the presence of organism, though it is unreliable in detecting UTI when the count of the organism is low yet it is clinically

significant in pyelonephritis, invasive UTI and for the immediate management.

Dip stick analysis (LN strip)

Which does microscopic enumeration of neutrophils, reflects pyuria,and is an easy, inexpensive means to provide information as to the nature of infecting organism (bacteria or yeast), a guide to emperic therapy,and a method to estimate bacteriuria was done.

Urine culture

Urine culture is a gold standard for diagnosis of UTI. culture was done according to the standard microbiological procedures with a calibrated one microlitre loop using the semi-quantitative method of plating onto blood agar, Mac conkey, CLED agar and Hichrome UTI agar plates to isolate the uropathogens.

Identification and confirmation of isolates was done by the standard identification method

Identification of Organisms on Hichrome UTI agar

Colonies on Hichrome UTI agar gives presumptive diagnosis of organisms. It is often added as mixture of two chromogens, the chromogen serves as substrate i.e., cleaved by specific enzymes produced by bacteria. The resulting chromophores colours the colony that can be differentiated on the basis of different colour reactions

Escherichia coli – pink red colour colonies • Klebsiella pneumonia – blue mucoid coloni•Pseudomonas spp – colour less • Staphylococcus aureus – white • Enterococcus faecalis – small bluish green. Mueller-Hinton agar was used for susceptibility testing and Nutrient agar for preservation of organism. The antibiotic sensitivity testing was performed using the Kirby-Bauer method as

per the Clinical Laboratory Standards Institute (CLSI)

For gram negative isolates the following discs were used

Amoxycillin [AX] 20 ug, Gentamycin (G) 10μg, Amikacin (AK) 30μg, Ceftrioxone (CTX) 30 μg, Cefotoxime (CF) 30 μg, Ceftazidime (CPZ) 10μg, Ciprofloxacin [CIP] 5 μg, Norfloxacin [NR] 10, Pipercillin + Tazobactum [PT] 100 μg / 10μg, Imipenum [IM]10 μg

For Gram positive isolates

Amoxycillin [AX] 20 ug, Oxacillin[OX} 1ug, Gentamycin [G] 10ug, Amikacin [AK] 30ug, Amoxyclav [AC] 30 UG, Cefotaxime [CA] 30 ug, Ceftrioxone [CTX]30ug, Cotrimoxazole [CO]25 ug, Ciprofloxacin

Results and Discussion

Of the 262 children with suspected UTI included in this study,157were males and 105 were females. From among the male(157) specimens 62 and from the females (105) specimens, 38 were found to be culture positive thus a total of 100 (38.16%) were culture positive. The age and gender of children from whom the culture positive samples were collected are shown in table 4 Maximum number of cases was seen in age group less than 2 years, where males were 79 and females were 23 and majority of female culture positive cases were in age group 6 to 14 years

The major uropathogens isolated were Escherichia coli(55%), with majority in female than that of male followed by Klebsiella pneumoniae (26%). Enterococci spp. (10%). Proteus sp, Pseudomonas, Enterobacter, (02%), Citrobacter (01%) were the other bacteria that have been isolated With

regards to the sensitivity the present study reveals that most sensitive antibiotic was Imipenem (95%), followed by Amino glycosides 75%, Amoxyclav 60%, Piper + Tazo 70% and 3rd generation cephalosporins 55%.

In this study, the highest resistance rate was to Amoxycillin, followed by Cotrimoxazole

The most antibiotic resistant organism was pseudomonas which was sensitive only to Imipenam and piperacillin + Tazobactum

In vitro sensitivity testing in our study showed many strains of E.Coli were resistant to amoxycillin 90%, gentamycin 66%, cotrimoxazole 82% and ciprofloxacin 85%.

Klebsiella spp., the second common organism causing UTI, showed the highest sensitivity to Imipenam 92% and piperacillin + Tazobactum 70% and amikacin 76%. and the highest resistance to amoxycillin 88% and cotrimoxazole 76%

Enterococcus species was sensitive to Vancomycin (90%) and Aminoglycosides and Amoxiclav (60%). They were partly sensitive to Amoxicillin (45%) and floroquinolones (40%) and least to cotrimoxal (22%)

As per our study among 262 urine samples tested males were 157(59.92%) and females were 107(40.08%)

In our study, out of the total samples. 100 samples(38.16%)were only culture positive.

Other studies by (Malla *et al.,* 2008) (39.5%), (Taneja *et al.,* 2010) (28.3%), (Rai *et al.,* 2008) (28.6%) and (Sharmin *et al.,* 2009) (46.6%), showed similar results, while some studies showed lower positive rate than present study (1.39%), (Rai *et al.,* 2008), (7.3%) and (Godwin *et al.,* 2010), (Ranjbar *et al.,* 2009) (16.2%).Such variations might be due to inclusion of only those having complication of renal disease in higher culture cases and lower rate positive culture due to inclusion of all children of certain locality and variation on sample size

UTI is found to be the common problem in children and depends upon the age and sex of the children.

Occurrence of UTI among male children is more than female in first year of life and female after one year of age. Present study shows high rate of UTI among male children as compared to that of female

In the present study, maximum number of cases i.e. 103 cases were seen in age group < 2 years with culture positivity of 39.21%, followed by 2-5 years age group 88 cases with culture positivity of 36.36% and 6-14 years age group 72 cases with culture positivity of 38.88%.

Grag (1966) reported in the age group of 0-2 years (32.79%) cases, in the age group of 2-5 years (31.17%) of cases and in the age group of 5-12 years (35.48%) of cases.

Shiela Ethraj *et al.,* (1976) reported (47%) of cases in the age group of 0-2 years, (24%) in the age group of 2-5 years and in the age group of 5-12 years. (29%) of case. The above studies are almost similar to our study.

In the present study, increase incidence of urinary tract infection in the male patients is due to higher incidence of urinary tract infection associated with vesicle calculus leading to stasis of urine and allowing the micro-organisms to multiply in urine. In the neonatal age group there is male predominance of the condition with 2.2% of males and 0.3% of females being affected.

Table.1 Showing distribution of male & female cases

Total no. of cases	Males (percent)	Females (percent)
262	157(59.92%)	105(40.08%)

Table.2 Showing culture positivity among total cases

Total no. of cases	culture positive	percent
262	100	38.16%

Table.3 Culture positivity among male &female cases

Gender	Total no. of cases	Culture positive	percent
Males	157	62	39.49%
Females	105	38	36.19%

Table.4 Incidence of UTI with regards to Age and Sex

Age	Sex		culture positive		Percent	
	Male	**female**	**Male**	**female**	**Male**	**female**
	(157)	**(105)**	(62)	(38)		
< 2 years	79	23	34	06	43.03%	26.08%
2-5 years	50	38	20	12	40%	31.57%
6-14 years	28	44	08	20	**28.57%**	**45.45%**

Table.5 Showing Organism isolated, number and percent

Organism Isolated	*TOTAL STRAINS ISOLATED*	*PERCENT*
Escherchia coli	55	55%
Klebsiella pneumonia	26	26%
Enterococci	***10***	***10%***
Proteus	02	02%
Pseudomonas	02	02%
Enterobacter	02	02%
Citrobacter	01	01%
CONS	01	01%

Table.6 Antibiotic sensitivity pattern amoung different organisms

ANTIBIOTIC	*E.coli* [55] S R	*Kleb.* [26] S R	*Proteus* [2] S R	*Pseudomonas* [2] S R	*Enterobater* [2] S R	*Citrobacter* [1] S R	*Enterococcus* [10] S R	CoNS [1] S R
Amoxycillin	9 91	12 88	0 100	0 100	0 100	0 100	45 55	0 100
Gentamycin	34 66	70 30	50 50	50 50	50 50	0 100	58 42	60 40
AmiKacin	66 34	76 24	100 0	50 50	100 0	100 0	67 33	60 40
Amoxyclav	60 40	60 40	100 0	0 100	100 0	100 0	56 44	60 40
Norfloxacin	37 63	40 60	100 0	50 50	100 0	100 0	44 56	65 35
Ciprofloxacin	15 85	62 38	100 0	50 50	100 0	0 100	34 66	70 30
Cotrimoxazol.	18 82	24 76	0 100	50 50	0 100	0 100	22 78	30 70
Piper+tazo	67 33	70 30	100 0	100 0	100 0	100 0	---	--
Vancomycin	--	--	--	--	--	--	90 10	80 20
Cefataxime	52 48	54 46	50 50	50 50	50 50	0 100	---	---
Ceftriaxone	56 44	56 44	50 50	50 50	50 50	100 0	---	--
Imipenem	96 4	92 08	100 0	100 0	100 0	100 0	....	...

UTI among girls who are more than one year old is more possibly because of the structural anomalies. (Afsharpaiman *et al.,* 2012) Incidence in first 3 months among boys is more. In this study, the frequencies of positive cultures were more for males than for females. Farajnia *et al.,* reported this ratio1:2 (Farajnia *et al.,* 2009) and Farrell *et al.,* reported this ratio 1:4 (Farrell *et al.,* 2003). The cause of this needs more study.

E. coli (55%) was the commonest bacterial isolates of all positive cases followed by klebsiella spp (26%), Enterococci (10%), Proteus (02%), Pseudomonas (02%), Enterobacter (02%), Citrobacter (01%), CONS (01%),

The bacterial isolates in the present study are similar to that of studies by Seyed Reza Mirsoleyman *et al.,* (2014) who reported an incidence of 57.7 % of E.coli and 36.2% of Klebsiella spp, Gautam *et al.,* (2013) reported 40%of isolatres as E.coli, Rima H. Hanna-Wakim *et al.,* (2015) also reported an incidence of E.coli at 79%, Palak Gupta *et al.,* (2015) also reported 68.3 % of E.coli and 21.2% of Klebsiella spp, Tankhiwale *et al.,* (2004), Sharan *et al.,* (2013) also reported similar incidence

E. coli was the principal isolate showing high susceptibility to Imipenem (96%) followed by Piperacillin + Tazobactum (67%).

Many strains of E.Coli in our study showed resistance to Amoxicillin 90%, Gentamycin 66%, Cotrimoxazole 82% and Ciprofloxacin 85%.

Kumari *et al.,* (2005) reported Amikacin as the most sensitive followed by Gentamycin. Other studies by Malla *et al.,* (2008) from Pokhara, Das *et al.,* from western part of Nepal showed Amikacin was the most sensitive drug.

Klebsiella species was found to be the causative agent in about 26% of cases and was highly sensitive to Imipenam (92%)) and sensitive to Aminoglycosides (75%), and Amikacin (76%) cotrimoxazole (76%) piperacillin + Tazobactum(70%) and ciprofloxacin (62%). and resistant to Amoxycillin (88%)

Enterococcus species was sensitive to Vancomycin (90%) and Aminoglycosides and Amoxiclav (60%). They were partly sensitive to Amoxycillin (45%) and floroquinolones (40%) and least to cotrimoxazole (22%). %.

The most resistant organism was pseudomonas which was sensitive only to Imipenam and piperacillin + Tazobactum.

Proteus showed 100 % sensitivity to Amikacin, Gentamycin, Ciprofloxacin & and Norfloxacin. The present study reveals that most of the gram negative organism (GNB) were sensitive to Imipenem (95%), Amino glycosides (75%,) Amoxyclav() Piperacillin + Tazo bactum (70%), and 3rd generation cephalosporins 55 The most resistant drugs were Amoxicillin, followed by Cotrimoxazole. These significantly higher bacterial resistance rates to antibiotics in our country in comparison with other countries seem to be the result of higher rate of antibiotic usage by families even in the absence of a prescription

Seyed Reza Mirsoleymani *et al.,* (2014), reported E. coli as the leading uropathogen but the resistance pattern of this organism to antibiotics was very different, specimens were 52.2% and 56.2% resistant to Ceftriaxone and Cefotaxime, respectively, study by Farajnia *et al.,* (2009) showed resistance rate of 90.7% to Ampicillin, 51.8% to Cotrimoxazol, and 26.5% to Cefalexin, whereas the highest percentages of susceptibility were seen for Amikacin (96.6%) and Gentamicin (92.9%).

Sharifian *et al.,* (2012). reported the highest susceptibility percentage of E.coli to Ceftriaxone (97.8%) and Cefotaxime (95.2%) in 2006 in Tehran [Sharmin *et al.,* 2009]. Studies by Palak Gupta *et al.,* (2015) reported both E.coli and Klebsiella were sensitive to nitrofurantoin, amikacin, cefoperazone-sulbactam followed by Meropenem. In a study conducted in Khartoum, Ali and Osman (2009) reported that the mean susceptibility of all isolates was too high to Gentamicin (96%), Ciprofloxacin (94%), and Ceftriaxone (90%) whereas the lowest percentages of susceptibility were reported for Amoxicillin clavulanate (19%) and Ampicillin (14%). In a study conducted in the US hospitals, resistance among E. coli was highest for Trimethoprim sulfamethoxazole (24%) but lower for Nitrofurantoin (<1%) and Cephalothin (15%) (Edlin *et al.,* 2013). This study revealed a high E.coli resistance rate to antibiotics.

Variation in susceptibility percentage is probably because of the pattern of the sensitivity of microorganisms to antibiotics varies over time and between different geographical locations.

UTI is a significant problem in children and it is a major cause of high morbidity and mortality in developing world. UTI varies with age and gender and, therefore, extensive evaluation is required in boys under one year of age with UTI. Enteriobacteriaceae family was found to be the most common pathogen that causes UTI among the children. Pediatric urine culture isolates were becoming increasingly resistant to commonly used antibiotics over the last 10 years, regular monitoring in large scale is required at regular intervals in order to identify the reliable information about the resistance patterns of urinary pathogen. Long term studies on a large sample of children need to be planned to understand the disease process better in terms

of the interplay between the host and microbial factors. This study also highlights the better efficacy of Imipenem, Amino glycosides, Amoxyclav Piperacillin + Tazo bactum and third generation cephalosporins to Gram–negative uropathogens.

Finally it is suggested that empirical antibiotic selection should be based on the knowledge of local prevalence of bacteria and their sensitivities rather than the Universal guidelines.

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