Original Research Article

Prevalence, Microbial Profile and Antimicrobial Sensitivity Pattern of Uropathogens Isolated from Paediatric Patients

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A B S T R A C T

Urinary tract infection (UTI) is one of the common infections in paediatric patients. Emergence of antibiotic resistance in bacteria is an increasing problem worldwide. The study was conducted in the Department of Microbiology, Jawaharlal Nehru medical College and Hospital, AMU Aligarh (India) over one year period. A total 534 urine samples were collected and processed. 95(17.8%) yielded significant bacterial growth, 8(1.5%) *Candida*. Majority of the patients were in 1-5 year age group. *E. coli* was isolated in 61(64.2%) followed by *Citrobacter koseri* 9(9.5%), *Enterococcus faecalis* 7(7.4%), *Klebsiella pneumoniae* & *Staphylococcus aureus* 5(5.3%). Amikacin and nitrofurantoin was found to be the most effective antibiotic. Majority of 1(100%) MRSA strains were found in group III and HLAR strains in group I [2 (100%)] and group II [1 (100%)]. ESBL were detected in 0(0%), 8(23.5%) and 3(11.1%) strains while AmpC were detected in 14(87.5%), 25(73.5%) and 16(59.3%) strains in group I, group II and group III respectively. In uncomplicated UTI nitrofurantoin should be prescribed as empirical treatment and in complicated UTI, amikacin should be prescribed as first line treatment till bacterial culture and antibiotic reports are awaited, after checking the renal function test.

K e y w o r d s

UTI,
Sensitivity,
ESBL

Introduction

Urinary tract infection (UTI) is an infection of any part of the urinary tract and this includes: kidneys (pyelonephritis), bladder (cystitis) and urethra. Usually UTI in children occurs due to ascending infection but in the first year of life hematogenous spread may be more common (Gautam *et al.,* 2013). It is one of the common infections in children but difficult to diagnose because symptoms are non-specific, more so in young childrens (Pooja

and Garala, 2014; Muoneke *et al.,* 2012). Moreover an episode of UTI increases risk of reinfection (Williams and Craig, 2011). UTI is more common in boys during the first year of life and subsequently it is more common in females (Gautam *et al.,* 2013). World health organisation (WHO) reported that UTI is diagnosed in 1% of boys and 3-8% of girls (WHO, 2005). Indiscriminate use of antibiotics without knowledge of sensitivity pattern of causative organism,

results in emergence of resistant strains making treatment difficult thus increasing morbidity and occasionally mortality rate. Treatment for UTI should be based on antibiotic sensitivity result as increasing numbers of uropathogens are developing resistance to commonly used antibiotics. This study was done to determine the prevalence and antimicrobial sensitivity pattern in bacterial isolates causing UTI in paediatric patients with special emphasis on MRSA (Methicillin Resistance *Staphylococcus aureus*), HLAR (High Level Aminoglycosides Resistance), ESBL (Extended Spectrum Beta Lactamases), AmpC beta lactamase and MBL (Metallo-Beta-Lactamases).

Materials and Methods

Study design: The study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, AMU Aligarh, Uttar Pradesh, India over a period of 1 year (Oct 2013–Sep 2014). Patients below the age of 14 years were included in the study and classified into three age groups: less than 1 year (Group I), 1–5 year (Group II), and more than 5 year age (Group III).

Patients and sample collection: All children (<14 years age) with sign and symptoms suggestive of UTI (fever, dysuria, burning micturition, urgency, frequency haematuria and change in character of urine like smoky, foul smelling or yellow urine) were included in the study. Urine samples were collected from paediatric wards and outpatient department (OPD) in the sterile wide mouth container by finger tap method or suprapubic aspiration (in infants and neonates), midstream (in toilet trained children) and transurethral catheterisation (in hospitalised and catheterized patients) and send to the microbiology department for culture and sensitivity testing.

Direct microscopy: Wet mount examination was performed to look for the presence of pus cells, red blood cells, crystal or microorganisms.

Bacterial identification: All urine specimens were cultured on 5% sheep blood agar and MacConky agar by standard loop method (Collee *et al.,* 2006) and incubated at 37^OC for 24 hours. The urine culture was considered positive if single type of bacteria was isolated at a concentration of colony count >100,000 colony forming units (CFU)/mL and culture showing growth of more than two types of bacteria were considered contaminated. Identification of isolates was done by examination of each pure bacterial colony by Gram staining, microscopy and standard biochemicals (Collee *et al.,* 2006).

Antibiotic susceptibility testing: Antibiotic susceptibility testing was performed by Kirby-Bauer's disk diffusion method on Mueller-Hinton agar (Hi Media, Mumbai, India) as per the Clinical Laboratory Standards Institute guidelines (CLSI, 2014) using the commercially available antibiotic discs from HiMedia (Mumbai, India).

Antibiotics used for Gram negative bacilli: Amikacin (30μg), gentamicin (10μg), ceftriaxone (30μg), cefepime (30μg),, cefixime (5μg) cefoperazone (75μg), cefoperazone-sulbactum (75/75μg), ofloxacin (5μg), imipenem (10μg), piperacillin (100μg), piperacillin +tazobactum (100/10μg), ceftazidime (30μg), tobramycin (10μg) and nitrofurantoin (300μg).

Antibiotics used for Gram positive cocci: Among the Gram positive cocci the antibiotics tested for *Staphylococcal* species were amikacin (30μg), gentamicin (10μg), azithromycin (15μg), ofloxacin (5μg),

cefaclor (30μg), cefazolin (30μg), oxacillin (1μg), nitrofurantoin (300μg) and vancomycin (30μg). For *Enterococcus* species, the antibiotics tested were azithromycin (15μg), amoxicillin (30μg), gentamicin (10μg), erythromycin (15μg), high content gentamicin (120μg), high content streptomycin (300μg), nitrofurantoin (300μg) and vancomycin (30μg).

S. aureus ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* 25873 were used as control strains.

Detection of MRSA: MRSA was detected by oxacillin disc diffusion test by using oxacillin disc (1μg) (Fatima khan *et al.,* 2013).

Detection of HLAR: High content gentamicin (120μg) and high content streptomycin (300μg) disc were used for the detection of HLAR in *Enterococci*.

Detection of ESBL: Screening of possible ESBL production was done by using ceftriaxone (30μg) and cefoperazone (75μg). Those isolates with zone diameters less than 25 mm for ceftriaxone and less than 22 mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone+sulbactum. An increase in diameter of 5 mm was considered positive for ESBL detection.

Detection of inducible and derepressed AmpC beta lactamase: Isolates resistant to ceftriaxone, cefixime, cefoperazone and cefoperazone sulbactam were tested for AmpC production. Organism resistant to cefoperazone and cefoperazone combination were considered to be AmpC producers (Rizvi *et al.,* 2009).

Detection of Metallo-beta-lactamases: If the zone of Imipenem was reduced to 16–20 mm or less or heaping occurred, we tested the isolate for MBL production. Hodge test and Double Disc synergy test using EDTA were used for detection of MBL. The method was as described by *Lee et al.* (2001).

Result and Discussion

Urine is the commonest specimen send to the laboratory from OPD of a hospital as well as from admitted cases (Sohely Sharmin *et al.,* 2009). During the study period, total 534 urine samples were collected and processed, out of which 95(17.8%) demonstrated significant bacterial growth, 8(1.5%) had *Candida* infection and 81(15.2%) were contaminated and thus excluded from the study. Raghubanshi *et al.* (2014) also reported similar (18.5%) bacterial culture positivity but some studies showed higher culture positivity rate (32.14%) (Pooja Patel and Garala, 2014) and (57%) (Malla *et al.,* 2008).

Amongst the three age groups majority of the culture positive patients were in group II [41(43.2%)] followed by group III [34 (35.8%)] and group I [20(21.1%)]. Other studies by Singh and Madhup (2013) Pooja Patel and Garala, (2014) and Neelam Taneja *et al.* (2010) also reported that majority of UTI cases occur in the age group 1–5 year and reported 47.7%, 44.4% and 38.7% prevalence respectively.

Prevalence of UTI was more in male patients in group I, However females predominated in other pediatric age groups. However, overall the prevalence was more in female patients than male patients.

Table.1 Antimicrobial Sensitivity Pattern of Gram Negative Bacterial Isolates

Antibiotics	Group I		Group II		Group III	
	Enterobacteriaceae (n=16)	*P. aeruginosa* (n=1)	Enterobacteriaceae (n=34)	*P. aeruginosa* (n=1)	Enterobacteriaceae (n=27)	*P. aeruginosa* (n=1)
Amikacin	9(56.3%)	1(100%)	18(52.9%)	0	21(77.8%)	1(100%)
Gentamycin	5(31.3%)	-	9(26.5%)	-	12(44.4%)	-
Tobramycin	-	1(100%)	-	0	-	1(100%)
Ofloxacin	3(18.8%)	0	2(5.9%)	0	17(62.9%)	-
Cefepime	3(18.8%)	1(100%)	5(14.7%)	-	6(22.3%)	1(100%)
Cefoperazone	2(12.5%)	-	1(2.9%)	-	6(22.3%)	-
Cefoperazone+Sulbactam	2(12.5%)	-	9(26.5%)	-	9(33.3%)	-
Ceftazidime	-	1(100%)	-	0	-	1(100%)
Ceftriaxone	4(25%)	-	2(5.9%)	-	5(18.5%)	-
Cefixime	1(6.3%)	-	1(2.9%)	-	4(14.8%)	-
Piperacillin	-	0	-	0	-	1(100%)
Piperacillin+tazobactam	-	1(100%)	-	0	-	1(100%)
Nitrofurantoin	7(43.9%)	0	18(52.9%)	0	17(62.9%)	0

Table.2 Antimicrobial sensitivity pattern of Gram positive bacterial isolates

Antibiotics	Group I		Group II		Group III	
	S. aureus	*E. faecalis*	*S. aureus*	*E. faecalis*	*S. aureus*	*E. faecalis*
Amoxicillin	-	0(0%)	-	0(0%)	-	0(0%)
Ofloxacin	1(100%)	0(0%)	0(0%)	1(50%)	0(0%)	1(33.3%)
Azithromycin	1(100%)	0(0%)	1(33.3%)	0(0%)	0(0%)	0(0%)
Amikacin	1(100%)	-	3(100%)	-	1(100%)	-
Gentamycin	1(100%)	-	2(66.7%)	-	1(100%)	-
High content gentamicin	-	0(0%)	-	0(0%)	-	1(33.3%)
High content Streptomycin	-	0 (0%)	-	0(0%)	-	2(66.7%)
Cefaclor	1(100%)	-	2(66.7%)	-	1(100%)	-
Cefazolin	1(100%)	0(0%)	1(33.3%)	0(0%)	0(0%)	1(33.3%)
Oxacillin	1(100%)	-	2(66.7%)	-	0(0%)	-
Vancomycin	1(100%)	2(100%)	3(100%)	2(100%)	1(100%)	2(66.7%)
Nitrofurantoin	1(100%)	1(50%)	3(100%)	2(100%)	1(100%)	1(33.3%)

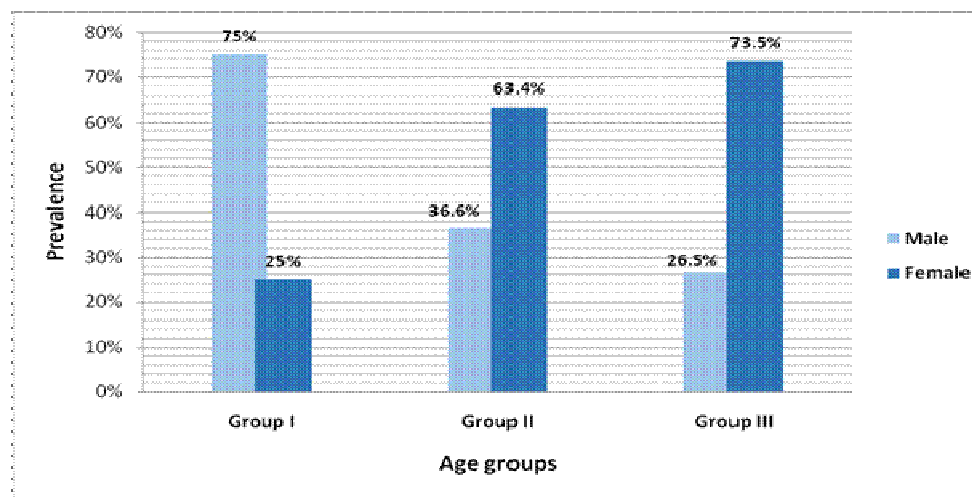**Fig.1** Gender wise distribution of cases

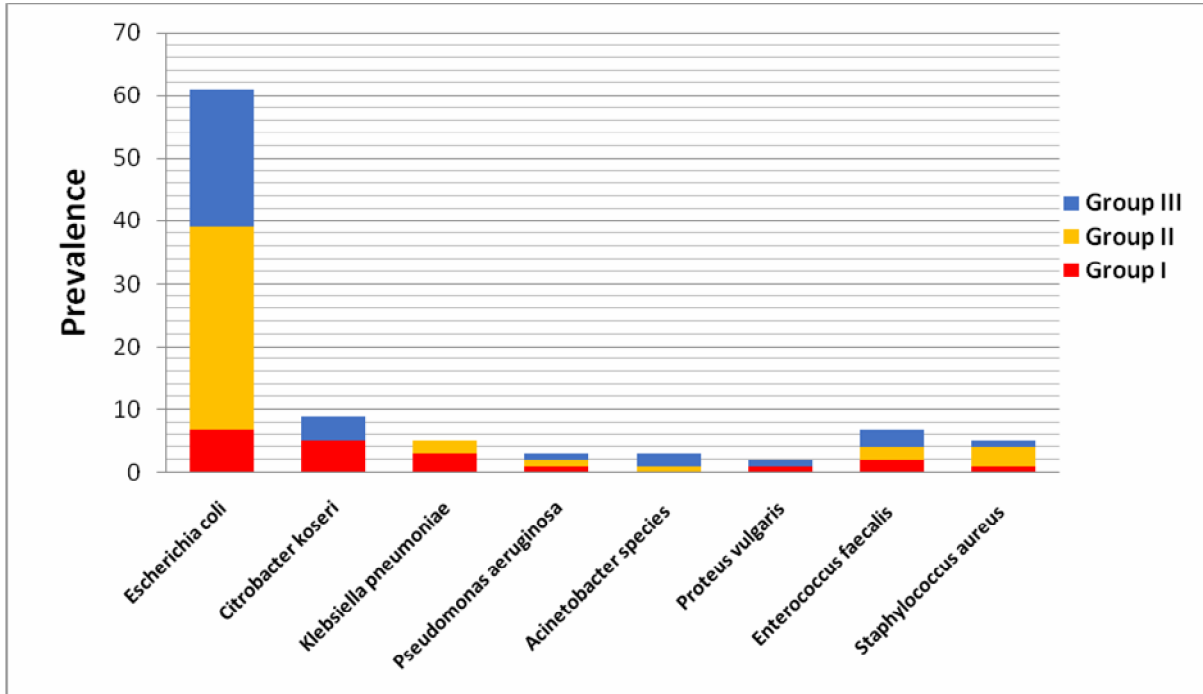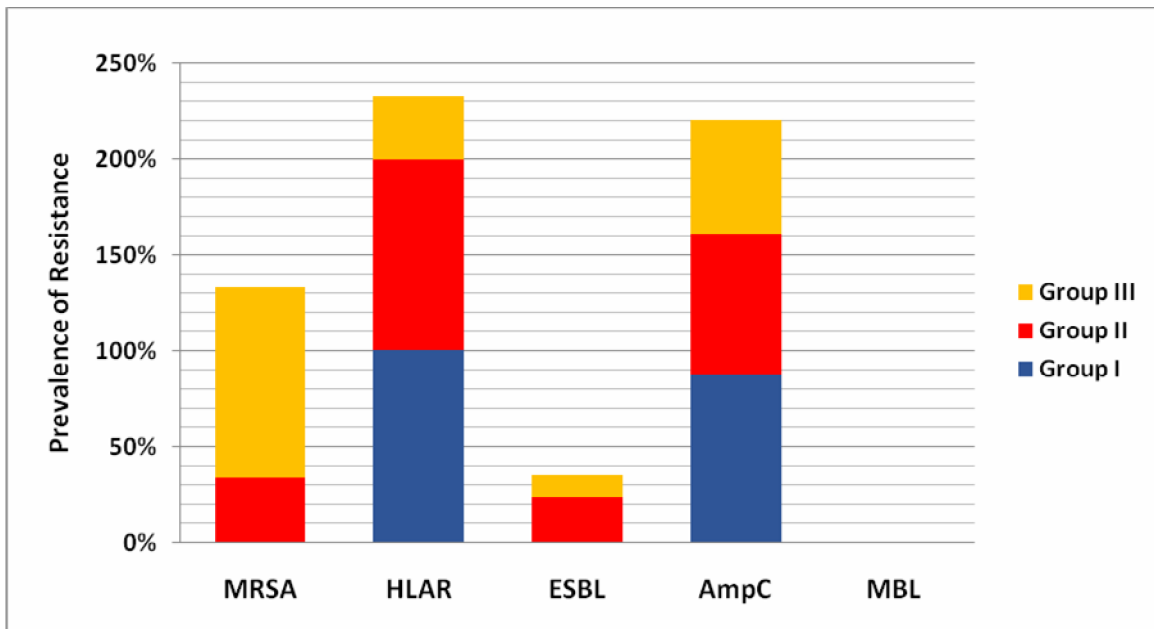**Figure.2** Age wise distribution of uropathogens

Figure.3 Distribution of different resistance pattern in uropathogens

Similar gender wise prevalence were reported by Sohely Sharmin *et al.* (2009) and Shahla Afsharpaiman *et al.* (2012) Females are more prone because their urethra is shorter and closer to the anus. Some studies however showed contrasting result (Pooja Patel and Garala, 2014; Neelam Taneja *et al.,* 2010).

Majority of uropathogens isolated were Gram negative bacilli 83(87.4%) followed by Gram positive cocci 12(12.6%). Amongst which members of enterobacteriaceae family predominated (81.1%), which is consistent with another study (Gautam *et al.,* 2013).

Overall the most common uropathogen isolated were *Escherichia coli* 61 (64.2%) followed by *Citrobacter koseri* 9 (9.5%), *Enterococcus faecalis* 7 (7.4%), *Klebsiella pneumoniae* and *Staphylococcus aureus* 5 (5.3%), *Pseudomonas aeruginosa* 3 (3.2%), *Acinetobacter* species 3 (3.2%) and *Proteus vulgaris* 2 (2.1%). Although *E. coli* was the predominant pathogen, prevalence of other isolates was different in the three age groups (Figure 2).

E. coli as in other studies was the most common isolate in all age groups. Raghubanshi *et al.* (2014) (64.66%), Alia and Osman (2009) (60.0%) and Shahla Afsharpaiman *et al.* (2012) (71.7%) also reported *E. coli* the most common isolate.

Citrobacter koseri was the second most common isolate in group I and group III while *S. aureus* was the second most common isolate in group II.

Antimicrobial sensitivity testing of Gram negative isolates showed, amongst aminoglycosides, amikacin was most effective antibiotic. Nitrofurantoin was the second most effective antibiotics in all the age groups with 40% sensitivity against all

the isolates. Cefixime was found to be least effective drug in all three age groups. Sensitivity of cephalosporins ranged from 2.9%–33.3% (Table 1). Flouroquinolones showed great variation in sensitivity in different age groups. In group I, group II and group III it showed 18.8%, 5.9%, and 62.9% sensitivity respectively. The sensitivity of ofloxacin was equal to nitrofurantoin in group III.

For gram positive isolates vancomycin, amikacin and nitrofurantoin were the most effective drugs (Table 2)

MRSA was detected in 1(33.3%) cases in group II and 1(100%) in group III. MRSA was not detected in infants. HLAR were detected in 2 (100%) cases in infants, 1 (100%) in group II and 1 (33.3%) in group III (Figure 3).

Amongst enterobacteriaceae isolates ESBL were detected in 0 (0%), 8 (23.5%) and 3 (11.1%) strains in group I, group II and group III respectively while AmpC were detected in far large numbers: 14 (87.5%), 25 (73.5%) and 16 (59.3%) in the three groups respectively. The higher rates of AmpC in infants could be due to higher incidence of instrumentation or other intervention done in the hospital and may represent nosocomial infection. MBL producers were not detected in any group.

Nitrofurantoin was found to be effective antibiotic in all MRSA strains while in AmpC producers it was effective in 7 (50%) and 13 (52%) strains in group I and group II respectively and slightly low 6 (37.5%) in group III.

Members of enteriobacteriaceae family were found to be the most common pathogens causing UTI in pediatric patients. Amikacin and nitrofurantoin were found to be most

active drugs for uropathogens. In uncomplicated UTI nitrofurantoin should be prescribed as first line treatment. Ofloxacin can also be prescribed in place of nitrofurantoin in more than 5 year age group. In complicated UTI, amikacin should be prescribed as first line treatment till bacterial culture and antibiotic reports are awaited, after checking the renal function test.

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