Antibiotic Sensitivity Pattern and Plasmid Profile Of Urinary Tract Infection Isolates among Children Below 10 Years Of Age

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ABSTRACT

Urinary tract infection (UTI) is defined as colonization of pathogen anywhere along the urinary tract. UTI has been classified by site of infection as Upper urinary tract infection and lower urinary tract infection and by severity as Complicated and uncomplicated UTI. This community based cross sectional study was conducted to determine the Antibiotic sensitivity pattern and plasmid profile of most prevalent urinary tract infection isolates among children below 10 years of age, from December 2013 to February 2014. Among the total 800 sample collected from the community 390(48.8%) were female and 410(51.2%) were male. The prevalence rate was found to be 44(5.5%) of total cases and was statistically significant ($p<0.05$). Among significant growth, 15(1.9%) and 29(3.6%) were male and female respectively ($p<0.05$). Out of 44 total isolates frequency of Escherichia coli (E. coli) was 20 followed by Staphylococcus aureus(12), which accounted for 45.5% and 27.3% respectively. Remaining were Pseudomonas(11.4%), Klebsiella(11.4%), Proteus(2.3%)and Citrobacter sps.(2.3%). Tobramycin(100%) and Amikacin(97.73%) were found to be the most sensitive antibiotics followed by Chloramphenicol(93.18%), Imipenem(90.91%) and Ciprofloxacin(75%) respectively.Out of 20 E. coli isolates, no plasmid was seen in 7(35%) while 8(40%) showed single plasmid which was present in 8 isolates. Plasmid copy number of 2, 3 and 4 were displayed by 2(10%), 2(10%) and 1(5%) of the isolates respectively. A common (>21 kilobases) plasmid was the most common among isolates under study. This study revealed that E. coli was the most prevalent organism causing community acquired pediatric UTI. Antibiotics that are commonly used for the management of UTI and other cases are being more resistant i.e., Ampicillin. Plasmid analysis showed the presence of plasmids in resistant E. coli isolates that might harbor resistant genes. So that further analysis is required for the detection of responsible genes.

Key words: *Antibiotic sensitivity pattern, Plasmid profile, Urinary tract infection*

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INTRODUCTION

Urinary tract infection (UTI) is defined as colonization of pathogen anywhere along the urinary tract. In children more simpler and practical approach of categorization of UTI as a first time infection versus recurrent infection. Recurrent infections can be further subdivided into unresolved bacteriuria, bacterial persistence, and re-infection. The recurrence of a UTI may be caused by several reasons. Unresolved bacteriuria is most commonly caused by inadequate antimicrobial therapy. Sub-therapeutic levels of the antimicrobial agents may be a result of noncompliance, mal-absorption, suboptimal drug metabolism, and resistant uropathogens unresponsive to attempted therapy.[1] In these cases, infection typically resolves after altering the therapy according to antimicrobial sensitivities determined by a proper urine culture. In the case of bacterial persistence, the nidus of infection in the urinary tract is not eradicated. Characteristically, the same pathogen is documented on urine cultures during subsequent episodes of UTI despite negative cultures after treatment. Re-infection is characterized by different pathogens documented on proper urine cultures with each new UTI, sometimes with same microorganism, which may have persisted in the vagina or feces create a new infection.

The true incidence of pediatric UTI is difficult to determine because there are varying presentations that range from an absence of specific urinary complaints to fulminant urosepsis. Urinary tract infection (UTI) occurs in approximately 8% of girls and 2% of boys by 7 years of age. The frequency of symptomatic UTI during the first 10 years of life is 3.0% in girls and 1.1% in boys.[2]

Microbial etiology of UTIs has been regarded as well as established, with *E. coli* being the causative pathogen in 50–80% of cases and other Enterobacteriaceae (*Klebsiella, Proteus, Enterobacter*) together with Enterococci, Streptococci, Staphylococci, and *Pseudomonas* sps account for most of the remaining positive urine cultures of children.[3] Children who have UTI do not necessarily present with the characteristic signs and symptoms although various clinical presentation includes fever, flank pain, dysuria, oligouria,

abdominal discomfort, anorexia, nausea and vomiting.

Female gender, social concerns, Infants, foreskin**,** fecal and perineal colonization**,** Urinary tract anomalies**,** Functional abnormalities, immune-compromised state, Sexual activity are regarded as the risk factors for urinary tract infection in children.

Bacteria enter into the urinary tract by the fecal-perineal-urethral route with subsequent attachment, colonization and ascend to the bladder and once the uropathogens reaches the bladder, it may ascend to the ureters and then to the kidneys. Additional pathways of infection include, hematogenous seeding in the setting of systemic infection, lymphatic route and direct extension caused by the presence of fistulae from the bowel or vagina.

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo***.** Commonly used methods of AST include diffusion, dilution and E-test. Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. It is one of the major causes of failure in the treatment of infectious diseases that results in increased morbidity, mortality, and costs of health care. Factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion.[4]

Plasmids are defined as double stranded, extra-chromosomal genetic elements that replicate independently of the host cell chromosome and are stably inherited that may encode products(protein) that aid in virulence, pathogenicity, and the spread of resistance among a wide spectrum of bacteria. Plasmids differ from chromosomes in being small and coding for genes that are non-essential for the bacterial survival. Absence of plasmids doesn't kill bacterium, but their presence provides additional benefits to the bacterial cell.

Urinary tract infection is the common and life threatening clinical condition which in case of community acquired infection usually remains undiagnosed. Untimely diagnosis and improper use of antibiotics for treatment of UTI results in re-current infection with subsequent resistant to the antibiotics commonly used in clinical practice. Antibiotic sensitivity of commonly isolated uropathogens and subsequent plasmid profile may correlate with the resistant pattern that helps for the detection of resistant genes and further drug discovery.

MATERIAL AND METHODS

A community based cross sectional descriptive study was carried out at School of Health and Allied Sciences, Pokhara University, Lekhnath-12, Kaski from December 2013 to February 2014 and plasmid profiling was done at Kantipur College of Medical Sciences, Sitapaila, Kathmandu, Nepal. Sample was collected from various schools of Kaski and Tanahun district namely Shree Brahma Rupa Primary School, Shree Gaun Farka Primary School, Shree Himalayan Primary School, Shree Chandra Jyoti Primary School, Shree Bishow Shanti Primary School, Kaski, and Shree Pancha Jyoti primary School, Shree Basanta Kali Primary School, Tanahun. Eight hundred Clean catch Mid-Stream Urine (CCMSU) sample (at least 5ml) was collected in sterile urine culture container from children below 10 years of age. Appropriate collection instructions were given at site and oral expressed consent was taken from the subjects. Sample was transported in ice pack and processed for isolation, identification and antimicrobial susceptibility testing using standard methodology and Plasmid isolation from Alkali lysis method.[5]

RESULTS

Table: 1. Growth pattern among gender

Growth	Frequency	Female	Male	Percent (%)
Insignificant	756	361(45.1%)	395(49.4%)	94.5%
Significant	44	29(3.6%)	15(1.9%)	5.5%
Total	800	390(48.8%)	410(51.2%)	100.0%

Out of 800 urine sample collected from the community 756(94.5%) showed insignificant growth. Overall prevalence rate was found to be 5.5% i.e. 44 out of total cases and was statistically significant (p=0.000). Among which 15(1.9%) and 29(3.6%) were male and female respectively with male: female ratio of 1:1.93(p=0.019).

Table: 2. Overall distribution of AST pattern in different uropathogens

Organisms N=44	*E. coli* N=20(45.5%)			*Proteus* sps. N=1(2.3%)			*Pseudomonas* sps. N=5(11.4%)			*Citrobacter* sps. N=1 (2.3%)			*Klebsiella* sps. N=5(11.4%)			*S. aureus* N=12(27.3%)		
Antibiotics	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	0	0	20	0	0	1	0	0	5	0	0	1	0	0	5	0	0	12
Amikacin	20	0	0	1	0	0	5	0	0	1	0	0	4	1	0	12	0	0
Ciprofloxacin	13	0	7	1	0	0	4	1	0	1	0	0	4	1	0	10	1	1
Chloramphenicol	20	0	0	1	0	0	2	1	2	1	0	0	5	0	0	12	0	0
Imipenem	16	3	1	1	0	0	5	0	0	1	0	0	5	0	0	12	0	0
Nitrofuratoin	17	3	0	0	0	1	0	1	4	1	0	0	1	2	2	12	0	0
Nalidixic acid	8	1	11	0	0	1	0	2	3	0	0	1	3	2	0	0	0	12
Vancomycin	0	0	20	0	0	1	0	0	5	0	0	1	0	0	5	8	4	0
Tobramycin	20	0	0	1	0	0	5	0	0	0	1	0	5	0	0	12	0	0
Cefotaxime	0	6	14	0	0	1	0	0	5	0	1	0	1	3	1	0	2	10
Ceftazidime	0	0	20	0	0	1	0	0	5	0	0	1	0	0	5	0	0	12
Erythromycin	0	5	15	1	0	0	1	3	1	0	1	0	0	3	2	9	3	0
Cotrimoxazole	14	0	6	0	0	1	0	2	3	1	0	0	3	0	2	8	2	2

(Note: – S=sensitive, I=intermediate, R=resistant)

Among 44 total isolates frequency of *E. coli* was 20 followed by *S. aureus* (12), which accounted for 45.5% and 27.3% respectively. Remaining were *Pseudomonas, Klebsiella, Proteus* and *Citrobacter* sps. Most sensitive drugs were Tobramycin (100%), Amikacin (97.73%), Chloramphenicol (93.18%) and Imepenem (90.91%) while most resistant drugs were Ampicillin (100%), Ceftazidime (100%), Vancomycin (72.73%) and cefotaxime (70.45%).

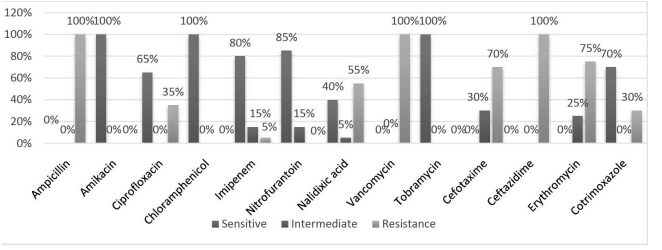


Figure: 1 Antibiotic Sensitivity pattern of *E. coli*

All *E. coli* isolates (20) were 100% sensitive to Amikacin, Chloramphenicol and Tobramycin followed by Nitrofurantoin (85%), Imepenem (80%) and Cotrimoxazole (70%). Ampicillin, Vancomycin, and Ceftazidime were 100% resistant followed by Erythromycin (75%), Cefotaxime (70%) and Nalidixic acid (55%).

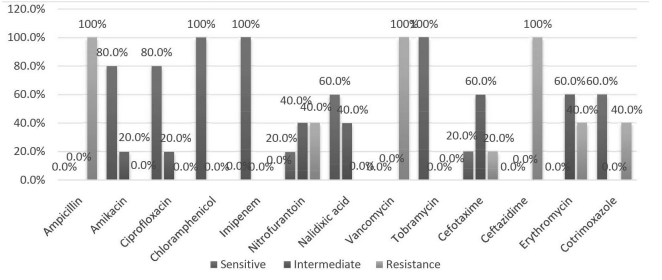


Figure: 2 Antibiotic sensitivity pattern of *Klebsiella* sps.

All *Klebsiella* sps. isolates were 100% sensitive to Chloramphenicol, Imepenem and Tobramycin followed by Amikacin (80%) and Ciprofloxacin (80%). Ampicillin, Vancomycin and Ceftazidime were 100% resistant followed by Cotrimoxazole (40%), Erythromycin (40%) and Nitrofurantoin (40%).

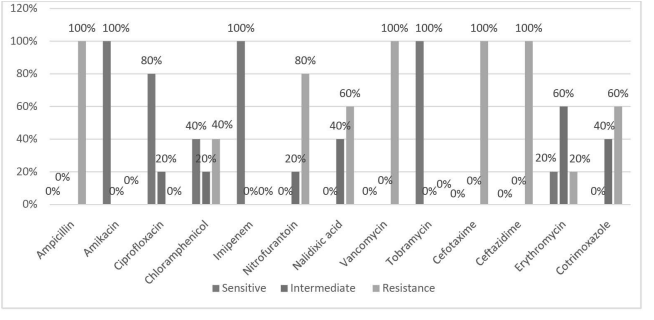


Figure: 3 Antibiotic Sensitivity patterns of *Pseudomonas* sps.

All *Pseudomonas* sps. isolates were 100% sensitive to Amikacin, Imepenem and Tobramycin followed by Ciprofloxacin (80%). Ampicillin, Vancomycin, Ceftazidime and Cefotaxime were 100% resistant followed by Nitrofurantoin (80%), Nalidixic acid (60%) and Cotrimoxazole (60%).

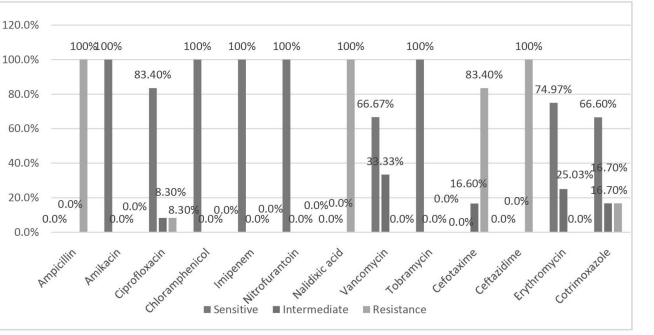


Figure: 4 Antibiotic Sensitivity pattern of *S. aureus.*

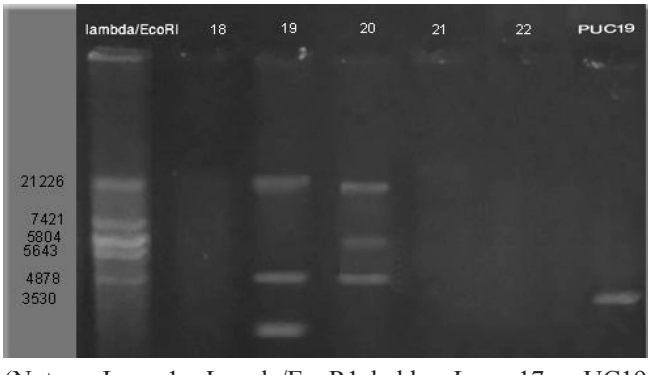
All *S. aureus* isolates were 100% sensitive to Amikacin, Chloramphenicol, Imepenem, Nitrofurantoin and Tobramycin followed by Ciprofloxacin (83.4%), Erythromycin (74.97%) and Vancomycin (66.67%). Ampicillin, Nalidixic acid and Ceftazidime were 100% resistant followed by Cefotaxime (83.4%).

Table 3: Plasmid copy number and prevalence among total *E. coli* isolates (N=20).

Plasmid copy number	No. of isolates (N=20)	prevalence
0	**7**	**35%**
1	**8**	**40%**
2	**2**	**10%**
3	**2**	**10%**
4	**1**	**5%**

Among the total *E. coli* isolates, no plasmid was seen in 7(35%) while 40% showed single plasmid which was present in 8 isolates. Plasmid copy number of 2, 3 and 4 were displayed by 2(10%), 2(10%) and 1(5%) of the isolates respectively.

Electrophoretic pattern of plasmid DNA of *E. coli* isolates Lane 1 Lamda/EcoR1



(Note: - Lane 1= Lamda/EcoR1 ladder, Lane 17= pUC19

control DNA (2.6 kb), lane 2-22 *E. coli* plasmid DNA).

Plasmid copy number of 0 to 4 were seen among *E. coli* isolates with different molecular size (0 to >21 kb).All *E. coli* having single copy number showed >21kb plasmid that were resistant to 4-6 antibiotics used.

Table: 4 Relation of AST with Plasmid profile of *E. coli* isolates

Resistant drugs	Lane no.	Plasmid copy number	Molecular weight(MW)
AMP,VA,NA,CAZ,E	2	1	>21kb
AMP,VA,CTX,CAZ,E	3	1	>21kb
AMP, IPM,CIP,VA,NA, TX,CAZ,E ,CO	4	4	>21kb,18kb, 6.5kb,5.8kb
AMP,NA,VA,CTX,CAZ,E	5	1	>21kb
AMP,VA,CAZ	6	0	
AMP,VA,CAZ,E	7	0	
AMP,VA,NA,CAZ,E	8	1	>21kb
AMP,NA,VA,CAZ,E,CTX,CO	9	2	>21kb,>21kb
AMP,VA,CAZ,CTX,E	10	0	
AMP,NA,VA,CAZ,E,CTX,CO	11	2	>21kb,14.5kb
AMP,VA,CTX,CAZ	12	1	>21kb
AMP,VA,CTX,CAZ,CO,E	13	1	>21kb
AMP,VA,CAZ,CTX,E	14	1	>21kb
AMP,CIP,NA,VA,CAZ,CTX	15	0	
AMP,CIP,NA,VA,CAZ,CTX,E	16	1	>21kb
AMP,CIP,VA,CAZ,CTX	18	0	
AMP,CIP,NA,VA,CTX,CAZ,E,CO	19	3	>21kb,4.8kb,<2.6kb
AMP,CIP,NA,VA,CTX,CAZ,E,CO	20	3	17.5kb,5.4kb,<4.8kb
AMP,CIP,NA,VA,CAZ	21	0	
AMP,VA,CAZ,E	22	0	

Note :-AMP(Ampicillin), CTX(Cefotaxime), CAZ(Ceftazidime), CIP(Ciprofloxacin), CO(Co-trimoxazole), E(Erythromycin), IPM(Imepenem), NA(Nalidixic acid), NIT(Nitrofurantoin) and VA(Vancomycin).

DISCUSSION

It was a community based cross sectional descriptive study. Sample was collected from various schools of Kaski and Tanahun district. Overall prevalence rate was found to be (5.5%) 44 out of total cases (800) and was statistically significant ($p<0.05$). Male: female ratio of 1:1.93($p<0.05$). Female cases were high as compared to male cases because short and close proximity of anus to urethra, relatively high moisture and socio-economic concerns determines the outcome of infection.

In this study prevalence of *E. coli* was higher (45.5%) as compared to other isolates from community acquired UTI. Ghadage *et al.,* also showed that *E. coli* was present in 45.12% of total isolates.[6] Similar research done by Gautam *et al.,* at Gandaki Medical College Teaching Hospital, Pokhara, Nepal showed slightly lower (39.4%) percentage of *E. coli*.[7] Overall sensitivity pattern against all isolates were Tobramycin (100%), Amikacin (97.73%), Chloramphenicol (93.18%) and Imepenem (90.91%) while most resistant were Ampicillin (100%), Ceftazidime (100%), Vancomycin (72.73%) and cefotaxime (70.45%). Antibiotic sensitivity pattern is different and changing among local population, nationally and globally. Previous results from Paudel *et al.,* have shown 94% sensitivity to Amikacin.[8] In the study carried out by Forouzan *et al.,* Ampicillin resistance rate was more than 90%.[9]

All *E. coli* isolates (20) were 100% sensitive to Amikacin, Chloramphenicol and Tobramycin followed by Nitrofurantoin (85%), Imepenem (80%) and Cotrimoxazole (70%). Ampicillin, Vancomycin, and Ceftazidime were 100% resistant followed by Erythromycin (75%), Cefotaxime (70%) and Nalidixic acid (55%). Increased resistance to third generation cephalosporins might be due to ESBL production. Acharya *et al.,* also reported 100% ampicillin resistance to uropathogenic *E. coli* from Bharatpur, Nepal.[10]

Antibiotics that were effective against *Klebsiella* sps in our study were Chloramphenicol, Tobramycin, Imepenem and Amikacin supported by previous study results.[11] However Cotrimoxazole and Nitrofurantoin showed moderate levels of resistance same as for *Pseudomonas* except for Nitrofurantoin which was higher (80%).

All *S. aureus* isolates were 100% sensitive to Amikacin, Chloramphenicol, Imepenem, Nitrofurantoin and Tobramycin followed by Ciprofloxacin (83.4%) whereas Vancomycin was 66.67% sensitive (33.33% were intermediate). Paudel *et al.,* also showed 100% sensitivity to Amikacin followed by Ciprofloxacin (96%).[8]

Among the total *E. coli* isolates, no plasmid was seen in 7(35%) while plasmids were present in 13(65%). Single plasmid was present in 8(40%) isolates while plasmid copy number of 2, 3 and 4 were displayed by 2(10%), 2(10%) and 1(5%) of the isolates respectively. Plasmid copy number of 0 to 4 were seen among *E. coli* isolates with different molecular size approximately (0 to >21 kb). All *E. coli* having single copy number showed >21kb plasmid which was the most prevalent one, that were resistant to 4-6 antibiotics used. In a study carried out by khadgi *et al.,* a band of approximately 23 kb was seen in most of the *E. coli* isolates.[12] In our study strains showing 2 copy number were resistant to same class of antibiotics but different plasmid profile was seen among which one strain showed two >21 kb plasmids while >21kb and 14.5kb plasmids were seen in another strain. Isolates showing 3 plasmid copy number were resistant to same eight antibiotics used but different profiles were observed. For isolate in which 4 plasmid copy number was present, >21kb, 18kb, 6.5kb and 5.8kb plasmids were seen which was resistant 9 antibiotics under study. According to Sadeghi *et al.,* on their study "Plasmid profile of *E. coli* strain isolated

from UTI of inpatient and outpatient". Ten strains (10%) lacked any plasmid and in 90 strains (90%), 1-7 plasmids were detected. Although some strains contained plasmids of >21kb molecular mass, but mainly isolated plasmids ranged from 0.9 kb to 21 kb.[13]

Our study suggests that *E. coli* is the predominant cause of community acquired pediatric UTI.

Antibiotics that are commonly used for the management of UTI cases are being less effective i.e., Ampicillin. Plasmid analysis showed the presence of plasmids in resistant *E. coli* isolates that might harbor resistant genes. So that further analysis of R-plasmid is required for the detection of responsible genes.

ACKNOWLEDGEMENT

We would like to acknowledge to the School of Health and Allied Sciences for his management and laboratory support during the research period. We would like to show our greatest appreciation to family of Shree Brahma Rupa Primary School, Shree Gaun Farka Primary School, Shree Himalayan Primary School, Lekhnath-13-TalloGagangauda, Shree Chandra Jyoti Primary School, lekhnath-15, Dhodbesi, Shree Bishow Shanti Primary School, Kaski and Shree Pancha Jyoti primary School, Shree Basanta Kali Primary School, Tanahun, for help and co-operation in sample collection process. We are thankful to Kantipur College of Medical Sciences, Kathmandu for co-operation and technical guidance during plasmid profiling.

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