Biomedical & Pharmacology Journal Vol. **9**(1), 377-383 (2016)

Pathogens Causing Urinary Tract Infection in Children and their *in vitro* Susceptibility to Antimicrobial Agents - A Hospital Based Study

BALAJI CHINNASAMI [1*], SUBHA SUNDARAMOORTHY [2$], KANIMOZHI SADASIVAM[3#] and SEKAR PASUPATHY[4*]

[1*]Associate Professor, [4*]Head of Department, Department of Pediatrics, [2$]Associate Professor of Microbiology, [3#]Associate Professor of Physiology, SRM Medical College, Hospital & RC, Potheri, Kattankulathur, India.

*Corresponding author E-mail: balajictriumphants@gmail.com

http://dx.doi.org/10.13005/bpj/949

(Received: February 01, 2016; accepted: March 14, 2016)

ABSTRACT

To identify common microbes causing urinary tract infection in pediatric population and their resistance pattern to commonly used antibiotics in a south Indian hospital. We retrospectively collected urine culture and sensitivity data of children admitted with suspected urinary tract infection from January 2015 to December 2015. Antimicrobial susceptibility testing was performed by antibiotic disc diffusion method and ESBL production confirmed by Double disk synergy test. About 130 were culture positive with E.coli being the predominant microbe grown (40.8%) and ESBL production was high to the tune of 83% in them. Most Gram negative bacteria were sensitive to aminoglycosides and carbapenams and Gram positive bacteria were susceptible to Vancomycin and Linezolid. ESBL mpositive *E.coli* was resistant to many drugs including Penicillins, Cephalosporins and Quinolones. Nitrofurantoin elicited good invitro response to ESBL positive *E.coli, Enterococcus* and Coagulase Negative Staphylococcus. Our study shows that E.coli is the most common cause of urinary tract infection and since 80% are ESBL producers they are multidrug resistant. Oral Nitrofurantoin is a good choice for uncomplicated urinary tract infection. Parenteral aminoglycosides, Piperacillin/Tazobactum, Cefoperazone/Sulbactum can be recommended for complicated UTI reserving carbapenams for those cases who don't respond to initial theraphy.

Key words: Antibiotic resistance, ESBL, *E.coli, Klebsiella,* Proteus, nitrofurantoin

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections in children associated with significant complications like scarring of kidney, hypertension, and chronic kidney disease[1]. Gram-negative bacilli are the most common cause of pediatric UTI especially *Escherichia coli* responsible for more than 90% of uncomplicated UTI[2]. Enterococcus, Klebsiella and Coagulase negative Staphylococcus aureus (CoNS) are other organisms sharing remaining burden of UTI. Antibiotics have been the mainstay of treatment of UTI. Antibiotic resistance has become a major global problem especially in India where 80% of *E.coli* is resistant to penicillins, cephalosporins and quinolones (http://www.cddep.org/resistancemap/). Treatment of UTI is becoming difficult because of the antibiotic resistance in bacteria especially with *E.coli* producing extended spectrum ß-lactamases (ESBLs). Our study aims to identify common pathogens causing UTI in children, their in vitro antibiotic susceptibility pattern to commonly used antibiotics in a clinical setting in India. These findings will help us in deciding the empirical choice of antibiotics for children presenting with UTI, thus

preventing treatment failure and decreasing the drug resistance rates.

Design and setting

It was a retrospective observational study collecting data from hospital records during the period of January 2015 - December 2015 in SRM medical college hospital & research centre. Urine culture reports of all pediatric patients with suspected UTI admitted in SRM hospital during that period were reviewed.

Inclusion criteria

All children with suspected UTI and whose urine culture shows single pathogen with colony counts higher than or equal to 10^5 colony forming units (CFU)/ml were included for the study.

Exclusion Criteria

Results of urine cultures without growth, multiple pathogens in urine culture, colony counts less than 10^5 colony forming units (CFU)/ml were excluded from the study.

Antibiotics and reagents

Urine culture was done semi-quantitatively on 5% sheep Blood Agar and MacConkey Agar plates incubated at 37°C overnight. Standard technique for semi-quantitative culture of urine was applied for colony count. After identification of organism antimicrobial susceptibility testing of the bacterial isolates was performed by Kirby Bauer's disk diffusion method using commercial antibiotic disks (Himedia, India) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines[3]. The list of antibiotics used was appropriate for the urine sample and the type of organism (Gram positive/gram negative). ESBL-producing *E.coli*, were detected using a double-disc synergy test.

It included Ampicillin (10µg), Amikacin (30µg), amoxicillin/ clavulanic acid (30µg+10µg), Cefotaxime (30µg), Cefepime (30µg), Ceftazidime (30µg), Ceftazidime+Clavulanic acid (30µg+10µg), Cefeperazone/sulbactam (75/30µg), Cefpodoxime (10µg), Ceftriaxone (30µg), Cefuroxime (30µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Co-trimoxazole (25µg), Gentamicin (10µg), Imipenem (10µg), Meropenem (10µg), Nitrofurantoin (300µg), Norfloxacin (10µg), Ofloxacin (5µg), Piperacillin/ Tazobactam (100µg).

The *Eschericia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 , *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 strains were used as controls for the susceptibility testing of Enterobacteriacea, Nonfermenters, Staphylococcus species and Enterococcus species respectively. The interpretation of the zone diameters was done as per the CLSI break points[13].

RESULTS

About 551 urine samples from suspected UTI children were subjected to culture and antibiotic susceptibility testing by Kirby Bauer Disc diffusion method. About 130 were culture-positive, had significant colony count and included in the final analysis. *E coli* was detected in 40.8 % of cultures, followed by Enterococcus spp (22.3%), Klebsiella spp (17.0%), Proteus spp (10.8%), Coagulase negative Staphylococcus (3.8%), Pseudomonas (3.1%), and Acinetobacter (0.8%). The detection rate of ESBL-producing *E coli* was 83%. *E.coli* & Klebsiella were almost equally distributed among both sexes but all cases of Proteus and most cases of Enterococcus (69%) occurred in males.

Most Gram negative bacteria were sensitive to Aminoglycosides and Carbapenams

Table 1: Number and proportion of isolated pathogens (Gram negative and Gram positive pathogen) from midstream urine species

Bacteria Species	n	Percent
Gram negative bacteria		
Escherichia coli	53	40.8%
Klebsiella species	22	17 %
Proteus species	14	10.8%
Enterobacter species	-	
Pseudomonas aeruginosa	4	3.1%
Acinetobacter species	1	0.8%
Gram positive bacteria		
Bacteria Species	n	Percent
Enterococcus species	29	24.6 %
Coagulase Negative Staphylococcus (CoNS)	5	3.8%

and Gram positive bacteria were sensitive to Vancomycin and Linezolid. ESBL positive *E.coli* was more problematic with resistance to Amoxycillin/ clavulanic acid, Cephalosporins and Quinolones. They were also not uniformly sensitive to Piperacillin/Tazobactum (77.3%), Cefoperazone/ Sulbactum (86.4%) as ESBL negative *E.Coli.* Nalidixic acid resistance which is considered as a

Table 2: The distribution of pathogens in patients according to gender

	Boys		Girls	
Bacteria	**Number of patients**	**Percent**	**Number of patients**	**Percent**
Escherichia coli	23	43.4 %	30	56.6 %
Klebsiella species	20	59.1 %	9	40.9 %
Proteus species	14	100 %	-	-
Enterobacter species	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Acinetobacter species	1	100 %	-	-
Enterococcus species	20	69 %	9	31.03 %
Coagulase Negative Staphylococcus (CoNS)	02	40%	3	60%

Table 3: Antibiotic susceptibility test results to antibiotics commonly used against ESBL- positive and ESBL-negative *Escherichia coli*

Antimicrobial	**ESBL-positive *E. coli* (n= 44)**			**ESBL-negative *E. coli* (n= 9)**		
drug	**S%**	**I%**	**R%**	**S%**	**I%**	**R%**
Imipenem	42(95.5)	-	2(4.5)	9 (100)	-	-
Ciprofloxacin	2 (4.5)	1(2.3)	41(93.2)	7 (77.8)	-	2 (22.2)
Nitrofurantoin	40(90.9)	-	4(9.1)	8 (88.9)	-	1 (11.1)
Meropenem	42 (95.5)	-	2 (4.5)	9(100)	-	-
Amoxycillin Clavulanic acid	-	-	44 (100)	3 (33.3)	-	6 (66.7)
Amikacin	42 (95.5)	1(2.3)	1(2.3)	9 (100)	-	-
Gentamicin	42(95.5)	-	2(4.5)	9 (100)	-	-
cefazolin	-	-	44 (100)	6 (66.7)	1(11.1)	2(22.2)
cefepime	4(9.1)	1(2.3)	39(88.6)	3 (33.3)	-	6 (66.7)
cefotaxime	-	-	44(100)	9 (100)	-	-
Ceftazidime	-	-	44 (100)	9 (100)	-	-
cefuroxime	1(2.3)	-	43(97.7)	9 (100)	-	-
Colistin	44 (100)	-	-	9 (100)		
Ertapenem	43 (97.7)	-	1(2.3)	9 (100)	-	-
Nalidixic acid	-	-	44(100)	2 (22.2)	-	7 (77.8)
Netilmycin	41(93.2)	1(2.3)	3(6.8)	9 (100)	-	-
Norfloxacin	13(29.5)	-	31(70.5)	9 (100)	-	-
Piperacillin/Tazobactum	34(77.3)	3(6.8)	7(15.9)	9 (100)	-	-
Co-trimoxazole	8(18.2)	-	38(86.4)	4 (44.4)	-	5 (55.5)
Ampicillin	-	-	44 (100)	3 (33.3)	-	6 (66.7)
Ceftriaxone		1 (2.3)	43 (97.7)	9(100)	-	-
Cefaperazone sulbactum	38(86.4)	3 (6.8)	3 (6.8)	9 (100)	-	-

*S%- Percentage sensitive, I%- Percentage Intermediate Sensitive, R%- Percentage Resistant

Table 4: Antibiotic susceptibility test results to antibiotics commonly used against Klebsiella spp and Proteus spp

Antimicrobial drug	Klebsiella spp, n= 22			Proteus spp, n=14		
	S%	I%	R%	S%	I%	R%
Imipenem	21 (95.5)	1 (4.5)	-	14 (100)	_	-
Ciprofloxacin	21(95.5)	-	1(4.5)	9 (64.3)	2 (14.3)	3 (21.4)
Nitrofurantoin	13 (59.1)	7 (31.8)	2 (9.1)	7 (50)	2 (14.3)	5(35.7)
Meropenem	22 (100)	-	-	14 (100)	-	-
Amikacin	21(95.5)	-	1(4.5)	13 (92.9)	1 (7.1)	-
Gentamicin	21(95.5)	-	1(4.5)	14(100)	-	-
Cefazolin	13 (59.1)	-	9 (40.9)	14 (100)	-	-
Cefepime	14 (63.6)	1(4.5)	7(31.8)	3 (21.4)	2 (14.3)	9(64.3)
Cefotaxime	14(63.6)	-	8(36.4)	11(78.6)	-	3(21.4)
Ceftazidime	14(63.6)	-	8(36.4)	12 (85.7)	-	2 (14.3)
Cefuroxime	15(68.2)	-	7(31.8)	14 (100)	-	-
Colistin	11(50)	-	-	7(50)	4(18.2)	3(21.4)
Ertapenem	21(95.5)	-	1(4.5)	14(100)	-	-
Nalidixic acid	15 (68.2)	2(9.1)	5(22.7)	9(40.9)	1(7.1)	4(18.2)
Netilmycin	20 (90.9)	1(4.5)	1 (4.5)	13(92.9)	-	1 (7.1)
Norfloxacin	18(81.8)	3(13.6)	1(4.5)	11(78.6)	1(7.1)	2(14.3)
Piperacillin/Tazobactum	19(86.4)	2(9.1)	1(4.5)	14 (100)	-	-
Co-trimoxazole	14(63.6)	-	8(36.4)	4(28.6)	1(7.1)	9(64.3)

*S%- Percentage sensitive, I%- Percentage Intermediate Sensitive, R%- Percentage Resistant

Table 5 : Antibiotic susceptibility test results to antibiotics commonly used against CoNS and Enterococcus spp

CoNS , n= 5				Enterococcus spp, n=29			
Antimicrobial drug	**S%**	**I%**	**R%**	**Antimicrobial drug**	**S%**	**I%**	**R%**
Amikacin	5 (100)	-	-	Ampicillin	20 (69)	3(10.3)	6(20.7)
Cefazolin	4 (80)	-	1(20)	Gentamicin (High level)	14(48.3)	-	15(51.7)
Ciprofloxacin	2(40)	-	3(60)	Linezolide	28(96.6)	-	1(3.4)
Gentamicin	5(100)	-	-	Nitrofurantoin	26(89.7)	1(3.4)	1(3.4)
Linezolide	5(100)	-	-	Penicillin	21(72.4)	-	8(27.6)
Nitrofurantoin	5(100)	-	-	Tetracyclin	28(96.6)	1(3.4)	-
Norfloxacin	5(100)	-	-	Vancomycin	26(89.7)	2(6.9)	1(3.4)
Penicillin	-	-	5(100)				
Rifampicin	5(100)	-	-				
Tetracyclin	4(80)	1(20)	-				
Cotrimoxzole	2(40)	1(20)	2(40)				
Vancomycin	5(100)	-	-				

*S%- Percentage sensitive, I%- Percentage Intermediate Sensitive, R%- Percentage Resistant CoNS- Coagulase Negative Staphylococcus

marker of fluoroquinolone resistance ranges from 100% in ESBL positive *E.coli* to 25% in Klebsiella. The surprise drug has been Nitrofurantoin with ESBL positive *E.coli,* Enterococcus & Coagulase Negative Staphylococcus being sensitive. Not only in ESBL positive *E.coli*, cephalosporin resistance was observed one in three times in Klebsiella and 80% resistance to Cefazolin was seen in Proteus. Also Trimethoprim/Sulfamethoxazole resistance was high in the range of 35%-86% among various enterobacteraciaea.

DISCUSSION

This study describes one year susceptibility pattern of microorganisms causing UTI in pediatric population in SRM hospital. Male preponderance (62%) was there in our study. *E.coli* (40.8%) was the most common bacteria grown followed by Enterococcus (24.6%) and Klebsiella (17.0%). Studies done earlier also suggest a similar pattern[4-6]. However in our study 83% of E.coli were ESBL producers, higher than what has been reported by other studies.

E.coli treatment options

E.coli arising from gut flora and causing UTI is a real challenge to clinicians because of the production of ESBL. Due to indiscriminate usage of antibiotics, *E.coli* in the gut are exposed to multiple drugs and due to selective pressure develop resistance against them. Plasmids transmit such drug resistance genes to remaining bacteria[7]. Our susceptibility results suggest that ESBL positive *E.coli* are resistant to more number of commonly used antibiotics than ESBL negative *E.coli.*

We are now facing a situation where we don't have a good oral antibiotic against *E.coli* with no option of quinolones, cephaosporins and penicillins. One ray of hope is orally available Nitrofurantoin which is still sensitive against ESBL positive *E.coli* (91%). At present Nitrofurantoin can be given empirically for uncomplicated UTIs but studies related to its efficacy in complicated UTI is not convincing.

Aminoglycosides can be recommended as initial empiric choice parenterally for ESBL positive *E.coli* although nephrotoxic potential has to be kept in mind. Usually a resistance of 20% is taken as the threshold for changing the empirical treatment of UTI[8]. Based on this recommendation, eventhough Piperacillin/Tazobactum (15.9% resistance) and Cefoperazone/Sulbactum (6.8% resistance) are not uniformly sensitive, still can be recommended as first choices for ESBL positive *E.coli.* Carbapenams should be reserved as the final choice when patients fail to respond to the above antibiotics.

Klebsiella treatment options

Nine percentage resistance with Nitrofurantoin makes it an oral option for UTI due to Klebsiella. Quinolones may be recommended with caution because Norfloxacin(4.5%) resistance is low but Nalidixic acid resistance (22.7%) is higher. Few patients may not respond invivo to quinolones. Cephalosporins are poor choices as more than 30% resistance to cephalosporins is seen. Aminoglycosides and Piperacillin/Tazobactum should be the empiric first choice parenteral antibiotics.

Proteus treatment options

Resistance to Nitrofurantoin (35.7%) is high and hence cannot be recommended as an oral option but Amoxycillin Clavulanic acid can be tried orally since it is 100% sensitive. Aminoglycosides, Cefazolin and Piperacillin/ Tazobactum should be the empiric parenteral choices.

Gram positive bacteria- treatment options

Coagulase Negative Staphylococcus (CONS) and Enterococcus were highly sensitive to Nitrofurantoin, Linezolid and Vancomycin. Hence uncomplicated UTI may be treated with oral Nitrofurantoin and complicated ones with parenteral Vancomycin and Linezolid.

Combating antibiotic resistance

The last time when an antibiotic was approved for gram negative sepsis was in 1973 and it was trimethoprim/sulfamethoxazole. Our research is unable to catch the pace at which bacteria are relentlessly developing resistance. With no new antibiotic classes in the near future it is just a matter of time that they will develop resistance against all current options also.

One option for combating resistance will be to explore promising drugs like fosfomycin, cycloserine and include them in our armamentarium. Fosfomycin is a phosphonic acid with broad spectrum Gram positive and Gram negative activity including Methicillin Resistant Staphylococcus Aureus (MRSA), Vancomycin Resistant Enterococcus (VRE) and Pseudomonas used often in Europe and US[9, 10]. However it has not yet been included in the list of antibiotics for susceptibility testing. Cycloserine which is used as second option for tuberculosis has been explored previously for UTI[11]. Since limited studies are available cycloserine can be studied further as an oral option.

Other strategies like vaccination with orally administered Urovaxon[R 12] and parenteral Strovac[R 13], Probiotics[15], Immune drugs[14] should be further researched as relying only on antibiotics may lead to disaster in the near future. Till then judicious and rational use of antibiotics based on culture and susceptibility patterns should be recommended.

CONCLUSION

Our study shows that ESBL producing *E.coli* is the major pathogen causing UTI among pediatric population. Major cause of concern is that most of the organisms grown are resistant to three or more antibiotics. Oral Nitrofurantoin seems to be a promising oral option for uncomplicated UTI. Parenteral aminoglycosides, Piperacillin/ Tazobactum and Cefoperazone/ Sulbactum can be given as first choice for Gram negative bacteria and parenteral Vancomycin, Linezolid for Gram positive bacteria. Carbapenams should be kept as reserve for patients who fail to respond with first choice antibiotics. Strict hospital antibiotic stewardship program should be in place which routinely reviews culture and susceptibility patterns and optimizes the selection and dosing accordingly.

ACKNOWLEDGEMENTS

We sincerely thank the head of the department Microbiology, Dr. S. Gomathi, for making the data available to us.

REFERENCES

1. Taneja N, Chatterjee SS, Singh M, Singh S, Sharma M. Pediatric urinary tract infections in a tertiary care center from north India. *Indian J Med Res* **131**:101-05 (2010).
2. Elder JS. urinary tract infection. In: Kliegman, et al. Nelson text book of Pediatrics.19 ed. *Philadelphia, Saunders.* 1829-34 (2011)
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; *twentieth informational supplement.* **30**(1): M100-S20 (2010).
4. Fatemeh A, Houman H, Masumeh S, Zahra A, Maryam RN, Morteza FK. Aetiologies and Antibiotic Resistance Patterns in Infants With urinary tract Infections Hospitalized in Children Medical Center. *Iranian J Neonatology.* **4**(2):21-25 (2013).
5. Akram M, Shahid M, Khan Au. Aetiology and antibiotic resistance patterns of community-acquired urinary tract infection in JNMC Hospital Aligarh. India. *Ann Clin Microbiol Antimicrob.* **6**: 4 (2007).
6. Sharma A, Shrestha S, upadhyay S, Rijal P. Clinical and bacteriological profile of urinary tract infection in children at Nepal Medical College teaching Hospital. *Nepal Med Coll J.* **13**(1):24-26 (2011).
7. Guangying Y, Xueyin Z, Anrong L. Development of a novel test identifying extended-spectrum beta lactamase producing strains relative to resistant plasmids. Chin J *Microecol* **14**:89–90 (2002).
8. Gupta, Kalpana, et al. "International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases." *Clinical infectious diseases* 52.5 e103-e120 (2011).
9. Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenemresistant

Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* (2011).

10. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect Dis*. **10**:43-50 (2010).
11. Kaltenis P. Cycloserine as a urinary tract antiseptic. *Int Urol Nephrol.* **18**:125-130 (1986).
12. H. Bauer, G.S. Bleßmann, D.B. Pitrow, V.W. Rahlfs. Prevention of recurrent urinary tract infections with immunoactive *E.* coli fractions. *Eur J Obstet Gyn*. **86**: 33 (1999)
13. G. Riedasch, K. Möhring. Immunisierungs the rapie rezidivierender Harnwegsinfekte der Frau. *Therapiewoche.* **6**: 896-900 (1986).
14. Lin AE, Beasley FC, Olson J, Keller N, Shalwitz RA, Hannan TJ, et al., Role of Hypoxia Inducible Factor-1α (HIF-1α) in Innate Defense against Uropathogenic *Escherichia coli* Infection. *PLoS Pathog* 11(4): e1004818 (2015).
15. Beerepoot MA, ter Riet G, Nys S, et al. Lactobacilli vs antibiotics to prevent urinary tract infections: a randomized, double-blind, noninferiority trial in postmenopausal women. *Arch Intern Med* **172**: 704–12 (2012).