Extended Spectrum β-lactamase (ESBL) Producing Multi Drug Resistant (MDR) Urinary Pathogens in a Children Hospital from Nepal

Sharma KR,[1] Bhandari P,[2] Adhikari N,[1] Tripathi P,[3] Khanal S,[2] Tiwari BR[4]

[1]Department of Microbiology,
Kantipur College of Medical Sciences,
Kathmandu, Nepal.

[2]Department of Microbiology,
National College (NIST),
Kathmandu, Nepal.

[3]Department of Pathology Laboratory,
Kanti Children's Hospital,
Kathmandu, Nepal.

[4]School of Health and Allied Sciences,
Pokhara University,
Pokhara, Nepal.

Corresponding Author

Khem Raj Sharma

Department of Microbiology,

Kantipur College of Medical Sciences,

Kathmandu, Nepal.

E-mail: khemrajsharma69@gmail.com

Citation

Sharma KR, Bhandari P, Adhikari N, Tripathi P, Khanal S, Tiwari BR. Extended Spectrum β-lactamase (ESBL) Producing Multi Drug Resistant (MDR) Urinary Pathogens in a Children Hospital from Nepal. *Kathmandu Univ Med J.* 2018;62(2):151-5.

ABSTRACT

Background

Multidrug resistant in clinical bacterial isolates has increasingly been reported through out the world and is associated with high morbidity, mortality and increased health care costs. It is important to determine the status of multidrug resistance pattern to understand the current resistance trend so that appropriate antibiotics can be used in practice.

Objective

To determine the antibiotic resistant profile and prevalence of extended spectrum β-lactamase producing multidrug resistant strains in pediatric patients of Kanti Children's Hospital, Kathmandu, Nepal.

Method

Urine sample was cultured by standard microbiological techniques and bacterial isolates were identified using different biochemical tests. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method and extended spectrum β-lactamase detection was carried out using combined disc method as recommended by Clinical Laboratory Standard Institute guidelines.

Result

All together 65 different bacteria were isolated and subsequently identified. *E. coli* was the most common isolate with 46 (71%) isolates 63% of these isolates were multidrug resistant. Gram negative isolates were most resistant to nalidixic acid (81.97%) followed by ampicillin (69.35%) and co-trimoxazole (69.35%). The extended spectrum β-lactamase producing isolates were 43% among total isolates.

Conclusion

Higher rate of Extended Spectrum β-lactamase production among multidrug resistant isolates suggested routine extended spectrum β-lactamase testing in clinical isolates.

KEY WORDS

Antimicrobial, Multidrug resistant, Urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections in children that occur in at least 1% of boys and 3% of girls during first ten years of life.[1] Urinary tract is normally sterile which gets contaminated by bowel flora resulting in ascending urinary tract infection, less commonly infection may result from haematogenous spread of an organism to the kidney known as descending infection.[2] Urinary tract infection is more common in female than in male because of the short female urethra and its close anal proximity.[3] Most common bacteria involved in urinary tract infection are *E. coli*, *Klebsiella spp, Proteus spp, Citrobacter spp, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus spp, Enterococcus fecalis,* fungi such as *Candida spp*.[4,5]

The incidence of urinary tract infection varies with age and sexes which may be high in the first six months, and more common in boys with estimation around 1% of boys and 3% of girls have UTI during the first decade and around 40% of girls suffer recurrent infection.[6] Correct diagnosis and prompt treatment is crucial in order to prevent morbidity and mortality associated with the disease which is further backed up by frequent changing pattern of antimicrobial resistance with development of various resistant mechanisms like drug efflux, reduced uptake and production of hydrolytic enzymes like extended spectrum β-lactamases.[7] This study was conducted with an aim to determine the prevalence and antibiotic resistance profile with focus on extended spectrum β-lactamases producing MDR isolates in clinically relevant urine isolates from children.

METHODS

This study was conducted from June 2013 to December 2013 at Department of Microbiology, Kanti Children's Hospital, Kathmandu, Nepal. Five hundred fifteen urine samples were collected from neonates, infants and children from birth to 15 years. In case of neonates and infants, genital area was first cleaned with sterile water and wiped from front to back until area is clean. For female, urine bag was affixed over genital area, starting from the perineum and working upwards. For male, urine bag was placed over the penis ensuring a tight seal all around the bag. Urine bag was checked frequently and removed as soon as the urine is passed. Parents were instructed to collect clean catch midstream urine sample from children. Samples that were leaked, improperly labeled or unlabelled were excluded in the study.

Culture and identification of isolates

Urine samples were cultured by semi quantitative culture technique, a loopful of well mixed and un-centrifuged urine sample was inoculated into MacConkey agar (MA) and Blood agar (BA) plate with sterile calibrated inoculating loop and agar plates were incubated at 37°C for 24 h aerobically. Plates were examined next day to count colonies and samples showing ≥105 colony forming unit (CFU/ml), as determined by using formula was taken as significant.[8] Isolates were then identified by colony characteristics, Gram staining, catalase, oxidase and other different biochemical tests.[9]

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI). Four to five different colonies of same morphological type were touched with sterile inoculating loop, transferred to 2 ml of sterile Mueller Hinton Broth and then incubated at 37°C for 4-6 h until it achieved turbidity equal to 0.5 McFarland standards. In case of overgrowth, normal saline was used to dilute the broth to adjust 0.5 McFarland tube. Using a sterile swab carpet culture from broth was made on Mueller Hinton Agar and allowed to dry for some time. Antibiotic discs (MAST Diagnostics Merseyside, England) were applied on the plates and then incubated at 37°C for 24 hours. Zone of inhibition was then measured and interpreted using the standard CLSI chart.[10]

Criterion for Multidrug Resistance

In this study, criteria for an isolate to be multi drug resistant (MDR) was set as resistance to three or more drugs belonging to different structural classes.[11]

Screening and Confirmatory ESBL testing

Strains showing zone of inhibition of ≤ 22 mm to ceftazidime (30µg) and) ≤ 27 mm to cefotaxime (30µg) were selected for confirmatory ESBL testing. Briefly, a lawn culture of the isolated bacteria on Mueller Hinton agar (MHA) was made and ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30µg + 10µg) or cefotaxime (30µg) and cefotaxime plus clavulanic acid (30µg + 10µg) disc was placed with 25 mm apart. An increase of ≥ 5 mm in zone of inhibition for combination drug compared to corresponding cefotaxime or ceftazidime alone was confirmed as ESBL producing strains, as per guidelines of CLSI.10 *E. coli* ATCC 25922 and K. pneumoniae ATCC 700603 were used as negative and positive controls respectively.

All the results were entered in the worksheet of Statistical Package for Social Sciences (SPSS) software (Version 16.0).

RESULTS

Out of 515 urine samples processed during the study, 294 were from male and 221 from female, 65 (12.6%) different bacteria were isolated and identified, among them 42 (64.6%) were MDR and 28 (43%) were ESBL producer. Results are presented in table 1.

Of the total 65 isolates, 62 (95.38%) were gram negative and only 3 (4.62%) were gram positive. Most common

Table 1. MDR and ESBL status on total growth positive isolates

Total sample number	Significant bacteriuria		MDR cases		ESBL test	
	n	(%)	n	%	Screen positive	Confirmatory positive
515	65	12.6	42	64.6	38	28

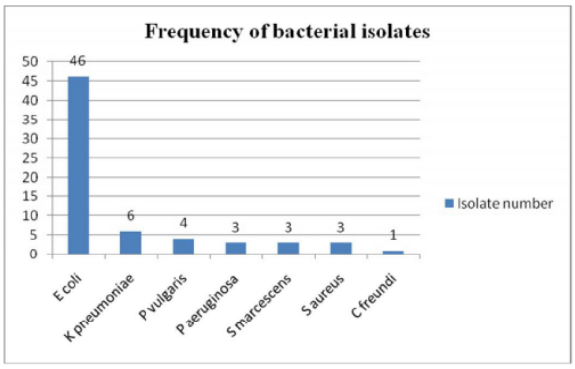

Figure 1. Frequency of total bacterial isolates

isolates of urinary tract infection was *E. coli* with 46 (70%) isolates followed by K. pneumoniae with 6 (9%) isolates. Details shown in figure 1.

Highest frequency of multi drug resistant and ESBL production both was seen in *E. coli* with 29 (69%) isolates and 22 (75.8%) isolates respectively. Increasing spectrum of drug resistance seen among ESBL producers was found statistically significant (P<0.005). P value was calculated by using SPSS software (v 16.0). Detail result is shown in Table 2.

Table 2. Frequency of ESBL producer among MDR

Bacteria	No. of MDR isolates	ESBL producer
E. coli	29	22
K. pneumoniae	3	3
P vulgaris	3	1
P aeruginosa	3	NT
S aureus	2	NT
S. marcescens	2	2
Total	42	28

NT: Not tested

Among 35 total screen positive ESBL isolates, only 28 (80%) were confirmed as true ESBL producer on confirmatory test. Details results are presented in table 3. ESBL were not tested against *P aeruginosa* and *S aureus* as methods for screening and phenotypic confirmatory testing of these isolates have not been determined by CLSI yet.[10]

Drugs to be tested were selected based on National Institute for Health and Clinical Excellence (NICE) guidelines, UK. Of the 14 different antibiotics used against gram negative isolates, Amikacin and Ofloxacin were found to most sensitive drugs with a susceptibility of 85.48% and 85% respectively. Results are shown in table 4.

Table 3. Frequency of ESBL producing bacterial isolates

Bacteria	Screen positive	Confirmed positive cases
E. coli	27	22
K. pneumoniae	3	3
P. vulgaris	3	1
S. marcescens	2	2
Total	35	28

Table 4. Antibiotic susceptibility pattern of Gram negative isolates (n=62)

Antibiotics used	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ampicillin	16	25.81	3	4.84	43	69.35	62
Amikacin	53	85.48	3	4.85	6	9.67	62
Co-trimoxazole	22	35.49	0	0	40	64.51	62
Ciprofloxacin	31	52.55	6	10.16	22	37.29	59
Cefixime	13	22.81	8	14.03	36	63.16	57
Ceftazidime	20	33.33	5	8.33	35	58.34	60
Cefotaxime	9	33.33	8	29.63	10	37.04	27
Norfloxacin	13	65	0	0	7	35	20
Nitrofurantoin	43	69.35	5	8.07	14	22.58	62
Nalidixic acid	9	14.76	2	3.27	50	81.97	61
Ofloxacin	17	85	0	0	3	15	20
Tobramycin	22	51.16	14	32.56	7	16.28	43

All three isolates of *Staphylococcus aureus* were 100% sensitive to Co-trimoxazole, Tobramycin and Vancomycin.

DISCUSSION

UTI is a common problem in children and prevalence varies with the age and sex of children and in this study significant bacteriuria was found only in 12.6 % (65/ 515) samples.[11] Out of these isolates, 64.6% were MDR that includes *E. coli* 69%; 7.1% each of *K. pneumoniae, P. vulgaris* and *P. aeruginosa*; and 4.7% each of *S. aureus* and *S. marcescens*. Similar results were found in another study carried out at same hospital that reported 15.8% significant growth and 69% of these isolates were resistant to more than two drugs with *E. coli* (66%) and *K. pneumonia* (56%).[12] However, another research showed a higher prevalence of 28.6% which could be attributed to seasonal variations, different age groups of children's and inclusion of every urine samples for culture regardless of their illness.[13]

Gram negative bacteria were the commonest cause of UTIs in this study, 95% of gram negative rods were isolated which are comparable with previous studies conducted in Iran (90.3%), Turkey (89%) and Australia (96%).[12] *E.coli* and *Klebsiella spp* were found as the major pathogens responsible for causing UTI in this study which was similar to many studies conducted before in similar settings.[13-15] *E. coli* is considered as normal flora of intestine and

contamination is important factor to cause urinary tract infection as its several strains can cause infection.[16]

Amikacin and ofloxacin were found to be most sensitive drug against Gram negative bacteria in the study with sensitivity of 85%. MDR was found in 64.6% of the urinary isolates among them major MDR producer was *E. coli.* Production of different β-lactamase, hydrolyze β-lactam ring of antibiotic, like TEM-1, TEM-2, SHV-1 and many other plasmid-mediated β-lactamases confers high level of resistance to drug in *E. coli.*[17] Furthermore, different efflux pumps and target site mutation at *gyrA* and *parC* are responsible for fluoroquinolones resistance.[18] Multiple resistances to antimicrobial drugs arising in *E. coli* isolates may complicate therapeutic management.[19]

Among Gram positives, only three isolates of *S. aureus* were found and 2 of them were MDR. Resistance in *S. aureus* is generally mediated by production of β-lactamase enzyme and presence of the *mecA* gene that codes for the modified penicillin-binding protein having a very low affinity for β-lactam antibiotics.[20]

The genes that code for production of ESBL are often linked to other resistance genes causing extended spectrum of drug resistance.[21] In this study too, all ESBL strains were found only in multi drug resistance cases. In another study all 16% ESBL producing urinary isolates were multi drug resistant.[22] Higher level of drug resistance seen among *E. coli* and *Klebsiella spp* is associated with the production of different kind of β-lactamases primarily ESBL, AmpC and Metallo β-lactamases and carriage of resistance trait for auinolones and aminoglycoside in the plasmid along with the gene for β-lactamases have had a great impact on the drug resistance character.[23]

The prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution and these differences may be due to geographical variations, local antibiotic prescribing habits, etc.[24] ESBL prevalence of 67%, 42% and 43% has been reported in *E. coli* from Iran, India and Bangladesh respectively. While less than 1% of *E. coli* isolates produce ESBL in the Scandinavian countries.[25] In this study, 43% isolates were ESBL producers. Though ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins, however, treatment with such antibiotics has been associated with high failure rates therefore carbapenems are the treatment of choice for serious infections caused by ESBL-producing organisms.[26] Seven month study period and lack of molecular tests to elucidate drug resistant mechanism are limitations of this study.

In this study only data from seven months period were included that can give narrow view on yearly prevalence rate and lack of molecular characterization of resistance isolates has limited the detail understanding about those isolates".

CONCLUSION

Multidrug resistance ESBL producing *E. coli* was major pathogens responsible for causing urinary tract infection in children during the study period.

REFERENCES

1. Sharma A, Shrestha S, Upadhyay S, Rijal P. Clinical and bacteriological profile of urinary tract infection in children at Nepal Medical College and Teacing Hospital. *Nepal Med Coll J.* 2011; 13.
2. Leigh D. Topley and Wilson's Principle's of Bacteriology, Virology and Immunity, Bacterial Diseases. 6th ed. Smith GR, Easmon CS, editors. London: Balter and Tanner Ltd.; 2002.
3. Heffner VA, Gorelik MH. Pediatric urinary infection. *Clin Ped Emerg Med.* 2008; 9.
4. Taneja N, Chattarjee SS, Singh M, Singh S, Sharma M. Pediatric urinary tract infections in a tertiary care center from North India. *Indian J Med Res.* 2010; 131.
5. Rai GK, Upreti HC, Rai SK, Shah KP, Shrestha RM. Causative agents of urinary tract infections in children and their antibiotic sensitivity pattern: a hospital based study. *Nepal Med Coll J.* 2008; 10.
6. Evans JH. Investigation of urinary tract infection in children. *Curr Pediatr.* 2006; 16.
7. Ghedira L, Messaoudi A, Ben MC, Guediche MN. Profile of antimicrobial resistance of agents causing urinary tract infections in children. *Tunis Med.* 2004; 82.
8. Pokhrel BM. A Handbook of Clinical Microbiology. 1st ed.: Goraknath Desktop and Printing Supports; 2004.
9. Collee JG, Miles RS, Watt B. Mackie and McCartney Practical Medical Microbiology Collee JG, Fraser AG, Marmion BP, Simmons A, editors. New York: Churchil Livingstone; 1996.
10. Wayne, P A. Performance standards for antimicrobial susceptibility testing. Clinical Laboratory Standard Institute; 2009. Report No.: M100-S19.
11. Bickerton MW, Ducket JW. Urinary tract infection in pedeiatric patients: American Urological Association. [Online].; 2011 [cited 2014 January 16. Available from: "http://www.auane.org" http://www.auane.org .
12.
13. Zafar A, Jamil I, Qamar MU, Ejaz H, Akhtar J, Waheed A. Multidrug resistant Klebsiella pneumoniae causing urinary tract infections in children in Pakistan. *African J Microbiol.* 2014; 8(4).
14. Dutta P, Gupta V, Sidhu S, Chander J. Community urinary tract infection due to ESBL producting E coli epidemiology and susceptibility to oral antimicrobials including Methicillin. *Nep J Med Sci.* 2014; 3(1).
15. Khadka KS, Khadka J, Lekhak B, Shrestha P, Tiwari BR. Incidence of urinary tract infection among the patients visiting Western Regional Hospital, Pohara, Nepal. *Journal of Health and Allied Sciences.* 2012; 2(1).
16. Bonacorsi S, Houdouin V, Kurkdjin PM, Messai FM, Bingen E. Comparative prevalence of virulence factors in Escherichia coli using urinary tract infection in male infants with and without bacterimia. *J Clin Microbio.* 2006; 44.
17. Livermore DM. Beta-lactamase in laboratory and clinical resistance. *Clin Microbiol Rev.* 1995; 8.

18. Ozeki S, Deguchi T, Yasuda M, Nakano M, Kawamura T, Nishino Y. Development of a rapid assay for detecting gyaA mutations in Eschechia coli and determination of incidence of gyaA mutations in clinical strains isolated from patients with complicated urinary tract infections. *J Clin Microbiol.* 1997; 35.

19. Santo E, Salvador MM, Marin JM. Multidrug resisistant urinary tract isolates of E coli from Ribeirao Preto Sao Paulo, Brazil. *Braz J Infect Dis.* 2007; 11.

20. Otter JA. Molecular Epidemiology of community-associated methiciliin resistant Staph aureus in Europe. Lancet Infect Dis. 2010; 10.

21. Sotto A, Lavigne JP, Chanal C, Mahamat A, Michaux SC. Molecular epidemiology of Enterobacteriace ae isolates producing extended spectrum beta-lactamases in a French Hospital. *J Clin Microbiol.* 2004; 42.

22. Pokhrel BM, Koirala J, Mishra SK, Dahal RK, Khadka P, Tuladhar NR. Multidrug resistance and extended specctrum betalactamase producing strains causing lower respiratory tract and urinary tract infection. *Journal of Institute of Medicine.* 2006; 28.

23. Poudyal S, Bhatta DR, Shakya G, Upadhyaya BR, Dumre SP, Buda G. Extended spectrum beta-lactamase producing multidrug resistant bacterial isolates at National Public Health Laboratory, Nepal. Nepal Med Coll J. 2011; 12(1).

24. Nag VL, Ayyagari A, Venakatesh V, Ghar M, Yadav V, Prasad KN. Drug resistant Haemophilus influenzae from respiratory tract infection in a tertirary care hospital North Inda. *Indian J Chest Dis Allied Sci.* 2001; 43.

25. Iqbal R, Majid A, Alvi I, Hayat A, Andaleeb F, Gul S. Multiple drug resistance and ESBL production in bacterial urine culture isolates. *Am J BioSci.* 2014; 2(1).

26. Paterson DL, Bonomo RA. Extended spectrum beta-lactamase: a clinical update. *Clin Microbiol Rev.* 2005; 18(4).