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Original Article

Multi Drug Resistant Pathogens Causing Urinary Tract Infections in Children at Kathmandu Model Hospital

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Abstract

Introduction: Antibiotic resistance of urinary tract pathogens has increased globally. Updated knowledge of the antibiotic resistance patterns of uropathogens in the health institutes is important for the selection of an appropriate empirical antimicrobial therapy. The aim of this study was to evaluate the multi drug resistant urinary isolates in the children from 1 to15 years and evaluate the options for empiric antibiotic therapy. **Materials and Methods:** The study was conducted from December 2011 to May 2012 in the Bacteriology laboratory, Kathmandu Model Hospital. Urine samples received in the laboratory were processed for routine, culture and its sensitivity. The antimicrobial susceptibility of bacterial isolates was determined following Clinical and Laboratory Standard Institute (CLSI) recommended Kirby-Bauer Disc Diffusion method. **Results:** Of the total 372 urine samples received in the laboratory, 60 (16.13%) showed significant growth; of which 55.0 % (33/60) were MDR isolates. *Escherichia coli were* the predominant isolate from urine sample. Out of 49 *Escherichia coli* isolates, 27 (45.0%) were Multi drug resistant. *Enterococcus faecalis* (N=3) was the most predominant Gram positive isolate and 66.67 % (2/3) of this organism were multi drug resistant. Among the first line drugs used against gram negative isolates, nitrofurantoin was the most effective drug followed by quinolones, while among the second line drugs; meropenem was the most effective drug followed by chloramphenicol and amikacin, whereas; nitrofurantoin (100%) was the most effective drug for Gram positive isolates followed by norfloxacin and cefotaxime. **Conclusion:** High percentages of multi drug resistant uropathogens were revealed in children. Nitrofurantoin was found to be the most effective drug for gram positive, gram negative and multi drug resistant isolates.

Key words: Children, Multi drug resistance, Empirical antimicrobial therapy, Escherichia coli

Introduction

Urinary tract infection (UTI) is defined as bacteriuria along with urinary symptoms and is identified by growth of a significant number of organisms of single species in the urine. Urinary tract infections are common medical problems in children and are important cause of morbidity. UTI during childhood varies by age and gender. UTI commonly affects boys during the first year of life[1], but thereafter 3-5% of girls are affected1, increasing to 10% by the teenage years[2]. Although several different microorganisms can cause UTI, including fungi and viruses, bacteria are the major causative organisms and responsible for more than 95% of UTI cases[3]. Treatment of urinary tract infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics[4]. Overuse and use of incomplete course of antibiotics as well as empirical antimicrobial therapy has been the major contributing factor in the development of Multi Drug Resistant(MDR) bacteria[5].

Multidrug resistance is defined as resistance to two or more different structural classes of antimicrobial agents[6]. There is growing concern regarding the resistance to uropathogens to antibiotics. The clinical impact of drug resistance may be great or insignificant, depending on the level of resistance, the site of infection, and the availability of effective, nontoxic therapeutic alternatives[7]. This prospective study was conducted to identify UTI due to multidrug resistant uropathogens among children and to evaluate empiric antibiotic therapy.

Materials and Methods

This is the prospective study conducted at Kathmandu Model Hospital on Pediatric patients (1-15 years) attending for the treatment of suspected UTI case. Three hundred and seventy two urine samples were examined from clinically suspected urinary tract infection during the research period of six months (December 2011- May 2012) using culture and sensitivity tests. All the patient or parents of the patient were instructed carefully for collection of morning mid-stream urine specimens. They were given a sterile, dry and clean collection bottles for urine collection. All the urine specimens were processed within 30 minutes of collection.

Culture of all urine specimens was done on 5% Blood Agar and Mac Conkey Agar plate utilizing semi-quantitative culture method (Fig 1). Shaking with hand to ensure a uniform suspension of bacteria vigorously mixed the urine specimens. Then, an inoculating loop of standard dimension was used to take up approximately fixed and known volume (0.001ml) of mixed urine and placed on the center of the plate. The drop was spread in a line and then over the entire surface of the agar plate. After inoculation, the culture plates were incubated in an inverted position at 37°C for 24 hours.[8] After 24 hours, the numbers of colonies were counted on each plate, which was multiplied by 1000 to calculate the number of organisms per ml in the specimen. Samples showing 10^5 or more organisms per ml of urine were taken as significant. Colony counts less than this was considered as non-significant[8].

Gram's staining and various biochemical tests identified pure culture of bacterial growth. Different biochemical media used were Triple Sugar Iron Agar, Sulphite Indole motility Agar, Urease agar, Simmons's Citrate Agar, Methyl Red / Voges Proskauer Test and Oxidation Fermentation medium. Catalase, Coagulase and Oxidase Tests were also performed. The antibiotics used as first line drugs for Gram negative bacteria were Amoxycillin (10 mcg), Cefotaxime (30 mcg), Ciprofloxacin (5 mcg), Cefixime (5 mcg), Cotrimoxazole (1.25/23.75 mcg), Norfloxacin (10 mcg), Nitrofurantoin (300 mcg) and Ofloxacin (5 mcg) and those used for Gram positive bacteria were; Amoxycillin (10 mcg), Cefotaxime (30 mcg), Ciprofloxacin (5 mcg), Norfloxacin (10 mcg), Cotrimoxazole (1.25/23.75 mcg), Nitrofurantoin (300 mcg), Gentamicin (10 mcg) and Ceftriaxone (30 mcg). The antibiotics used as second line drugs were Ceftriaxone (30 mcg), Ceftazidime (30 mcg), Gentamicin (10 mcg), Amikacin (30 mcg), Chloramphenicol (30 mcg), Piperacillin/Tazobactam (100/10 mcg), Cefoperazone/ Sulbactam (50/50 mcg) and Meropenem (10 mcg). All the antibiotics were tested by Kirby Bauer's Disc Diffusion Technique. The colonies picked up and suspended in Nutrient broth and adjusted turbidity to 0.5 Mc Farland standard.

Within 15 minutes, a sterilized cotton swab was dipped into the adjusted suspension. Carpet culture was done by streaking the swab over the entire sterile Mueller Hinton agar plate (Fig 2). The antibiotic impregnated discs were placed on the surface of the agar plate and then incubated at 37°C for 18 hours[9]. The different inhibition zones were measured and interpreted the results on the basis of zone size compared with standard interpretive table given by manufacturer. The organisms which showed resistant to all first line antibiotics except Nitrofurantoin were tested for second line drugs.

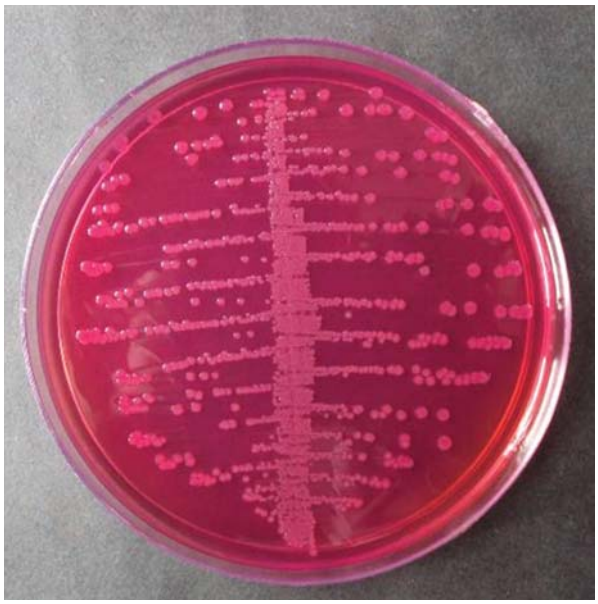

Fig 1: Significant Growth of *E. coli* in Mac Conkey Agar

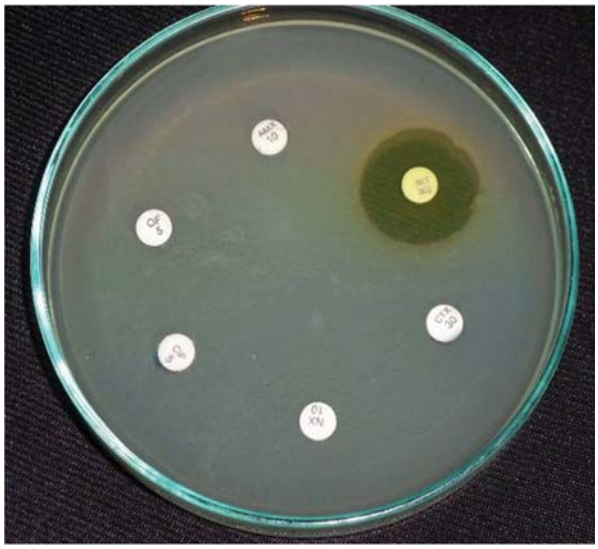

Fig 2: Antibiotic Susceptibility Test of MDR *E. coli* in Mueller Hinton Agar

Results

Of the 372 urine samples processed, 60 (16.13%) samples showed significant growth where as majority of samples i.e. 284 (76.34%) showed no growth and 28 (7.53%) showed growth of no significance (Table 1).

Out of 372 patients, 192 (51.61%) were male, while 180 (48.39%) were female. Of the total samples, 350 (94.09%) were from outdoor patients, 7 (1.88%) were from indoor patients and 15 samples (4.03%) were from emergency Department. The age group of 1-5 years had the maximum requests of 179 (48.12%) for urine culture, while the age group 11-15 years was second with 102 (27.42%) requests. Age group of 6-10 years was the least with 91 (24.46%) request.

The age and gender wise distribution of children with UTI is shown in Table 2. UTI was commonly found in young female children of age 1-5 years. MDR isolates were common in young children (46.67%) of the 1-5 years age group. Children of age group 6-10 years showed three (5.0%) and age group 11-15 years showed only two (3.34%) MDR isolates. Of the total MDR isolates, 31.67 occurred in young female children of age 1-5 years. UTI was significantly high in female children (p=0.004) in comparison to male. There was no significant difference seen in MDR infection between male and female (p=0.8). However, the significant difference was seen in MDR infection between age group 1 to 5 and above 5 year's group (p=0.0001)

Gram negative bacteria were predominant; constituting 56 (93.33%) of the total 60 isolates and 33(55.0%) were MDR. Among Gram negatives, *Escherichia coli* were the most frequently isolated species with 49 (81.67%). Gram positive organisms constituted only 4 (6.67%) of total isolates, and 2 (3.33%) of them were MDR. Both MDR isolates were Enterococcus *faecalis* (Table 3).

Table 3: Pattern of microbial isolates

Organisms	No. of Isolates (%)	MDR (%)
Gram Positive Bacteria		
Staphylococcus epidermidis	1(1.67)	
Enterococcus faecalis	3(5.0)	2 (3.33)
TOTAL (Gram Positive)	**4 (6.67)**	**2(3.33)**
Gram Negative Bacteria		
Escherichia coli	49 (81.67)	27(45.0)
Citrobacter spp.	2 (3.33)	1(1.67)
Enterobacter aerogenes	2(3.33)	1(1.67)
Enterobacter cloacae	1(1.67)	1(1.67)
Proteus vulgaris	1(1.67)	1(1.67)
Salmonella Paratyphi A	1(1.67)	0
TOTAL (Gram Negative)	**56 (93.33)**	**33 (55.0)**
Grand Total	**60 (100)**	

Among the common antibiotics used as first line against gram negative isolates, nitrofurantoin showed a susceptibility of 55/56 (98.21%). Quinolones (Ciprofloxacin, Norfloxacin and Ofloxacin) followed Nitrofurantoin with susceptibility of 38/56 (67.86%). Among the second line antibiotics used, Meropenem was found to be most effective drug with susceptibility of 16/17 (94.12%) followed by chloramphenicol and Amikacin with a susceptibility of 15/17 (88.24%). Most of the Gram negative isolates i.e. 45 (80.36%) were resistant to Amoxycillin (Table 4, 5).

Among the gram positive isolates, Nitrofurantoin was the most effective drug with susceptibility of 4/4 (100%) which was followed by Cefotaxime and Norfloxacin (Table 6).

Out of 60 isolates, 24 (40.0%) isolates were resistant to >3 drugs where as only 3 isolates of *Escherichia coli* and one isolate of *Salmonella* Paratyphi were sensitive to all antibiotics used. Among 56 gram negative isolates, 31(51.67%) isolates were MDR whereas of the 4 gram positive isolates, 2 (50.0%) isolates were MDR (Table 7).

Table 1: Growth profile of urine sample

Specimen	Total no. of samples	Significant Growth		No Significant Growth		No Growth	
		No.	%	No.	%	No.	%
Urine	372	60	16.13	28	7.53	284	76.34

Table 2: Age and gender wise distribution of infected patients with MDR isolates

Age Group (yrs)	Growth with MDR isolates				Total MDR (%)
	Male		Female		
	Isolates (%)	MDR (%)	Isolates (%)	MDR (%)	
1-5	14 (23.33)	9 (15.0)	24 (40.0)	19 (31.67)	28 (46.67)
6-10	2 (3.33)	0	9(15.0)	3 (5.0)	3 (5.0)
11-15	3 (5.0)	1 (1.67)	8 (13.33)	1 (1.67)	2 (3.34)
Total	**19 (31.67)**	**10 (16.67)**	**41 (68.33)**	**23 (38.33)**	**33 (55.0)**

Table 4: Antibiotic Susceptibility Pattern of Gram-negative Isolates towards first line antibiotics

Antibiotic used	Susceptible		Intermediate		Resistant		Total
	Frequency	%	Frequency	%	Frequency	%	
Amoxycillin	9	16.07	2	3.57	45	80.36	56
Cefotaxime	26	46.43	2	3.57	28	50.0	56
Ciprofloxacin	38	67.86	2	3.57	16	28.57	56
Cotrimoxazole	28	50.0	1	1.79	27	48.21	56
Cefixime	33	58.93	1	1.79	22	39.28	56
Nitrofurantoin	55	98.21	0	0	1	1.79	56
Norfloxacin	38	67.86	2	3.57	16	28.57	56
Ofloxacin	38	67.86	1	1.79	17	30.35	56

Table 5: Antibiotic Susceptibility Pattern of gram negative isolates towards second line antibiotics

Antibiotic used	Susceptible		Intermediate		Resistant		Total
	Frequency	%	Frequency	%	Frequency	%	
Amikacin	15	88.24	0	0.00	2	11.76	17
Gentamicin	10	58.82	0	0.00	7	41.18	17
Chloramphenicol	15	88.24	0	0.00	2	11.76	17
Ceftriaxone	1	5.88	0	0.00	16	94.12	17
Ceftazidime	1	5.88	1	5.88	15	88.24	17
Cefoperazone/Sulbactam	11	64.71	2	11.77	4	23.52	17
Piperacillin/Tazobactam	13	76.47	1	5.88	3	17.65	17
Meropenem	16	94.12	0	0.00	1	5.88	17

Table 6: Antibiotic Susceptibility Pattern of Gram-positive Isolates

Antibiotic used	Susceptible		Intermediate		Resistant		Total
	Frequency	%	Frequency	%	Frequency	%	
Amoxycillin	2	50.0	0	0	2	50.0	4
Ciprofloxacin	2	50.0	1	25.0	1	25.0	4
Cotrimoxazole	2	50.0	0	0	2	50.0	4
Cefotaxime	3	75.0	0	0	1	25.0	4
Norfloxacin	3	75.0	0	0	1	25.0	4
Nitrofurantoin	4	100.0	0	0	0	0	4
Gentamicin	2	50.0	0	0	2	50.0	4
Ceftriaxone	2	50.0	0	0	2	50.0	4

Table 7: Status of antibiotic resistance among MDR isolates

Organism	Total Isolates	Resistance to							
		0 Drug	1 Drug	2 Drugs	MDR isolates				
					2 Drugs (different classes)	3 Drugs	> 3 Drugs	Total	%
Escherichia coli	49	3	18	7	7	0	20	27	45.0
Citrobacter spp	2	0	1	0	0	0	1	1	1.67
Ent. aerogenes	2	0	1	0	0	0	1	1	1.67
Ent. Cloacae	1	0	0	1	1	0	0	1	1.67
Proteus vulgaris	1	0	0	1	1	0	0	1	1.67
Salmonella Paratyphi	1	1	0	0	0	0	0	0	0
Staph. epidermidis	1	0	1	0	0	0	0	0	1.67
E. faecalis	3	0	0	0	0	0	2	2	3.33
Total	**60**	**4**	**21**	**9**	**9**	**0**	**24**	**33**	**55.04**

Discussion

The emergence of multi drug resistance in uropathogens is of great public health concern. Prevalence of these organisms varies according to species, antibiotic use and geographical area. The increasing prevalence of infections caused by antibiotic resistant bacteria makes the empirical treatment of UTIs difficult. In the current study, we isolated 60 (16.13%) uropathogens among 372 urine culture samples. The study demonstrates that *E. coli* (81.67%) remains the leading uropathogen responsible for UTIs which was supported by several previous studies.[10,11] The frequency of UTI is greater in female children as compared to male[10,11,12] and our results were similar to these reports showing 68.33% of patients were female.

Paediatric UTI causing bacteria are becoming increasingly resistant to commonly used antibiotics such as fluroquinolones and third generation cephalosporins. Cotrimoxazole (Trimethoprim-sulphamethoxazole), fluroquinolones, or nitrofurantoin are recommended for empirical treatment of uncomplicated UTI[13,14]. However, several reports from worldwide indicated the excessive increase in the emergence of trimethoprim-sulphamethoxazole resistant *E. coli*[15,16]. Cotrimoxazole was replaced by fluroquinolones and cephalosporins because of high level of resistance to this drug but unfortunately after sometime resistance to these drugs was also detected and published. Our study also showed the similar findings with 28.5-30.35% resistance to quinolones, 39.2-50.0% resistance to cephalosporins and 48.21% resistance to cotrimoxazole for gram negative isolates[17]. The results showed a considerable increase in resistivity of gram negative isolates to amoxycillin (80.36%) which was supported by several studies[11,12,18]. Nitrofurantoin demonstrated better activity against gram negative (98.21% susceptible) as well as gram positive isolates (100.0% susceptible), in agreement with data published by others[17,18,19]. The high level susceptibility of uropathogens to nitrofurantoin may be the narrow spectrum of activity, narrow tissue distribution (low or undetectable serum concentration) and limited contact with bacteria outside the urinary tract[20]. According to our study, the first line antibiotics to be used for the treatment of UTI is nitrofurantoin.

In our study, we defined those organisms as MDR which were resistant to two or more different structural classes of antibiotics[6]. According to this, 33 (55.0%) MDR isolates were detected. Of the 33 MDR isolates, 27(45.0%) were *E. coli*. This result was supported by previous other studies[21,22,23]. Our study demonstrated the highest resistance to Ceftriaxone (94.12%) among 17 MDR isolates. This may be due to the production of ESBL enzymes or other resistance mechanisms which could not be addressed because of limited resources. The resistance to cephalosporin is explained though the enzymatic mechanisms and efflux pumps[24]. It has been reported that pathogenic *E. coli* isolates have relatively high potential for developing resistance.[25] Among the antibiotics used in the second line, meropenem was the most active drug with susceptibility of 94.12% followed by amikacin and chloramphenicol with susceptibility of 88.24%. These findings reveals stronger propensity of uropathogens towards multiple drugs resistance limiting few therapeutic options for the treatment.

Conclusion

The results of the present study suggest that prevalence of MDR *E. coli* is alarmingly high and the most appropriate first line oral antibiotic for empiric treatment of urinary tract infection at our hospital is nitrofurantoin and meropenem, amikacin and chloramphenicol as second line agents. Antibacterial resistance patterns need to be updated periodically to ensure proper empiric treatment of UTI.

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References

1. Hellerstein S. Antibiotic treatment for urinary tract infections in Pediatric patients. *Minerva Pediatr* 2003;55(5):395-406.
2. Ahmed SM. Evaluation and treatment of urinary tract infections in children. *Am Family Physician* 1998;57: 7.
3. Bonadio M, Meini M, Spitaleri P, Gigli C. Current Microbiological and Clinical Aspects of Urinary Tract Infections. *Eur Urol* 2001; 40: 439-445.
4. Alekshun MN and Levy SB. Molecular Mechanism of Antimicrobial Multidrug Resistance. *Cell* 2007;128(6):1037-1050.
5. Brad GF, Sabau I, Marcovici T, Maris I, Daescu C, Belei O, Vetesi T, Nilima K, Hodut A, Popoiu CM. Antibiotic resistance in urinary tract infections in children. *Jurnalul Pediatrului* 2010;13(51-52):73-77.
6. Bartoloni A, Pallecchi L, Benedetti M, Fernandez C, Vallejos Y, Guzman E, Villagran AL, Mantella A, Lucchetti C, Bartalesi F, Strohmeyer M, Bechini A, Gamboa H, Rodriguez H, Falkenberg T, Kronvall G, Gotuzzo E, Paradisi F and Rossolini GM. Multidrug resistant Commensal Escherichia coli in children, Peru and Bolivia. *Emerg Infect Dis* 2006;12(6):907-913.
7. Bronzwaer SL, Cars O, Buchholz U, Moldstad S, Goettsch W, Veldhuijzen IK, Kool JL, Sprenger MJ, Degener JE. The relationship between antimicrobial use and antimicrobial resistance in Europe. *Emerg Infect Dis* 2002;8(3):278–282.

8. Cheesbrough M. District Laboratory Practice in tropical Countries Part 2. 2nd ed. UK: Cambridge University Press; 2006.

9. Clinical Laboratory Standard Institute: Performance standards for antimicrobial susceptibility testing; Twentieth informational supplement document. M100-S20.Wayne, PA: Clinical Laboratory Standard Institute; 2010.

10. Sharifan M, Karimi A, Rafiee-Tabatabaei S, et al. Microbial sensitivity pattern in urinary tract infections in children: a single center experience of 1177 urine cultures. *Jpn J Infec Dis*.2006; 59: 380-382.

11. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community- acquired urinary tract infections in India: a multicentered study. *J Infect Develop Countr* 2008;2:354-358.

12. Keah S, Wee E, Chng K et al. Antimicrobial susceptibility of community-acquired uropathogens in general practice. *Malaysian Family Physician* 2007;2:64-69.

13. Echols RM, Tosiello RL, Haverstock DC et al. Demographic, clinical and treatment parameters influencing the outcome of acute cystitis. *Clin Infec Dis* 1999;29:113-119.

14. Warren JW, Abrutyn E, Hebel JR et al. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute Pyelonephritis in women. Infectious Disease Society of America(IDSA). *Clin Infect Dis* 1999;29:745-758.

15. Karlowsky JA, Jones ME, Thornsberry C. Prevalence of antimicrobial resistance among urinary tract pathogens isolated from female outpatients across the USA in 1999. *Int J Antimicrob Agents* 2001; 18: 121-127.

16. Dias Neto J, Martins ACP, da Silva LDM, Tiraboschi RB, Domingos ALA, Colonga AJ, Paschoalin ED, Tucci Jr S. Community- acquired urinary tract infections: etiology and bacterial susceptibility. *Acta Cirurgica Brasileira* 2003;18(5):33- 35.

17. Guidoni EB, Berezin EN, Nigro S, Santiago NA, Benini V, Toporovski J. Antibiotic resistance patterns of paediatric community acquired urinary infection. *Braz J Infect Dis* 2008;12:321-323.

18. Kashef N, Djavid GE, Shahbazi S. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran. *Iran J Infect Dev Ctries* 2010;4(4):202-206.

19. Kahlmeter G. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis in Europe. The ECO-SENS study.*Int J Antimicrob Agents* 2003;2(2):49-52.

20. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF. Trends in antimicrobial resistance among urinary tract isolates of Escherichia coli from female outpatients in the United States. *Antimicrob Agents Ch* 2002;46(8):2540-45.

21. Koljalg SK, Truusalu K, Vainuma I, Stsepetova J and Mikelsaar M. Persistence of Escherichia coli Clones and Phenotypic and Genotypic Antibiotic Resistance in Recurrent Urinary Tract Infections in Childhood. *J Clin Microbiol* 2009;47:99-105.

22. Jan N, Meshram SU, Kulkarni A. Plasmid profile analysis of multidrug resistant E. coli isolated from UTI patients of Nagpur City, India. *Rom Biotechnol Lett* 2009;14(5):4635-40.

23. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Res Notes* 2012;5:38.

24. Joumana N, Kfoury S, Araj GF. Recent developments in beta lactamases and extended spectrum beta lactamase. *BMJ* 2003;327:1209-213.

25. Karlowsky JA, Jones ME, Draghi DC, Thornberry C, Sahm DF, Volturo GA. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002 *Antimicrob Agents Ch.* 2004;3:1-8.

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