

Beta Lactamase Producing *Escherichia coli*, *Klebsiella pneumoniae* and Methicillin Resistant *Staphylococcus aureus* among Uropathogens

V.K.Singh¹, R. Tuladhar² and M.K. Chaudhary³

¹Nepal Academy of Science and Technology, Lalitpur, Nepal

²Central Department of Microbiology, Tribhuvan University, Nepal

³Department of Microbiology, KIST MCTH, Lalitpur, Nepal

e-mail: viveksingh1424@gmail.com

Abstract

Urinary tract infection (UTI) is the leading cause of morbidity and health care expenditures in persons of all the ages. The impact of disease is high in the low-resource developing countries due to lack of awareness about the UTIs caused by extended spectrum beta lactamase producing pathogens and methicillin resistant *Staphylococcus aureus*. This study represents a cross-sectional analysis of UTI prevalence in patients attending at KIST Medical College Teaching Hospital, Imadole, Lalitpur. Bacterial isolates were identified using standard biochemical tests. The antimicrobial susceptibility to various drugs and confirmation of the extended spectrum β -lactamase (ESBL) and methicillin resistant *Staphylococcus aureus* (MRSA) production was done following Clinical & Laboratory Standards Institute (CLSI) guidelines. During the study period, 303 (24.08% of urine submissions) UTIs were confirmed. Among these 303 strains, *E. coli* was the most common, representing 65.34% of the isolates, followed by *S. aureus* (6.93%) and other pathogens (27.72%). Prevalence of ESBL *E. coli*, *K. pneumoniae* and MRSA in the urinary isolates were found to be 38.38%, 35% and 47.61% respectively. Empiric treatment for UTIs in Nepal should be reconsidered and prudent use of antibiotics, particularly Gentamycin, is highly recommended. The recent spread of ESBL-producing Enterobacteriaceae and MRSA is striking and underlines the need for further studies.

Key words: antimicrobial susceptibility pattern, ESBL, MDR, MRSA, Urinary tract infection

Introduction

The term urinary tract infection (UTI) refers to the invasion of the urinary tract by a non-resident infectious organisms. UTI encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus (Pokhrel 2004). Bacteriuria which may lead to the infection of the prostate, epididymis or the testes are also included in the definition of UTI (Fowler & Mariano 1990). UTI is among the most common reasons patients seek medical care. It is estimated that approximately 10% of humans will have UTI at some time during their lives (Boscia & Kaye 1987).

Bacteria of only a limited number of species are able to initiate infection in the urinary tract. The causative agents are listed below (Cheesbrough 2010):

Gram negative *E. coli*, *Proteus mirabilis*, *P. vulgaris*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp, *Serratia* spp., *M. morgani* and *P. aeruginosa*

Gram positive *S. aureus*, *S. saprophyticus*, Group B streptococci and *E. faecalis*

Other pathogens *Chlamydia* (*Chlamydia trachomatis*), *Mycoplasma*, *Ureaplasma urealyticum*, *Candida* spp. and *Mycobacterium tuberculosis*

Extended spectrum beta lactamases (ESBLs) are a group of enzymes with the ability to hydrolyze and cause resistance to the oxymino-cephalosporins (i.e. cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) and monobactams (i.e. aztreonam) (Peirano & Pitout 2010). Increasing resistance to third-generation cephalosporins amongst *E. coli* and

Klebsiella spp. is predominantly due to the production of extended-spectrum beta lactamases (ESBLs). ESBL producers are associated with increased morbidity and mortality, especially amongst patients on intensive care and high-dependency units. Accurate laboratory detection is important to avoid clinical failure due to inappropriate antimicrobial therapy.

Methicillin-resistant *Staphylococcus aureus* is a bacterium responsible for difficult-to-treat infections in humans. It may also be referred to as multidrug-resistant *Staphylococcus aureus* or oxacillin-resistant *Staphylococcus aureus*. MRSA is by definition a strain of *Staphylococcus aureus* that is resistant to a large group of antibiotics called the beta-lactams, which include the penicillins and the cephalosporins. MRSA has evolved an ability to survive treatment with beta-lactam antibiotics, including methicillin, dicloxacillin, nafcillin, and oxacillin (Maucky 2011). This group of organisms has since emerged as a serious concern in human medicine (Barrett *et al.* 1968, Lee 2003, Pinho *et al.* 2001, Weese *et al.* 2005).

Methodology

Specimen collection

The patient was given a sterile, dry, wide-necked leak-proof Hi-Media container of 100 ml capacity for collection of 10-20 ml of clean-catch mid-stream urine. The patient was given instructions for the collection of CC-MSU.

Culture of specimen

The urine sample was cultured onto the MacConkey agar and Blood agar medium by the semi-quantitative culture technique using a standard loop (Sapkota *et al.* 2014).

Identification of the isolates

Identification of significant isolates was done by using microbiological techniques which involves morphological appearance of the colonies, staining reactions and biochemical properties (Cheesbrough 1984, Collee *et al.* 1999, Forbes *et al.* 2002).

Antibiotic susceptibility testing

The antibiotic sensitivity testing was performed according to the recommended Kirby-Bauer sensitivity testing method (CLSI 2013).

Quality control for test

Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was tested by incubating one plate of each lot on the incubator. During identification of organism, for each test ATCC control positives and control negatives was taken simultaneously. Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4mm and the pH at 7.2-7.4. Similarly antibiotic discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all these procedures.

Screening test for ESBLs

Isolates were screened for ESBL production by using disc diffusion of cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CTR) and Aztreonam (AT) placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations.

Confirmatory test for ESBLs

Phenotypic confirmatory test for ESBL was done by combination disc method for all the ESBL producing isolates as per CLSI 2013 guidelines.

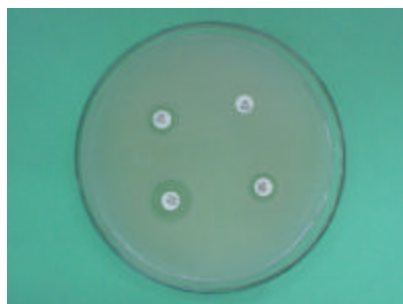


Fig.1. Phenotypic confirmatory test for ESBL *E. coli* (CAZ= Ceftazidime, CAC= Ceftazidime+Clavulanic acid, CTX= Cefotaxime, CEC= Cefotaxime+Clavulanic acid)

In this test a disc of ceftazidime (30 μ g), cefotaxime (30 μ g) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10 μ g) were used for each isolates. Both the discs were placed 25 mm apart, centre to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37 $^{\circ}$ C. A \geq 5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive.

Interpretation: An increase in inhibition zone diameter of ≥ 5 mm around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disk alone, confirms ESBL production.

Identification of the MRSA

Identification of *S. aureus* was done based on colony morphology on 5% sheep blood agar, gram stain appearance and catalase test.

Detection of MRSA was done using cefoxitin disc. Bacterial colonies was suspended in saline to a turbidity of 0.5 McFarland standards and inoculated on a Muller Hinton agar plate. Plates were incubated at 35°C. All isolates resistant to cefoxitin were considered as MRSA (Mshana *et al.* 2009).

Statistical analysis

Statistical package for social sciences (SPSS) version 16 was used to analyze the data.

Results and Discussion

Of the 1258 specimens examined in this study, 303 (24.08%) showed significant bacteriuria. Of the 303 bacterial isolates, the gram positive and negative bacteria accounted 15 % and 83. % respectively. The rest were yeast (0.99 %). The gram negative bacteria included: *Escherichia coli* 198, *Klebsiella pneumoniae* 20, *Klebsiella oxitoca* 1, *Proteus mirabilis* 6, *Proteus vulgaris* 4, *Citrobacter koseri* 3, *Citrobacter freundii* 2, *Enterobacter* species 1, *Pseudomonas aeruginosa* 4, *Acinetobacter* species 14 and the gram positive were *Staphylococcus aureus* 21, Coagulase negative *Staphylococcus aureus* 10, *Streptococcus* species 7, *Enterococcus* species 9 and the yeast included *Candida albicans* 1, Non *albicans Candida* species 2.

The in-vitro antibiotic sensitivity pattern of the isolates to common anti-microbial agents is shown in Table 1 and 2. Results indicated that gentamycin and nitrofurantoin were the most potent of all the antibiotics. Norfloxacin, ampicillin, ciprofloxacin, ceftriaxone and cotri-moxazole were poorly effective.

Table 1. Antibiotic susceptibility pattern of gram positive bacteria isolated from urine samples.

Antibiotic used	Sensitive	Intermediate	Resistant	Total
Ampicillin	25		22	47
Gentamicin	32		15	47
Norfloxacin	21		26	47
Nitrofurantoin	38	2	7	47
Ciprofloxacin	23	1	23	47
Cotrimoxazole	24		23	47
Ceftriaxone	24		23	47

Table 2. Antibiotic sensitivity pattern against gram negative bacteria isolated from urine samples.

Antibiotic used	Sensitive	Intermediate	Resistant	Total
Ampicillin	50	2	201	253
Gentamicin	192	3	58	253
Nalidixic acid	62	3	188	253
Norfloxacin	111	3	139	253
Nitrofurantoin	148	39	66	253
Ciprofloxacin	123	1	129	253
Cotrimoxazole	107	1	145	253
Ceftriaxone	129	1	123	253

Out of the 303 isolates, 58.33% were MDR. 59.09 % *E. coli* isolated and 78.57.0% of *S. aureus* isolated were MDR strains. 100% isolates of *C. koseri*, *K. oxitoca*, *Enterobacter* species and *P. aeruginosa* were MDR-strains.

Of the 98 ESBL screen positive *E. coli* isolates, 76 isolates were ESBL producers and out of 9 ESBL

screen positive *K. pneumoniae* isolates, 7 isolates were ESBL producers by Combination disc method. *E. coli* and *K. pneumoniae* showed maximum ESBLs production in CAZ/CAZC combination than CTX/CTXC combination. The in-vitro antibiotic sensitivity pattern of the ESBL *E. coli* and *K. pneumoniae* isolates to common anti-microbial agents is shown in Table 3 and 4.

Table 3. Antibiotic profile in ESBL producing *E. coli* strain

S.No.	Name of antibiotics	Sensitive	Resistant	Total
1	Ampicillin	4	72	76
2	Nalidixic acid	4	72	76
3	Norfloxacin	9	67	76
4	Ciprofloxacin	3	73	76
5	Ofoxacin	17	59	76
6	Nitrofurantoin	62	14	76
7	Gentamicin	48	28	76
8	Amikacin	75	1	76
9	Cotrimoxazole	20	56	76
10	Ceftriaxone	0	76	76
11	Ceftazidime	0	76	76
12	Amoxicillin/Clavulanic acid	0	76	76
13	Imipenem	76	0	76

Table 4. Antibiotic profile in ESBL producing *K. pneumoniae* strain

S.No.	Name of antimicrobials	Sensitive	Resistant	Total
1	Ampicillin	0	7	7
2	Nalidixic acid	0	7	7
3	Norfloxacin	0	7	7
4	Ciprofloxacin	0	7	7
5	Ofoxacin	0	7	7
6	Nitrofurantoin	0	7	7
7	Gentamicin	0	7	7
8	Amikacin	6	1	7
9	Cotrimoxazole	0	7	7
10	Ceftriaxone	0	7	7
11	Ceftazidime	0	7	7
12	Amoxicillin/Clavulanic acid	0	7	7
13	Imipenem	7	0	7

Overall, the prevalence of MRSA in the urinary *S. aureus* isolates was found to be 47.61% using ceftazidime disc. The in-vitro antibiotic sensitivity pattern of the

ESBL *E. coli* and *K. pneumoniae* isolates to common anti-microbial agents is shown in Table 5.

Table 5. Antibiotic profile in MRSA strain

S. No	Antibiotic used	Sensitive	Resistant	Total
1	Penicillin	0	10	10
2	Cefoxitin	0	10	10
3	Ampicillin	0	10	10
4	Gentamicin	7	3	10
5	Norfloxacin	3	7	10
6	Nitrofurantoin	10	0	10
7	Cotrimoxazole	7	3	10
8	Vancomycin	10	0	10

Urinary tract infection is a serious health problem affecting millions of people each year. Urinary tract infections are a common problem in general practice and are usually treated empirically. Empirical treatments should be based on local data regarding common pathogens and their susceptibility to available antibiotics.

The prevalence of UTI in the population was 24.08%. This figure is higher than the 20.2% rate recorded by Getachew (2010) and 21.8% rate recorded by Mahmood (2011). The high prevalence may be due to genuine population susceptibility because factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status, are common among Nepali men

and female. It is lower than prevalence rate of 60% significant bacteriuria recorded by Kolawole *et al.* (2009) and 80% by Durgesh *et al.* (2012).

Out of 303 cases with significant bacteriuria, 83.49% were due to gram negative rods and 15.51% were due to gram positive cocci. In a similar study performed by Mahmood (2011) out of 100 urinary isolates, 85% constituted Gram negative isolates whereas 15% Gram Positive Isolates.

The study revealed *E. coli* and *S. aureus* to be the dominant organisms among other uropathogens. The most common organism isolated in these patients was *E. coli* (65.3%) and this finding is similar to other reports which indicated that a gram negative bacterium, particularly *E. coli*, was the commonest pathogen isolated in patients with UTI (Mahmood 2011, Kolawole *et al.* 2009, Devanand & Saxena 2013, Sasirekha *et al.* 2010). This was due to the fact that strains of *E. coli* affecting the urinary tract possess a variety of virulence characteristics that facilitate their intestinal carriage, persistence in vagina and then ascension and invasion of the anatomically normal urinary tract (Annabelle *et al.* 1999).

Among the common antibiotics used against all Gram negative isolates, Gentamycin was the drug of choice with susceptibility of 75.9% because 192 out of 253 isolates were found to be susceptible to the drug followed by Nitrofurantoin with a susceptibility of 58.5%. Most of the Gram negative isolates, i.e. 79.5% (201 out of 253) were resistant to Ampicillin. Nitrofurantoin was found to be the most effective drug against urinary pathogens also in other similar studies by Jha and Yadav (1992).

Among the Gram positive isolates, most of them i.e. 80.9% were susceptible to Nitrofurantoin followed by Gentamycin with the susceptibility of 68.1%. Nalidixic acid was found to be the least effective as only 14.9% isolates were sensitive to the drug.

Resistance rates among common uropathogens to many commonly used antimicrobial agents have increased over the years and these resistance rates vary from country to country. Among the 198 *E. coli* isolates, 117 (59.1%) were isolates were MDR strains. Similarly 100% of *C. freundii*, *P. aeruginosa*, *Klebsiella oxitoca*, *Enterobacter* species were MDR.

ESBLs are paradigmatic as a mechanism of resistance because of the impact they have had on the therapy of infections and the insight they have offered on the relationship between structures and function, in antibiotics and in their determinants of resistance. Of the 198 Uropathogenic *E. coli* (UPEC) isolates, 98 (49.5%) were shown resistance to ceftriaxone and all were subjected for phenotype confirmatory test for ESBL, combination disc method (CLSI 2013). Total 76 isolates were identified as ESBL producers. Poudel (2013) also observed similar results in which 67 out of 222 *E. coli* isolates were ESBL producers. Out of 20 isolates of *K. pneumoniae*, 9 isolates showed a resistance to ceftriaxone and 7 were confirmed as ESBL producers.

In our study, resistance to third generation cephalosporins was found to coexist with resistance to two or more antibiotics like ampicillin, nalidixic acid, ciprofloxacin, Ofloxacin, Amoxicillin/Clavulanic acid as also reported by several studies indicating multidrug resistant pattern. Mechanisms of co-resistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids (Barber 1948, Levy 1982, Levy 1998, Crofton & Mitchison 1948, Bradford 2001).

Above 90% ESBL positive isolates were found resistant to ampicillin, quinolones, fluoroquinolones and amoxicillin/clavulanic acid. High sensitivity of ESBL positive isolates towards imipenem and amikacin advocates the usage of amikacin and carbapenem antibiotics as the therapeutic alternative to beta-lactam antibiotics as indicated in many studies (Bradford 2001, Al-Agamy *et al.* 2006). Nitrofurantoin was considered the most effective of the drugs that can be orally administered; which represent an alternative for oral empiric therapy of uncomplicated UTI and is recommended by the Infectious Disease Society of America (IDSA) (Gupta *et al.* 2011). Nitrofurantoin in this study demonstrates excellent activity against UPEC isolates i.e. 68 % sensitive; both in complicated and uncomplicated UTIs and also in community acquired and hospital acquired infections. This study showed good co-resistance between nitrofurantoin and ESBL. Moreover, the production of ESBLs has been associated with decreased susceptibility to nitrofurantoin (Carattoli 2009, Laupl and *et al.* 2008, Rodriguez-Bano & Navarro 2008). As in UPEC

infection, rising rates of resistance to antimicrobial drugs limits the choice of drugs that can be used to treat infections with these potent pathogens.

Aztreonam was 100% resistant in our study, which agrees with those of Sasirekha *et al.* (2010). Most of the ESBL producing organisms were found to be coresistance to fluroquinolones, aminoglycosides and co-trimoxazole, which correlates with the study done by Denholm and Jabeen (Denholm *et al.* 2009, Jabeen *et al.* 2005). This was due to the genes encoding β -lactamases were often located on large plasmids that also encode genes for resistance to others antibiotics, including aminoglycosides, tetracycline, sulfonamides, trimethoprim and chloramphenicol (Perez *et al.* 2007). We found such associated resistance with fluroquinolones.

In this study we used two combinations with clavulanic acid (CAZ/CAZC and CTX/CTXC) and found that *E. coli* and *K. pneumoniae* showed maximum ESBLs production in CAZ/CAZC combination, which correlates with other studies (Rahman *et al.* 2004, Thomson *et al.* 1991).

The overall methicillin-resistant *S. aureus* among *S. aureus* urinary tract infection was found to be 47.61%. The prevalence of MRSA in this study was higher (27.4%) than the reported prevalence of MRSA among uropathogens by Sashirekha *et al.* 2010. The high prevalence of MRSA among UTI patients in this study may suggest that MRSA is more prevalent in *S. aureus*.

Conclusion

Based on our study, it is concluded that UTI affected females more than males. The main organism causing UTI is *E. coli* followed by *S. aureus*. Almost all isolates are resistant to commonly prescribed antibiotics. Therefore, antibiotics should only be commenced after performing culture sensitivity test because most of the UTI patients are treated empirically with different antibiotics.

Infection-control practitioners and clinicians need the clinical laboratory to rapidly identify and characterize different types of resistant bacteria specially ESBLs and MRSA efficiently to minimize

the spread of these bacteria and help to select more appropriate antibiotics.

Acknowledgements

We like to express our special thanks of gratitude to Central Department of Microbiology, Tribhuvan University and KIST Medical College Teaching Hospital for the support to complete this research work.

References

- Al-Agamy, M.H., M.E. Ashour and I. Wiegand. 2006. First description of CTXM β -lactamase-producing clinical *Escherichia coli* isolates from Egypt. *Int. J. Antimicrob. Agent.* **27**: 545-548.
- Annabelle, T., M.D. Dytan, A. Jennifer and M.D. Chua. 1999. Surveillance of pathogens 15 and resistance patterns in urinary tract infections. *Phil. J. Microbiol. Infect. Dis.* **28(1)**: 11-14.
- Barber, M. 1948. Infection by penicillin resistant *Staphylococci*. *Lancet* **2**:641-644.
- Barrett, F.F., R.F. McGhee and M. Finland. 1968. Methicillin-resistant *Staphylococcus aureus* at Boston city hospital. *New England Journal of Medicine* **279**: 448.
- Boscia, J.A. and D. Kaye. 1987. A symptomatic bacteriuria in the elderly. *Infect Dis. Clin. North Am.* **1**: 893-905.
- Bradford, P.A. 2001. Extended-spectrum β -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.* **14**: 933-951.
- Carattoli, A. 2009. Resistance plasmid families in Enterobacteriaceae. *Antimicrob Agents Chemother.* **53**: 2227-2238.
- Cheesbrough, M. 1984. Medical Laboratory Manual for Tropical Countries Microbiology. 1st ELBSed. Cambridge: University Press.
- Cheesbrough, M. 2000. *District Laboratory Practice in Tropical Countries*. Part 2, Cambridge University Press.
- CLSI. 2013. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Third Informational Supplement, CLSI Document M100-S23, Wayne, PA: Clinical and Laboratory Standards Institute.
- Collee, J.G., A.G. Fraser, B.P. Marmion and A. Simmons. 1999. *Mackie and McCartney Practical Medical Microbiology*, 14th edition, Churchill Livingstone, New York, USA.
- Crofton, J and D.A. Mitchison. 1948. Streptomycin resistance in pulmonary tuberculosis. *Br. Med. J.* **2**: 1009-1015.
- Denholm, J.T., M. Huysmans and D. Spelman. 2009. Community acquisition of ESBL-producing *Escherichia coli*: a growing concern. *Medical Journal Australia* **190**: 45-46.
- Devanand, P and Saxena. 2013. Prevalence and distribution of gram negative bacteria of Enterobacteriaceae causing Urinary tract infections among hospitalized patients. *J. Acad. Indus. Res.* **1(11)**: 50-53.

- Durgesh, D., Wasnik, P.M. Tumane. 2012. Prevalence and antibacterial susceptibility pattern of Urinary Tract Infection Causing Human Pathogenic Bacteria. *Asian Journal of Biomedical and Pharmaceutical Sciences* **2** (15): 1-3.
- Forbes, B.A., D.F. Sahm and A.S. Weissfeld. 2002. *Bailey and Scott's Diagnostic Microbiology*, 11th edition. Mosby, Inc. USA.
- Fowler, J.E. and M. Mariano. 1990. Immunologic response of the prostate to bacteriuria and bacterial prostates: Antigen specific immunoglobulin in men with bacterial prostatitis. *J Urol.* **131**: 363.
- Getachew, T. 2010. Bacterial pathogens implicated in causing urinary tract infection (UTI) and their antimicrobial susceptibility pattern in Ethiopia Revista CENIC. *Centro Nacional de Investigaciones Cientificas Cuba* **41**: 1-6.
- Gupta, K., T.M. Hooton, K.G. Naber, B. Wullt, R. Colgan, L.G. Miller, G.J. Moran, L.E. Nicolle, R. Raz, A.J. Schaeffer and D.E. Soper. 2011. *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 **52**: 103-120.
- Jabeen, K., A. Zafar, R. Hasan. 2005. Frequency and sensitivity pattern of extended spectrum beta lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. *Journal Pakistan Medical Association* **55**: 436-439.
- Jha, V.C. and J.N. Yadav. 1992. Bacterial species isolated from urine of UTI patients and their sensitivity to commonly available antibiotic. *J Nep Med Assoc.* **30**: 222-225.
- Kolawole, A.S., O.M. Kolawole, Y.T. Kandaki-Olukemi, S.K. Babatunde, K.A. Durowade and C.F. Kolawole. 2009. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital. *International Journal of Medicine and Medical Sciences* **1**: 163-167.
- Laupland, K.B., D.L. Church, J. Vidakovich, M. Mucenski and J.D. Pitout. 2008. Community-onset extended-spectrum β -lactamase (ESBL) producing *Escherichia coli*: importance of international travel. *J Infect.* **57**: 441-448.
- Lee, J.H. 2003. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl Environ Microbiol.* **69(11)**: 6489-6494.
- Levy, S.B. 1982. Microbial resistance to antibiotics. An evolving and persistent problem. *Lancet* **2**: 83-88.
- Levy, S.B. 1998. The challenge of antibiotic resistance. *Sci. Am.* **278**: 46-53.
- Mahmood, M.A. 2011. Department of Basic Sciences, College of Dentistry, Baghdad University, Baghdad-Iraq.
- Maucky, H.F. 2011. Methicillin resistant *Staphylococcus aureus* skin, soft tissue infection among HIV infected individuals attending HIV care and treatment clinics in Dar es Salaam city, Tanzania. Muhimbili University of Health and Allied Sciences (MUHAS). 15.
- Mshana, S.E., E. Kamugisha, *et al.* 2009. Prevalence of clindamycin inducible resistance among methicillin-resistant *Staphylococcus aureus* at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania Journal of Health Research* **11(2)**: 59-64.
- Peirano, G., J.D.D Pitout. 2010. Molecular epidemiology of *Escherichia coli* producing CTX-M β , Lactamases: worldwide emergence of clone ST131 O25:H4. *International journal of Antimicrobial Agents* **35**: 316-321.
- Perez, F., A. Endimiani, K.M. Hujer and R.A. Bonomo. 2007. The continuing challenge of ESBLs. *Current Opinion in Pharmacology* **7**: 459-469.
- Pinho, M.G., H. de Lencastre and A. Tomasz. 2001. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. *Proc Natl Acad Sci U S A.* **98(19)**: 10886-10891.
- Pokhrel, B.M. 2004. *A Handbook of Clinical Microbiology*. 1st ed. Gorakhnath Desktop and Printing Supports, Kathmandu, Nepal.
- Poudel, S. 2013. Status of extended spectrum beta lactamase producing Enterobacteriaceae among bacterial uropathogens. Central Department of Microbiology, TU, Kirtipur, Nepal. 23-55.
- Rahman, M.M., J.A. Haque, M.A. Hossain, R. Sultana, F. Islam and S. Islam. 2004. Prevalence of extended spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in an urban hospital in Dhaka Bangladesh. *International journal of antimicrobial agents.* **24**: 508-510.
- Rodriguez-Bano, J., M.D. Navarro. 2008. Extended-spectrum β -lactamases in ambulatory care: a clinical perspective. *Clin Microbiol Infect.* **14**: 104-110.
- Sapkota, B., R.K. Bhandari and B.R. Tiwari. 2014. Susceptibility Pattern of Gram Negative Urine Pathogens with Exclusion of *Escherichia coli* to Quinolone/Fluoroquinolone Antibiotics. *Nepal Journal of Science and Technology.* **15(2)**: 77-84.
- Sasirekha, B., R. Manasa, P. Ramya and R. Sneha. 2010. Frequency and antimicrobial sensitivity pattern of extended spectrum β -Lactamases producing *E. coli* and *Klebsiella pneumoniae* isolated in a tertiary care hospital. *Al Ameen journal Medical science.* **3**: 265-271.
- Thomson, K.S., C.C. Sanders and J.A. Washington. 1991. High-level resistance to cefotaxime and ceftazidime in *Klebsiella pneumoniae* isolates from C level and, Ohio. *Antimicrob Agents Chemother.* **35**: 1001-1003.
- Weese, J.S., M. Archambault, B.M. Willey, P. Hearn, B.N. Kreiswirth, B. Said-Salim, A. McGeer, Y. Likhoshvay, J.F. Prescott and D.E. Low. 2005. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. *Emerg Infect Dis.* **11(3)**: 430-435.

