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Extended Spectrum Beta-Lactamase (ESBL) Producing Multi Drug Resistant Gram-Negative Isolates Causing Urinary Tract Infection in a Tertiary Care Hospital

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Abstract:- Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*. MDR is the growing concern nowadays which is increasing significantly because of the unwanted use of antibiotics, bacteria to be more resistance producing ESBL in the near future. The aim of this study is to detect ESBL production in MDR Gram-negative bacteria causing UTIs. Among total samples received, 100 (22.0%) were reported to be gram negative bacilli. *E. coli* (n=75) was the dominant isolate. Out of total gram negative bacilli, 52 (52.0%) were found to be MDR. Highest rate of susceptibility of Gram negative bacteria was seen towards Piperacillin/Tazobactam (84.7%) and Amikacin (80.8%) and highly resistant to Amoxicillin (90.3%) followed by Cefixime (82.7%) and Imipenem (80.8%) in case of Enterobacteriaceae. In addition, *Acinetobacter* spp. highly resistant (100%) to all the antibiotics used except for the Polymixin B and Tigecycline 100% susceptible. This study demonstrates the high prevalence of ESBL producers among *E. coli* followed by *Citrobacter* spp. Hence, controlling antibiotic resistant bacteria and subsequent infections more efficiently necessities the sensible and responsible use of antibiotics.

Keywords:- *Urinary Tract Infection, MDR, ESBL.*

I. INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common infectious diseases often associated with significant morbidity and mortality, frequently occurring in both males and females of all ages, more commonly in women than men and almost half of the women have at least one UTI in their lifetime [1,2]. It is expected that about 35% of healthy women experiences warning signs of UTIs [3].

The dominance of this disease is additional in developing countries owed to deprived sanitation, living method, undernourishment, and ecological stipulation. Mostly, neonates, girls, young women, infants, young children and older men are mainly vulnerable to UTIs [4]. Serious sequelae include frequent recurrences, pyelonephritis with sepsis, renal damage in young children, pre-term birth and complications caused by frequent antimicrobial use, such as high-level antibiotic resistance and *Clostridium difficile* colitis [5]. UTIs causing etiological agent *Escherichia coli* is the predominant both in community and hospital settings accounting for 70-90% of UTI [6]. Other pathogens commonly isolated are *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* [5].

Their microbial spectrum and susceptibility pattern against different antibiotic vary with different geographical regions and the previous use of antibiotic is important predictor of resistance. The high incidence of UTI and need of starting treatment before availability of microbiological results leads to adoption empirical therapy which is based on the local susceptibility pattern [7].

Bacteria are being resistance to antibiotics by various mode of action such as biochemical aspects and genetic aspects. Biochemical aspects include antibiotic inactivation, efflux pumps, peptidoglycan structure alteration and target modification and genetic aspects include mutation and horizontal gene transfer. Unfortunately, the recent emergence of multidrug-resistant (MDR) pathogens seriously threatens this class of life saving drugs [8, 9].

Many authors in Nepal have reported the alarming problems of UTI due to uropathogens that are resistant to commonly prescribed antibiotics (Amoxicillin, Co-trimoxazole, Ciprofloxacin), reducing therapeutic possibilities [10]. Globally accelerating antimicrobial resistance has revived interest in use of Nitrofurantoin more recently in UTI [11]. Nitrofurantoin is also an old drug used for uncomplicated UTI, but its use is limited because of nephrotoxicity [12].

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Antibiotic resistance pattern of uropathogens should be updated periodically to ensure proper empirical treatment of UTI and to avoid emergence of drug resistance.

II. MATERIALS AND METHODS

This study was hospital based prospective on Urine sample of urinary tract infection cases in Manmohan Memorial Community Hospital, Kathmandu from May 2018 - November 2018. A total of 454 non repeat samples were collected from patients clinically suspected of UTI and referred for urine culture and AST by physicians.

A freshly void midstream urine samples (10-20 ml) were collected in a sterile wide mouth container by the patients. Semi-quantitative culture technique was used to culture urine sample. Urine specimens were mixed well and aseptically inoculated on Blood and Mac-Conkey agar using a standard calibrated nichrome loop. The culture plates were incubated aerobically at 37°C for 24 hours. A single bacterial species from the urine sample with a colony count of > 10^5 CFU/ml was considered significant bacteriuria and reported as significant growth. Uropathogens were identified based on standard laboratory procedures including, morphological characteristics, Gram's stain and biochemical tests.

The antibiotic sensitivity test was performed on Mueller-Hinton agar media by modified Kirby Bauer's disc diffusion method as described in the guidelines of CLSI (2018). In this study, resistances to two or more than two antibiotics of different structural classes were considered to be multidrug resistance.

➢ *Initial screening test for ESBL*

The isolates were screened for ESBL production by disc diffusion method as described by Clinical Laboratory Standards Institute (CLSI 2018) for Enterobacteriaceae. Isolates with Ceftazidime (30µg) zone diameter equal to or less than 22 mm were identified as ESBL producers (CLSI 2018). Although ESBL detection for *Pseudomonas* spp. and *Acinetobacter* spp. were not mentioned, zone diameter less than or equal 17 were considered as possible ESBL producers.

➢ *Phenotypic confirmatory test for ESBL*

Isolates were confirmed for ESBL production by combined disc method as described by CLSI guidelines (CLSI 2018). Prepared Mueller-Hinton agar was inoculated with the test organism (0.5McFarland standard) to give a semi-confluent growth. *E. coli* ATCC 25922 was used as control strain. A Ceftazidime (30µg) disc with Ceftazidime-Clavulanic acid (30/10 µg) combination disc were then placed at 20 to 25 mm distance. Following incubation at 35°C ± 2 in ambient air for 18-24 hours, a ≥5mm increase in a zone diameter for both antimicrobial agent tested in combination with Clavulanate versus the zone diameter of the agent when tested alone confirmed the isolate as an ESBL producer (CLSI 2018).

➢ *Statistical analysis*

All the study data were entered into computer using standard format, checked for errors and verified. Statistical programmed statistical package for social science (SPSS 20.0) and Microsoft word were used to analyze all the obtained data. A value of P≤0.05 was assumed statistically significant and 95% confidence intervals along with the exact p-values were presented.

III. RESULTS

Out of 454 urine samples processed, 25.0% showed significant growth and among them 86.9% were confirmed as Gram-negative bacilli. The most common isolates were *E. coli* (65.2%). After antimicrobial susceptibility test, 52 (52.0%) were found to be MDR. Among the total MDR 34 (65.3%) *E. coli* were found to be the dominant one followed by *Citrobacter* spp. 6 (11.5%) as shown in Figure 1.

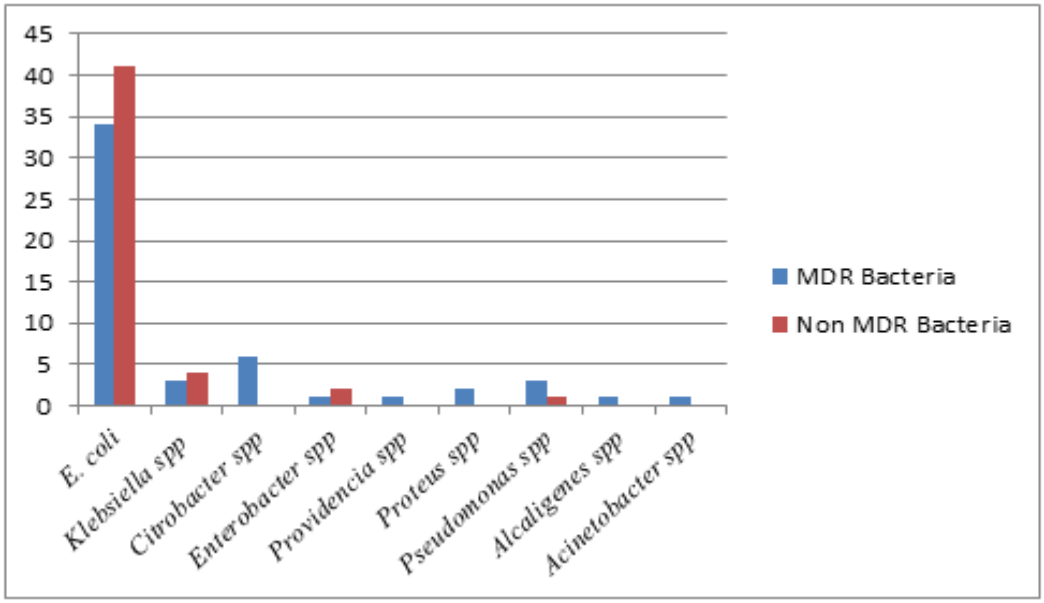

Fig 1: Distribution of MDR Gram-negative bacteria

Among the Gram-negative isolates after antimicrobial susceptibility test, 52 (46.5%) were found to be MDR. Of all the MDR isolates, enterobacteriaceae were highly resistant to Amoxicillin (90.3%) followed by Cefixime (82.7%) and Imipenem (80.8%). In contrast to resistance, most Enterobacteriaceae were sensitive to Piperacillin/Tazobactam (84.7%) and Amikacin (80.8%) (Table 1).

Table 1: Antibiogram of MDR Enterobacteriaceae

Antibiotics Used	Sensitive (%)	Resistant (%)
Amoxycillin	5(9.7)	47(90.3)
Levofloxacin	15(28.9)	37(71.1)
Ceftriaxone	17(32.7)	35(67.3)
Cefixime	9(17.3)	43(82.7)
Cotrimoxazole	20(38.4)	32(61.6)
Nitrofurantoin	35(67.3)	17(32.7)
Norfloxacin	15(28.9)	37(71.1)
Ofloxacin	14(26.9)	38(73.1)
Gentamicin	30(57.7)	22(42.3)
Amikacin	42(80.8)	10(19.2)
Imipenem	10(19.2)	42(80.8)
Meropenem	39(75.0)	13(25.0)
Ampicillin/sulbactam	31(59.7)	21(40.3)
Piperacillin/Tazobactam	44(84.7)	8(15.3)

Pseudomonas spp. was highly sensitive to Piperacillin/tazobactam (100.0%), Amikacin, Ciprofloxacin, Levofloxacin, Gentamicin, Meropenem (75.0%) where as resistant to Carbenicillin and Ceftazidime (75.0%) and Imipenem (50.0%). In addition, *Acinetobacter* spp. (1) isolate was found to be highly resistant (100%) to all the antibiotics used except for the Polymixin B and Tigecycline 100% susceptible (Table 2).

Table 2: Antibiotic susceptibility pattern of *Pseudomonas* spp. and *Acinetobacter* spp.

Oraganism	***Pseudomonas* spp.**		***Acinetobacter* spp.**	
Antibiotics Used	**Susceptible (%)**	**Resistance (%)**	**Susceptible (%)**	**Resistance (%)**
Carbenicillin	1 (25.0)	3 (75.0)	0 (0)	1 (100.0)
Amikacin	3 (75.0)	1 (25.0)	0 (0)	1 (100.0)
Ceftazidime	1 (25.0)	3 (75.0)	0 (0)	1 (100.0)
Ciprofloxacin	3 (75.0)	1 (25.0)	0 (0)	1 (100.0)
Levofloxacin	3 (75.0)	1 (25.0)	0 (0)	1 (100.0)
Gentamicin	3 (75.0)	1 (25.0)	0 (0)	1 (100.0)
Imipenem	2 (50.0)	2 (50.0)	0 (0)	1 (100.0)
Meropenem	3 (75.0)	1 (25.0)	0 (0)	1 (100.0)
Piperacillin/Tazobactam	4 (100.0)	0 (0.0)	0 (0)	1 (100.0)
Polymixin B	-	-	1 (100.0)	0 (0)
Tigecycline	-	-	1 (100.0)	0 (0)

Among the total MDR Gram-negative bacteria, 47 were resistant to Ceftazidime (≤22mm) in screening test which later were processed for confirmed test. Thirty seven bacilli were confirmed to be ESBL producers, most common as *E. coli* (25) followed by *Citrobacter* spp. (5).

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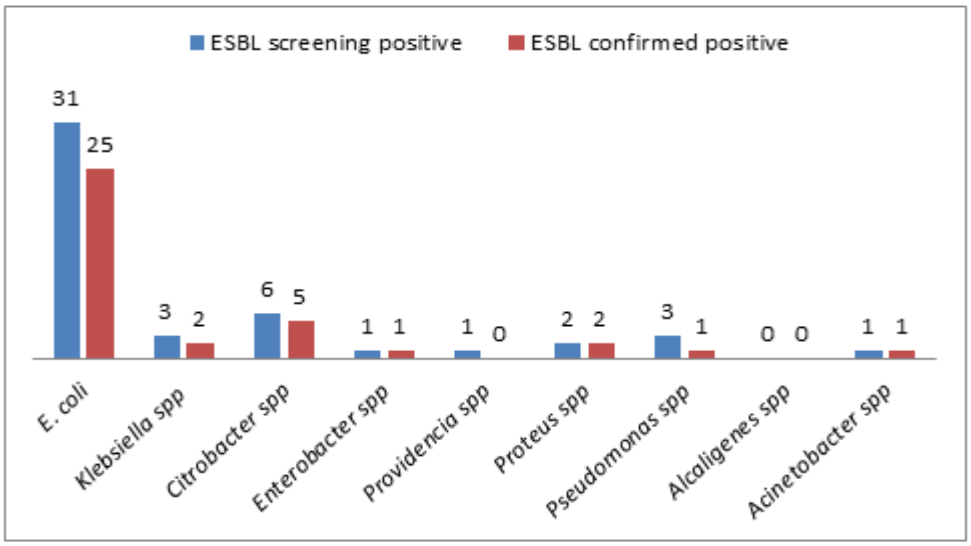

Fig 2: Frequency of ESBL among Gram negative MDR isolates causing UTI

Out of total confirmed ESBL producers, 33/88 was from OPD and 4/12 from IPD patients. *E. coli* (66.7%) and *Citobacter* spp. (15.2%) from OPD and *E. coli* (75.0%) from IPD represented the majority of ESBL producers. Of the screening test positive isolates 78.7 % (37/47) were found to be confirmed ESBL producers (Table 3).

ESBL producer *E. coli* was mostly found in age group of 21-40 years old female patients. Also, *Citrobacter* spp. was found in female with different age group. *Acinetobacter* spp. and *Pseudomonas* spp. (both in 21-40 years age group) were found among males. Similarly, *Klebsiella* spp. was found in females only where as 3 isolates of other Gram-negative ESBL producers were from the males (61-80 years old) (Table 4).

Table 3: Distribution of ESBL producers among OPD and IPD

Organism	**ESBL producers**		**Total ESBL producers**
	OPD (n=88)	**IPD (n=12)**	
E. coli	22 (66.7)	3 (75.0)	25 (67.5)
Klebsiella spp.	2 (6.0)	0 (0)	2 (5.4)
Citrobacter spp.	5 (15.2)	0 (0)	5 (13.5)
Pseudomonas spp.	1 (3.0)	0 (0)	1 (2.7)
Acinetobacter spp.	1 (3.0)	0 (0)	1 (2.7)
All other Gram-negative bacteria	2 (6.0)	1 (25.0)	3 (8.1)
Total	33	4	37

Table 4: Distribution of ESBL producers according to age and gender

Organisms	**Gender**	**Age (Years)**				
		≤20	**21-40**	**41-60**	**61-80**	**≥81**
E. coli	Male	0	1	1	2	2
	Female	1	12	3	3	0
Klebsiella spp.	Male	0	0	0	0	0
	Female		1	1	0	0
Citrobacter spp.	Male	0	1	0	0	1
	Female	1	0	0	1	1
Pseudomonas spp.	Male	0	1	0	0	0
	Female		0	0	0	0
Acinetobacter spp.	Male	0	1	0	0	0
	Female	0	0	0	0	0
All other Gram-negative bacteria	Male	0	0	1	2	0
	Female	0	0	0	0	0
Total		2	17	6	8	4

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IV. DISCUSSION

Out of 454 urine samples processed, 25% samples showed significant growth, similar to other reports shown in Nepal [14, 15]. Occurrence of Gram negative bacteria among the total uropathogen was found to be 86.9% which is in agreement with the other results reported [14].

MDR can also be defined as insensitivity or resistance of a microorganism to the administered antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to it [16]. In this study, 52% Gram-negative isolates were found to be MDR which is lower than reported by other researcher [17, 18]. Highest frequency of MDR isolates were *E. coli* (45.3%) which is lower in compared to the results from previous study in Nepal [18] while it is higher than reports from other study in Nepal [10] and in USA [19].

Non-fermenter MDR *Alcaligenes* spp. was isolated and *Acinetobacter* spp. showed complete resistance to Amikacin, Imipenem, Meropenem and Piperacillin where as Polymixin-B and Tigeycline found to be effective drug which is similar to the finding for the treatment of complicated UTIs [20].

ESBL producer were detected among the MDR Gram-negative bacteria (n=52) by screening and confirmative tests. Forty seven isolate were resistant to Ceftazidime that might be possible ESBL producers, on further analyzing 37(78.7%) were sensitive to Ceftazidime/Clavulanic acid, that is confirmed ESBL producers which is higher than the study carried out [18, 21]. ESBLs production among Gram-negative organisms was tested following CLSI (2018) recommendation. Among 37 isolates of possible ESBL producers, 33 were from OPD where as 4 isolate were from IPD. UTIs are the most common hospital-acquired infection accounting for almost 40% of all nosocomial infections [22]. The organisms harbored in the hospital environment are usually MDR capable of producing many antibiotic degrading enzymes.

ESBLs are a rapidly evolving group of β-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid, sulbactam, and which are encoded by genes that can be exchanged between bacteria. ESBLs work by antibiotic inactivation, target by group bypass, target modification, mutation and horizontal gene transfer [8]. Twenty five ESBL producing *E. coli* (67.5%) was the dominant isolate found in this study which have also been reported [18, 23] along with *Klebsiella* spp. (5.4%) and *Citrobacter* spp. (13.5%). Other Gram-negative bacteria: *Pseudomonas* spp. (2.7%), *Alcaligenes* spp. (2.7%), *Acinetobacter* spp. (2.7%) were observed which have also been reported [18]. ESBL producers are mostly isolated in the age group of 21-40 years of female patients except *Pseudomonas* spp. (21-40 years), *Proteus* spp. (61-80 years) and *Acinetobacter* spp. (21-40 years) which were isolated from males only.

Prevalence of multidrug resistance observed in this study may be due to the irrational and haphazard use of antibiotics. The limitations in this study included the lack of molecular test for the identification of uropathogens as well as for the confirmation of ESBL.

V. CONCLUSION

In the present study, the increasing pattern of the drug resistance seen among ESBL producers was seen. ESBL producing strains are creating significant therapeutic problems since these pathogens are resistant to a wide range of beta-lactams, including plasmid mediated Quinolone resistance. Hence, ESBL detection in routine laboratory practice is necessary to limit the rapid spread of ESBL producing MDR Gram-negative bacteria.

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REFERENCES

[1]. Mengistu A., Forster, N., Gaeseb, J., Habimana, G., Kauhondamwa, S., Uaaka, G., Ndjavera, C., Ugbaro, E., Indongo, L., & Evans Sagwa, E. (2014). A Review of Empirical Treatment of Urinary Tract Infections Based on National Antimicrobial Sensitivity Data. Enliven:Pharmacovigil Drug Saf1(1): 002.

[2]. Al-Badr, A., & Al-Shaikh, G. (2013). Recurrent urinary tract infections management in women: A review. Sultan Qaboos Uni Med J 13: 359–367.

[3]. Haque, R., Akter, M.L., & Salam, M.A. (2015). Prevalence and susceptibility of uropathogens: a recent report from a teaching hospital in Bangladesh. BMC Res Notes 8:416.

[4]. Yadav, K., Prakash, S., Serayi, R.C., Shilpkar, T., & Shrestha, S. (2014). Antimicrobial susceptibility test of pathogens isolated from urinary tract infection suspected cases. JMCJMS 2 (1): 28-34.

[5]. Flores-Mireles, A.L., Walker, J.N., Caparon, M. & Hultgren, S.J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol **13**:269-284.

[6]. Tajbakhsh, E., Ahmadi, P., Abedpour-Dehkordi, E., Arbab-Soleimani, N., & Khamesipour, F. (2016). Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrob Resist Infect Control 5: 11.

[7]. Cunha, M.A., Assunção, G.L., Medeiros, I.M., & Freitas, M.R. (2016). Antibiotic resistance patterns of urinary tract infections in a northeastern brazilian capital. Rev Inst Med Trop Sao Paulo 58: 2.

[8]. Thenmozhi, S., Moorthy, K., Sureshkumar, B.T., & Suresh, M. (2014). Antibiotic Resistance Mechanism of ESBL Producing Enterobacteriaceae in Clinical Field: A Review. Int J Pure App Biosci 2 (3): 207-226.
[9]. Queenan, A.M., Shang, W., Flamm, R., & Bush, K. (2010). Hydrolysis and inhibition profiles of beta-lactamases from molecular classes A to D with doripenem, imipenem, and meropenem. Antimicrob Agents Chemother 54(1): 565–569.
[10]. Baral, P., Neupane, S., Marasini, B., Ghimire, K., Lekhak, B., & Shrestha, B. (2012). High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Res Notes 5: 38.
[11]. Wali, N., Butt, T., Wali, U., & Hussain, Z. (2016). Fosfomycin versus Nitrofurantoin efficacy against multi- drug resistant gram negative urinary pathogens. J Rawal Med Coll 20: 265–268.
[12]. Seo, M.R., Kim, S.J., Kim, Y., Kim, J., Choi, T.Y., Kang, J.O., Wie, S.H., Ki, M., Cho, Y.K., Lim, S.K., Lee, J.S., Kwon, K.T., Lee, H., Cheong, H.J., Park, D.W., Ryu, S.Y., Chung, M.H., & Pai, H. (2014). Susceptibility of *Escherichia coli* from community-acquired urinary tract infection to Fosfomycin, Nitrofurantoin, and temocillin in Korea. J Korean Med Sci 29: 1178–1181.
[13]. Clinical and Laboratory Standards Institute (CLSI) (2018). Performance standards for Antimicrobial Susceptibility Testing. USA: CLSI: M100-S28 Wayne, PA.
[14]. Singh, V., Tuladhar, R., & Chaudhary, M. (2016). Beta Lactamase Producing *Escherichia coli*, *Klebsiella pneumoniae* and Methicillin Resistant *Staphylococcus aureus* among Uropathogens. Nepal Journal of Science and Technology, 16(1): 105-112.
[15]. Sherchan, J., Gurung, P., Karke, P., Ranabhat, N., Shrestha, N., & Ohara, H. (2016). Microbiological and Clinical Profile of Uropathogenic *Escherichia coli* Isolates in Kathmandu University Hospital. J Nepal Health Res Counc 14(32): 33-8.
[16]. Tanwar, J., Das, S., Fatima, Z., & Hameed, S. (2014). Multidrug Resistance: An Emerging Crisis. Interdisciplinary perspectives on infectious diseases. Hindawi 541340: 1-7.
[17]. Poudyal, S. (2010). Prevalence of β-lactamase producing multidrug resistant bacterial pathogens isolated from different clinical samples at National Public Health Laboratory. M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University 5-74.
[18]. Shrestha, A., Manandhar, S., Pokharel, P., Panthi, P., & Chaudhary, K.D. (2016). Prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing multidrug resistance gram-negative isolates causing urinary tract infection. EC Microbiol 4: 749-755.
[19]. Khawcharoenporn, T., Vasoo, S., & Singh, K. (2013). Urinary tract infections due to multidrug-resistant enterobacteriaceae: Prevalence and risk factors in a Chicago Emergency Department. Emerg Med Int page 1-7.
[20]. Taneja, N., Singh, G., Singh, M., & Sharma, M. (2011). Emergence of tigecycline & colistin resistant *Acinetobacter baumanii* in patients with complicated urinary tract infections in north India. Indian J Med Res 133(6): 681-4.
[21]. Sultan, A., Rizvi, M., Khan, F., Sami, H., Shukla, I., & Khan, H. (2015). Increasing antimicrobial resistance among uropathogens: Is Fosfomycin the answer? Urol Ann 7: 26.
[22]. Saint, S., Kowalski, C.P., Kaufman, S.R., Hofer, T.P., Kauffman, C.A., Olmsted, R.N., Forman, J., Banaszak-Holl, J., Damschroder, L., & Krein, SL. (2008). Preventing hospital-acquired urinary tract infection in the United States: A National Study. Clin Infect Dis 46: 243–250.
[23]. Yadav, K., & Prakash, S. (2017). Screening of ESBL Producing Multidrug Resistant *E. coli* from Urinary Tract Infection Suspected Cases in Southern Terai of Nepal. J Infect Dis Diagn 2: 116.