

Prevalence of Extended Spectrum Beta Lactamases (ESBL) and Metallo Beta Lactamases (MBL) Mediated Resistance in Gram Negative Bacterial Pathogens

Pramila Pathak¹, Nandalal Jaishi², Binod Kumar Yadav², Pradeep Kumar Shah¹

¹Department of Microbiology, Tri-Chandra Multiple Campus, Ghantaghar, Kathmandu,

²Department of Microbiology, Shahid Gangalal National Heart Center, Bansbari, Kathmandu, Nepal

* **Corresponding author:** Pradeep Kumar Shah, Department of Microbiology, Tri-Chandra Multiple College, Ghantaghar, Kathmandu, Email: pkshah210@gmail.com

ABSTRACT

Objectives: This study was conducted to determine the prevalence of multi-drug resistance (MDR) along with Extended Spectrum β -lactamase (ESBL) and Metallo β -lactamase (MBL) producing gram negative bacterial isolates among the patients attending Shahid Gangalal National Heart Centre, Kathmandu, Nepal.

Methods: This cross-sectional study was carried out from June to December; 2016. Altogether 977 clinical specimens were processed for analysis of bacteriological profile and the isolates were identified by culture, morphological and biochemical tests. Antibiotic susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion methods following Clinical and Laboratories Standard Institute guideline and the isolates were tested for ESBL and MBL by combined disk method.

Results: out of 977 clinical specimens, 254 (25.99%) were found to be gram negative bacterial isolates, among them *Klebsiella pneumoniae* 83 (32.67%) was the most predominant organism followed by *E. coli* 51 (20.07%), *Pseudomonas aeruginosa* 36 (14.17%), *K. oxytoca* 32 (12.59%), *Proteus mirabilis* 13 (5.11%) and *P. vulgaris* 13 (5.11%), *Acinetobacter* spp. 11 (4.33%), *Citrobacter* spp. 10 (3.93%) and *Enterobacter* spp. 5 (1.96%) respectively. 83 (32.67%) isolates were found to be MDR, 38(14.96%) were positive for ESBL while 19 (7.48%) were MBL producer.

Conclusion: The deterrent drug resistance among ESBL and MBL producers, reflect the extensive use of antibiotics possessing difficulties in therapeutic potions in hospital setting which might be overcome by proper microbiological analysis of pathogenic isolates and judicious use of antibiotics for emergence of resistance strains.

Key words: Clinical Specimen, Gram negative bacteria, MDR, ESBL, MBL

INTRODUCTION

Antibiotics have been critical in the fight against infections caused by bacteria and other microbes. The most common and important mechanism of resistance in Gram-negative bacteria is production of β -lactamases. Antibiotic resistance is particularly rising because of inappropriate use of antibiotics in human medicine (Tiersma 2013). Multidrug resistance has been increasing among Gram negative bacteria (Bush 2010). Beta- Lactamases are enzymes produced by some bacteria that hydrolyze the amide bond of the four-membered characteristic β -lactam ring (Peshattiwar

and Peerapur 2011) of β -lactam antibiotics (penicillin, cephalosporin, monobactams, and carbapenems) (Noyal et al. 2009), result in an inactive product when the ring is broken (Simoens et al. 2006). In recent years, there has been an increase in incidence and prevalence of ESBL producing microbial diseases (Thokar et al. 2010). More than 200 types of extended spectrum β -lactamases (ESBLs) have been found worldwide, most belonging to the Enterobacteriaceae family (Yazdi et al. 2012). MBLs have been globally isolated from various bacteria and more than 80 types of MBLs have been identified worldwide, with over 75% occurring

as plasmid encoded enzymes (Bush and Jacoby 2010). Early detection of MBL and ESBL producing organisms is crucial to establish appropriate antimicrobial therapy and to prevent their interhospital and intrahospital dissemination (Pandey et al. 2011). Thus, the present study was conducted with an objective to find out the presence of ESBL and MBL producing gram negative bacterial isolates and multidrug resistant strains to formulate effective antibiotic strategy on the basis of the local epidemiological data to control infection and to prevent the spread of these strains.

MATERIALS AND METHODS

A total of 977 clinical specimens including blood, urine, sputum, pus, ET tip secretion, CVP tip, suction tube, Foley's tip, pericardial fluid, body fluid (peritoneal fluid), throat swab, pleural fluid, wound swab, mitral valve vegetation, mediastinal drain culture, aortic valve, tracheal aspirate was collected in a clean, leak-proof sterile container during June, 2016 to December, 2016 from the cardiac patients attending Shahid Gangalal National Heart Center, Bansbari, Kathmandu, Nepal.

The specimens were subjected for routine culture and susceptibility testing in the microbiology department. For blood sample 3-5 ml of blood was inoculated into brain-heart infusion broth and incubated at 37°C and sub-cultures were made on blood agar (BA) and McConkey agar (MA) after 24, 48 and 72 hours. BHI broth was incubated aerobically for upto 7 days at 37 °C whereas others samples were inoculated onto BA and MA plates. The plates were incubated at 37 °C for 24 hrs. The significant bacterial isolates were identified

on the basis of morphology, gram staining & various biochemical tests. Antibiotic susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method following Clinical and Laboratories Standard Institute (CLSI) guideline and they were tested for ESBL and MBL by combined disk method. *K. pneumoniae* ATCC 700603 positive controls for ESBL and *P. aeruginosa* PA 105663 positive control for MBL were used. Statistical analysis was done by using SPSS version 16. Frequency and percentages were calculated and Chi-square test was done whenever applicable with $P < 0.05$ regarded as significant.

RESULTS

Among the total 977 clinical specimens for culture, 295 (30.19%) showed significant bacterial growth of which 254 (25.99%) were gram negative bacteria while 41 (4.19%) were gram positive bacteria. Out of 254 gram negative bacterial isolates, 123 (48.42%) were from the samples of female and 131 (51.57%) were from male patients. Likewise, 52 (5.32%) were from outpatients and remaining 202 (20.67%) were from inpatients. However, there was significant difference in bacterial growth between the samples of outpatients and inpatients (P value = 27.16). The age of the patients ranged from 49 days to 87 years. The highest percentage of bacterial growth 7 (18.50%) was obtained in samples of age group <10yrs, followed by 44 (17.32%) in age group 51-60yrs, whereas least 6 (2.36%) was from age group 81-90 yrs. Highest percentage of growth was found to be in samples of male 11 (4.33%) in age group 51-60 yrs. whereas for Female, highest % of growth 14 (5.51%) was in age group 21-30 yrs.

Table 1: Distribution of clinical specimens

Clinical samples	Gender		In patients		Out patients		Significant growth		Total No.
	Female	Male	No.	%	No.	%	No.	%	
Blood	96	166	144	54.96	118	45.03	22	8.39	262
Body Fluid	1	8	7	77.78	2	22.22	2	22.22	9
CVP tip	8	19	27	100	0	0	10	37.03	27
ET Tip	26	55	62	76.54	19	23.45	40	49.38	81
Foley Tip	3	9	11	91.66	1	8.33	8	66.67	12
Others	19	35	43	79.62	11	20.38	11	20.37	54
Pericardial fluid	15	22	29	78.37	8	21.62	7	18.91	37
Pus	32	15	35	74.46	12	25.53	13	27.65	47
Sputum	44	40	48	57.14	36	42.85	37	44.04	84
Suction Tip	6	10	14	87.5	2	12.5	10	62.5	16
Throat Swab	5	6	8	72.73	3	27.27	2	18.18	11
Urine	189	138	211	64.52	116	35.47	92	28.13	327
Total	447 (45.75)	530 (54.24)	647 (66.22)		330 (33.77)		254 (25.99)		977

In this study, out of 7 different bacterial genera isolated, *Klebsiella pneumoniae* 83 (32.67%) was being the most predominant whereas *Enterobacter* spp. was being least 5 (1.96%). *Pseudomonas aeruginosa* was most predominant

from indoor patients 34 (94.44%) while *Citrobacter* spp. was being least 4 (40%). Similarly, from outpatient, *Citrobacter* spp. was most predominant 6 (60%) whereas *P. aeruginosa* showed least growth 2 (5.56%).

Table 2: distribution pattern of gram negative bacterial isolates from various clinical samples

Clinical samples	<i>Acinetobacter</i>	<i>Citrobacter</i>	<i>Enterobacter</i>	<i>E. coli</i>	<i>Klebsiella</i>		<i>Proteus</i>		<i>P. aeruginosa</i>	Total	
					<i>oxytoca</i>	<i>pneumoniae</i>	<i>mirabilis</i>	<i>vulgaris</i>		No.	%
Blood	0	0	3	5	3	6	0	1	4	22	8.66
Body fluid	0	0	0	0	1	0	0	0	1	2	0.78
CVP tip	1	0	1	2	1	4	0	0	1	10	3.93
ET tips	2	1	0	8	3	11	2	1	12	40	15.74
Foley's tip	0	0	0	1	2	2	0	0	3	8	3.14
Others	0	1	0	1	2	4	2	0	1	11	4.33
Pericardial fluid	1	0	0	1	0	1	0	0	4	7	2.75
Pus	0	1	0	1	5	4	1	1	0	13	5.11
Sputum	0	0	0	2	1	24	2	1	7	37	14.56
Suction tip	2	1	0	1	0	2	3	0	1	10	3.93
Throat swab	0	0	0	1	0	1	0	0	0	2	0.78
Urine	5	6	1	28	14	24	3	9	2	92	36.22
Total	11	10	5	51	32	83	13	13	36	254	100
	4.3	3.9	1.9	20.1	12.6	32.7	5.1	5.1	14.2		100

The most effective drug for gram negative pathogenic bacteria was nitrofurantoin followed by Gentamicin, ciprofloxacin and cotrimoxazole. The highest MDR

strain was found to be *Acinetobacter* spp. 6 (54.54%) whereas *Enterobacter* spp. (0%) did not show any MDR pattern.

Table 3: Antibiotic resistance pattern of gram negative bacterial isolates

Bacterial isolates / total no.	Antibiotics (% Resistance)						
	Amp	Caz	Cipro	Cotri	Genta	Nali	Nitro
<i>Acinetobacter</i> (11)	7 (63.63)	8 (72.73)	2 (18.18)	4 (36.36)	3 (27.27)	7 (63.63)	0 (0)
<i>Citrobacter</i> (10)	7 (70.0)	6 (60.0)	3 (30.0)	4 (40.0)	3 (30.0)	6 (60.0)	0 (0)
<i>Enterobacter</i> (5)	4 (80.0)	1 (20.0)	2 (40.0)	0 (0)	0 (0)	3 (60.0)	0 (0)
<i>E. coli</i> (n=51)	43 (84.31)	36 (70.58)	21 (41.17)	21 (41.17)	17 (17.64)	31 (60.78)	4 (14.28)
<i>K. oxytoca</i> (32)	23 (71.87)	14 (63.85)	11 (34.37)	10 (31.25)	9 (28.12)	27 (84.37)	0 (0)
<i>K. pneumoniae</i> (83)	68 (79.06)	53 (63.85)	17 (20.48)	30 (36.14)	13 (15.66)	48 (57.83)	2 (8.33)
<i>P. mirabilis</i> (13)	5 (38.46)	6 (46.15)	2 (15.38)	4 (30.76)	2 (15.38)	4 (30.76)	0 (0)
<i>P. vulgaris</i> (13)	4 (30.76)	8 (61.53)	5 (38.46)	5 (38.46)	8 (61.53)	8 (61.53)	1 (11.11)
<i>P. aeruginosa</i> (36)	29 (80.56)	26 (72.22)	14 (38.89)	9 (25.0)	10 (27.78)	27 (75.0)	0 (0)
Total (254)	190 (74.48)	158 (62.2)	77 (30.31)	87 (34.25)	65 (25.59)	161 (63.38)	7 (2.75)

Table 4: Distribution of MDR, ESBL and MBL producer among gram negative bacterial isolates

Bacterial isolates	No.	MDR strains No. (%)	ESBL producer No. (%)	MBL producers No. (%)
<i>Acinetobacter</i>	11	6 (54.54%)	0	1 (9.09)
<i>Citrobacter</i>	10	3 (30%)	2 (20%)	0
<i>Enterobacter</i>	5	0	0	0
<i>E. coli</i>	51	19 (37.25%)	6 (54.54%)	2 (3.92%)
<i>K. oxytoca</i>	32	7 (21.87%)	5 (15.62%)	0
<i>K. pneumonia</i>	83	29 (34.93%)	13 (15.66%)	7 (8.43%)
<i>P. mirabilis</i>	13	2 (15.38%)	1 (7.69%)	0
<i>P. vulgaris</i>	13	1 (7.69%)	1 (7.69%)	0
<i>P. aeruginosa</i>	36	16 (44.44%)	4 (11.11%)	9 (25%)
Total	254	83 (32.67%)	38 (14.96%)	19 (7.48%)

The highest no. of ESBL producer was isolated from ET Tip 10 (25%) and least from pus 1 (7.69%). Similarly, for MBL producers, it was throat swab 1 (50%) and least from urine 3 (3.26%). The Antibiotic susceptibility pattern of ESBL producers revealed that, they were 100% resistant against ampicillin and ceftazidime but sensitive towards nitrofurantoin (100%) followed by imipenem (81.58%), amikacin (73.7%), ofloxacin (63.2%) gentamicin (57.9%) and cotrimoxazole (50%). MBL producers showed resistance towards most of the drug used and were sensitive towards polymyxin.

DISCUSSION

This study was aimed to examine the status of MDR among different gram negative bacterial pathogens and underlying production of ESBLs and MBLs. The highest percentage of sample obtained was urine (33.46%) whereas body fluid i.e. peritoneal fluid (0.92%) was found in a least percentage which was correlated with the study done where higher percent of sample collected was urine (Sherchan et al. 2016). The frequency of growth pattern among individual specimens were highest from Foley tips (66.67%) similar to the study done by Sherchan et al. 2016 which shows 61.11% from catheter, whereas least growth obtained from blood sample (8.39%), a similar study conducted in western Nepal in 2007 showed the isolation rate from blood was 10.28% (Easow et al. 2010) and a study by (Vanitha et al. 2012) also showed 8.39%. This least growth from blood sample might be due to patient may have already taken antibiotics or he or she may not have systemic infection so far. and high % of urinary catheter use is a risk factor for inoculation of bacteria into bladder. No significant difference in growth number between male and female patients revealed that both of them have equal chance of having heart diseases. *Enterobacter* spp.

were least isolates to cause infection predominated from blood sample whereas *E. coli* (20.07%) was second predominant organism from urine after *Klebsiella pneumonia* (32.67%) from urine and sputum sample contrast to a study (Chander and Shrestha 2013), reported low prevalence of *K. pneumoniae* from urine sample. More isolation rate of *K. pneumoniae* due to contamination of invasive devices, weakened immune system, nosocomial infection. Whereas *E. coli* was predominant in urine sample, resembled the study done by various others workers viz: (Shrestha et al. 2012), (Mishra et al. 2012) and (Manandhar et al. 2006) in Nepal. This high incidence of the *E. coli* is a commensal of the bowl and infection due to poor hygiene and anatomy proximity to the genito-urinary area. Highest percentage of resistance towards first line antibiotic ampicillin, Nalidixic acid, and ceftazidime with considerable resistivity to ampicillin (74.48%) which was found to be congruous with the study done by (Bhatt et al. 2012).

In this study, no MDR strains were observed in *Enterobacter* spp. while highest number of MDR was found in *Acinetobacter* spp. (54.54%) whereas lowest from *P. vulgaris* (7.69%). These results were less than outcomes of previous studies (Karn et al. 2016) which showed 42.91% were MDR, and *Acinetobacter* spp. account for 60%. This is due to drugs are easily available without doctor's prescription from pharmacy and in developing countries like Nepal self-medication is a common practice. ESBLs were predominantly present among *E. coli* (23.52%) followed by *Citrobacter* spp. similar to the study done by Pokhrel et al. in which 16.0% isolates were found to be ESBL producing with *E. coli* being the predominant one (11.60%) (Pokharel et al. 2006) and also study done by (Ahmed et al. 2014) in which 18.95% were

ESBL producers. 3rd generation Cephalosporins were combined with β -lactamases inhibitor Clavulanic acid (i.e. CTX30 + Clav10 and CAZ 30+ Clav10), structural analog of β -lactamase and antibiotics inhibits the action of β -lactamases and antibiotic can act on the cell wall of the bacteria, result confirmed by at least or more than 5mm increase in zone of inhibition than Cephalosporins alone (Rawat and Nair 2010). *K. pneumoniae* responsible for ESBL and MBL production resulting a potential threat to hospitalized patients by limiting therapeutic option (Bora et al. 2014). ESBLs are mostly produced by *E. coli* and *Klebsiella* spp. but may also occur in other Gram-negative bacteria including *Citrobacter* spp., *Morganella* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., *Serratia* spp. and *Shigella* spp. (Akujobi and Ewuru 2010). A (Dalela 2012) study showed that imipenem is the most active drug for the treatment of infections which are caused by ESBL producers, followed by Amikacin which is similar to our finding. 7.48% isolates were found to be MBL producers which is higher than Mishra et al. 2012 (1.3%) and lesser than Haider et al. 2014 (17.93%) highest MBL producer being *Pseudomonas aeruginosa* (Mishra et al. 2012) and (Haider et al. 2014). And similar to previous study conducted at SGNHC which showed 8.4% were MBL producers (Chaudhary et al. 2016). Our findings of *P. aeruginosa* (25%) as the most common MBLs producer followed by *Acinetobacter* spp., *K. pneumoniae* and *E. coli* being least MBL producer which is exactly similar with Kamble 2015 (Kamble 2015) in which *P. aeruginosa* (23.62%), higher than study done in India by Agrawal 2008 which showed only 8.05% (Agrawal et al. 2008). It is a nosocomial pathogen of particular clinical concern not only because of its extraordinary resistance mechanisms but also for its formidable ability to adept very well to the hospital environment.

CONCLUSION

The prevalence of Gram negative bacteria based upon observed in 977 clinical samples was found to be 25.99% and an antibiogram, revealed the presence of ESBL and MBL enzyme in multidrug resistant gram-negative isolates which reflects the extensive use of antibiotics for the treatment in hospitals. Therefore, proper identification of isolates using microbiological tools should be undertaken. These types of study could help to estimate and to employ effective antimicrobial strategy so that the emergence of resistant strains could be reduced.

ACKNOWLEDGEMENTS

We would like to thank all the patients whose samples were used in the study, all the staff of Shahid Gangalal National Heart Centre, all the faculties and Staffs of Department of Microbiology, Tri-Chandra Multiple Campus.

REFERENCES

- Agrawal P, Ghosh AN, Kumar S, Basu B and Kapila K (2008) Prevalence of extended-spectrum beta-lactamases among *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital. *Indian J Pathol Microbiol* **51**(1): 139-42.
- Ahmed SM, Rajeevan S, Jasmin P T and Shakir V A (2014) Detection of ESBL among the gram negative uropathogens and their antibiotic resistance pattern in a rural medical college hospital North Kerala, India. *Int J Curr Microbiol App Sci* **3**(2): 561-567.
- Akujobi CN and Ewuru CP (2010) Detection of extended spectrum beta-lactamases in gram negative bacilli from clinical specimens in a teaching hospital in South eastern Nigeria. *Niger Med J* **51**(4): 141-146.
- Bhatt C P, Shrestha B, Khadka S, Swar S, Shah B and Pun K (2012) Etiology of Urinary tract infection and drug resistance cases of uropathogens. *Journal of Kathmandu Medical College* **1**(2): 114-120.
- Bora A, Sanjana R, Jha BK, Mahaseth SN and Pokhrel K (2014) Incidence of metallo-beta-lactamase producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in central Nepal. *BMC Research Notes* **7**: 557.
- Bush K (2010) Bench-to-bedside review: The role of beta-lactamases in antibiotic-resistant Gram-negative infections. *Critical Care* **14**(3): 224.
- Bush K and Jacoby GA (2010) Updated functional classification of β -lactamases. *Antimicrob Agents Chemother* **54**: 969-976.
- Chander A and Shrestha CD (2013) Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu, Nepal. *BMC Research Notes* **6**: 487.
- Chaudhary AK, Bhandari D, Amatya J, Chaudhary P and Acharya B (2016) Metallo-Beta-Lactamase Producing Gram-Negative Bacteria among

- Patients Visiting Shahid Gangalal National Heart Centre. *Austin J Microbiol* **2**(1):1010.
- Dalela G (2012) Prevalence of extended spectrum beta lactamase (ESBL) producers among Gram negative bacilli from various clinical isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. *Journal of Clinical and Diagnostic Research* **6**(2): 182-187.
- Easow MJ, Joseph MN, Dhungel AB, Chapagain B and Shivananda PG (2010) Blood steams infections among fabrile patient attending a teaching hospital in western regions, Nepal. *AMJ* **3**: 633-37.
- Haider M, Riziv M, Fatima N, Shukla I and Malik A (2014) Necessity of detection of extended spectrum beta-lactamase, AmpC and metallo-beta-lactamases in Gram-negative bacteria isolated from clinical specimens. *Muller J Med Sci Res* **5**(1): 23-8.
- Kamble DS (2015) Phenotypic detection of ESBL and MBL in Gram negative bacilli isolated from clinical specimens. *Int J Med Res Rev* **3**(8): 866-870.
- Karn S, Pant ND, Neupane S, Khatiwada S, Basnyat S and Shrestha B (2016) Prevalence of carbapenem resistant bacterial strains isolated from different clinical samples: study from a tertiary care hospital in Kathmandu, Nepal. *Journal of Biomedical Sciences* **3**(1): 11-15.
- Manandhar T, Koirala J, Pokhrel BM and Ghimire P (2006) Status of extended spectrum beta lactamase producing. *Journal of Institute of Medicine* **28**(2): 24-29.
- Mishra SK, Acharya J, Kattel HP, Koirala J, Rijal BP and Pokhrel BM (2012) Metallo-beta-lactamase producing gram-negative bacterial isolates. *J Nepal Health Res Counc* **10**(22): 208-13.
- Noyal MJC, Menezes GA, Harish BN, Sujatha S and Parija SC (2009) Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. *Indian J Med Res* **129**(6): 707-12
- Pandey N, Prajapati SB, Mehta SJ, Kikani KM and Joshi PJ (2011) Evaluation of various methods for detection of metallo- β -lactamase production in gram negative bacilli. *Int J Biol Med Res* **2**(3): 775-777.
- Peshattiwar PD and Peerapur BV (2011) ESBL and MBL Mediated Resistance in *Pseudomonas aeruginosa*: An Emerging threat to clinical therapeutics. *Journal of Clinical and Diagnostic Research* **5**(8): 1552 - 1554.
- Pokharel BM, Koirala J, Mishra SK, Dahal RK, Khadga P and Tuladhar NR (2006) Multidrug-resistant and extended-spectrum beta-lactamase producing strain causing lower respiratory tract and urinary tract infections. *Journal of Institute of Medicine* **28**(3): 19-27.
- Rawat D and Nair D (2010) Extended-spectrum β -lactamases in Gram negative bacteria. *J Glob Infect Dis* **2**(3): 263-274.
- Sherchan S, Shrestha D, Gurung K, Sherchand SP, Manandhar S, Shrestha B (2016) Prevalence of multidrug resistant extended-spectrum β -lactamase-producing. *EC Microbiology* **4**(2): 676-698.
- Shrestha B, Gurubacharya RL, Maharjan B and Shrestha S (2012) Multi drug resistant pathogens causing urinary tract infections in children at Kathmandu Model Hospital. *J Nepal Paediatric Soc* **32**(3): 233-238.
- Simoens S, De Corte N and Laekeman G (2006) Clinical practice and costs of treating catheter-related infections with teicoplanin or vancomycin. *Pharmacy Practice* **4**(2): 68-73.
- Thokar MA, Fomda BA, Maroof P, Ahmed K, Bashir D and Bashir G (2010) Proliferation of extended spectrum β -lactamase (ESBL) producing Gram negative bacteria, diagnostic inputs and impact on selection of antimicrobial therapy. *Physician Academy* **4**(3): 25-31.
- Tiersma Y (2013) β -Lactamase-producing bacteria How can I resist you?, M.Sc. dissertation submitted to department of Biology Science Utrecht University.
- Vanitha RN, Kannan G, Venkata NM, Vishwakanth D, Nagesh VD and Yogitha M (2012) A retrospective study on blood stream infections and antibiotic susceptibility patterns in a tertiary care teaching hospital. *Int J Pharm Pharm Sci* **4**: 543-48.
- Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M and Sharifi SH (2012) Genotypic versus Phenotypic methods to detect Extended-Spectrum Betalactamases (ESBLs) in Uropathogenic *Escherichia coli*. *Annals of Biological Research* **3**(5): 2454-2458.