Original article

Co-existence of aminoglycosides and β-lactam-resistant *Escherichia coli* phenotypes in a Tertiary care center of Nepal

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Abstract

Introduction: Multidrug-resistant *Escherichia coli* isolates conferring simultaneous resistance to both aminoglycosides and β-lactam drugs have serious implications for clinicians worldwide. This study was designed to evaluate the co-existence of various β-lactamases in aminoglycoside-resistant *Escherichia coli* amongst hospitalized subjects in a tertiary care center of Kathmandu, Nepal, between December 2013 and December 2014.

Methods: Standard microbiological techniques were used for isolation and identification of the isolates. The antimicrobial susceptibility of bacterial isolates was determined following Clinical and Laboratory Standard Institute recommended Kirby-Bauer Disc Diffusion method. The defining criterion in this study for an isolate to be MDR, resistance to at least one agent in three or more than three different structural classes was taken.

Results: Among 302 MDR *E. coli* isolates, 174 (58.0 %) were resistance to gentamicin and 138 (46.0 %) were resistance to amikacin. Maximum aminoglycoside-resistant 9/11(82.0%) strains were isolated from body fluids followed by 7/10 (70.0%) from bile, 6/9 (67.0%) from blood and 2/3 (67.0%) from tissue. Out of 174 aminoglycosides-resistant *E. coli* isolates, the simultaneous occurrence of Extended-spectrum- b- lactamase (ESBL) and AmpC β–lactamaseswas noted in 13.0 % isolates and Metallo-β-lactamase (MBL) and AmpC β–lactamasesin 8.0 % isolates. None *E. coli* isolates were positive for all 3 types of β–lactamases in combinations. In amikacin-resistant isolates, ESBL+ AmpC observed in 12% and MBL+AmpC seen in 10% isolates.

Conclusion: Our results show a high frequency of aminoglycoside- resistance phenotypes. Strict application for appropriate use of antimicrobials in medical settings should be essential to minimize the emergence of multidrug-resistance among *E. coli* in hospitalized patients.

Keywords: Aminoglycoside-resistant *E. coli*, Amp C β–lactamases, Metallo-β-lactamase, MDR *E.coli*

Introduction

An increase in the emergence of multidrug-resistant (MDR) bacteria in recent years is a leading public health problem. To combat antibiotic resistance; a broad array of potent antibiotics is available to present-day clinicians. However, growing problems with antimicrobial drug resistance are beginning to erode our antibiotic armamentarium.[1] The β-lactam antibiotics, in combination with aminoglycosides, are among the most widely prescribed antibiotics and are important components of empirical therapy. Because of extensive and unnecessary use in developing countries, resistance to these drugs has become a major problem

especially after the introduction of newer broad-spectrum cephalosporins, β-lactamase inhibitor/β-lactam antibiotics, monobactams, and carbapenems.[2] A major feature in the emergence of multidrug-resistant Gram-negative bacilli is the production of various β-lactamases including AmpC β-lactamases,extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLS) along with enzymatic modification of aminoglycosides, which are responsible for resistance to β-lactam antibiotics and aminoglycosides, respectively.[3]

Escherichia coli is usually a commensal bacterium of humans. Pathogenic variants cause intestinal and extraintestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia.[4]Surveillance data show that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine.[5]The past 2 decades have witnessed major increases in emergence and spread of MDR bacteria and increasing resistance to newer compounds, such as fluoroquinolones and certain cephalosporins.[6]Infections with MDR bacteria are difficult, and in some cases impossible to treat and have been associated with mortality rates up to 50%.[7]To our knowledge, status of phenotypic co-existence of aminoglycoside and β-lactam-resistant *E. coli* isolates is not assessed in Nepal. To better understand the current status of co-existence of aminoglycosides and β-lactam resistant *E. coli* isolates among hospitalized patients, we conducted this prospective study at a tertiary care center of Kathmandu, Nepal.

Methods

Participants and clinical isolates: A cross-sectional study was conducted prospectively during the period from December 2013 to December 2014. Total of 302 MDR *E. coli* isolates were recovered from various clinical samples such as urine (n=149), pus (n=75), sputum (n=44), blood (n=9), body fluid (n=11), bile (n=10), tissue (n=3) central venous pressure (CVP) line (n=1), among hospitalized patients of Tribhuvan University Teaching Hospitalwere included in the study after obtaining approval from institution review board. Details of antibiotics used and clinical outcome of patients were collected. Samples were processed immediately using standard microbiological procedures as described by American Society for Microbiology (ASM).[8] Isolates were identified based on colony morphology on Blood agar, MacConkey agar, Gram reaction and by standard biochemical tests.[9]

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done by the modified Kirby-Bauer disk diffusion method in accordance with CLSI guidelines.[10]The antibiotic discs used were amikacin (30 µg), amoxycillin (10 µg), amoxycillin/ clavulanic acid (20/10 µg), cefepime (30µg), cefoperazone /sulbactam (75/30µg) ceftazidime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), chloramphenicol (30µg), ciprofloxacin (5 µg), colistin sulphate (10 µg), doxycycline (30µg), levofloxacin (5 µg), gentamicin (10 µg), imipenem(10 µg), meropenem (10 µg), nitrofurantoin (300 µg), ofloxacin (5µg), piperacillin-tazobactam (100/10 µg), polymyxin- B (300 units) and tigecycline(5 µg).

Screening for MDR *E. coli* isolates

Multidrug-resistant (MDR)*E.coli*were determined as the isolates of *E.coli* resistant to at least three classes of antimicrobial agents-all penicillins and cephalosporins (including inhibitor combinations), aminoglycosides, cephamycins, fluoroquinolones, folate pathway inhibitors, glycylcyclines, phenicol, polymyxins andtetracyclines.[11]

Screening test for ESBL productions

The isolates showing zone of inhibition (ZOI) of ≤22 mm for ceftazidime (CAZ) (30 µg), ≤27 mm for cefotaxime (CTX) (30 µg), and ≤25 mm for ceftriaxone (CRO) (30 µg) were considered as potential ESBL-producer and were phenotypically confirmed for ESBL production.[10]

Phenotypic confirmation for ESBL production

The potential ESBL producer isolates were tested by CLSI phenotypic confirmatory test of combined disc assay method. Briefly,CAZ (30 µg) and CTX (30 µg) disks alone and in combination with clavulanic acid (10 µg) were placed 25 mm apart. An increase of ≥5 mm in ZOI for ceftazidime-clavulanic acid (30/10 µg) and cefotaxime-clavulanic acid (30/10 µg) compared to CAZ and CTX alone was confirmed as ESBLproducers.[10]*Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Screening for carbapenemase production

E. coli isolates that showed the zone of inhibition ≤19 mm against meropenem and imipenem were suspected as carbapenemase producers.[10]

Phenotypic confirmation for metallo-β-lactamase production

All carbapenemase producers *E. coli* were phenotypically confirmed for Metallo-β-lactamase (MBL) production. MBLin carbapenemase producing strains were detected as described by Franklin et al.[12]In the combined-disk test, two IPM disks (10 µg), one containing 10 µl of 0.1 M (292 µg) anhydrous EDTA (Sigma Chemicals, St. Louis, MO), were placed 25 mm apart (center to center),on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37°C. An increase in zone diameter of >4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for MBL production.*P. aeruginosa* ATCC 27853 was used as a negative control strain.

Screening for AmpC production

A 30-µg cefoxitin disk was placed on inoculated Mueller-Hinton agar. The isolates with zone diameters less than 18 mm were suspected as AmpC producers[13]

Phenotypic confirmationfor AmpC production

The AmpC producer suspected isolates were phenotypically confirmedas described by Yagi et al [14]. Discs containing boronic acid were prepared as follows: 120 mg phenylboronic acid (Sigma chemicals) was dissolved in 3ml of dimethyl sulfoxide. Three milliliters of sterile distilled water was added to this solution. Twenty microliters of the stock solution was dispensed onto disks containing 30 µg of cefoxitin. Discs were dried for 30 minutes and used immediately. Remaining discs were stored in airtight vials with desiccant at 4°C. The boronic acid disc test was performed by placing a disc containing 30 µg of cefoxitin and a disc containing 30 µg of cefoxitin with 400 µg of boronic acid onto Mueller- Hinton agar which was inoculated with AmpC producer suspected isolate. Inoculated plates were incubated overnight at 35°C. An organism that demonstrated a zone diameter around the disc containing cefoxitin and boronic acid that was 5 mm or greater than the zone diameter around the disc containing cefoxitin was considered as an AmpC producer. *E. coli* ATCC 25922 was used as a negative control strain.

Results

A total 302 specimens with MDR *E. coli* infection were included in this study. These included 149(49.0%) urine, 75 (25.0 %) pus, 44 (15.0%) sputum, 9 (3.0 %) blood, 10 (3.3%) bile, 11 (3.6 %) body fluid, 3 (0.9%) tissues, and 1(0.3%) CVP line (Table 1).

Table 1. Specimen wise distribution of MDR *E. coli* isolates

Specimen	No	Percent (%)
Urine	149	49.0
Pus	75	25.0
Sputum	44	15.0
Body fluid	11	3.6
Bile	10	3.3
Blood	9	2.9
Tissue	3	0.9
CVP line	1	0.3
Total	302	100.0

A total of 302 MDR *E. coli* isolates were analyzed for resistance ability and were found to resist most of the antimicrobial agents. Among first line antibiotics, MDR isolates were found to be 100% resistant toward amoxycillin, ceftriaxone, cefotaxime, cefixime, ciprofloxacin and cotrimoxazole. 76% (n=230) of isolates were resistant to levofloxacin. Likewise, among second line of antibiotics, 100.0% of isolates were resistant to ceftazidime and ceftriaxone followed by amoxycillin/clavulanic acid (77.0%), cefoperazone/ sulbactam (70.0%), piperacillin/tazobactam (61.0%) and doxycycline (56.0%). Colistin sulphate, polymyxin-B and tigecycline were 100% effective followed by nitrofurantoin (95.0%), imipenem (87.0%) and meropenem (86.0%) indicating these as the most potent antimicrobials (Table 2).

Table 2:Antibiotic susceptibility pattern of MDR *E. coli* isolates with first and second line antibiotics

Antibiotics	Sensitive		Resistant	
	No	%	No	%
First line antibiotics				
Amoxycillin	0	0.0	302	100.0
Cefixime	0	0.0	302	100.0
Cefotaxime	0	0.0	302	100.0
Ciprofloxacin	0	0.0	302	100.0
Cotrimoxazole	0	0.0	302	100.0
Levofloxacin	72	24.0	230	76.0
*Nitrofurantoin	132	95.0	17	5.0
*Norfloxacin	0	0.0	149	100.0
Ofloxacin	0	0.0	302	100.0
Second line antibiotics				
Amikacin	164	54.0	138	46.0
Amoxycillin/clavulanic acid	68	23.0	234	77.0
Ceftazidime	0	0.0	302	100.0
Ceftriaxone	0	0.0	302	100.0
Cefoperazone/sulbactam	90	30.0	212	70.0
**Chloramphenicol	83	54.0	70	46.0
Colistin sulphate	302	100.0	0	100.0
Doxycycline	134	44.0	168	56.0
Gentamycin	128	42.0	174	58.0
Imipenem	262	87.0	41	13.0
Meropenem	261	86.0	40	14.0
Piperacillin/tazobactam	118	39.0	184	61.0
Polymyxin-B	302	100.0	0	0.0
Tigecycline	302	100.0	0	0.0

*only for urine isolates

**except urine isolates

All 302 MDR strains were resistance to third generation cephalosporins (ceftazidime, ceftriaxone and cefotaxime). Among 302 isolates, 65% (n=196) were found to be ESBL producers, 14% (n=41) were MBL producers and 40% (n=122) were AmpC producers. Out of 302 isolates, 46% (n=138) were resistance to amikacin and 58% (n=174) were resistance to gentamycin.(Figure 1)

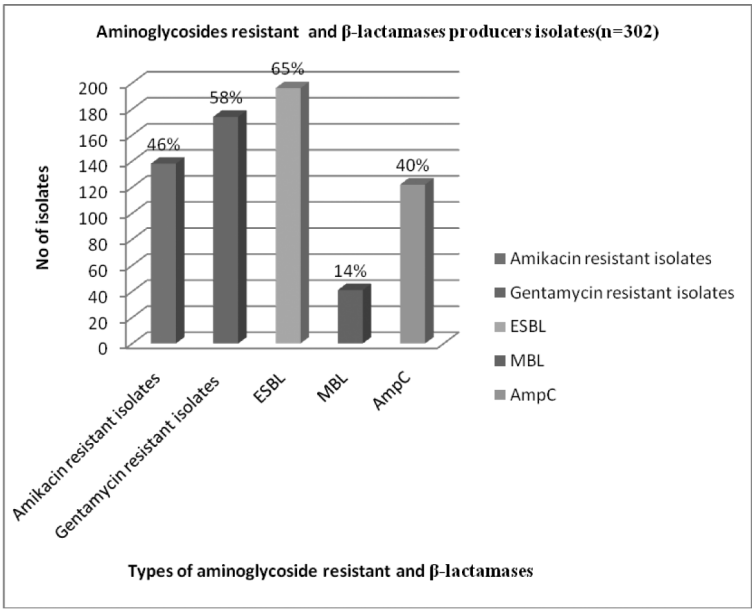

Figure 1. Distribution of aminoglycoside resistant and β-lactamases in MDR *E .coli*

Table 3:Specimen wise distribution of aminoglycosides-resistantand various β-lactamases producers *E. coli*isolates (n=302)

Clinical Specimens	Total No.	Amikacin resistant isolates No.(%)	Gentamycin resistant isolates No.(%)	ESBL No. (%)	MBL No. (%)	AmpC No. (%)
Urine	149	84(56.0)	84 (56.0)	110 (74.0)	19 (13.0)	66 (44.0)
Pus	75	26(35.0)	44 (59.0)	45 (47.0)	8 (11.0)	29 (39.0)
Sputum	44	15(34.0)	22 (50.0)	32 (50.0)	8 (18.0)	20 (45.0)
Body fluid	11	5(45.0)	9 (82.0)	4 (36.0)	1 (9.0)	2 (18.0)
Bile	10	4(40.0)	7 (70.0)	1(10.0)	4(40.0)	2 (20.0)
Blood	9	3(33.0)	6 (67.0)	2 (22.0)	2 (22.0)	2 (22.0)
Tissue	3	1(33.0)	2 (67.0)	2 (67.0)	1(33.0)	0 (0.0)
CVP tip	1	0(0.00	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	302	138(46.0)	174 (57.0)	196 (65.0)	43 (14.0)	121 (40.0)

Out of total 302 MDR*E. coli* strains, maximum gentamycin-resistant 9/11(82.0%) strains were isolated from body fluids followed by 7/10 (70.0%) from bile, 6/9 (67.0%) strains from blood and 2/3 (67.0%) from tissue. Among 196 ESBL producers, 110/149 (74.0%)were isolated from urine .The highest amikacin-resistant isolates 84/149 (56.0%) were obtained from urine. Out of 43 MBL producers, 4/10 (40.0%) were isolated from bile and 1/3 (33.0%) were isolated from tissue. Maximum AmpC β – lactamase producer were isolated from sputum 22/44 (45.0%) followed by urine 66/149 (44.0%). None of β-lactamases producer was isolated from CVP line.

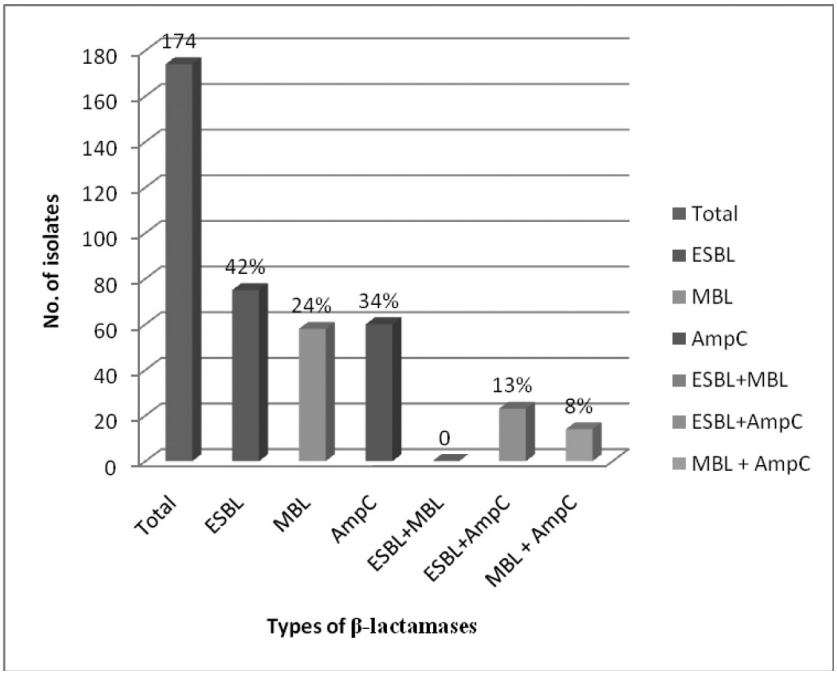

Figure 2. Co-existence of β-lactamases in gentamycin-resistant MDR *E .coli* (n=174)

Out of 174 gentamycin-resistant*E. coli* strains studied, almost all strains produced any of the 3 types of β – lactamases i.e. MBL, ESBL and Amp C β–lactamases, either alone or in combinations (Figure 2).75 (42.0 %) were ESBL producers, 41 (24.0%) were MBL producers and 60 (34.0 %) were AmpC producers. ESBL and AmpC producers in combination were 23 (13.0%) and 14(8.0%) isolates produced both MBL and AmpC in combination. In our study, none *E. coli* isolates were positive for all 3 types of β – lactamases i.e. ESBL, MBL and AmpC β – lactamases in combination (Figure 2).

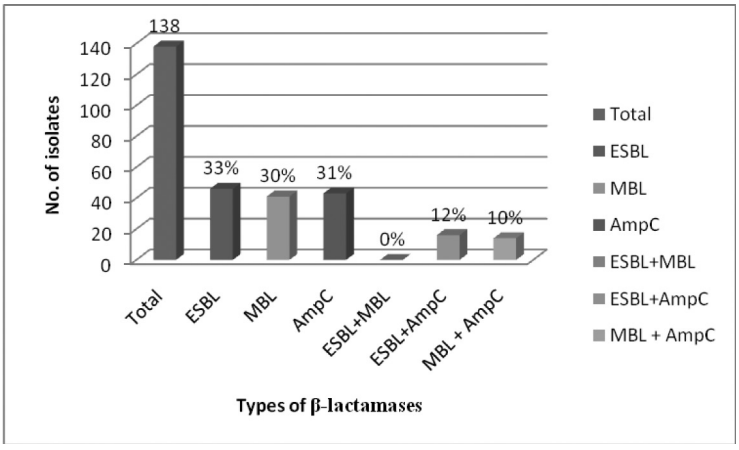

Figure 3. Co-existence of β-lactamases in amikacin-resistant MDR *E .coli* (n=138)

All amikacin-resistant isolates (n=138) were simultaneously resistant to gentamycin. Out of 138 amikacin-resistant *E. coli* studied, all isolates produced any of the 3 types of β – lactamases i.e. MBL, ESBL and Amp C β–lactamases, either alone or in combinations (Figure 3).46 (33.0 %) were ESBL producers, 41 (30.0%) were MBL producers and 43 (31.0 %) were AmpC producers. ESBL and AmpC producers in combination were 16 (12.0%) and 14 (10.0%) isolates produced both MBL and AmpC in combination. None amikacin-resistant *E. coli* isolates were positive for all 3 types of β – lactamases i.e. ESBL, MBL and AmpC β – lactamases in combination.

Discussion

Previous studies on the mechanisms of aminoglycoside resistance have shown the production of aminoglycoside-modifying enzymes, including (i) aminoglycoside acetyltransferases, (ii) aminoglycoside phosphotransferases, and (iii) aminoglycoside adenyltransferases, to be the primary mechanism of resistance. However, any one of these enzymes alone cannot confer resistance to all aminoglycosides because of their narrower substrate specificities. Because gentamicin-modifying enzymes have poor activity against amikacin and because amikacin was developed from kanamycin to block the access of a variety of kanamycin-modifying enzymes to their target sites, a relatively low prevalence of amikacin resistance is usually observed amongmembers of the family *Enterobacteriaceae*[15]. Among the various aminoglycoside-modifying enzymes, acetyltransferases [AAC (6')-I and AAC (6')-APH (2')], adenyltransferases [ANT(4')-I and ANT(4')-II], and phosphotransferases [APH(3')-II and APH (3')-III] have been shown to result in the modification of amikacin[16].

Co-existence of aminoglycoside modifying enzymes along with β-lactamases such as ESBL, AmpC and MBL are of increasing clinical concern. ESBLs are most commonly produced by *E. coli* and *Klebsiella* spp. but may also present in other gram negative bacteria[17]. Many multidrug resistant bacteria produce multiple β-lactamases including combinations of these different enzymes.We noted high prevalence (65.0%) of MDR *E.coli* isolates were ESBL positive. This rate is similar to other studies that reported in some other developing countries (50-70%)[18, 19]. By contrast, the prevalence of ESBL-producing isolates among Enterobacteriaceae in developed nations is much lower:<1% in Sweden [20] and 1.7% in France .[21] This issue is evidently a challenge in developing nations, and our study clearly shows that it is a matter of urgency in our region. Furthermore, the MDR isolates that were obtained from body fluid, blood, sputum and urineshowed resistance to aminoglycosides (amikacin, and gentamicin), thus limiting the treatmentoptions. β-Lactamase-producing Enterobacteriaceae often harbor other enzymes (AmpC, MBL, and aminoglycoside modifying enzymes) that confer resistance to other antibiotics, including carbapenems, cephems, and aminoglycosides.[22, 23]

A previously published report suggested the spread of aminoglycoside modifying enzymes like ArmA and RmtB in Enterobacteriaceae isolates globally[24], and these enzymes were often reported to coexist with other resistance determinants, such as ESBL and MBL.[25, 26]Newer 16S ribosomal RNA methylases have been identified recently and added to the database of aminoglycoside resistance mechanism.[22, 27]

To our knowledge, no data is available on the prevalence of aminoglycoside-resistant MDR *E.coli* isolates in our country. Therefore, we studied the association of β-lactamases in aminoglycoside-resistant isolates. We observed that the most of the aminoglycoside-resistant MDR isolates produced any one of β-lactamases i.e. ESBL (42.0 %), MBL (33.0 %) and AmpC (34.0 %). This rate is higher than those reported in a Taiwanese study[28] and a Belgian study [29].The respective studies suggested an increasing resistance against aminoglycosides and β-lactams but the contradiction inresults is due to different geographical areas and various numbers of different isolates.

In agreement with previous reports [30, 31], β-lactamase positive isolates were susceptible to colistin, polymyxins and tigecycline, which were the only available therapeutic options for them.

Conclusion

In conclusion, β-lactamases-producing MDR *E.coli* isolates were common in our medical settings and they are usually resistance to aminoglycosides. Our findings underline the emerging threat of pan-drug resistant pathogens that produce various β-lactamases disseminating in this region. The increase in multidrug-resistant *E. coli* producing ESBLs, MBLs and AmpC enzymes has limited therapeutic options.Therefore, early identification of infections due to these organisms is necessary for prompt institution of appropriate treatment and to reduce the mortality in hospitalized patients. Further experiments will be needed to evaluate other b-lactamase types and novel antibiotic resistance mechanisms. It is important to follow antibiotic restriction policies to control increasing resistance against aminoglycosides, carbapenems and other broad spectrum antibiotics

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Conflict of interests: None Declared

References

1. Laxminarayanan R, Heymann DL. Challenges of drug resistance in the developing world. BMJ.2012; 344: e1567.
2. Centers for Disease Control and prevention (CDC).Vital signs: carbapenem-resistant Entero bacteriaceae. MMWR Morb Mortal Wkly Rep. 2013; 62: 165-170.
3. Davies J, Davies D. Origin and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010; 74: 417-433.
4. Von Baum H, Marre R. Antimicrobial resistance of *Escherichia coli* and therapeutic implications. Int J Med Mocrobiol. 2005: 295: 503-511.
5. US Food and Drug Administration.National antimicrobial resistance monitoring system-enteric bacteria (NARMS):2008 executive report.
6. Levy SB.Antimicrobial resistance worldwide: causes, challenges and responses. Nat Med. 2004; 10: S122-S129.
7. Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A et al.. Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated infections Forum. Antimicrob Resist Infect Control. 2013; 2: 31.doi: 10.1186/2047-2994-2-31.
8. Isenberg HD. Clinical Microbiology Procedures Handbook. 2nd ed. Washington DC: ASM Press. 2004.
9. Mahon CR, ManuselisGA. A textbook of Diagnostic Mocrobiology. 5th ed. Maryland; Elsevir Inc.2015.
10. Clinical Laboratory Standard Institute (CLSI): Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement document. Wayne, PA: CLSI: 2012; M100-S20.
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18: 268–81.
12. Franclin C, Liolios L, Peleg AY.Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing Gram-negative bacilli in clinical laboratory. J Clin Microbiol. 2006; 44:3139-44.
13. Coudron PE. Inhibitor-based methods for detection of plasmid-mediated Ampc-b-lactamases in *Klebsiella spp*, *Escherichia coli* and *Proteus mirabilis*. J Clin Microbiol. 2005; 43: 4163-4167.
14. Yagi T, wachino J, Kurokawa H, suzuki S, Yamane K, Doi Y et al.Practical Methods Using Boronic Acid Compounds for Identification of Class C β-Lactamase-Producing Klebsiella pneumoniae and *Escherichia coli*.J Clin Microbiol. 2005; 43:2551-58.
15. Kondo S, Hotta K. Semisynthetic aminoglycoside antibiotics: development and enzymatic modifications. J Infect Chemother. 1999; 5:1-9.
16. Shaw KJ, Rather RS, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev. 1993; 57: 138-163.
17. Bradford BA. Extended-spectrum -b-lactamases in 21st Century: Characterization, epidemiology and detection of this important resistant threat. Clin Microbiol Rev. 2001; 14: 933-951.
18. Sharma S, Bhat GK, SS. Virulence factors and drug resistance in Escherichia coli isolated from extraintestinal infection. Indian J Med Microbiol. 2007; 25: 369-373.
19. Goyal A, Prasad KN, Prasad A, Gupta S, Ghosal U and AA. Extended-spectrum- -b-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*& associated risk factors. Indian J Med Res. 2009; 129: 695-700.
20. Ostholm-Balkhed A, Tarnberg M, Nilsson M, Johansson AV, Hanberger H, Monstein HJ, et al..

Prevalence of extended-spectrum-b-lactamase-producing Enterobacteriaceae and trends in antibiotic consumption in a county of Sweden. Scand J Infect Dis. 2010; 42: 831-838.

21. Galas M, Breton N, Godard T, Allouch PY, Pina P, et al. Nationwide study of the prevalence, characteristics and molecular epidemiology of extended-spectrum- -b-lactamase-producing Enterobacteriaceae in France. Antimicrob Agents Chemother. 2008; 52: 786-789.
22. Krishnappa LG, John J, Marie MAM, Gopalkrishnan S, Pradeep CS, Rani SRB. Detection of pan-amino glycosideresistant Gram negativebacteria using a molecular method. South Asian J Exp Biol. 2012; 2: 256-258.
23. Doi Y, Yokoyama K, Yamane K, Wachino J-I, Shibata N, Yagi T, et al. Plasmid- mediated 16S rRNA methylase in Serratiamarcescensconferring high-level resistance toaminoglycosides. Antimicrob Agents Chemother. 2004; 48: 491-496.
24. Bogaerts P, Galimand M, Bauraing C, Deplano A, Vanhoof R, De Mendonca R, et al. Emergence of ArmAand RmtBaminoglycoside resistance 16S rRNA methylases inBelgium.J Antimicrob Chemother.2007; 59: 459-464.
25. Hidalgo L, Hopkins KL, Gutierrez B, Ovejero CM, Shukla S, Douthwaite S, et al.Association of the novel aminoglycosideresistance determinant RmtF with NDMcarbapenemase in Enterobacteriaceae isolated in India and the UK. J AntimicrobChemother. 2013; 68: 1543-1550.
26. Berpot B, Poirel L, Nordmann P.Plasmid-mediated 16S rRNA methylases among extended-spectrum beta-lactamaseproducingEnterobacteriaceae isolates. Antimicrob Agents Chemother. 2008; 52: 4256-4257.
27. Yamane K, Rossi F, Barberino MGMA, Adams-Haduch JM, Doi Y, Paterson DL. 16S ribosomal RNA methylase RmtDproduced by *Klebsiella pneumoniae* in Brazil.J AntimicrobChemother. 2008; 61: 746-747.
28. Yan J-J, Wu J-J, Ko W-C, Tsai S-H, Chuang C-L, Wu H-M, et al. Plasmid-mediated 16S rRNA methylases conferringhigh-level aminoglycoside resistance in *Escherichia coli*and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals. J AntimicrobChemother. 2004; 54: 1007-1012.
29. Bogaerts P, Galimand M, Bauraing C, Deplano A, Vanhoof R, De Mendonca R, et al.. Emergence of ArmAand RmtBaminoglycoside resistance 16S rRNA methylases inBelgium. J AntimicrobChemother. 2007; 59: 459-464.
30. Marie MAM, John J, Krishnappa LG, Gopalkrishnan S. Molecular characterization of theb-lactamase in *Escherichia coli*and Klebsiella pneumoniae from a tertiary care hospitalin Riyadh, Saudi Arabia. MicrobiolImmunol. 2013; 57: 805-810.
31. Li J1, Nation RL, Milne RW, Turnidge JD, Coulthard K.Evaluation of colistin as anagent against multi-resistant gram-negativebacteria. Int J Antimicrob Agents. 2005; 25: 11-25.