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PHENOTYPIC DETECTION OF AMPC β-LACTAMASE AND METALLO-β-LACTAMASE PRODUCTION AMONG UROPATHOGENIC E. COLI

Microbiology

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ABSTRACT

Background: Urinary tract infections occur when microorganisms invade the urinary tract. Entry of microorganisms to the urinary tract occurs via the urethra and they multiply in the bladder. Urinary tract infection often affects women and causes cystitis and urethritis. The most important agent causing UTIs is usually E. coli, isolated from urine samples; pose a major concern to society as it produces some beta-lactamase enzymes which help the organism to resist action of antibiotics.

Aims and objectives: To detect AmpC ß-lactamase and Metallo-ß-lactamase production among uropathogenic E. coli isolates and to detect Antibiotic susceptibility patterns of uropathogenic E. coli.

Materials and methods: A total of 130 E. coli urinary isolates were collected for the study. Antibiotics susceptibility testing for the isolates was performed by Kirby bauers disc diffusion method. The isolates were further subjected for phenotypic detection of AmpC β-lactamase and MBL production by AmpC disc test and Modified Hodge test. The study of antibiogram for AmpC and MBL producers was also considered.

Results: MBL was detected in 18.46 % (24/130) and AmpC in 10.77 % (14/130). Among the MBL producers, maximum susceptibility was given by colistin, followed by amikacin, gentamycin, nitrofurantoin, cotrimoxazole. These isolates also showed highest resistance to ampicillin, amoxyclav, cefipime, then cefotaxime, ceftazidime, cefoxitin, piperacillin/tazobactum, ceftriaxone, ciprofloxacin, ertapenem, imipenem, meropenem, and nalidixic acid. AmpC β-lactamase producers showed highest susceptibility to colistin followed by cotrimoxazole, gentamycin, amikacin, nalidixic acid and nitrofurantoin. Most of the AmpC producers were resistant to cefotaxime, ampicillin, amoxyclav, piperacillin/tazobactum, ceftazidime, cefipime, cefoxitin, ceftriaxone, imipenem, meropenem and ertapenem.

Conclusion: Early detection of AmpC and MBL lactamases in uropathogenic E. coli isolates with performance of antibiotic susceptibility testing is suggested and is considered to prevent the spread of resistance in clinical situations.

KEYWORDS

AmpC, MBL, uropathogenic E. coli.

INTRODUCTION

Urinary tract infection (UTI) is a disease caused by microbial invasion of the urinary tract, extending from the renal cortex of kidneys to the urethral meatus[1]. It remains the second, most common infection in humans, both in community and hospital settings; affecting more than 150 million annually worldwide[2]. UTIs affect all age groups and gender. However, women are found to be at higher risk than men in developing UTI; this is due to short and wider urethra and its closeness to the anal orifice than in men[3]. Various risk factors contributing to the UTIs include sex, age, presence of underlying disease, hospitalization and obstruction[4,5]. Most frequent organism causing UTIs being uropathogenic *Escherichia coli* (UPEC), accounts for about ~80% of UTIs[1,3]

UPEC have specific virulence factors that help the organism to colonize and adhere to uro-epithelial cells such as Fimbricase (P fimbriae, type -1 fimbria) [6,7] Antimicrobial resistance in UPEC has increased and hence poses difficulty in treatment of UTIs[5]. This is due to production of ß-lactamases such as extended spectrum-beta lactamase (ESBL), AmpC ß-lactamases (AmpC) and Metallo-ß-lactamases (MBL)[8,9].

Metallo ß-lactamases are enzymes belong to Class B family of ß-lactamases that hydrolyse all classes of ß-lactam antibiotics except monobactam [10,11]. Therefore, only toxic Polymixin B and Colistin save as optional drugs for treatment. Nonetheless, the enzyme activity is inhibited by metal-chelating agents such as Ethylene diamine tetra acetic acid (EDTA) [11,12,13] Screening of MBL producers is required for early therapy. Phenotypic methods are used widely to detect MBLs, which includes MBL E-test, Double-disc synergy test, combined disc (CD) assay and Modified Hodge Test (MHT) [10].

AmpC

AmpC ß-lactamases belongs to class C or group I cephalosporinase which are sensitive to Carbapenems and cefipime; poorly inhibited by clavulanic acid but confer resistance to a wide range of ß-lactams such as alpha-methoxy ß-lactam and narrow and broad spectrum cephalosporins and aztreonam [8,14,15]. AmpC ß-lactamases can be detected phenotypically or genotypically by AmpC Disk Test, Ceftazidime-Imipenem Antagonism Test (CIAT), Disc Antagonism Test and Modified Three-dimensional Test and PCR[16].

The aim of this study was to detect AmpC ß-lactamase and Metallo-ß-lactamase production among uropathogenic E. coli isolates and to detect Antibiotic susceptibility patterns of uropathogenic E. coli.

Materials and Methods

130 *E. coli* isolates from urine samples were used for the study.

Kirby-Bauer's disk diffusion method was used to test for antibiotic susceptibility of uropathogenic *E. coli* isolates on Muller-Hinton agar (MHA) plates in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [17] Antibiotics used included; ampicillin(10mcg), nitrofurantoin (300 mcg), Gentamycin (10 mcg), Cotrimoxazole (25mcg), ciprofloxacin (5mcg), amoxycillin - clavulanic acid (30/10µg), Colistin (10mcg), imipenem (10mcg), Ertapenem (10mcg) Meropenem (10 mcg), Amikacin (30 mcg), cefotaxime (30 mcg), cefoxitin (30 mcg), cefepime (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), nalidixic acid (30 mcg) and Piperacillin/tazobactum (100/10 mcg). E. coli ATCC 25923 was used as control. Results were recorded and interpreted as per CLSI recommendations[17]

Detection of MBL production

Phenotypic detection of MBL was carried out by Modified Hodge Test [11]. A lawn of E. coli ATCC 25922 was prepared on Mueller Hinton agar and a 10 µg imipenem antibiotic disk was then placed at the centre of the plate. In a straight line, test organisms were streaked from the edge of the disk to the edge of the plate. Up to four organisms were tested on the same plate. The plates were incubated overnight at 37°C in ambient air for 16–24 hours.

Interpretation of Results:
Plates were examined for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the imipenem disk.

Positive MHT test: clover leaf-type indentation at the intersection of the test organism and the ATCC *E. coli* 25922, within the zone of inhibition of the imipenem disk. Negative MHT test: shown by no indentation or growth of the ATCC E. *coli* 25922 along the test organism growth streak within the disc diffusion.

Detection of AmpC production
All *E. coli* isolates were subjected to AmpC Disk Test using cefoxitin (30µg) disc. A lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. Sterile disks (6 mm) moistened with sterile saline (20 µl) and inoculated with colonies of test organism. The inoculated disk was then placed besides a cefoxitin disk (almost touching) on the inoculated plate, with the inoculated side facing the surface of the media. Plates were incubated overnight at 37°C [8,18,19].

Interpretation of results:
A positive test: appearance of a flattening or indentation of the cefoxitin inhibition zone in the area of the test disk. A negative test: shown as an undistorted zone.

RESULTS
In the present study, 98.46 % of the isolates were resistant to amoxyclav, 93.08 % to ampicillin, followed by 77.69 % to cefipime, 76.15 % to ceftazidime, cefoxitin and piperacillin/tazobactum, 66.15 % to cefotaxime and 50.8 % to imipenem. Most of the isolates were sensitive to colistin (98.46 %), followed by cotrimoxazole (86.92 %), gentamycin (83.85 %), amikacin (82.31 %), ceftriaxone (76.92 %), nitrofurantoin (76.15 %), ciprofloxacin (67.69 %), nalidixic acid (62.31 %), meropenem (61.54 %) and ertapenem (58.46 %) [Table 1]
Out of 130 isolates, 24 (18.46) were phenotypically identified as MBL producers [Table 2/Fig. 1]. MBL producers showed highest sensitivity towards colistin (95.83 %), followed by amikacin (79.17 %), gentamycin, nitrofurantoin (66.97%) and cotrimoxazole (54.17 %). Higher resistance of MBL producers was seen against ampicillin (100 %), followed by amoxyclav (95.83 %), cefipime (91.67), cefotaxime, ceftazidime (79.17 %), cefoxitin (75.0 %), piperacillin/tazobactum (70.83), ceftriaxone (66.67 %), ciprofloxacin, ertapenem (58.33 %), imipenem, meropenem (54.17 %) and nalidixic acid (50.0 %). [Table 3]
AmpC production was detected in 14 (10.77 %) isolates by AmpC disc test [Table 4/Fig. 2]. Among AmpC β-lactamase producers, colistin showed 100 % sensitivity, followed by cotrimoxazole, gentamycin (71.43 %), amikacin (64.23 %), nalidixic acid, nitrofurantoin (57.14 %). However, resistant to cefotaxime (100 %), followed by ampicillin, amoxyclav (92.86 %), piperacillin/tazobactum, ceftazidime, cefipime (85.71 %), cefoxitin (78.57 %), ceftriaxone (64.23 %), imipenem, meropenem and ertapenem (57.14 %) was also observed [Table5].

DISCUSSION
In the present study, uropathogenic *E. coli* isolates showed higher resistance towards amoxyclav (98.46 %), followed by ampicillin (93.08 %), cefepime (77.69 %), cefoxitin (76.16 %), ceftazidime (76.15 %), piperacillin/tazobactam (75.85 %), cefotaxime (66.15 %) and imipenem (50.8 %). In our study, uropathogenic *E. coli* isolates has shown highest resistance (98.46 %) to amoxyclav. Similarly, other studies reported higher resistance of 100 % and 93.3 % to amoxyclav [16,20,21].

A study conducted in Iran showed resistance of 68.2 % to amoxyclav, which is lower than that obtained in the present study [22]. On contrary, another study conducted in Libya has showed susceptibility of *E. coli* isolates to amoxyclav to be 60.92 % [15].

Resistance of *E. coli* isolates to ampicillin as reported in the present study is 92.55 %, which is similar to that as reported by Amit et al [2]. In addition, Bora et al reported 100 % resistance to ampicillin, which is higher than that obtained in our study [1].

Our study has shown resistance towards cefepime, cefoxitin, cefotaxime and ceftazidime. Other studies showed similar cases in resistance to the same drugs [1,23]. However, other studies reported isolates resistance towards cefepime, cefoxitin and ceftazidime[1,22]. Yet, higher resistance of 100 % was reported in Mangalore[16]. Isolates were also found to be resistant to imipenem while sensitive to meropenem and ertapenem [2,10,21,22].

Colistin had the highest susceptibility of (98.46 %). These results are in agreement to studies conducted by other researchers[1]; followed by cotrimoxazole (86.92 %), gentamycin (83.85 %), amikacin (82.31 %), meropenem (61.54 %) and ertapenem (58.46 %). Chandigarh, Libya and Iran reported susceptibility between 62% and 71 % towards cotrimoxazole [10]In the present study, 85 % susceptibility was obtained for gentamycin. Similar results were reported in Iran [10], whereas lower susceptibility of 60% - 74 % was reported in other parts of India and Pakistan [1,18,22]. In contrast to these results, gentamycin was reported resistant in Iran, Iraq and Nigeria [5,21,22,23].

Nitrofurantoin, a drug commonly used for UTIs showed susceptibility to *E. coli* isolates in our study. Similar results were reported from other studies[2,10,20]. However, a study conducted in Nigeria showed resistance of *E. coli* isolates towards nitrofurantoin[5].

In our study, isolates were susceptible to ciprofloxacin and nalidixic acid. Studies from Iran, Libya, Pakistan and Nigeria has also shown ciprofloxacin to be effective towards *E. coli* isolates, with higher value from Nigeria (96.4 %) [5,20] and These results are comparable to other as reported by other studies whereby E. coli isolates exhibited resistance to ciprofloxacin and nalidixic acid of 60 % -78%[1,2,19].

In our study, the prevalence of MBL producers among uropathogenic *E. coli* was found to be 18.46 % which is consistent with the study by Bora et al who reported MBL production to be 18.98 %.[13] Lower incidence of MBL production between 7.0 % and 12.5 % was reported by Manoj et al., Hamed et al., and Mita et al. [10,11,25] In contrast, Anwa et al., reported high incidence of MBL production ranging between 45.45 % and 69.69 %.[26] A study by Javed Humera et al., detected at least 32.84 % by both CDT and DDST.[26]

All isolates of *E. coli* found to be MBL producers in our study showed higher (up to 100 %) resistance to ampicillin, followed by all cephalosporins, quinolones and piperacillin/tazobactum combination tested. About 50 % MBL producers have shown resistance to Carbapenems tested. These findings are similar to as those reported by Bora et al., and Nepal et al.[13]. Higher resistance of 70 % towards Imipenem was also reported by Hamed et al.[10] Moreover, a study by Javed Humera et al., showed highest susceptibility for colistin among MBL producers, which is in accordance with our study[26].

There were 10.77 % AmpC β-lactamase producers in our study. Varying results in other studies, 20.5 % and 35.9 % as reported by Manasi et al., Nahid et al., showed the prevalence of AmpC production among uropathogenic *E. coli* isolates[16,27]. Very low incidence was seen from other studies compared to our results[15]. In addition, Khan et al. detected 44.5 % and 21.9 % AmpC production by CDT and AmpC disc test[19]. Yet, Muna et al., reported 0 % of AmpC production[15].

Our study showed 100 % resistance against cefotaxime among the AmpC β-lactamase producers. Higher resistance was also seen towards ampicillin, amoxyclav, cefepime, ceftazidime, cefoxitin and piperacillin/tazobactum. Similar results were reported by Manasi et al. and Liu et al. reported high resistance towards gentamycin[16].

Table1: Antibiotic Susceptibility patterns of Uropathogenic E. coli isolates (n=130) to the selected antibiotics

Antibiotic Name	Resistance Number (%)	Intermediate Number (%)	Sensitive Number (%)
Amikacin (AK)	8 (6.15)	15 (11.54)	107 (82.31)
Ampicillin (Amp)	121 (93.08)	6 (4.62)	3 (2.31)
Amoxyclav (Amc)	128 (98.46)	2 (1.54)	0
Cefipime (Cpm)	101 (77.69)	12 (9.23)	17 (13.08)
Cefotaxime (CTX)	86 (66.15)	21 (16.15)	23 (17.69)
Cefoxitin (Cx)	99 (76.15)	12 (9.23)	19 (14.62)
Ceftazidime (Caz)	99 (76.15)	11 (8.46)	20 (15.38)
Ceftriaxone (Ctr)	27 (20.8)	3 (2.31)	100 (76.92)
Ciprofloxacin (Cip)	30 (23.08)	12 (9.23)	88 (67.69)
Colistin (Cl)	2 (1.54)	-	128 (98.46)

Cotrimoxazole (Cot)	14 (10.8)	3 (2.31)	113 (86.92)
Ertapenem (Etp)	29 (22.31)	25 (19.23)	76 (58.46)
Gentamycin (Gen)	11 (8.46)	10 (7.69)	109 (83.85)
Imipenem (Imp	66 (50.8)	13 (10)	51 (39.23)
Meropenem (Mrp)	24 (18.46)	26 (20)	80 (61.54)
Nalidixic acid (Na)	33 (25.38)	16 (12.31)	81 (62.31)
Nitrofurantoin (Nit)	16 (12.31)	15 (11.54)	99 (76.15)
Piperacillin/Tazobactum (Pit)	99(76.15)	11 (8.46)	20 (15.38)

Table 2: Distribution of MBL production

MBL production results	Number	%
MBL Positive	24	
MBL Negative	106	

Table 3: Distribution of AmpC β-lactamase

AmpC production results	Number	%
AmpC Positive	14	10.77
AmpC Negative	116	89.23

Table 3: Antibiotic Susceptibility pattern of MBL producers (n = 24)

Antibiotic Name	Resistance Number (%)	Intermediate Number (%)	Sensitive Number (%)
Amikacin	4 (16.67)	1 (4.17)	19 (79.17)
Ampicillin	24 (100)	0	0
Amoxyclav	23 (95.83)	1 (4.17)	0
Cefipime	22 (91.67)	2 (8.33)	0
Cefotaxime	19 (79.17)	3 (12.5)	2 (8.33)
Cefoxitin	18 (75.0)	1 (4.17)	5 (20.83)
Ceftazidime	19 (79.17)	2 (8.33)	3 (12.5)
Ceftriaxone	16 (66.67)	0	8 (33.33)
Ciprofloxacin	14 (58.33)	4 (16.67)	6 (25)
Colistin	1 (4.17)	-	23 (95.83)
Cotrimoxazole	10 (41.67)	1 (4.17)	13 (54.17)
Ertapenem	14 (58.33)	2 (8.33)	8 (33.33)
Gentamycin	8 (33.33)	0	16 (66.67)
Imipenem	13 (54.17)	1 (4.17)	10 (41.67)
Meropenem	13 (54.17)	1 (4.17)	10 (41.67)
Nalidixic acid	12 (50)	3 (12.5)	9 (37.5)
Nitrofurantoin	4 (16.67)	4 (16.67)	16 (66.67)
Piperacillin/ Tazobactum	17 (70.83)	4 (16.67)	3 (12.5)

Table5: Antibiotic Susceptibility pattern of AmpC producers (n = 14)

Antibiotic Name	Resistance No (%)	Intermediate No (%)	Sensitive No (%)
Amikacin	5 (35.71)	0	9 (64.23)
Ampicillin	13 (92.86)	1 (7.14)	0
Amoxyclav	13 (92.86)	1 (7.14)	0
Cefipime	12 (85.71)	1 (7.14)	1 (7.14)
Cefotaxime	14 (100)	0	0
Cefoxitin	11 (78.57)	0	3 (21.43)
Ceftazidime	12 (85.71)	1 (7.14)	1 (7.14)
Ceftriaxone	9 (64.23)	0	5 (35.71)
Ciprofloxacin	6 (42.86)	2 (14.29)	6 (42.85)
Colistin	0	0	14 (100)
Cotrimoxazole	4 (28.57)	0	10 (71.43)
Ertapenem	8 (57.14)	0	6 (42.86)
Gentamycin	4 (28.57)	0	10 (71.43)
Imipenem	8 (57.14)	0	6 (42.86)
Meropenem	8 (57.14)	0	6 (42.86)
Nalidixic acid	6 (42.86)	0	8 (57.14)
Nitrofurantoin	4 (28.57)	2 (14.29)	8 (57.14)
Piperacillin/ Tazobactum	12 (85.71)	2 (14.29)	0

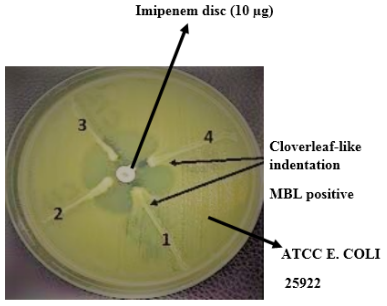

Fig 1: 1 - Positive control (MBL E. coli), 2- Negative MBL test, 3- Negative control (ATCC E. coli 25922), 4 - Positive MBL test

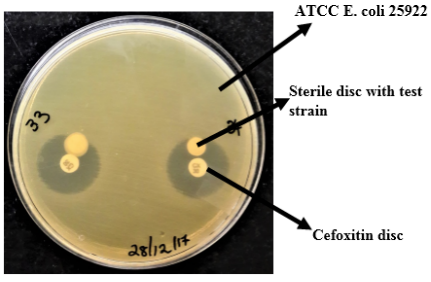

Fig 2: AmpC disc test using 30 µg Cefoxitin disc. a) Positive result and b) Negative result.

CONCLUSION

Cases due to Uropathogenic E. coli producing AmpC and MBL lactamases associated with UTIs are increasing in both hospital and community based settings.

In the present study, the prevalence of MBL and AmpC β-lactamase production was not very high when compared to most of the studies carried out earlier. The study has also showed coexistence of AmpC producers among MBL producing strains.

To conclude, the results of this study indicates incidence of MBL and AmpC β-lactamase production among uropathogenic E. coli isolates. For this reason, it is suggested that early detection of β-lactamase enzymes should be considered to prevent the spread of resistance in clinical situations. Therefore, there should be a rational use of antibiotics to prevent such calamity from occurring.

REFERENCE

1. Bora A, Ahmed G.U. Hazarika NK. Phenotypic detection of extended spectrum ß-lactamase and AmpC ß-lactamase in urinary isolates of Escherichia coli at a tertiary care referral hospital in Northeast India. Journal of College of Medical Sciences- Nepal, 2012, 8 (3) 1-8.
2. Amit A. Rangari, Sachin Sharma, Nidhi Tyagi, Paramjit Singh, Garima Singh, Rameshwari Thakur. Antibiotic susceptibility pattern of bacterial Uropathogens isolated from patients at a tertiary care hospital in Western Uttar Pradesh, India. Int.J.Curr.Microbiol.App.Sci. 2015, 4 (10): 646-657.
3. Dennis L. Kasper, Anthony S. Fauci. Harrison's Infectious Diseases, 3rd Ed. (305-312). Mc Graw Hill Education, New York.
4. Agbawa O.E, Ifeanacho Emeka J.U. The prevalence of UTI pathogens in urine specimen obtained from a hospital in Rivers State Nigeria. Journal of Microbiology Research. 2015; 5(5): 143-148.
5. Kolawole A.S, Kolawole O.M., Kandaki-Olukemi Y.T, Babatunde S.K, Durowade K.A,Kolawole C.F. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medicine and Medical Sciences. May 2009; 5 (163-167).
6. Raksha R, Srinivasa H, Macaden R.S. Occurrence and characterization of Uropathogenic Escherichia coli in urinary tract infections. Indian Journal of Medical Microbiology. 2003; 21 (2): 102-107).
7. Agarwal J, Srivastava S, Singh M. Pathogenesis of Uropathogenic Escherichia coli. Indian Journal of Medical Microbiology, 2012; 30 (2): 141-9.
8. Mohamudha Parveen R, Harish B.N, Parija S.C. AmpC beta lactamases among Gram-negative clinical isolates from a tertiary hospital, South India. Brazilian Journal of Microbiology, 2010; 41: 596-602.
9. Kalaivani R, Shashikala P, Sheela Devi C, Prashanth K, Saranathan R. Phenotypic assays for detection of ESBL and MBL producers among the clinical isolates of multidrug resistant Pseudomonas Aeruginosa from a tertiary care hospital. Int. J. Cur, Res. Rev, Sep. 2013; 5(17).
10. Hamed Ghadiri, Hamid Vaez, Kamal Razavi-Azarkhiavi, Ramin Rezaee, Mehdi Haji-Noormohammadi, Ali Asghar Rahimi, Vahid Vaez, Enayatollah Kalantar. Prevalence and Antibiotic Susceptibility patterns of Extended-spectrum ß-lactamase and Metallo-

ß-lactamase-producing Uropathogenic Escherichia coli isolates. Lab Med Fall, 2014; 45 (4): 290-294).
11. Manoj Kumar, Renu Dutta, Sonal Saxena, Smita Singhal. Evaluation of different phenotypic methods to detect ESBL and MBL production in Escherichia coli and Klebsiella species with Detection of NDM-1 by PCR. Intl. J. Microbiol. Res. 6(1): 20-26, 2015.
12. Patel G, Bonomo R.A. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol, 4:48, 2013.
13. Arijit Bora, Rajkumari Sanjana, Brajesh Kumar Jha, Surya Narayan Mahaseth, Khilasa Pokharel. Incidence of Metallo-beta-lactamase producing clinical isolates of Escherichia coli and Klebsiella pneumoniae in Central Nepal. BMC Research Notes, 2014; 7: 557.
14. Mehvash Haider, Meher Rizvi, Nazish Fatima, Indu Shukla, Abida Malik. Necessity of detection of extended beta-lactamase, AmpC and Metallo-beta-lactamases in Gram-negative bacteria isolated from clinical specimens. Muller Journal of Medical Science and Research. Jan-Jun 2014; 5(1).
15. Muna Mohammed Buzayan, Fauzia Rajab El-Garbulli. Detection of ESBL and AmpC ß-lactamases producing in Uropathogenic Escherichia coli isolates at Benghazi Centre of Infectious Diseases and Immunity. Int. J. Curr. Microbiol. App. Sci. (2014); 3(2): 145-153).
16. Manasi R and Anitha K.B. AmpC Beta Lactamase detection in Escherichia coli and comparison of two phenotypic methods. International Journal of Current Microbiology and Applied Sciences. 2017; 6 (2): 1123-1128
17. Clinical Laboratory Standards Institutes. Performance Standards for Antimicrobial Susceptibility testing, XXI International Supplement (M100-S21). Wayne, Pennsylvania, USA: National Committee for Clinical Standards.
18. Sunitha B.U. and Navaneeth B.V. Inducible and plasmid mediated AmpC beta-lactamase among Klebsiella pneumoniae in a Tertiary Care Teaching Hospital of South India. Int. J. Curr. Microbiol. App. Sci. (2017) 6(2): 1665-1672.
19. Khan Mohammed Nasir, Srivastava Preeti, Chophi Vikili and Nirwan Prem Singh. Prevalence of ESBL and AmpC ß-lactamase in Gram-negative bacilli in various clinical samples at tertiary care hospital. Int. Res. J. Medical Sci. August 2015; 3(8): 1-6.
20. Varsha Gupta, Hena Rani, Nidhi Singla, Neelam Kaistha and Jagdish Chander. Determination of Extended Spectrum ß-lactamases and AmpC production in Uropathogenic isolates of Escherichia coli and susceptibility to Fosfomycin. J Lab Physics. 2013 July-Dec; 5 (2): 90-93.
21. Shayan S and Bokaeian M. Detection of ESBL and AmpC-producing E. coli isolates from urinary tract infections. Adv Biomed Res 2015; 4: 220.
22. Khosro Issazadeh, Seyedeh Nasrin Naghibi, Mohammad Reza Majid Khoshkholgh-Pahlaviani. Drug resistance and serotyping of Uropathogenic Escherichia coli among patients with urinary tract infection in Rasht, Iran. Zahedan J Res Med Science. 2015 June; 17 (6): e989.
23. Ahmed A.A. Hadi Almutairy, Thekra Abdul-Aali Alka' aby and Anwa A. Abdulla. Detection of Metallo-ß-lactamases and their association with integrons among Multidrug Resistant clinical isolates of Escherichia coli. International Journal of PharmTech Research, 2016; 9(12): 740-753.
24. Nadia Saad, Tehmina Munir, Maliha Ansari, Mehreen Gilani, Mahwish Latif and Amira Haroon. Phenotypic identification and Antibiotic susceptibility pattern of AmpC beta-lactamase producing Escherichia coli and Klebsiella pneumoniae isolated from Urinary tract infections from a tertiary care hospital of Rawalpindi, Pakistan. J Med Microbiol Infec Dis, 2014; 2(4): 143-146.
25. Mita D. Wadekar, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int. J. Curr. Res. Aca. Rev. 2013; 1 (3): 89-95.
26. Humera javed, Hadan Ejaz, Aizza Zafar, Ahsan Waheed Rathore, Ikran'ul Haq. Metallo-beta-lactamase producing Escherichia coli and Klebsiella pneumoniae: A rising threat for hospitalized children. J Pak Med Assoc. Sep 2016; 6(9).
27. Nahid Hoseini, Iraj Sedighi, Amir Sasan Mozaffari Nejad and Mohammad Yousef Alikhani. Phenotypic and Genotypic detection of AmpC enzymes in clinical isolates of Escherichia coli and Klebsiella pneumoniae. Journal of Krishna Institute of Medical Sciences University. July-Sept. 2017; 6 (3)