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Phenotypic Detection and Antimicrobial susceptibility Profile of ESBL, AmpC and Carbapenemase producing Gram-negative isolates from Outpatient clinic specimens

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A B S T R A C T

Infection with resistant organisms is a major public health issue. Evolution of resistance to beta lactam antibiotics in Gram negative pathogens, especially *E.coli*, frequently results from the production of B-lactamase enzymes able to hydrolyze B-lactam ring. The aim of this study was to detect the different types of resistance in Gram-negative bacilli to understand the disease burden and the antimicrobial susceptibility pattern. In addition we aimed to formulatean effective antibiotic strategy and to be the basis for a proper infection control strategy to prevent the spread of these stains .A total of 141 Gram negative bacilli isolates were identified and processed for the detection of ESBL, AmpC and Carbapenemase production using various methods according to Clinical Laboratory Standards Institute. Out of 141 Gram negative bacilli; *E. coli* and *Klebsiella pneumoniae* were the commonest two organisms identified (79.4% and17.7% respectively) and 27.7%, 3.5% and 0.7% showed the presence of ESBL, AmpC and Carbapenemase respectively. Among *E. coli* 32/112 (28.6%) were ESBL producers compared to 6/25 (24%) *in Klebsiella pneumoniae* and 1/1 (100%)in *Pseudomonas aeruginosa* with statistically insignificant difference (P=0.55). Among *E. coli* 4/112 (3.6%) were AmpC producers and 1/1(100%) in *Enterobacter Cloacae* witha highly significant statistical difference (P=0.000). All ESBL and AmpC producing isolates were sensitive to Ertapenem, Imipenem, Meropenem and Amikacin. Carbapenemase producing organisms were resistant to all antibiotics except Gentamicin and Fosfomycin. From our results we can conclude that ESBL producers are increasing in the patients visiting primary healthcare clinics. So, routine ESBL detection should be mandatory done. The different antimicrobial resistance patterns of GNB must be taken in consideration by local physicians to ensure appropriate empiric use of antibiotics and hopefully help in treatment of CA-acquired infections.

Introduction

Antimicrobial resistance is a growing threat worldwide. Resistance mechanisms have been found for every class of antibiotics (Doddaiah and Anjaneya, 2014). β-Lactamase production is perhaps the single most important mechanism of resistance to

Penicillins and Cephalosporins (Chaudhary and Aggarwal, 2004). These enzymes are thought to have been evolved from Penicillin binding proteins. In early 1960, TEM-1 was the first plasmid mediated B-Lactamase described in Gram- negative organisms. Another common plasmid mediated B- Lactamase is SHV-1 (Bradford, 2001).

Extended spectrum B- Lactamases (ESBLs),enzymes that show increased hydrolysis of oxyimino-B-Lactams, which include Cefotaxime, Ceftriaxone, Ceftazidime and aztreonam, have been reported increasingly in recent years (Rodrigues et al., 2004). They cannot hydrolyze Cephamycin and are inhibited by Clavulanic acid (Shoorashetty et al., 2011). They belong to Ambler molecular class A and Bush-Jacoby functional group 2be (Bush and Jacoby, 2010). These enzymes have been identified in large numbers from different regions and are significantly detected in various *E. coli* strains. They have also been found in other members of Enterobacteriaceae such as *Klebsiella spp*., *Citrobacter spp*., *Enterobacter spp*., *Proteus spp*., and non- lactose fermenters like *Pseudomonasaeruginosa*(Bradford, 2001). Nowadays over 200 different ESBLs have been described (Kumar et al., 2014).

AmpC B- Lactamases are clinically significant because they may confer resistance to Penicillins, Cephalosporins (oxyimino-cephalosporins eg. Ceftriaxone, Cefotaxime, Ceftazidime and Cephamycins eg. Cefoxitin and Cefotetan) and monobactams. They are not affected by the ESBL inhibitor Clavulanic acid (Tan et al., 2009). They are susceptible to advanced spectrum Cephalosporins eg. Cefepime, Cefepirome and carbapenems (Jacoby, 2009). In the Ambler structural classification of B- Lactamases AmpC enzymes belong to class C (Ambler, 1980).While in the functional classification scheme of Bush et al., 1995, they were assigned to group 1. In Gram- negative bacteria, AmpC B-Lactamase production is chromosome or plasmid mediated (El-Hady and Adel, 2015).

Although Carbapenems are considered the antibiotic class of choice to treat ESBLand ampC producing Enterobacteriaceae, the ability of these organisms to produce carbapenemases has now become apparent. Recent surveillance indicates increasing resistance to all currently available antibiotics, against many strains where only, Polymyxins retain activity; however, resistance has also been reported to these agents (Nicasio et al., 2008).

The aim of our study was to detect the different types of resistance in Gram-negative bacilli (GNB)isolated from the outpatients' specimens in our clinic to be able to understand the disease burden and the antimicrobial susceptibility pattern. In addition, we aimed to formulate the effective antibiotic strategy and to be the basis for a proper infection control strategy to prevent the spread of these stains.

Materials and Methods

This study was conducted on patients visiting aprimary healthcare outpatient clinic in UAE presenting with different types of infections (urinary tract, vaginal, respiratory tract, wound infections) in the period from August 2014 to August 2015. All age groups of either sex were included in the study. Mixed types of infection were excluded. Ethical clearance had been obtained from the institution.

A total of 141 GNB were isolated from various specimens received during the study period.

Urine samples were collected before the start of antibiotics and were cultured on MacConkey agar and blood agar by calibrated loop (1ul) and incubated for 24 hrs in 37^{o}C. urine was examined microscopically and chemically by Iris iQ200 2nd generation Automated Urine Microscopy Analyzer- HVL (High volume laboratories) which can process 60 samples/h (Iris Diagnostic. Chatsworth, CA).

Iris iQ200 2nd Generation

It is an in-vitro diagnostic use device composed of the iQ200 Automated Urine Microscopy Analyzer, connected physically and electronically to the AUTION MAX TM AX- 4280 Automated Urine Chemistry Analyzer and a workstation. It is a walk-away system that uses flow imaging analysis technology and Auto-Particle Recognition (APR, Iris Diagnostic) software to classify particles based on multiple parameters. Images are stored and can be viewed on the workstation screen, thereby eliminating the need for manual microscopy.

High vaginal swab (HVS) samples were cultured on blood, MacConkey,& Chocolate agars (in 5-10% Co_2) for 24 hrs and Sabouraud ' s agar for 48 hrs in 37^{o} C. Wet smears were examined microscopically for *Trichomonas*, WBCs, RBCs, epithelial cells or yeast. Direct film stained with Gram stain was examined for Clue cell, Gram Negative diplococci or yeast.

Sputum samples were cultured on blood, Chocolate, MacConkey and Sabouraud ' s agar. We did direct Gram stain smear for WBCs, RBCs, epithelial cells and bacteria or yeast.

Pus swab were cultured on blood, MacConkey agar for 24 hs and Sabouraud ' s agar for 48 hs and direct Gram stain was examined.

After 24 hrs we did Gram stain from isolated colonies and Automated identification and susceptibility on VITEK 2 compact (BioMerieux, France) machine were done for fast (5-8 hs) and accurate microbial identification of gram negative isolates.

VITEK 2 Compact Machine

It includes an expanded identification database, andreads every 15 min for greater speed in identification.

It uses Advanced Colorimetry ^{TM}.

The following items were used for this study VITEK2 AST-N 204 for GN susceptibility, the panel includes: Amikacin (AN), Amoxicillin/Clavulanic acid (Augmentin), Ampicillin (AM), Cefepime (FEP), Cefotaxime (CTX), Ceftazidime (CAZ), Ciprofloxacin (CIP), Ertapenem (ETP), Fosfomycin (FOS), Gentamicin (GM), Imipenem (IPM), Meropenem (MEM), Nitrofurantoin (FT), Norfloxacin (NOR), Piperacillin/ Tazobactam (TZP), Trimethoprim/ Sulfamethoxazole (TMP/SMZ).

Screening Test for ESBL, AmpC ad Carbapenemase

Each isolate was swabbed into Mueller Hinton agar plate (MHA).Amoxicillin Clavulanic acid disc (20µg+10µg) was placed in the centre of petridish and cefotaxime (30µg) and ceftazidime (30µg) were placed on either side of amoxyclav disc at a distance of 20mm. Cefoxitin (30µg) disc was placed at a distance of 15 mm from cefotaxime and ceftazidime disc. Meropenem (10µg) disc was also placed in the same plate at a distance of more than 25mm from other discs (HI media India). Plates were incubated at 37^{0}C for 16 to 18

hours. Organism which showed extension of zone of inhibition of cefotaxime or ceftazidime towards amoxyclav disc was taken as ESBL screen positive. Blunting of zone of inhibition of ceftazidime towards cefoxitin was taken as AmpC screen positive. Blunting of zone of inhibition of ceftazidime towards amoxyclav was taken as inducible AmpC positive. Zone of inhibition to meropenem disc less than 21mm was taken as carbapenemase screen positive.

Confirmatory Test for ESBL, AmpC and Carbapenemase

ESBL

1) By VITEK 2 compact: FEP (Cefepime) 1, CTX (Cefotaxime) 0.5, CAZ (Ceftazidime) 0.5, FEP/CA (Cefepime/Clavulanic) 1/10, CTX/CA (Cefotaxime/Clavulanic) 0.5/4, CAZ/CA (Ceftazidime/Clavulanic) 0.5/4 for *E. coli*, *K. pneumoniae*, *K. oxytoca*.

E test (BioMerieux)

The ceftazidime/ceftazidime-clavulanate (TZ/TZL) ESBL E test strip generates a stable concentration gradient of ceftazidime (MIC test range, 0.5-32 mg/L) on one end and the remaining end generates a gradient of ceftazidime (MIC test range, 0.064-4mg/L) plus 4 mg/L clavulanic acid.

The cefotaxime/cefotaxime-clavulanate (CT/CTL) E test ESBL strip contains cefotaxime (MIC test range, 0.25 –16 mg/L) and cefotaxime (MIC test range, 0.016 – 1mg/L) plus 4 mg/L clavulanic acid.

The cefepime/ cefepime-clavulanate (PM/ PML) Etest ESBL strip contains cefepime (MIC test range, 0.25-16 mg/L) and cefepime (MIC test range, 0.064 –4 mg/L) plus 4 mg/L clavulanic acid.

The E test procedure, reading, and interpretation were performed according to the manufacturer's instructions. . Isolated colonies from an overnight plate were suspended in saline (0.85% NaCl) to achieve an inoculum equivalent to 0.5 McFarland standard. This suspension was swabbed on a Mueller-Hinton agar plate and allowed to dry completely. An ESBL E test strip was then applied to the agar surface with sterile forceps and the plate was incubated at 35ºC overnight. ESBL results were read either as MIC values or observation of "phantom zones" or deformation of inhibition ellipses.

ESBL Positive: CT≥ 0.5 and CT/CTL≥ 8 or TZ≥ 1 and TZ/TZL≥ 8 or PM≥ 0.25 and PM/PML≥ 8 or Phantom zone or deformation of the CT,TZ or PM ellipse.

AmpC E test *(BioMerieux):* for Cefotetan susceptibility, we did it as the methodology of ESBL E test. It consists of a strip containing Cefotetan (CN) on one end and Cefotetan/Cloxacillin (CNI) on the other end. Ratio of the MICs of CN and CNI of ≥8, Deformation of ellipse, Phantom zone are considered positive for AmpC B-lactamase production.

Carbapenemase*:* By Modified Hodge Test: we prepared 0.5 MacFarland standard suspension of E. Coli ATCC 25922 in broth or saline and dilute 1:10 in saline or broth, then inoculate an MHA plate as for the routine disk diffusion procedure, then allowed it to dry 3-10 min. Ertapenem or Meropenem disks were put on the plate. By using a swab, 3-5 colonies of the tested organism were picked and inoculated in a straight line out from the edge of the disk. The streak should be at least 20-25 mm in length. It was incubated in 35^0C for 16-20 hs. The plate was examined for the enhanced growth around the test streak. Enhanced growth= positive for Carbapen*e*mase

production, No enhanced growth= negative for Carbapene*m*ase.

Statistical Analysis

Data were analyzed using SPSS (Statistical Package for Social Science) version 19. Qualitative data was presented as number and percentage. Quantitative data was presented as mean and standard deviation. The Chi-square was used to compare between variables of qualitative data. The P value of< 0.05 indicates a significant difference while P value of < 0.001 indicates a highly significant difference.

Results and Discussion

The study included one hundred forty one patients; 16 males (11.3%) and 125 females (88.7%), the age ranged from 3 to 80 years with mean 40± 19.5.Types and microscopic examination of the studied samples are shown in table (1). The majority of our samples were urine samples (92.2%) followed by HVS (5.7%). A total of one hundred forty one GNB isolates were recovered during the study period, which included *E. coli*112/141(79.43%), *Klebsiella pneumoniae* 25/141 (17.73%), *Proteus mirabilis*, *Citrobacter koseri*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* each of them isolated once (0.71%) (Table 2).

We detected 39/141 (27.7%) ESBL producers, most of them were*E. coli*, followed by *Klebsiella pneumonia spp.* and only one *Pseudomonas aeruginosa* with statistically insignificant difference (P = 0.55). AmpC producerswere5/141 (3.5%), most of them were *E. coli* and only one was *Enterobacter Cloacae* with statistically highly significant difference (P= 0.00). From 141 isolates, only one Carbapenemase was detected (0.7%) which is *Klebsiella pneumoniae spp.* (Tables 3).

Antimicrobial susceptibility pattern of ESBL producing GNB showed that all were sensitive to Ertapenem, Imipenem, Meropenem, Amikacin (100%) and Piperacillin/ Tazobactam (97.5%). The susceptibility to Fosfomycin, Nitrofurantoinwere 89.7%, 87.2% respectively. The rate of resistance to Gentamicin, Amoxicillin/ Clavulanic (Amoxaclav.), Ciprofloxacin, Norfloxacin and Trimethoprim/ Sulfamethoxazole were 30.8%, 38.5%, 61.5%, 64.1% and 69.2% respectively with statistically highly significant difference (P= 0.00) (Table 4).

Antimicrobial susceptibility pattern of AmpC producing GNB showed that all were sensitive to Ertapenem, Imipenem, Meropenem, Cefepime, Amikacin and Nitrofurantoin. The susceptibility to Gentamicin and Fosfomycin were 80% and the least sensitivity was to Ciprofloxacin and Norfloxacin (60%) (Table 5).

Carbapenemase producing organism was resistant to all antibiotics except Gentamicin and Fosfomycin (susceptibility,100% each).

E. coli and *Klebsiella spp*. were highly susceptible to Ertapenem, Imipenem, Meropenem, Amikacin. Generally all studied GNB exhibited high susceptibility to Amoxacillin/ Clavulanic acid than Ampicillin alone and also the same trend was observed with Piperacillin/ Tazobactam. The *E.Coli* resistance rates were higher than *klebsiella spp.* to Ciprofloxacin, Norfloxacin, Gentamicin 29.5%, 29.5%, 15.2% respectively versus12%, 16%, 8% respectively. The *Klebsiella spp* .resistance rates were higher than *E.Coli* to Fosfomycin, Nitrofurantoin 36%, 28% versus 1.8% each respectively. The overall resistance rates to 3rd generation (Cefotaxime, Ceftazidime) and 4th generation cephalosporins (Cefepime) were comparable to each other

31.3%, 32.1%, 28.6% for *E. coli* versus 28%each, respectively in *Klebsiella spp.* There was similar rate of resistance to Trimethoprim/Sulfamethoxazole for both *E. coli* and *Klebsiellae spp*. 37.5%, 36% respectively (Table 6).

Drug resistance poses a therapeutic problem not only in the hospital settings, but also in the community as most of the bacteria have acquired resistance to multiple antibiotics. In the clinical laboratory settings, the commonly detected enzymes causing resistance are AmpC B-lactamases and ESBLs (El-Hady and Adel, 2015). Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failure (Singhal et al., 2005).

Most of our samples were urine (92.2%) because UTI is the second most common community acquired infection in clinical practice worldwide (Sharma and Paul, 2013). The incidence of UTI was higher in female than male patients (88.7% versus 11.3%) due to physical factors, absence of prostatic secretions, and anatomical urethral shortage (Alzohairy and Khadri, 2011).Accurate and prompt diagnoses of UTI are important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract (pyelonephritis) sites.

In our study *E. coli* accounted for 79.4% (112/141), followed by *Klebsiella pneumoniae* 17.7% (25/141) and other GNB 2.9% (4/141). A result which is in agreement with other study in Sothern Saudi Arabia which showeda percentage of77%, 16% and 7% for the isolated organisms in outpatient UTI(El-Kersh et al., 2015). Previous studies (Gupta et al., 2011; Al-Jiffri et al., 2011; Pondei et al., 2012; Ahmad, 2012) have shown lower frequency of *E. coli* causing community-acquired infections 36%,44%,54%, 66% respectively. Other studies have shown higher percentage of *Klebsiella pneumonia* 57.4%, 50%, 54.1% (El-Hady and Adel, 2015; Doddaiah and Anjaneya, 2014; Mohanty et al., 2010)in the hospital-acquired infections

In our study, 27.7% of the organism was ESBL producer; mostly in *E. coli* (28.6%) followed by *Klebsiella pneumoniae spp*. (24%). Dutta et al, 2014[11] in a tertiary care hospital and Lu et al., 2012inoutpatient UTI detected the same percentage (27.3%, 28.2%),In contrast, higher percentages were shown in a tertiary care hospital (Doddaiah and Anjaneya, 2014), nosocomial infection (Tsering et al., 2009) and outpatients UTI (Kashyap et al., 2013; El-Kersh et al., 2015).

El-Kersh et al., 2015and Kumar et al., 2014 had detected higher prevalence (44.2%, 55.6%) of*E. coli* ESBL producer in outpatient's infections than our results. Also for *Klebsiella pneumonia* ESBL producer, a very high prevalence (53.5%, 96%) had been shown (El-Kersh et al., 2015; Muzaheed et al., 2008).

AmpC was detected in 3.5% of the total samples, most of them was *E. coli* 3.6% (4/112). This result was in agreement with Wassef et al, 2014 who had detected a similar percentage for AmpC producer (4.4%). Doddaiah and Anjaneya, 2014 had showed slightly higher percentage (14.24 %). El-Hady and Adel, 2015had showed higher prevalence in ICU admitted patients (33.8%).

Carbapenemase was detected in our study only in 1/141 (0.7%) in *Klebsiella pneumonia spp*., Doddaiah and Anjaneya, 2014 had detected a higher percentage of Carbapenemase (18.25%) mostly in *E. coli* (50%) and *Klebsiella* (32.35%) in rural tertiary care teaching hospital. Carbapenem-

resistant Enterobacteriaceae (CRE) has an overall prevalence of 2-7% in ICUs in Europe, Asia and the United states. This issue appears especially critical in *Klebsiellae pneumonia spp*. (Ruppe et al., 2015). The existing data showed a wide variation in the prevalence of ESBL, AmpC and Carbapenemase from region to region or even from hospital to hospital in the same region (Babypadmini and Appalaraju, 2004). The ESBL producing isolates showed a highly significant resistance to Ampicillin, 3rd and 4thgeneration cephalosporin (P=0.000) and high sensitivity to Ertapenem, Imipenem, Meropenem, Amikacin and Piperacillin/ Tazobactam compared to non-ESBL producers. The rate of resistance of ESBL producing isolatesto Gentamicin, Amoxaclav, Ciprofloxacin, Norfloxacin and Trimethoprim/ Sulfamethoxazole were 30.8%, 38.5%, 61.5%, 64.1%, 69.2% respectively with statistically highly significant difference (P= 0.00).

Table.1 Types and Microscopic Examination of the Studied Samples (n=141). Values are Numbers (%) or Mean ± SD (range)

Samples	Number (%)	WBCs/HPF	RBCs/HPF
Urine	**130 (92.2)**	**175± 412.5 (1-3152)**	**40 ± 184 (1-1526)**
HVS	**8 (5.7)**	**3.5± 1.4 (2-5)**	**1.3± 0.5 (1-2)**
Sputum	**2 (1.4)**	**3 ± 0.00**	**0.5± 0.7 (0-1)**
Pus	**1 (0.7)**	**2**	**1**

HVS= High vaginal swab
WBCs= White blood cells
RBCs = Red blood cells
HPF = High power field

Table.2 Types of the Isolated Organisms in the Studied Samples (n=141)

Types of organisms	Number	Percentage
E. coli	**112**	**79.43**
Klebsiella pneumoniae	**25**	**17.73**
Proteus mirabilis	**1**	**0.71**
Citrobacter koseri	**1**	**0.71**
Enterobacter cloacae	**1**	**0.71**
Pseudomonas aeruginosa	**1**	**0.71**
Total	**141**	**100**

Table.3 Distribution of ESBL, AmpC and Carbapenemase among the Studied Gram Negative Bacilli (n=141)

Type of organisms	ESBL			AmpC			Carbapenemase		
	-ve N (%)	+ve N (%)	P-value	-ve N (%)	+ve N (%)	P-value	-ve N (%)	+ve N (%)	P-value
E. coli (112)	80 (71.4)	32 (28.6)		108 (96.4)	4 (3.6)		112 (100)	0	
Klebsiella (25)	19 (76)	6 (24)		25 (100)	0		24 (96)	1 (4)	
Proteus (1)	1 (100)	0		1 (100)	0		1 (100)	0	
Citrobacter (1)	1 (100)	0	0.55	1 (100)	0	0.00	1 (100)	0	0.45
Entrobacter Cloacae (1)	1 (100)	0		0	1 (100)		1 (100)	0	
Pseudo (1)	0	1 (100)		1 (100)	0		1 (100)	0	
Total (141)	102 (72.3)	39 (27.7)		136 (96.5)	5 (3.5)		140 (99.3)	1 (0.7)	

ESBL: Extended spectrum B-Lactamase

Table.4 Antimicrobial Susceptibility Pattern of ESBL Producing Organisms. Values are Numbers (%)

Antibiotics	ESBL –ve (n=102)			ESBL +ve (n=39)			P-value
	S	R	I	S	R	I	
Ampicillin	44 (43.1)	57 (55.9)	1 (1)	0	39 (100)	0	0.000
Amoxacillin/ Clavu acid	86 (84.3)	7 (6.9)	9 (8.8)	16 (41)	15 (38.5)	8 (20.5)	0.000
Piperacillin/Tazobactam	91 (89.2)	9 (8.8)	2 (2)	32 (82.1)	1 (2.6)	6 (15.4)	0.005
Cefotaxime	96 (94.1)	5 (4.9)	1 (1)	0	39 (100)	0	0.000
Ceftazidime	96 (94.1)	6 (5.9)	0	0	39 (100)	0	0.000
Cefepime	97 (95.1)	5 (4.9)	0	0	39 (100)	0	0.0000
Amikacin	101 (99)	1 (1)	0	39 (100)	0	0	1.0000
Gentamicin	94 (92.2)	7 (6.9)	1 (1)	26 (66.7)	12 (30.8)	1 (2.6)	0.000
Ciprofloxacin	90 (88.2)	12 (11.8)	0	12 (30.8)	24 (61.5)	3 (7.7)	0.000
Norfloxacin	90 (88.2)	12 (11.8)	0	14 (35.9)	25 (64.1)	0	0.000
Fosfomycin	94 (92.2)	8 (7.8)	0	35 (89.7)	4 (10.3)	0	0.74
Nitrofurantoin	80 (78.4)	6 (5.9)	16 (15.7)	29 (74.4)	5 (12.8)	5 (12.8)	0.38
TMP/SMZ	76 (74.5)	26 (25.5)	0	12 (30.8)	27 (69.2)	0	0.000
Ertapenem Imipenem Meropenem	101 (99)	1 (1)	0	39 (100)	0	0	1.000

S=Sensitive R=Resistant I= Intermediate; TMP/SMZ: Trimethoprim/Sulfamethoxazole; ESBL: Extended spectrum B-Lactamase

Table.5 Antimicrobial Susceptibility Pattern of AmpC Produing Organisms
Values are Numbers (%)

Antibiotics	AmpC –ve (n=136)			AmpC +ve (n=5)			P-value
	S	R	I	S	R	I	
Ampicillin	44 (32.4)	91 (66.9)	1 (0.7)	0	5 (100)	0	0.30
Amoxacillin/ Clavu acid	102 (75)	17 (12.5)	17 (12.5)	0	5 (100)	0	0.000
Piperacillin/Tazobactam	123 (90.4)	5 (3.7)	8 (5.9)	0	5 (100)	0	0.000
Cefotaxime	96 (70.6)	40 (29.4)	0	0	4 (80)	1 (20)	0.000
Ceftazidime	96 (94.1)	40 (29.4)	0	0	5 (100)	0	0.003
Cefepime	96 (70.6)	40 (29.4)	0	5 (100)	0	0	0.18
Amikacin	135 (99.3)	1 (0.7)	0	5 (100)	0	0	1.000
Gentamicin	116 (85.3)	18 (13.2)	2 (1.5)	4 (80)	1 (20)	0	0.88
Ciprofloxacin	99 (72.8)	34 (25)	3 (2.2)	3 (60)	2 (40)	0	0.73
Norfloxacin	101 (74.3)	35 (25.7)	0	3 (60)	2 (40)	0	0.61
Fosfomycin	125 (91.9)	11 (8.1)	0	4 (80)	1 (20)	0	0.36
Nitrofurantoin	106 (77.9)	11 (8.1)	19 (14)	3 (60)	0	2 (40)	0.25
TMP/SMZ	85 (62.5)	51 (37.5)	0	3 (60)	2 (40)	0	1.000
Ertapenem Imipenem Meropenem	135 (99.3)	1 (0.7)	0	5 (100)	0	0	1.000

S=Sensitive R=Resistant I= Intermediate
TMP/SMZ: Trimethoprim/Sulfamethoxazole

Table.6 Antimicrobial Susceptibility Pattern of the Studied Organisms (n=141). Values are Numbers (%)

Antibiotics	***E. Coli* (n=112)**			***Klebsiella* (n=25)**			***Proteus* (n=1)**		***Citrobacter* (n=1)**		***Enterobacter* (n=1)**			***Pseudomonas* (n=1)**		**P-value**
	S	R	I	S	R	I	S	R	S	R	S	R	I	S	R	
Ampicillin	42 (37.5)	69 (61.6)	1 (0.9)	0	25 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0	1 (100)	**0.04**
Amoxacillin/ Clavu acid	80 (71.4)	17 (15.2)	15 (13.4)	20 (80)	3 (12)	2 (8)	1 (100)	0	1 (100)	0	0	1 (100)	0	0	1 (100)	0.25
Piperacillin/ Tazobactam	100 (89.3)	8 (7.1)	4 (3.6)	20 (80)	1 (4)	4 (16)	1 (100)	0	1 (100)	0	0	1 (100)	0	1 (100)	0	0.03
Cefotaxime	76 (67.9)	35 (31.3)	1 (0.9)	18 (72)	7 (28)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0	1 (100)	0.8
Ceftazidime	76 (67.9)	36 (32.1)	0	18 (72)	7 (28)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0	1 (100)	0.37
Cefepime	80 (71.4)	32 (28.6)	0	18 (72)	7 (28)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	0	1 (100)	0.6
Amikacin	112 (100)	0	0	24 (96)	1 (4)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0.5
Gentamicin	93 (83)	17 (15.2)	2 (1.8)	23 (92)	2 (8)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0.1
Ciprofloxacin	79 (70.5)	33 (29.5)	0	19 (76)	3 (12)	3 (12)	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0.05
Norfloxacin	79 (70.5)	33 (29.5)	0	21 (84)	4 (16)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0.6
Fosfomycin	110 (98.2)	2 (1.8)	0	16 (64)	9 (36)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	0	1 (100)	0.00
Nitrofurantoin	103 (92)	2 (1.8)	7 (7)	5 (20)	7 (28)	13 (52)	0	1 (100)	1 (100)	0	0	0	1 (100)	0	1 (100)	0.00
TMP/SMZ	70 (62.5)	42 (37.5)	0	16 (64)	9 (36)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0	1 (100)	0.5
Ertapenem Imipenem Meropenem	112 (100)	0	0	24 (96)	1 (4)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0.6

S=Sensitive R=Resistant I= Intermediate
TMP/SMZ: Trimethoprim/Sulfamethoxazole

Similarly, Tsering et al,2009showed a significant resistance to Ampicillin, Trimethoprim/ Sulfamethoxazole, Ciprofloxacin but not in agreement with our results for Piperacillin/ Tazobactam and Gentamicin which they documented significant resistance.Lu et al, 2012 showed nearly similar results to usregarding antibiotic sensitivity as Amikacin was the most effective antibiotic (91.7%) followed by Ertapenem (86.9%), Imipenem (86.6%) and Piperacillin/Tazobactam (84.9%). For Ciprofloxacin and Levofloxacin, the susceptibility rates were higher than our results (51.4% and 54.4%) respectively.

Mohanty et al, 2010 showed that all 70 ESBL-producing isolates were susceptible to Imipenem and Meropenem but Ertapenem was active against 97.14% of ESBL-producing isolates. However for Amikacin, Gentamicin, Ciprofloxacin, Piperacillin/

Tazobactam, they were only active against 52.8%, 15.7%, 27.1 and 32.8% of ESBL-positive isolates respectively. Kumar et al, 2014 documented that antimicrobial sensitivity pattern of ESBL-producing *E. coli* showed that it was 100% susceptible to Imipenem however their results were not in agreement with our results in that the susceptibility to ESBL inhibitor combination drugs was almost the same as compared to non-ESBL producing *E. coli*.

In our study, the antimicrobial susceptibility pattern of AmpC producing GNB showed that all were susceptible to Imipenem, Meropenem, Ertapenem, Cefepime, Amikacin and Nitrofurantoin. Dutta et al, 2014observed that all the ESBL and AmpC producing isolates were sensitive to Imipenem, thereby reiterating the continued efficacy of Carbapenems as the first line agents for treatment of healthcare associated infections caused by the members of Enterobacteriaceae producing ESBL and AmpC B-lactamases.

Treatment of UTI is often started empirically (Kurtaran et al., 2010).Conveniently, the most frequently prescribed antibiotics are oral broad spectrum beta lactam antibiotics as Amoxicillin, Ampicillin/ Clavulanate, and oral Cephalosporins, Trimethoprim/ Sulfamethoxazole (TMPSMZ), Nitofurantoin, or quinolones for lower uncomplicated UTI (cystitis). Common misuse, underuse, and/or overuse, as well as often neglected local community susceptibility profiles of these agents, especially in developing countries, invariably resulted in the emergence of multidrug resistant (MDR) isolates among all uropathogenic bacteria including *E coli*, thereby making treatment options very limited (Sharma and Paul, 2013).
Antimicrobial susceptibility pattern of our carbapenemase producing organisms were resistant to all antibiotics and only sensitive to Gentamicin and Fosfomycin. Nicasio et al, 2008 documented that although still rare, *Klebsiella species* retain susceptibility only to Tigecycline, Polymyxins and occasionally Aminoglycosides.

From our results we can conclude thatESBL producers are increasing in the patients visiting primary healthcare clinics. So, routine ESBL detection should be mandatory done. The different antimicrobial resistance patterns of GNB must be taken in consideration by local physicians to ensure appropriate empiric use of antibiotics and hopefully help in treatment of CA-acquired infections. We recommend to do culture and sensitivity before the start of antibiotic and to follow the culture results.

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