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PREVALENCE OF ESBL PRODUCING GRAM NEGATIVE BACTERIA AND DETERMINE THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESBL PRODUCING *E. COLI* IN ADEN CITY-YEMEN

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ABSTRACT

Background: Extended spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* has tremendously increased worldwide and it is one of the most common causes of morbidity and mortality associated with hospital and community-acquired infections. This could be attributed to association of multidrug resistance in ESBL producing bacteria. The present study was aimed to determine the prevalence of ESBL among Gram negative bacteria isolated from private clinical laboratories in Aden city-Yemen and determine the antimicrobial sensitivity profile of ESBL producing *E. coli* isolates from various clinical samples. Since there no such published data. **Materials And Methods:** A cross-sectional study was conducted during the study period from 12th January to 12th April 2018. Clinical samples, which consist of pus, urine, stool, sputum, throat swabs, and semen, were collected according to standard procedures and transported without delay. Samples were processed and identified as per routine laboratory protocol. ESBL screening test and confirmation by double disk diffusion test (DDDT) along with antimicrobial susceptibility test was done according to the Clinical Laboratory Standards Institute (CLSI) guidelines. **Results:** A total number of 114 clinical specimens of Gram negative bacteria were obtained from different clinical samples. The age of such patients were ranged from less than one year up to 40 years of age. Seventy nine (69.3%) were male and 35(30.7%) were female. Out of 114 Gram negative isolates in this study majority was *Escherichia coli* 85(74.6%) followed by *Klebsiella spp.* 14(12.3%), while *Pseudomonas spp.* and *Proteus spp.* were obtained in 8(7%) and 7(6.1%), respectively. In the combined disk test, 75(65.8%) isolates were ESBL producers. The higher occurrence of ESBL producing *E. coli* 60(80%), *Klebsiella spp.* 8(10.7%), *Proteus spp.* 4(5.3%), and *pseudomonas spp.* 3(4%) from various clinical isolates. Out of the 75 positive ESBL and the 39 negative ESBL the resistance pattern to Beta-Lactam drugs were: Cefuroxime (81.3% & 41%), Cefixime (88% & 38.5%), Cefpodoxime (100% & 94.9%), Ceftriaxone (100% & 41%), Cefotaxime (89.3% & 56.4%), Aztreonam (98.7% & 25.6%) and Imipenem (10.7% & 7.7%), while Aminoglycosides: Amikacin (20% & 10.3%) and Gentamycin (25.3% & 17.9%), in addition, the Fluoroquinolones: Ciprofloxacin (34.7% & 30.8%), Ofloxacin (52% & 33.3%) and Norfloxacin (37.3% & 23.1%). **Conclusion:** Results indicate that routine ESBL detection should be made mandatory by both combined methods and strains should be taken both 3rd Generation Cephalosporins sensitive and resistant. Irrational use of 3rd generation cephalosporins must be discouraged to reduce multidrug resistant bacteria, to increase patients` compliance and to make an antibiotic policy.

KEYWORDS: ESBL, *E. coli*, ESBL producing *E. coli*, DDDT.

INTRODUCTION

Antibiotic resistance is increasing at alarming levels and has emerged as a major public health concern of the 21st century *(Addo and Odonkor, 2011)*.

Extended spectrum β-lactamase (ESBL) isolates were first detected in Western Europe in the mid-1980s. Since then, their incidence has been increasing steadily. ESBLs are able to hydrolyze 3rd and 4th generation Cephalosporins and Monobactams (e.g., Aztreonam) but do not affect Cephamycins (e.g., Cefoxitin and Cefotetan) or Carbapenems (e.g., Imipenem or Meropenem). ESBL producing strains are inhibited by β-lactamase inhibitors (Clavulanic acid, Sulbactam and Tazobactam) *(Bradford, 2001; Fosse and Giraud-Morin, 2003)*.

ESBLs are a group of enzymes encoded by genes described predominantly on plasmid that are common among *Enterobacteriaceae* ***(Poole, 2004)***. Although most ESBLs are mutants of temoneira (TEM) and sulfhydryl variable (SHV) enzymes, the cefotaximase

(CTX-M) type-lactamases which have become important, originated from β-lactamases found in environmental species of the genus *Kluyvera*, and this enzyme hydrolyzes cefotaxime and cefriaxone but is weakly active against ceftazidime ***(Bonnet, 2004 and Perez, et al., 2007)***.

ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. For example, one enzyme may actively hydrolyze ceftazidime, resulting in ceftazidime minimum inhibitory concentrations (MICs) of 256 µg/ml, but have poor activity on cefotaxime, producing MICs of only 4 µg/ml. If an ESBL is detected, all penicillins, cephalosporins, and aztreonam should be reported as resistant, even if in vitro test results indicate susceptibility ***(Briissow, et al., 2004)***.

The aim of the study to determine the prevalence of extended spectrum beta lactamase (ESBL) among Gram negative bacteria using double disc diffusion methods and the isolated *E. coli* were subjected to routine antimicrobial susceptibility testing.

MATERIALS AND METHODS

Study design and Methods: The type of the study was cross sectional study. The study was conducted from 1st of January 2018 up to the 1st of April at the same year. The total of 114 samples were studied. Clinical samples, which consist of pus, urine, stool, sputum, throat swabs, and semen, were collected according to standard procedures and transported without delay ***(Collee, and Marr, 1996)***. All samples were inoculated onto blood agar and MacConkey agar except urine which was inoculated onto Cysteine Lactose Electrolyte Deficient agar (CLED) and incubated aerobically at 37°C. Bacterial pathogens were identified as per the standard protocol ***(Collee, et al., 1996 and Forbes, et al., 2007)***. The isolates were screened for ESBL production by using disc Diffusion of Cefixime (CFM), Cefpodoxime (CPD), Ceftriaxone (CRX), Cefotaxime (CTX), Ceftazidime (CAZ) and Aztreonam (AZM) placed on inoculated plates containing Muller Hinton agar according to the CLSI recommendations. Isolates showing inhibition zone size of ≤15mm with Cefixime (30µg), ≤17mm with Cefpodoxime (10µg), ≤25 mm with Ceftriaxone (30µg), ≤27mm with Cefotaxime (30µg), ≤22mm with Ceftazidime (30µg) and ≤27mm with Aztreonam (30µg) were suspected for ESBL production. In addition, phenotypic confirmatory test for ESBL producers were done by double disc diffusion test (DDDT) using: Ceftriaxone + Tazobactam (30µg/10µg). Increase in zone diameter (≥5 mm) for Ceftriaxone in combination with Tazobactam versus its zone when tested alone was designated as ESBL positive ***(Bradford, 2001)***. The isolated *E. coli* either positive or negative ESBL producers were subjected to routine antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method according to CLSI guidelines ***(Vemula, and Vadde, 2011)***. According to CLSI guidelines ***(CLSI, 2012)*** the following discs were used for antibiotic susceptibility testing of *E. coli* which isolated - Amikacin (AK, 30µg), Gentamycin (GEN, 10µg), Ciprofloxacin (CIP, 5µg), Ofloxacin (OF, 5µg), Cefuroxime (CXM, 30µg), Cefixime (CFM, 30µg), Cefpodoxime (CPD, 10µg), Ceftriaxone (CTR, 30µg), Cefotaxime (CTX, 30µg), Ceftazidime (CAZ, 30µg), Aztreonam (AT, 30µg), Imipenem (IPM, 10µg) and Norfloxacin (NX, 10µg). Norfloxacin was tested against urinary isolates only.

Data analysis: Data were analyzed by using computer based programmed excel and statistical package for social science (SPSS) version 20.0.

RESULTS

During the study period from 1st of January 2018 up to the 1st of April at the same year, 114 gram negative bacteria were evaluated for the presence or absence of ESBLs. The age of such patients were ranged from less than one years to 40 years of age. Seventy nine (69.3%) were male and 35(30.7%) were female. The most common specimens of such isolated bacteria were obtained from urine, stool, pus, semen, throat swab and sputum, (50.8%, 38.6%, 5.3%, 2.6%, 1.8% and **0.9%), respectively, table (1) and Figure (1).**

Table 1: Frequent Types of Specimens.

Type Specimen	Frequency	Percent
Urine	58	50.8
Stool	44	38.6
Pus	6	5.3
Semen	3	2.6
Throat swab	2	1.8
Sputum	1	0.9
Total	**114**	**100**

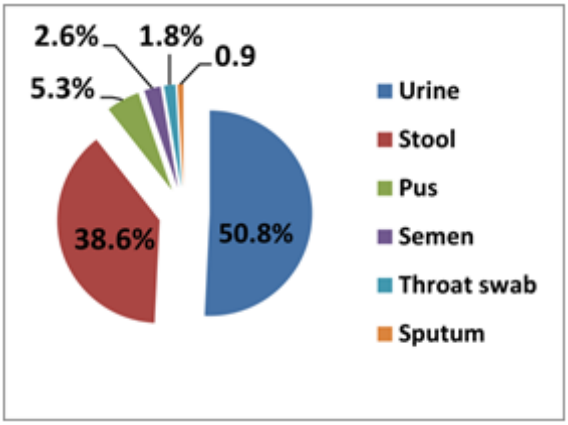

Figure 1: Frequency of specimens.

Out of 114 Gram negative isolates in this study majority was *Escherichia coli* 85(74.6%) followed by *Klebsiella spp.* 14(12.3%), while *Pseudomonas spp.* and *Proteus spp.* were obtained in 8(7%) and 7(6.1%), respectively, table (2).

Table 2: Type of bacteria isolated.

Type of bacteria	Frequency	Percent
E. coli	85	74.6
Klebsiella spp.	14	12.3
Pseudomonas	8	7
Proteus spp.	7	6.1
Total	**114**	**100**

There was a highly statistical significant association between the clinical isolates and the type of specimens obtained (P=0.000). Out of the 85 clinical isolates of *E. coli* 43(50.6%) were from urine, 38(44.7%) from stool, 3(3.5%) and 1(1.2%) were from pus and throat swab respectively. *Klebsiella spp.* and *Pseudomonas* mostly isolated from urine 9(64.3%), 4(50%), respectively, while Proteus spp. distributed in stool, urine and pus 3(42.9%), 2(28.6%) and 2(28.6%), respectively. Table (3).

Table 3: Distribution of the Clinical Isolates in Relationship with the Type of Specimens.

Type of bacteria	Type of Specimens						Total
	Urine	Stool	Pus	Semen	Throat Swab	Sputum	
E. coli	43(50.6%)	38(44.7%)	3(3.5%)	0(.0%)	1(1.2%)	0(.0%)	85(100%)
Klebsiella spp.	9(64.3%)	0(.0%)	0(.0%)	3(21.4%)	1(7.1%)	1(7.1%)	14(100%)
Pseudomonas	4(50%)	3(37.5%)	1(12.5%)	0(.0%)	0(.0%)	0(.0%)	8(100%)
Proteus spp.	2(28.6%)	3(42.9%)	2(28.6%)	0(.0%)	0(.0%)	0(.0%)	7(100%)
Total	58(50.8%)	44(38.6%)	6(5.3%)	3(2.6%)	2(1.8%)	1(0.9%)	114(100%)

X^2=48.000, df=15, P=0.000

Out of the 114 clinical isolates ESBLs was detected by screening test and double disc diffusion test (DDDT) using: Ceftriaxone + Tazobactam (30µg/10µg) in 75(65.8%). The association between the positive and negative ESBLs producers with the clinical samples and the type of bacteria isolated were evaluated (P=0.4 and 0.2), respectively. Table (4) and (5).

Table 4: Association between ESBL and the Type of the Specimens.

ESBLs	Type of Specimens						Total
	Pus	Urine	Sputum	Stool	Throat swab	Semen	
Positive	2(2.7%)	39(52%)	0(.0%)	31(41.3%)	1(1.3%)	2(2.7%)	75(100%)
Negative	4(10.3%)	19(48.7%)	1(2.6%)	13(33.3%)	1(2.6%)	1(2.6%)	39(100%)
Total	6(5.3%)	58(50.8%)	1(.9%)	44(38.6%)	2(1.8%)	3(2.6%)	114(100%)

X^2=5.434, df=5, P=0.4

Table 5: Association between ESBL and the Type of the Clinical Isolates.

ESBLs	Type of the Clinical Isolates				Total
	E. coli	***Klebsiella***	***Proteus***	***Pseudomonas***	
Positive	60(80%)	8(10.7%)	4(5.3%)	3(4%)	75(100%)
Negative	25(64.1%)	6(15.4%)	3(7.7%)	5(12.8%)	39(00%)
Total	85(74.6%)	14(12.3%)	7(6.1%)	8(7%)	114(100%)

X^2=4.412, df=3, P=0.2

Susceptibility testing was performed using the disk diffusion method as per the guidelines of Clinical and Laboratory Standards Institute (CLSI). thirteen antimicrobial disks were used as indicated in Table 6.

Table 6: The Antimicrobial Susceptibility Disks.

Antibiotic Family	The Disks & its Concentration
Beta-lactams	Cefuroxime (CXM 30µg), Cefixime (CFM 30µg), Cefpodoxime (CPD 10µg), Ceftriaxone (CTR 30µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Aztreonam (AT 30µg) and Imipenem (IPM 10µg).
Aminoglycosides	Amikacin (AK 30µg) and Gentamycin (GEN 10 µg).
Fluoroquinolones	Ciprofloxacin (CIP 5µg), Ofloxacin (OF 5µg) and Norfloxacin (NX 10µg)

Out of the 75 positive ESBL and the 39 negative ESBL the resistance pattern was depicted in Table 7 and represented in Figure 2.

Table 7: The Results Of Resistance pattern of Disk Diffusion Test Compared Between (ESBL Positive and Negative) Isolates.

Antibiotic Family	Disks and Resistance (ESBL Positive and ESBL Negative)
Beta-Lactamase	Cefuroxime (81.3% & 41%), Cefixime (88% & 38.5%), Cefpodoxime (100% & 35.9%), Ceftriaxone (100% & 41%), Cefotaxime (89.3% & 56.4%), Ceftazidime (100% & 15.4%), Aztreonam (98.7% & 25.6%) and Imipenem (10.7% & 10.3%).
Aminoglycosides	Amikacin (20% & 10.3%) and Gentamycin (25.3% & 17.9%).
Fluoroquinolones	Ciprofloxacin (34.7% & 30.8%), Ofloxacin (52% & 33.3%) and Norfloxacin (37.3% & 23.1%).

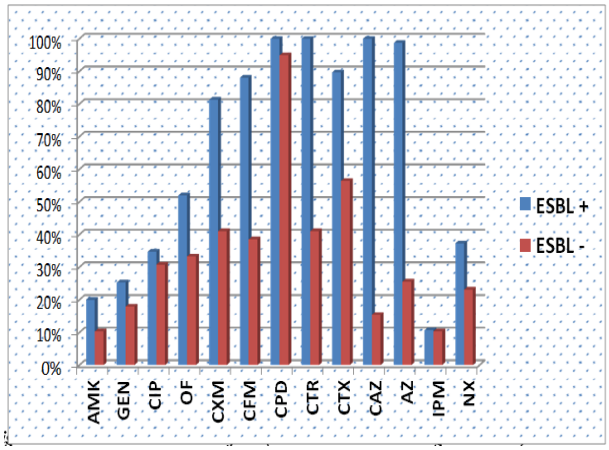

Figure 2: The Resistance Pattern of the (Positive ESBL & the Negative ESBL).

Out of the 85 *E. coli* the 60 cases were ESBL positive and 25 cases were negative as mentioned before. The susceptibility testing profile between positive and negative ESBL-*E. coli* strains is depicted in Table 8. The resistance pattern of the positive & negative ESBL-*E. coli* to the Beta lactam classes: Cefuroxime (80% & 32%), Cefixime (88.3% & 32%), Cefpodoxime (100% & 36%), Ceftriaxone (100% & 20%), Cefotaxime (90% & 56%), Ceftazidime & Aztreonam were same (98.3% & 20%) and Imipenem (11.7% & 4%). Moreover, the sensitivity pattern of *E. coli* regardless to be ESBLs-producers was showed a highest sensitivity to Imipenem followed by Amikacin and Gentamycin 77(90.6%), 70(82.4%) and 66(77.6%), respectively, while Ciprofloxacin and Ofloxacin showed a moderate susceptibility 57(67.1%) and 47(55.3%), respectively. Table (8).

Table 8: The susceptibility testing profile between ESBL positive and negative E. coli:

E. coli	Amikacin		Total	Gentamycin		Total	Ciprofloxacin		Total	Ofloxacin		Total	Cefuroxime		Total
	S	R		S	R		S	R		S	R		S	R	
ESBLs +	47 (78.3%)	13 (21.7%)	60 (100%)	45 (75%)	15 (25%)	60 (100%)	39 (65%)	21 (35%)	60 (100%)	28 (46.7%)	32 (53.3%)	60 (100%)	12 (20%)	48 (80%)	60 (100%)
ESBLs -	23 (92%)	2(8%)	25 (100%)	21 (84%)	4(16%)	25 (100%)	18 (72%)	7(28%)	25 (100%)	19 (76%)	6(24%)	25 (100%)	17 (68%)	8(32%)	25 (100%)
Total	70 (82.4%)	15 (17.6%)	85 (100%)	66 (77.6%)	19 (22.4%)	85 (100%)	57 (67.1%)	28 (32.9%)	85 (100%)	47 (55.3%)	38 (44.7%)	85 (100%)	29 (34.1%)	56 (65.9%)	85 (100%)
P-Value	X^2=2.268, df=1, P=0.1			X^2=0.824, df=1, P=0.4			X^2=0.391, df=1, P=0.5			***X^2=6.143, df=1, P=0.01***			***X^2=18.089, df=1, P=0.000***		
E. coli	Cefixime		Total	Cefpodoxime		Total	Ceftriaxone		Total	Cefotaxime		Total	Ceftazidime		Total
	S	R		S	R		S	R		S	R		S	R	
ESBLs +	7 (11.7%)	53 (88.3%)	60 (100%)	0(.0%)	60 (100%)	60 (100%)	0(.0%)	60 (100%)	60 (100%)	6 (10%)	54 (90%)	60 (100%)	1(1.7%)	59 (98.3%)	60 (100%)
ESBLs -	17(68%)	8(32%)	25(100%)	16(64%)	9(36%)	25(100%)	20(80%)	5(20%)	25(100%)	11(44%)	14(56%)	25(100%)	20(80%)	5(20%)	25(100%)
Total	24 (28.2%)	61 (71.8%)	85 (100%)	16 (81.8%)	69 (81.2%)	85 (100%)	20 (23.5%)	65 (76.5%)	85 (100%)	17 (20%)	68 (80%)	85 (100%)	21 (24.7%)	64 (75.3%)	85 (100%)
P-Value	***X^2=27.638, df=1, P=0.000***			***X^2=47.304, df=1, P=0.000***			***X^2=62.769, df=1, P=0.000***			***X^2=12.750, df=1, P=0.000***			***X^2=58.211, df=1, P=0.000***		
E. coli	Aztreonam		Total	Imipenem		Total	Norfloxacin		Total						
	S	R		S	R		S	R							
ESBLs +	1(1.7%)	59 (98.3%)	60 (100%)	53 (88.3%)	7 (11.7%)	60 (100%)	8 (13.3%)	23 (38.3%)	60 (100%)						
ESBLs -	20(80%)	5(20%)	25(100%)	24(96%)	1(4%)	25(100%)	7(28%)	5(20%)	25(100%)						
Total	21 (24.7%)	64 (75.3%)	85 (100%)	77 (90.6%)	8 (9.4%)	85 (100%)	15 (17.6%)	28 (32.9%)	85 (100%)						
P-Value	***X^2=58.211, df=1, P=0.000***			X^2=1.217, df=1, P=0.3			X^2=4.000, df=1, P=0.2								

Regarding Norfloxacin ESBLs positive E. coli not done (ND)= 29(483%), while ESBLs negative E. coli not done (ND)= 13(52%). S=Sensitive, R=Resistance.

DISCUSSION

Resistance to different antibiotics used varies between countries and communities, and may also change with time and geographic location ***(Mégraud et al., 1999)***.

However, the prevalence of multi-drug resistant strains, especially in developing countries ***(Smith, et al. 2001, and Poon, et al. 2002)*** makes culture and antibiotic sensitivity testing become a pre-requisite for patients with persistent infection after initial or repeated treatment failure ***(Krogfelt, et al. 2005)***.

Misuse and or overuse of antimicrobials in hospital or in community will not only be expensive but also cause unforeseen menace of drug resistance in the future ***(Harakuni, et al., 2011)***.

An extensive use of β-lactam antibiotics in hospital and community has created a major problem leading to increased morbidity, mortality and health care costs. Proper use of antibiotics is very important for various reasons. Development of bacterial resistance against newer antibiotics makes the main focus of research ***(Taslima, 2012)***.

Infections by ESBLs producing organisms have emerged as a major problem and the failure of therapy with broad spectrum antibiotics are creating serious problems ***(Sharma, 2013)***.

Out of 114 Gram negative isolates in this study majority was *Escherichia coli* (74.6%) followed by *Klebsiella spp.* 14(12.3%), while *Pseudomonas spp.* and *Proteus spp.* were obtained in 8(7%) and 7(6.1%), respectively, table (2). Contras to the current study was done by ***Taslima, (2012),*** in which she found that out of 300 Gram negative isolates in her study majority were *E. coli* 156 (52%), followed by *Proteus spp.* 55 (18.3%), *Klebsiella spp.* 45 (15%), *Pseudomonas spp.* 9 (3%) and others (*Enterobacter spp., Citrobacter spp.*) 35 (11.7%).

There was a highly statistical significant association between the clinical isolates and the type of specimens obtained (P=0.000). Out of the 85 clinical isolates of *E. coli* 43(50.6%) were from urine, 38(44.7%) from stool, 3(3.5%) and 1(1.2%) were from pus and throat swab respectively. *Klebsiella spp.* and *Pseudomonas* mostly isolated from urine 9(64.3%), 4(50%), respectively, while Proteus spp. distributed in stool, urine and pus 3(42.9%), 2(28.6%) and 2(28.6%), respectively, table (3). This study was similar to that done by ***Nipa, et al., (2016)*** in which maximum number of *E. coli* were isolated from urine. ***Taslima, (2012)*** In her study found that the isolation of *E. coli* was 55.6% and 61.2% from both urine and pus respectively. In Bangladesh, it has been reported that 65-92% of commensal *Enterobacteriaceae* isolated from urine ***(Chowdhury, et al., 1989)***.

Initially ESBL producing organisms were usually isolated from nosocomial infections but these organisms are now also being isolated from community ***(Helfand and Bonomo, 2005)***. Occurrence and distribution of ESBLs differs from country to country and from hospital to hospital ***(Ali, 2009)***.

In the current study, the level of ESBL production was higher 75(65.8%), it was isolated from the community. It correlates with the study done in India by ***Sanjo and Veena, (2017)*** in which the prevalence was (68%). Another study reported by ***Das and Borthakur, (2012)*** from South India on ESBL production in uropathogens showed (81.9%) ESBL producers, and ***Nojoomi, et al., (2017),*** in Iran showed that the combined disk test was (82%) isolates were ESBL producers; in which both study were higher than our finding. In addition, In Uganda ESBLs- producing bacteria was found to be higher than our study too (89%). Less than our study the prevalence of ESBLs was seen in Hungary, Poland, Romania, Russia and Turkey (10%) ***(Edelstein, et al., 2003)***, in Lebanon (15.4%) ***(Shaikh, et al., 2015)*** and in Sudan (45.1%) ***(Ibrahim, et al., 2013)***.

The association between the positive and negative ESBLs producers with the type of bacteria isolated were evaluated in our study, there no a statistical significant (P=0.2), but *E. coli* represented the highest prevalence (80%) followed by *Klebsiella spp., Proteus spp. and Pseudomonas* (10.7%, 5.3% and 4%), respectively. This was in agreement with findings done by ***Taslima, (2012)*** in which *E. coli* and *Klebsiella spp.* showed maximum ESBLs production.

A study in rural Thailand by ***Ulzii-Orshikh, et al., (2011)*** on performing bacterial identification for ESBL-producing *Enterobacteriaceae*, they found that *E. coli* was the predominant followed by *Citrobacter, Klebsiella* and *Enterobacter*. Another study by ***Sanjo and Veena, (2017)*** from India, was revealed that out of the 150 *Enterobacteriaceae* isolates, a majority were *E. coli* (40%), followed by *Klebsiella pneumoniae* (28%), *Citrobacter spp.* (13.3%), *Proteus spp.* (12%), *Enterobacter spp.* (6.6%).

In the present study ESBL producing isolates were more resistance to the 3rd generation cephalosporins and it was ranged from 88%-100% (Table 7), it was similar with the study done by ***Sasirekha, et al., (2010)*** found 75%-85% and ***Haque and Salam (2010)*** found 72%-100% resistant. This was due to irrational and wide use of third generation cephalosporins in both the hospital and community. Ceftazidime and Aztreonam were found 98.7% resistant in the ESBL producing isolates in our study, which nearly correlates with the study done by ***Nojoomi, et al., (2017)*** 97.3% for Aztreonam, while Ceftazidime was less than our finding 82%.

In our study, the susceptibility pattern of the strains to antibiotics demonstrated that among beta-lactam classes,

the most effective drug was Imipenem (89.3% & 89.7%) for the positive & negative ESBL producers, respectively. In addition, among Aminoglycosides class of drug, Amikacin exhibited the highest activity against the isolates (80% & 89.7%), followed by Gentamycin (74.7% & 82.1%) for the positive & negative ESBL producers respectively, while the Fluoroquinolones groups (Ciprofloxacin) showed a moderate susceptibility (65.3% & 69.2%) for the positive & negative ESBL producers, respectively, table (7). Similar to these results were conducted in Tehran by ***Nojoomi et al., (2017)*** who found that the susceptibility of the strains to antibiotics demonstrated that among beta-lactam classes, the most effective drugs were Imipenem (86.5%), Meropenem (67%) and Piperacillin (85%) and among non-beta-lactam antibiotics, Amikacin exhibited the highest activity against the isolates (63%).

Most of the ESBL producing organisms were found to be co-resistance to Fluoroquinolones and Aminoglycosides which correlates with the study done by ***Denholm, et al., (2009)*** and ***Jabeen, et al., (2005)***. This was due to the genes encoding these β-lactamases were often located on large plasmids that also encode genes for resistance to others antibiotics, including aminoglycosides, tetracycline, sulfonamides, trimethoprime and chloramphenicol ***(Perez, et al. 2007)***.

Moreover, the sensitivity pattern of *E. coli* irrespective to be ESBL producing isolates were showed a highest sensitivity to Imipenem followed by Amikacin and Gentamycin 77(90.6%), 70(82.4%) and 66(77.6%), respectively, while Ciprofloxacin and Ofloxacin showed a moderate susceptibility 57(67.1%) and 47(55.3%), respectively. Table (8). ***Dinesh, et al., (2014)*** in India found that antimicrobial susceptibility pattern of *E. coli* isolates demonstrated high susceptibility rates to imipenem (100%), followed by Amikacin and Gentamycin (80% and 74.7%), while Ciprofloxacin and Ofloxacin was (54.7% and 53.3%), respectively.

CONCLUSIONS

The ESBL producing Gram negative bacteria are a cause of concern to the microbiologist as well as to the clinicians, particularly the multidrug resistant strains. Correct choice of antimicrobial agents according to the sensitivity profile is essential for appropriate empirical treatment.

ESBL producers may have spread through communities, especially those with poor hygienic and sanitation conditions, through fecal contamination of soil and water, since most patients with ESBL producers may have had their urinary and gastrointestinal tracts colonized.

Considering various findings of the present study, it can be concluded that Extended spectrum beta lactamases are increasing in Aden city-Yemen with co-resistance to some other classes of antibiotics are very alarming. There was a limited number of drugs sensitivity for this bacteria and drug of choice is imipenem, followed by amikacin. But most probably in near future, if this irrational use is not stopped, infection with that Gram negative bacteria increase the rate of resistant to drugs that are now sensitive, resulting increase morbidity and mortality.

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