Research Article

Prevalence of Bla_{ctx-M} Gene Among MDR Escherichia Coli Isolated from Wound Infections in Minia University Hospital, Egypt

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Abstract

Multi-drug resistant (MDR) Escherichia coli is one of the most prevalent gram-negative pathogenic bacteria. Human skin is the first barrier against different pathogens. The invasion of the destructed skin with such MDR E. coli cause serious wound infections. Treatment of these infections is very difficult due to the ability of the bacteria to utilize different resistance mechanisms. Production of extended spectrum β-lactamases is the most important mechanism as they confer resistance to almost used antibiotics. The study aimed to detect the prevalence bla_{CTX-M} gene among MDR E. coli isolated from wound infections. In this study out of 150 wound specimens, 66 (44%) E. coli were isolated. Their susceptibility to different classes of antibiotics were tested by Kirby Bauer disk diffusion method. The isolates exhibited high resistance level to cefotaxime, ceftriaxone, ceftazidime and amoxicillin /clavulanic. Piperacillin/ Tazobactam and Azithromycin were found to be the most effective antibiotics. The ESBLs production was detected by combined disc method. Twenty-five isolates (37.87%) were found to be ESBLs producers. Their MARI ranged from 0.32 to 0.93. Out of the 25 ESBLs producers, 14 (56%) isolates were positive for bla_{CTX-M} gene. Their MARI ranged from 0.57 to 0.89. **In conclusion**, the high prevalence of MDR ESBL producer E. coli sounds the alarm. There is a need to apply strict infection control and continuous antimicrobial surveillance studies.

Key words: Escherichia Coli, Wound Infections, antimicrobial

Introduction

Escherichia coli is one of the most common commensals present in the human gut however, outside the gut it could be serious cause of extraintestinal infections.

Extraintestinal pathogenic Escherichia coli (ExPEC) is one of the most common gram-negative pathogenic bacteria. It causes a variety of clinical infections as: bacteremia, meningitis in neonates and urinary tract infections (Russo, Johnson et al., 2003).

The human skin barrier is one of the most important defense mechanisms against pathogenic microorganisms. Penetration of broken skin with Extraintestinal pathogenic Escherichia coli may lead to wound infection (Robson, Lea et al., 1968). It was found to be the causative agent of neonatal omphalitis (Fraser, Davies et al., 2006) , cellulitis, necrotizing fasciitis, surgical site infections, burns and traumatic wounds (Petkovšek, Eleršič et al., 2009).

The explosive spread of the antibiotic resistance to almost all antibiotic classes among the E. coli strains is considered a global threat. One of the most threatening resistance mechanisms is the Extended spectrum β-lactamases (ESBLs) production. The extended-spectrum β-lactamases are group of β-lactamases have the ability to hydrolyze the extended-spectrum cephalosporin and monobactam antibiotics (Peirano and Pitout 2010). There are several categories of ESBLs, according to Ambler classification, they are

divided into four molecular classes (A, B, C and D) (Medeiros 1997). ESBls of Ambler Class A are divided into several main groups: Sulphydryl variable (SHV), Temoneira (TEM) and Cefotaximases (CTX-M) (Stürenburg and Mack 2003) (Mathers, Peirano et al., 2015).

Prevalence of CTX-M type ESBls is in contentious increase since late 1990, they have been found in both hospital and community acquired infections(Hara, Sato et al., 2015). CTX-M types have emerged as new forms of ESBL that, unlike TEM and SHV, are more active against cefotaxime and ceftriaxone rather than ceftazidime (Chong, Ito et al., 2011). The CTX-M family is divided into five groups based on similarities in amino acid sequence i.e. CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (Kiratisin, Apisarnthanarak et al., 2007). All CTX-M enzymes contain a serine at position-238 that plays a significant role in increasing β-lactamase activity (Rossolini, D'andrea et al., 2008).

This study was carried out to determine the prevalence of bla_{CTX-M} in multidrug resistant Extraintestinal pathogenic Escherichia coli (ExPEC) isolated from wound infections from outpatients at Minia University Hospital.

Material and Methods

Collection of isolates:

one hundred and fifty clinical specimens of wound infections were collected from outpatients in Minia University Hospital. Sixty-six isolates of Escherichia coli have been isolated. All clinical samples were cultured on trypticase soy agar (Lab M, UK) and then E. coli were isolated on MacConkey agar and Eosin methylene blue (EMB) (lab M, UK) and were identified by regular microbiological biochemical tests.

Antibiotic susceptibility tests

The isolates sensitivity against different classes of antibiotics were tested by the Kirby-Bauer Disk Diffusion method as illustrated before(Hudzicki 2009) on Muller-Hinton agar (MHA) (LAB M,UK). The used antibiotics discs were ready cartilages purchased from Oxoid; Basingstoke, UK. The following antibiotic discs were used Cefpodoxime (10μg), Streptomycin (10μg), Aztreonam (30μg), Ceftriaxone (30μg), Gentamycin (10μg), Amoxycillin/clavulanic (20/10μg), Piperacillin/Tazobactam (100/10μg), Ceftazidime (30μg), Imipenem (10μg), Meropenem (10μg), Cefoperazone (75μg), Doxycycline (30μg), Ciprofloxacin (5μg), Amikacin (30μg), Nalidixic acid (30μg), Cefotaxime (30μg), Cefepime (30μg), Ampicillin/sulbactam (10/10μg), Norfloxacin (10μg), Tobramycin (10μg), Sulfamethoxazole/ trimethoprim (23.75 /1.25μg), Chloramphenicol (30μg). Isolates classified as sensitive, intermediate and resistant according to inhibition zones interpretation standards of Clinical Laboratory standards Institute (CLSI) 2018 (Wayne 2018).

Phenotypic detection of extended spectrum β-lactamases

The sixty-six *E. coli* isolates were tested for ESBLs production by combined disc test. Difference between inhibition zones of cefotaxime and cefotaxime/clavulanic discs or ceftazidime and ceftazidime/ clavulanic discs was observed. The test considered positive when the difference between the inhibition zones is ≥ 5mm **(Wayne 2018)**.

Determination of Multiple Antibiotics Resistance Index (MARI)

Multiple Antibiotic Resistance Indices (MARI) of the ESBL producers were calculated as described by **Christopher, Hora et al., (2013)** and **Subramanian, Shanmugam et al., (2012)**. It was calculated by dividing the number of antibiotics to which the tested isolate was resistant to (a) by the total number of antibiotics that was tested on the isolates (b) (**MARI=a/b).**

PCR amplification

The DNA of isolates extracted by available commercial kit (QIAquick; QIAGEN, Courtaboeuf, France) by following the manufacturer instruction. The CTX-M gene was detected by conventional PCR technique using primers CTX-M F 5'TCTTCCAGAATAA GGAATCCC 3', R 5'CCGTTTCCGCTATT

ACAAAC3' that amplify a fragment of 909 bp. The PCR cycling conditions was as mentioned in study of Ghorbani-Dalini et al., The amplified products were analyzed by electrophoresis in 1.5% agarose gel at 100 V for 30 minutes in Tris–Borate–EDTA buffer containing ethidium bromide under ultraviolet light (Ghorbani-Dalini, Kargar et al., 2015).

Results

Bacterial isolation:

Out of 150 collected specimens, 66 (44%) isolates of *Escherichia coli* have been isolated.

Antibiotic Susceptibility:

Antibiotic susceptibility testing of the isolated *E. coli* was done using twenty-two types of antibiotic discs. The isolates were found to be almost completely resistant to cefotaxime (96.97%) and amoxycillin/clavulanic (95.45%). The isolates also were highly resistant to ampicillin/sulbactam (89.39%), cefepime (87.88%) and ceftazidime (81.82%). About 39.3% and 31.82% of the isolates were resistant to gentamycin and tobramycin respectively. Piperacillin/Tazobactam was found to be the most effective antibiotic.

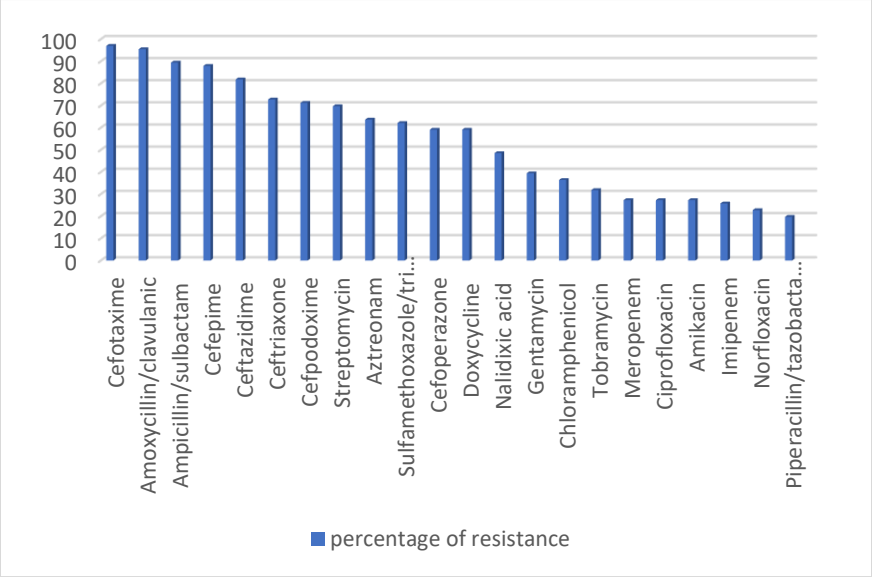

Fig (1): Antibiotic Susceptibility of all isolated *E. coli*

Phenotypic detection of extended spectrum β-lactamases

Out of the 66 tested E. coli, 25 strain (37.87%) considered ESBL producers. They have showed an inhibition zone difference more than 5mm between cefotaxime (CTX) or ceftazidime (CAZ) discs and cefotaxime/clavulanic (CTL) or ceftazidime/clavulanic (CAL) discs respectively.

Multiple Antibiotics Resistance Index (MARI)

The multiple antibiotics resistance indices for the 25 ESBL producers were calculated. They ranged from 0.32 to 0.93 with an average value of 0.7. The mean value of the MARI of the bla_{CTX-M} gene positive isolates was 0.71 range from (0.57 to 0.89) **(Table 1).**

PCR amplification

All the phenotypic positive isolates were subjected to PCR amplification. Out of 25 strain, 14 (56%) strain have the bla_{CTX-M} gene. All the 14 samples were resistant to ceftriaxone (CRO), Cefotaxime (CTX) and ceftazidime (CAZ) (Table1).

Table (1) : Distribution strains of the bla$_{CTX-M}$ gene positive among the 25 phenotypic positive E. coli

E. coli strain	Resistance*			MARI***	Combined disc**		PCR
	CRO	CTX	CAZ		CAL	CTL	CTX-M
9w	R	R	R	0.93	+	+	-
13w	R	R	R	0.89	+	+	+
14w	R	R	R	0.86	-	+	+
15w	R	R	R	0.89	-	+	+
18w	R	R	R	0.82	+	+	-
21w	R	R	R	0.89	+	+	+
25w	R	R	R	0.71	-	+	+
27w	R	R	R	0.71	-	+	+
30w	R	R	R	0.68	-	+	+
31w	R	R	R	0.79	-	+	+
32w	R	R	R	0.75	+	+	-
37w	R	R	R	0.75	+	+	-
40w	R	R	R	0.57	+	+	+
41w	R	R	R	0.82	+	+	-
42w	R	R	R	0.75	+	+	-
54w	R	R	R	0.57	+	+	+
59w	S	S	R	0.32	+	+	-
60w	R	R	R	0.75	+	+	-
62w	R	R	R	0.57	+	+	-
48w	R	R	R	0.61	+	+	+
39w	R	R	R	0.64	+	+	+
50w	R	R	R	0.64	+	+	-
52w	R	R	R	0.46	+	+	-
8w	R	R	R	0.57	+	+	+
7w	R	R	R	0.61	-	+	+

***R=resistant, s=sensitive; **CTL=cefotaxime/clavulanic, CAL=ceftazidime/clavulanic**
*****MARI= Multiple Antibiotics Resistance Index**

Discussion

Multidrug resistant (MDR) Extraintestinal pathogenic Escherichia coli (ExPEC) has become one of the global most threating infectious agents that cause community and hospital acquired infections. These bacterial infections pose a therapeutic dilemma due to the ability of the bacteria to utilize various resistance mechanisms against most of the used antibiotic. The production of the Extended spectrum β-lactamases (ESBLs) is the most prominent and threating mechanism.

ESBLs confer resistance toward extended spectrum β-lactam and monobactam drugs owing to the ability to hydrolyze oxyimino cephalonsporins at a rate equal to or higher than 10% of that for benzylpenicillin and have an active-site serine. They are generally inhibited by b-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam. ESBLs are mostly encoded by plasmids that are transferable from strain to strain and between bacterial species (Livermore 1995).

This study is conducted to detect the presence of bla_{CTX-M} type extended spectrum β-lactamases in multi-drug resistant E. coli isolated from wound infections. Out of 150 sample 66 (44%) E. coli strains have been isolated. Iroha, Okoye et al., (2017) showed lower prevalence 26.8% than this study.

The Antibiotic susceptibility test showed that the E. coli isolates were multi drug resistant (which are resistant to at least three different classes of antimicrobial agents) with very high resistance to cefotaxime (96.97%) and amoxycillin/clavulanic (95.45%), also were highly resistant to ampicillin/ sulbactam (89.39%), cefepime (87.88%) and ceftazidime (81.82%).

These results are too high when compared with the results of Petkovšek, Eleršič et al., (2009) that showed only 5% resistance toward the extended spectrum cephalosporins. About 39.3% and 31.82% of the isolates were resistant to gentamycin and tobramycin respectively this considered very high in comparison with Petkovšek, Eleršič et al., (2009) study that showed only 1% resistance against the aminoglycosides.

The study held by Chakraborty, Saralaya et al., (2017) have reported a higher gentamycin resistance of 47%. Iroha, Okoye et al., (2017) study showed a higher resistance level against different antibiotic cefotaxime (99%), ceftazidime (96%) and aztreonam (95%). (Chakraborty, Saralaya et al., 2017). Piperacillin/Tazobactam and Azithromycin were found to be the most effective antibiotics. This is consistent with the study held by Petkovšek, Eleršič et al., (2009) while Chakraborty, Saralaya et al., (2017) and Iroha, Okoye et al., (2017) reported that Meropenem and Imipenem were the most effective drugs respectively.

This study revealed that 25 (37.87%) E. coli isolates were detected as ESBLs producers using the combined disc test. Jayanthi and Soumya (2017) have reported in their study a lower percentage of 24% E. coli ESBLs producers. Ghorbani-Dalini, Kargar et al., (2015) studies also reported a quite lower percentage of 12.96%. The study by Zamani, Emami et al., (2015) has showed a little higher results of 40 % of ESBL producers among their wound isolates.

CTX-M β-lactamases are relatively newer family of ESBLs which have rapidly spread worldwide among Enterobacteriaceae. They able to hydrolyze the extended spectrum cephalosporins, another characteristic of the bla_{CTX-M} enzymes is that they are inhibited by tazobactam almost 10-fold time than clavulanic acid (Bauernfeind, Schweighart et al., 1990). This could explain that piperacillin/tazobactam was one of the most effective drugs on the isolates while isolates exhibited high level of resistance toward amoxycillin/clavulanic (95.45%). PCR experiment was conducted to detect bla_{CTX-M} gene in the all phenotypically positive strains. It was found that 56% of the tested strains were bla_{CTX-M} positive. This was higher than the results obtained by Zamani, Emami et al., (2015) as 50% of their wound isolates was bla_{CTX-M} positive. Chakraborty, Saralaya et al., (2017) have reported in their analysis a little higher bla_{CTX-M} prevalence of 60%.

CTX-M β-lactamases are known for their activity against cefotaxime and ceftriaxone rather than ceftazidime. Exceptions to this rule are four recently described enzymes, CTX-M-15, CTX-M-16, CTX-M-19 and CTX-M-27,which have significant hydrolytic activity against ceftazidime (Bonnet, Dutour et al., 2001, Poirel, Naas et al., 2001). This could explain that all the bla_{CTX-M} positive strains were resistant to cefotaxime, ceftriaxone and ceftazidime. This was the same as reported by Hara, Sato et al., (2015) but, partially agree with Zamani, Emami et al., (2015) as isolates where resistant to both cefotaxime and ceftriaxone but more susceptible to ceftazidime.

The MARI of the all 25 ESBL producers ranged from 0.32 to 0.93 while the mean value of bla_{CTX-M} gene positive isolates was 0.71. This was higher than results obtained by Iroha, Okoye et al., (2017) (0.20 to 0.85) . These high values of the MARI indicate an environment where several antibiotics are used and/or misused. This also, supports the presence of circulating plasmids that confer different antibiotic resistance genes

leading to the spread of resistance traits (Daini, Ogbolu et al., 2005).

Conclusion

The Extraintestinal pathogenic Escherichia coli (ExPEC) have become one of the most threatening pathogens owing to their ability to use several resistance mechanisms particularly the production of ESBLs. The continuous increase in the bla_{CTX-M} prevalence leading to the spread of the extended spectrum cephalosporins and the monobactams which, in its turn, increases the bacterial MARI. All these findings indicate the need continuous surveillance of antibiotic susceptibility patterns and effective infection control. MDR bacterial strains will continue to emerge unless the misusing of the antibiotics is curtailed and strict infection control practices are maintained.

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