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The frequency of CTX-M gene in Enterobacteriaceae ESBL of hospital origin

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ABSTRACT: A total of **37** clinical isolates of enterobacteriaceaes (16 *Escherichia coli*, 10 *Klebsiella pn*., 7 *Enterobacter Cloacae*, 2 *Morganella Morganii*, 1 *Proteus Mirabilis* and 1 C*itrobacter Freundi*) were recovered during 2014 from four Moroccan regions to study their resistance profile. Among these 37 strains, 19 tell ESBL *Enterobacteriaceae*. Over this period, the bacterial species most often ESBL producing was *Escherichia coli* (9 strains), followed by *Klebsiella pneumoniae* (7 strains), *Enterobacter cloacae* (2 strains), and *Morganella Morganii* (1 strains). The ESBL resistance was screened using disc diffusion method, while the resistance genes were detected by polymerase chain reaction (PCR). The results marked the high prevalence of gene CTX-M (16 strains). This study points out the high rate of transmission of CTX-M gene among *Escherichia coli,Klebsiella pneumonia, Enterobacter cloacae* strains and shows a reduced sensitivity to Penicillins (Amoxicillin-clavulanic acid, Amoxicillin, and Ticarcillin (0%)), to Cepholosporins 3 rdgeneration Cefotaxim (7,7%), Ceftazidim (11,5%), and to Fluoroquinolones (Ciprofloxacin, Norfloxacin, and nalidixic acid (3,8%). In addition, all tested strains were highly susceptible to imipenem (96,2%) and Trimethoprim-sulfamethoxazole (73,1%).

KEYWORDS: ESBL, Enterobacteriaceae, CTX- M, PCR, Escherichia coli, Klebsiella pneumonia.

1 INTRODUCTION

Plasmid-mediated extended-spectrum Betalactamases (ESBLs) is becoming increasingly frequent among clinical isolates of the family *Enterobacteriaceae* throughout the world [1].

There are so many types of ESBLs like TEM, SHV, CTX, AmpC, etc. The most prevalent ESBLs are included in three groups: TEM, SHV and CTX-M [2].

They derived from mutations in the classic TEM (Temoneira) and SHV (Sulphydryl variable) genes by one or more amino acid substitution around the active site [3].

The first plasmid-mediated β-lactamase in Gram-negative bacteria, TEM-1, was described in the early 1960s. TEM-1 is able to hydrolyze penicillins and early cephalosporins [4]. Another common plasmid-mediated β-lactamase that is found in *K. pneumoniaeand E. coli* is SHV-1 (for sulphydryl variable) [5]. Members of the SHV family of β-lactamases trace their descent to SHV-1, a plasmid encoded β-lactamase that confers to *K. pneumoniae* high levels of resistance against ampicillin [6]. The emergence of new enzyme groups that have a typical ESBL resistance phenotype but that are non-TEM and non-SHV derivatives have recently been reported [1]. CTX-M-type Betalactamases constitute a novel group of enzymes encoded by transferable plasmids. This novel family of plasmid-mediated ESBLs has been classified in Ambler class A (2) and in group 2be of the Bush, Jacoby, and Medeiros classification [3].

The name of CTX-M type Betalactamases, owing to their high activity against cefotaxime. In contrast with TEM and SHV ESBLs, most of the CTX-M enzymes preferentially hydrolyze and confer resistance to cefotaxime and ceftriaxone rather than

ceftazidime. In recent years a new family of plasmid mediated CTX-M extended spectrum b-lactamases (ESBLs) called CTX-M has arisen and reported in the literature with increasing frequency from Europe, Africa, Asia, South America and North America [7].

2 MATERIALS AND METHODS

2.1 BACTERIAL ISOLATES

37 strains of Enterobacteriaceae (16 *Escherichia coli*, 10 *Klebsiella pn*., 7 *Enterobacter Cloacae*, 2 *Morganella Morganii*, 1 *Proteus Mirabilis* and 1 C*itrobacter Freundi*), were isolated from four Moroccan regions during 2014.

2.2 PREPARATION OF DNA

Total DNA was extracted by thermal shock: for each strain, two to three colonies were dissociated in 300 µl of ultrapure water which is a water purity and quality for molecular biology; this suspension was heated at 100 ° C in a dry bath (Block Heater) for 10 min, and then cooled immediately in ice. After 10 min of centrifugation at 12 000 revolutions / min, the supernatant is transferred into a sterile Eppendorf tube of 1.5 ml, and then stored at -20 ° C until use.

2.3 GENOTYPIC IDENTIFICATION OF ESBLS [8]

In our study, CTX- M type of ESBL Producer was sought. (Tab. 1) specifies the primers and (tab. 2) the operating conditions. CTX- MF and CTX-MR primers were defined in the laboratory from consensual areas.

The amplification was made into a thermocycler, the mix for PCR reactions being composed of a unit of Taq polymerase, 0.4 mM of each primer, 100 mM of each deoxynucleoside triphosphate, 0.5 mM MgCl2, 10 mMTris - HCl pH 8.3 and 50 mMKCl. One microliter of the DNA test was added to a final volume of 50 µl.

The reaction was made in a thermal cycler (PROGEN), on terms of amplifications.

The PCR products were visualized after migration in agarose gel 0.8% and staining with ethidium bromide.

The reference strain (positive control) is used: E. coli U2A 1790 expressing blaCTX-M gene.

Table 1. Primers used in PCR techniques

CTX-M	CTX-MF CTX-MR	5′ - ATGTGCAGYACCAGTAARGT - 3′ 5′ - ACCGCRATRTCRTTGGTKGT - 3′

Table 2. Amplification Conditions

	Initial stage	Denaturation/hybridation/elongation	Number of cycles	Final stage
CTX-M	94 °C 5 min	94°C 30 sec/56 °C 30 sec/72°C 45 sec	30	72 °C 7 min

3 RESULTS AND DISCUSSION

3.1 DIFFERENT BACTERIAL SPECIES TESTED

The ESBLE are variously distributed according to continents and, within the same continent, according to the geographical areas [9].

They are increasingly involved in both community that nosocomial infections and constitute a real public health problem.

During 2014, 37 strains of enterobacteriaceae were isolated from four Moroccan regions to study their resistance profile. Among these 37 strains, 19 tell ESBL Enterobacteriaceae. Over this period, the bacterial species most often ESBL producing was Escherichia coli (9 strains), followed by Klebsiella pneumoniae (7 strains), Enterobacter cloacae (2 strains), and Morganella Morganii (1 strains) (fig.1). Those 19 ESBL producers were selected for CTX-M gene detection by PCR. One study in Morocco, found that 237 enterobacterial strains were isolated from community setting. Seven strains 3% (7/237) show an ESBL phenotype [10]. It can be concluded that the ESBL Enterobacteriaceae represent a higher rate in the hospital setting than in the community.

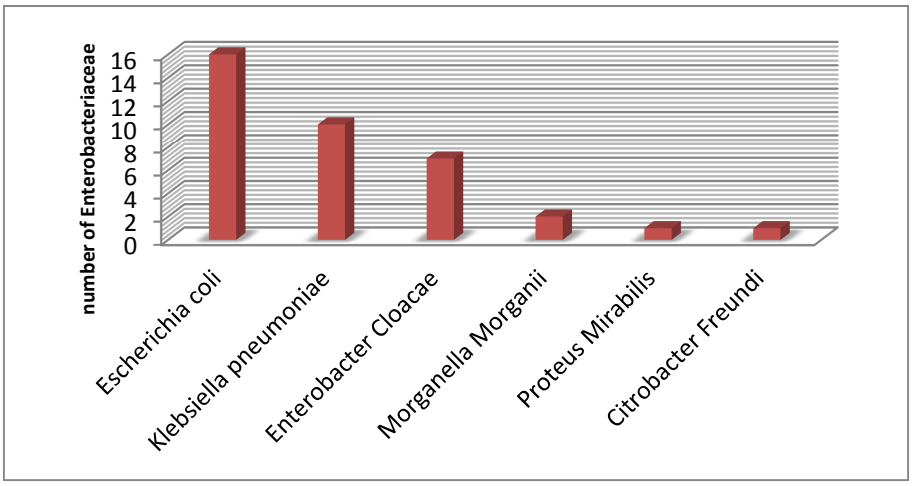

Fig. 1. Different bacterial species tested

In our study, the bacterial species most often ESBL producing was *Escherichia coli* (9 strains), and *Klebsiella pn*. (7strains). In Egypt, a high prevalence of infections caused by ESBL-producing *E. coli* and *Klebsiella spp.* have been reported in both academic teaching and general hospitals [11].

We can estimate that these two bacteria represent a high rate of hospital-acquired infections.

3.2 ANTIBIOTICS SUSCEPTIBILITY

It is important to note that our isolates represent a high resistance to Penicillins (amoxicillin – clavulanic acid, amoxicillin, and Ticarcillin (100%) acid), which is probably due to the use frequent of this antibiotic for treating infections caused by different families of enterobacteriaceae [12]. This rate of resistance would also report in an Iranian study [13]. The Resistance show high to the 3rd generation Cepholosporins (cefotaxime (92.3%), Ceftazidime (88.5%)) and Fluoroquinolons (ciprofloxacin, norfloxacin and nalidixic acid (96.2%)), our results are similar to a study carried out in the South of India [14] and with another Egyptian study [15]. In addition, all strains tested were highly susceptible to imipenem (96.2%) this shows a similarity with a study conducted by Tabbouche Sana et al. [16] (tab.3). Similar results were found in another study for Mohsen Mirzaee et al. [17].

Table 3. 37 strains tested antibiotic sensitivity

Antibiotic	**% sensitivity**
Amoxicillin	0
Amoxicillin + Clavulanic Acid	0
Piperacillin + Tazobactam	73,10
Trimethorim/Sulfamethoxazole	11,50
Imipenem	96,20
Norfloxacin	3,80
Ticarcillin	0
Cefotaxime	7,70
Ceftazidime	11,50
Gentamicin	42,30
Tobramicin	15,40
Ciprofoxacin	3,80
Nalidixic Acid	3,80

In recent years, many studies have reported the increasing resistance of Enterobacteriaceae to ampicillin, cotrimoxazole and first-generation cephalosporins. The resistance to third-generation cephalosporins has also been increasing, though less than the first-generation, in many countries. Currently, among the beta-lactam antibiotics, carbapenems are the most

effective drugs [18]. This may be due to routine and extensive use of third-generation cephalosporins in treating the infections.

3.3 THE CTX-M TYPE PREVALENCE

CTX-M type ESBLS are easily recognized on antibiotic susceptibility testing in agar because CTX is usually the most affected molecule with a very good inhibition around the disk containing the tazobactam. These primers were used to amplify all strains with this profile [8].

We used primers specific to CTX-M enzyme. We obtained amplification of all the strains, confirming the presence of enzyme.

In our study, 16 of 19 ESBLs strains were found to express CTX-M type ESBL. Escherichia coli representing (7 strains) CTX-M type ESBL, followed by Klebsiella pneumoniae (6 strains), and Enterobacter cloacae (3 strains) (fig.2). It appears clearly that The CTX-M gene predominates in our ESBLs strains. The same result was found in Europe [19], while in other countries, the ESBL genes are more diverse [19].

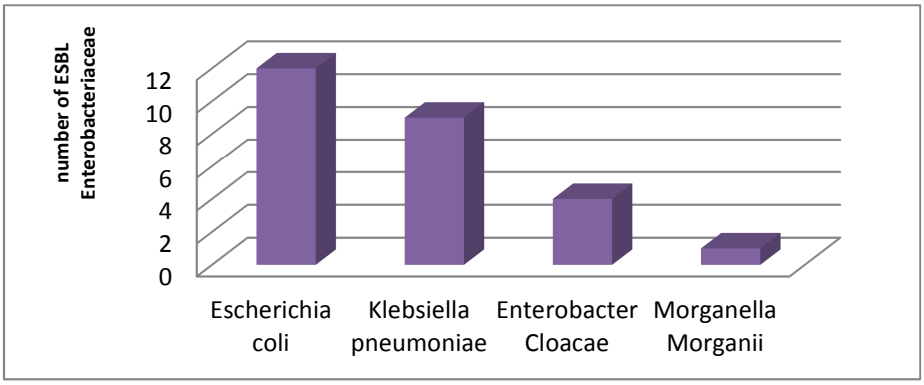

Fig. 2. Number of Enterobacteriaceae ESBL producing

The ESBLs were inhibited by clavulanate and tazobactam. Importantly, the isolates were more resistant to cefotaxime and aztreonam than to ceftazidime, suggesting that they were CTX-M producers. Some CTX-M ESBLs confer high-level resistance to ceftazidime [20].

In one study in south India, of 600 ESBL positive isolates which were analysed for the presence of blaCTX-M gene by PCR, 95 (15.83%) isolates were positive for blaCTX-M. These 95 strains were further analysed for the presence of CTX-M-1 gene using primers specific for blaCTX-M-1. Among the 95 CTX-M producing isolates, CTX-M-1 gene was positive in 45 (47.3%) of the isolates. They were found in 36% of E. coli isolates, 36.3% of Enterobacter spp. and 58.3% of Klebsiella spp. CTX-M [21].

In another study, 67 isolates were positive using PCR. CTX-M type ESBL was observed in 22.72% of E. coli isolates [22].

Elisabete Machado et al. describe a complex ESBL epidemiology in Portugal, including widespread dissemination of known strains and plasmids coding for TEM-24 and CTX-M-15 enzymes as observed in other European countries [23].

Antimicrobial susceptibility testing on transconjugants revealed that 26 out of 53 (49%) ESBL-producing Enterobacteriaceae were able to transfer antimicrobial resistance to the recipients. Transfer of high-level resistance to the transconjugants encoded by the blaCTX-M-15 gene downstream the ISEcp1 insertion sequence against 3rd generation cephalosporins, and of low-level resistance against ciprofloxacin [24].

The ESBL genes were detected in 19 Klebsiella spp. and in 49 of Escherichia coli isolates. The communist frequent ESBL gene was CTX-M which was 48 and was observed in 35 and 13 of Escherichia coli and Klebsiella spp., respectively [25]. The prevalence of ESBL-producing Enterobacteriaceae found to be higher in Sudan in comparison to other countries. And it is high in Asia particularly in China and India [26].

As elsewhere in Europe, CTX-M enzymes are accumulating with the new century, especially in the north of France. An E. coli strain with CTX-M-15 b-lactamase caused an outbreak [19].

In the past several years, the emergence of new variants of ESBL producers, especially CTX-M has suggested the involvement of the co-resistance to other drug classes during endemic condition. This co-resistance is due to the transmission of different types of resistance genes within the same clone. Several studies showed that blaCTX-M genes are commonly found on large plasmids that often carry other genes conferring resistance to other antimicrobial agents including aminoglycosides, fluoroquinolones, chloramphenicols, tetracyclins and others (particularly, blaOXA-1, blaTEM-1, tetA, aac (6')-lbcr). This may explain the high rate of transmission of CTX-M gene among the E. coli strains by acquiring R-plasmid, and often the high prevalence of the CTX-M resistance gene is combined with another resistance gene in these strains [24].

4 CONCLUSION

1. A total of 37 clinical isolates of enterobacteriaceae recovered during 2014 from four Moroccan regions to study their resistance profile.
2. 19 tell ESBL Enterobacteriaceae
3. It is important to note that our isolates represent a high resistance to Penicillin, to the 3rd generation Cepholosporins, and to Fluoroquinolons. In addition, all strains tested were highly susceptible to imipenem
4. The bacterial species most often ESBL producing was Escherichia coli (9 strains), and Klebsiella pn. (7strains).We can estimate that these two bacteria represent a high rate of hospital-acquired infections.
5. In our study, 16 of 19 ESBLs strains were found to express CTX-M type ESBL.

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