The Mediterranean Region: A Reservoir for CTX-M-ESBL-Producing Enterobacteriacae

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Received: December 13, 2013        Revised: December 30, 2013       Accepted: January 2, 2014

Abstract

The incidence of ESBL-producing bacteria is increasing worldwide, which represents a challenge for the healthcare systems. More recently, the emergence of the CTX-M group has been frequently reported as being associated with both nosocomial and community acquired infections. CTX-M-15 was identified as being the most prevalent in a large geographic area including Europe and the Middle East, suggesting the presence of a community reservoir of CTX-M enzymes disseminating in the Mediterranean area. Thus, this review will focus on the Mediterranean region to highlight the increasing prevalence of CTX-M-ESBL producing Enterobacteriacae.

Keywords: ESBL, CTX-M, Mediterranean, Enterobacteriacae, Multi-drug Resistance, E. coli, Klebsiella pneumonia, UTI

1. Introduction

Since the initial report in Germany in 1983, extended-spectrum \(\beta\)-lactamase (ESBL)-producing Escherichia coli emerged as a major pathogenic threat worldwide (Paterson and Bonomo, 2005; Livermore et al., 2007; Pitout and Laupland, 2008; Qian-Hong et al., 2011). The increasing prevalence of ESBLs producing pathogens and their alarming evolution can be attributed to the frequent prescription of \(\beta\)-lactam agents such as penicillins, cephalosporins, monobactams and carbapenems. ESBLs were identified shortly after the introduction of oxyimino-\(\beta\)-lactam antibiotics (Medeiros, 1997; Pitout et al., 2005). Initially, the classic ESBLs occurred due to mutations in the genes encoding the common plasmid-mediated SHV-1, TEM-1, or TEM-2 beta-lactamases (Jacoby and Munoz-Price, 2005). More recently, a novel group of ESBLs, the CTX-M family, have emerged and rapidly disseminated worldwide (Canton and Coque, 2006). This CTX-M emergence led to further dissemination of ESBLs in both community and hospital settings (Woerther et al., 2011).

2. Overview of ESBLs

These enzymes are termed ESBLs since they confer bacterial resistance to penicillins, all cephalosporins and aztreonam and frequently resistant to fluoroquinolones and aminoglycosides but not to cephamycin, carbapenems or \(\beta\)-lactamase inhibitors (Tham et al., 2012; Patterson and Bonomo, 2005; Drawz and Bonomo, 2010). ESBLs are mostly encoded by genes found on large plasmids which include as well genes encoding resistance for a variety of antimicrobial agents including aminoglycosides, sulphonamides, trimethoprim, chloramphenicol and tetracyclines (Paterson, 2000). Consequently, ESBLs are characterized by a broad antibiotic resistance extending to multiple classes (Bradford, 2001). This resistance is even extending to carbapenems which represent the treatment of choice for ESBL associated serious infections (Rahal, 2008; Doumith et al., 2009). Historically, mutations in the genes encoding the common plasmid-mediated TEM-1 and SHV-1 enzymes were the initial cause of the occurrence of ESBLs (Jacoby and Munoz-Price, 2005). Currently, 175 different TEM enzymes and 127 SHV different enzymes are described (www.lahey.org/studies/). Nevertheless, a new group of ESBLs, the CTX-M family, have emerged and spread rapidly worldwide (Canton & Coque, 2006). These enzymes are prominent among Enterobacteriacae from Europe, Africa, Asia, South America and North America (Bonnet, 2004). The number of CTX-M-type ESBLs is rapidly escalating and they were identified in every inhabited continent (Patterson and Bonomo, 2005). At present, the number of CTX-M \(\beta\)-lactamases identified exceeds 140 allelic variants (http://www.lahey.org/Studies/other.asp#table1) which can be classified, based
on their amino acid structure, into six distinct phylogenetic groups, namely groups 1, 2, 8, 9, 25 and 45 (Rossolini et al., 2008). For a period of time, CTX-M ESBLs were mainly detected in three geographic regions: South America, the Far East, and Eastern Europe, while they appeared to be sporadic in Western Europe and North America; however, more recently, the arrival of CTX-M-type ESBLs in these regions have been reported extensively (Dechamps et al., 2000). Furthermore, CTX-M-type ESBLs were largely reported in China and India, and hence CTX-M-type ESBLs can be considered as the most prevailing ESBL type (Pitout et al., 2005). In addition, some of the CTX-M enzymes were identified in particular countries, like CTX-M-1 in Italy, CTX-M-9 and CTX-M-14 in Spain, and CTX-M-2 in the majority of South American countries and Japan, while CTX-M-15 was detected worldwide (Bonnet, 2004; Ben-Ami et al., 2006; Canton and Coque, 2006). However, CTX-M-15 was identified as being the most prevalent in a broad geographic area which includes North America, Europe, The Middle East, and India, while a number of CTX-M-32-producing strains were recently detected in both humans and farm animals in Spain, Italy, Greece and Portugal (Eckert et al., 2006). This suggested the presence of a community reservoir of CTX-M enzymes disseminating in the Mediterranean area (Cartelle et al., 2004; Oteo et al., 2006).

3. Predominant ESBLs Circulating in European Countries on the Mediterranean

All published studies have confirmed that the prevalence of ESBL producing isolates is higher in southern Europe than in the rest of European countries and the wide dissemination of CTX-M-15 enzyme is increasingly reported from both hospital and community settings. A nationwide Spanish study including 40 medical centers reported CTX-M-9 (27.3%), SHV-12 (23.9%), and CTX-M-14 (20.5%) as the most prevalent ESBL-producing E. coli, and clonal dissemination was not detected among the 170 isolates included (Hernandez et al., 2005). In another Spanish study, Oteo et al. (2006) reported the dissemination of blaCTX-M-15 alleles linked to an ISEcp1-like element between the community, long-term care centers, and hospitals, and the majority of the tested isolates harbored the same three virulence genes: iutA and fyuA (siderophores), and traT (serum survival factor). Moreover, Valverde et al. (2008) identified high rates of colonization with ESBL-producing Enterobacteriaceae in patients with community infections and their household members with 66% of the ESBL-producing isolates being indistinguishable between patients and their healthy household contacts. These results highlight the high risk of the spread of these multi-drug resistant isolates to healthy individuals and their dissemination in both hospital and community settings (Valverde et al., 2008). In 2009, Valverde et al. demonstrated that the dissemination of IncK plasmids is responsible for the high incidence CTX-M-14-producing E. coli isolates which represents a frequent cause of community-acquired urinary tract infections in Spain (Valverde et al., 2009). In 2010, ESBL-producing E. coli was collected from 27 Spanish centers as part of European Antimicrobial Resistance Surveillance Network for susceptibility testing and typing and an increase in fosfomycin resistance was shown. The higher rate of fosfomycin resistance was 15.3% belonging to CTX-M-15-ESBL-producing isolates (Oteo et al., 2010). In a recent study, Dahbi et al. (2013) highlighted the emergence of new variants of ST131 clonal group among extra-intestinal pathogenic CTX-M-15 ESBL producing E. coli with a significantly elevated virulence score and novel virulence profile. In France, before 2008 the prevalence of ESBL producing Enterobacteriaceae was under 1% (Galas et al., 2008). However, since then, this prevalence has increasingly been reported in both community and nosocomial settings. In a study that aimed to report the evolution of ESBL production between 1999 and 2007 at a general hospital from south France, ESBL prevalence in E. coli augmented during this episode from 0.3 to reach 2.5% (Anastay et al., 2013). Concurrently, the predominant ESBL in 1999, TEM-24 was substituted by CTX-M in 2007, with CTX-M-15 being the most prevalent (88% of CTX-M) (Anastay et al., 2013). CTX-M variants were also identified predominantly in the southern coastal region of France, and the zoonotic spread was detected through gulls carrying bacteria that harbored mostly the CTX-M-1 group (Bonnedahl et al., 2009). In 2006, a nationwide survey was conducted in Italy and the widespread of CTX-M-producing ESBLs was described. Although the rate of CTX-M production varied between hospitals (1.2 to 49.5% of ESBL producers), all these isolates belonged to CTX-M group 1 with CTX-M-15 and CTX-M-1 being the most prevalent variants (60% and 35%, respectively) and CTX-M-32 carried only by a minority (5%). In 2011, the dominance of CTX-M group 1 ESBL-producing E. coli causing urinary tract infections among outpatients was reported (Huemer et al., 2011). More recently, an outbreak of colonization by ESBL producing E. coli sequence type 11 was identified in a neonatal intensive care unit. This epidemiological investigation highlighted the importance of detecting the silent spread of ESBLs (Giuffre et al., 2013). In addition, the emergence of K. pneumoniae clone carrying both VIM-1-MBL and CTX-M-15-ESBL was reported from different hospitals in Italy (Nucleo et al., 2013). In Greece, a multiclonal epidemic of K. pneumonia producing both VIM-1 and SHV-5 was reported to be under way in the major hospitals (Psichogiou et al., 2008). Sequencing analysis of ESBL-producing Enterobacteriaceae, collected between 2007 and 2011, showed that blaCTX-M-3 gene is predominant, followed by the blaCTX-M-15 gene and blashv-5 gene (Kristo et al., 2013). In 2008, Turkey was added to the list of countries concerned by community-acquired CTX-M-15-ESBL E. coli clone O25-ST131 among outpatients with E. coli urinary tract infection (Yumuk et al., 2008). More recently, in a nationwide study including ESBL-producing E. coli isolates collected from 10 different Turkish hospitals, CTX-M-1 was the most prevalent (366/440) followed...
by TEM (194/440) and CTX-M-2 (140/440) (Ciccek et al., 2013).

4. Prevalence and Distribution of ESBL Production in African Countries on the Mediterranean

In 2006, CTX-M production was initially detected and the high rate of ESBLs was confirmed in Egypt reaching 60% among urinary tract infections patients; three different enzymes were found CTX-M-14, CTX-M-15 and CTX-M-27 (Alagamy et al., 2006). Later on, Hassan et al. (2013) reported the high prevalence of quinolone resistance determinants qnr, aac(6’)-Ib-cr, qep A4, and their association with CTX-M positive E. coli isolates from Egypt (Hassan et al., 2013). The extensive community acquired CTX-M-15 carriage reaching 57% was also described in a recent study (Newire et al., 2013). In Tunisia, the initial identification of a CTX-M-producing strain (CTX-M-3) was recovered in 2001. Later on, CTX-M-27 originated a nosocomial outbreak in a Tunisian neonatal center. The first report of CTX-M-15 and CTX-M-16-producing Enterobacteriaceae in Tunisia was submitted in 2006 (Mamlouk et al., 2006). Later, Dahmen et al. (2010) corroborated the high prevalence of CTX-M-15 with 91% of the isolates producing this enzyme. The majority of CTX-M-15-ESBL-producing E. coli belonged to B2 phylogenetic group and to the sequence type 131 and was associated with Qnr-like determinants (Dahmen et al., 2010). It was also found that this CTX-M-15-B2-ST131 E. coli clone is also highly disseminating in community-acquired urinary tract infections in Tunisia (Hammami et al., 2013). The molecular analysis of a collection of ESBL producers isolated between 1989 and 2009 confirmed the prominence of blaCTX-M-15 gene followed by blaCTX-M-14 gene, blaSHV-12 gene, blaSHV-2a gene and blaTEM-26, with the frequent dissemination of CTX-M-15 producing E. coli being attributed to the spread of various IncF-type plasmids (Mnif et al., 2013). In Algeria, the results obtained were similar to the other Mediterranean countries, and in 2008 the prevalence of ESBLs belonging to CTX-M-1 group was noticed (Messai et al., 2008). It was determined that the size of the self-transferable plasmid reached 85 kb and included in addition to the blaTEM and blaCTX-M genes, determinants for aminoglycosides and sulfonamides resistance (Messai et al., 2008). Similarly, in a study that tried to determine the overall incidence of ESBL production among Enterobacteriaceae, predominance of CTX-M-1 group was revealed, TEM and SHV were also detected. But the conjugative plasmids carrying these genes were of higher molecular weight (≥125kb) (Nedjai et al., 2012). Ahmed et al. (2012) detected the CTX-M-15 ESBLs in the intensive care unit of an Algerian hospital and determined that this gene was genetically linked to insertion sequence IS6301B (Ahmed et al., 2012). More recently, in a study that aimed at characterizing environmental ESBLs and quinolone resistance, it has been found that the antibiotic resistance mechanisms are similar in both the environment and the clinical setting, and wastewater treatment plant might represent a cause of dissemination of resistance genes (Alouache et al., 2013). Morocco is another country where the production of CTX-M-type beta-lactamase blaCTX-M-15 is the most recurrently found mechanism of resistance to beta-lactams. In a nationwide study conducted over a 2-year period in the Moroccan community, blaCTX-M-15 was the most frequent gene detected among ESBLs causing urinary tract infections, followed by blaCTX-M-1, SHV-12 and PER-2. The blaOXA-48 and blaIMP-1 carbapenemases genes were also detected and qnr genes were harbored by only a small percentage of the isolates (Barquiqua et al., 2013).

5. ESBL-Producing E. coli in the Middle-East

In Lebanon, an increase in the prevalence of ESBL-producing bacteria was observed from 2.3% in 2000 to reach 8% in 2005 and 16.8% in 2009 (Kanaan et al., 2005; Daoud and Affif, 2011). The escalating numbers of ESBL-producing bacteria were not only noted for E. coli, but also for K. pneumoniae, Salmonella spp. and Shigella spp (Araj et al., 2012). The predominance and fast emergence of the CTX-Ms has been reported in Lebanon (Moubareck et al., 2005; Kanj et al., 2008) and sequence analysis indicated that the blaCTX-M-15 is the most prominent (Matar et al., 2007; Baroud et al., 2013). In a recent study, resistance to carbapenemase production was detected in ESBL E. coli and K. pneumoniae isolates (Baroud et al., 2013). There is a lack of sufficient information about the prevalence of ESBLs and their molecular characterization in Syria; however, in a recently published article, the high prevalence of ESBL producing E. coli was revealed reaching 52% among urinary tract infections patients (Al-Assil et al., 2013). In Jordan, the prevalence of ESBLs has been reported to be relatively high compared to other reported data worldwide (Youssef et al., 1999; Shehabia et al., 2000). More recently, clinical specimens have been collected from three major hospitals in Northern Jordan, and ESBL-producing gram negative bacteria comprised 22.9% of all isolates which included E. coli, K. pneumoniae, K. oxytoca and Enterobacter cloacae as the most prominent (Batchou et al., 2009). A study by Aqel et al. (2013) suggested the endemicity of ESBL producing bacteria in Jordanian hospitals, where CTX-M-1 and CTX-M-9 ESBLs were detected in two geographically distant hospitals. Moreover, the results of another recent study revealed that ESBL urinary E. coli isolates with high levels of sul2, blaCTX-M and blaTEM are circulating in the Jordanian community with associated multidrug resistance profile (Nimri and Azaiez, 2012). Similarly, in a recently published study, ESBL producers were isolated from patients with urinary tract infections from three hospitals in the West Bank and the results showed that all isolates harbored CTX-M, TEM was also detected but none harbored the SHV gene (Adwan et al., 2013). The dissemination of CTX-M sequence type 131 ESBLs was also detected in Israel among patients with community-onset bacteremia (Karfunkel et al., 2013). Moreover, in an investigation conducted over 7 years, the association...
between the increase of such infections and CTX-M ESBLs was suspected, and specifically it appeared to be related to the clonal extension of blal CTX-M-15 or blal CTX-M-14 carrying ST131 (Karfunkel et al., 2013).

6. Conclusion

The data provided in a large number of studies conducted in the Mediterranean area show that the dissemination of CTX-M genes, in both community and hospital settings, have been increasing extensively. This expansion is enhanced by environmental and zoonotic spread. Moreover, it is associated with a significantly elevated virulence score and multidrug resistance profiles, which highlights the importance of identifying the silent spread of ESBLs, and of implementing additional measures to prevent ESBL associated infections.

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