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Dissemination of multidrug-resistant $bla_{CTX\text{-}M\text{-}15}$/IncFIIk plasmids in *Klebsiella pneumoniae* isolates from hospital- and community-acquired human infections in Tunisia

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ABSTRACT

This study investigated the molecular features of extended-spectrum β-lactamase (ESBL)–producing *Klebsiella pneumoniae* from hospital- and community-acquired (HA/CA) infections in the region of Mahdia, Tunisia. Among 336 *K. pneumoniae* isolates recovered from both clinical contexts between July 2009 and December 2011, 49 and 15 were ESBL producers and originated from clinical and community sources, respectively. All isolates produced the CTX-M-15 enzyme. As shown by Southern blot on S1 nuclease treatment followed by pulsed-field gel electrophoresis (PFGE) gels, the $bla_{CTX\text{-}M\text{-}15}$ gene was carried on IncFII (n = 4), IncFIIk (n = 25), IncL/M (n = 4), IncK (n = 1), or untypeable (n = 15) plasmids in HA isolates. In CA isolates, the $bla_{CTX\text{-}M\text{-}15}$ gene was carried on IncFIIk (n = 6), IncFII (n = 1), IncHI1 (n = 1), or untypeable (n = 7) plasmids. In all, 23 and 11 PFGE types were found among the HA and CA isolates. Multilocus sequence typing on representative isolates shows diverse sequence types (STs), such as ST307, ST101, ST39, ST4, ST140, ST15, and ST307 in HA isolates and ST101, ST664, and ST323 in CA isolates. This study is the first comprehensive report of ESBL plasmids in *K. pneumoniae* from HA and CA infections in Tunisia.

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1. Introduction

Enterobacteriaceae are among the most common human pathogens causing community- and hospital-acquired infections. In particular, *Klebsiella pneumoniae* is responsible for various types of infections, including bacteremia and infections of the urinary and respiratory tracts. In parallel, in *K. pneumoniae*, the prevalence of resistance to antibiotics and especially to β-lactams has been increasing over time and has become a major issue for public health.

To this respect, production of extended-spectrum β-lactamases (ESBLs) is a worldwide cause of concern in Enterobacteriaceae, such as *K. pneumoniae*. ESBLs are usually plasmid-encoded enzymes derived initially from the classical TEM- and SHV-type β-lactamases and confer different levels of resistance to ceftazidime, cefotaxime, and other broad-spectrum cephalosporins and monobactams. However, this pattern changed owing to the emergence and expansion of the CTX-M enzymes, which have largely replaced and outnumbered other types of ESBLs (Rossolini et al., 2008).

In Tunisia, several reports highlighted to what extent the spread of ESBL producers has become of serious concern (Abbassi et al., 2010a, 2010b; Denton, 2007; Ferjani et al., 2014; Hammami et al., 2013; Mnif et al., 2013; Paterson and Bonomo, 2005). Similarly to other countries, the clonal dissemination of CTX-M-15–producing *K. pneumoniae* isolates has been observed in Tunisia (Abbassi et al., 2010a, 2010b; Calhau et al., 2014; Chouchani et al., 2012; Dahmen et al., 2010). Moreover, those strains are commonly resistant to multiple antibiotics, such as aminoglycosides, fluoroquinolones, and sulfonamides. The international spread of the CTX-M-15 enzyme has also been associated with the dissemination of specific *Escherichia coli* clones, such as of sequence type (ST) 131 harboring $bla_{CTX\text{-}M\text{-}15}$ on IncFII plasmids (Nicolas-Chanoine et al., 2014; Peirano et al., 2010). IncF is one of the most frequent plasmid families reported in Enterobacteriaceae and is frequently associated with the spread of many resistance and virulence determinants (Carattoli, 2013; D'Andrea et al., 2013). In Tunisia, the dissemination of CTX-M-15–producing *E. coli* was associated with various IncF-type plasmids harbored by the ST131 clone (Ferjani et al., 2014; Mnif et al., 2013). On the contrary, there are no data available on $bla_{CTX\text{-}M\text{-}15}$-carrying plasmids circulating in clinical *K. pneumoniae* isolates in Tunisia.

The aim of this retrospective study was to characterize ESBL-producing *K. pneumoniae* isolates recovered in Taher Sfar University Hospital in Mahdia, Tunisia, in order to further investigate the

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epidemiology of ESBL genes and plasmids and to highlight possible commonalities between healthcare-associated and community-acquired clinical *K. pneumoniae* isolates.

2. Materials and methods

2.1. Bacterial strains

From July 2009 to December 2011, a total of 338 nonrepetitive *K. pneumoniae* isolates were consecutively obtained both from clinical specimens of different wards of the Taher Sfar Hospital in Mahdia, Tunisia, a 800-bed university hospital, and from samples recovered from community-acquired infections at the external consultations and urgency units. All isolates were identified by using Api20E system (bioMérieux, Marcy l'Etoile, France).

2.2. Antimicrobial susceptibility testing and ESBL screening

Antimicrobial susceptibility was determined by the disk diffusion method on Mueller–Hinton agar plates with β-lactam and non–β-lactam antibiotic-containing disks (Bio-Rad, Marnes-la-coquette, France) according to the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (www.sfm-microbiologie.fr). *E. coli* ATCC 25922 was used as quality control. MICs were determined by E-Test® (bioMérieux). Double-disk synergy test (DDST) was used to confirm ESBL production.

2.3. Identification of the resistance genes

K. pneumoniae isolates with a positive DDST (n = 64) were screened by PCR for genes encoding ESBLs using primers targeting bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, and bla_{CMY} genes (Arlet et al., 1997; Eckert et al., 2004; Lavollay et al., 2006; Shibata et al., 2006).

The $bla_{CTX-M-1}$ group gene was identified using an additional PCR using external primers ISEcp1 and P2D to detect the presence of the insertion sequence ISEcp1 in association with the bla_{CTX-M} gene (Literacka et al., 2009). For ESBL-producing isolates with reduced susceptibility to nalidixic acid, enrofloxacin, and ofloxacin, detection of plasmid-mediated quinolone resistance genes was performed using primers targeting *qnrA*, *qnrB*, *qnrS* (Robicsek et al., 2006), *qnrC* (Wang et al., 2009), *qnrD* (Cavaco et al., 2009), *qepA* (Ma et al., 2009), *aac(6′)-ib-cr* (Park et al., 2006), and *oqxAB* genes (Kim et al., 2009). To identify the genes detected in the PCR assays, DNA sequence analysis of the amplicons were performed (Kim et al., 2009; Robicsek et al., 2006). PCR products were purified with QiAquick PCR purification kit (Qiagen, Courtaboeuf, France), and bidirectional sequencing was performed (Beckman Coulter, London, UK). The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information Web site (www.ncbi.nlm.nih.gov).

2.4. Transferability of ESBL genes

Conjugation and transformation experiments were carried out to determine whether the resistance determinants were transferable. Conjugative transfer of ESBL genes was tested in liquid medium using sodium azide-resistant *E. coli* J53 as a recipient cell. Transconjugants were selected on plates containing sodium azide (100 mg/L) and cefotaxime (4 mg/L). Transformation experiments were performed by electroporation of plasmid DNA from *K. pneumoniae* isolates extracted by Kieser method (Kieser, 1984) into competent *E. coli* TOP 10. Presence of ESBLs gene in transformants and transconjugants was confirmed by PCR experiments.

2.5. Plasmid identification and typing

Plasmids were typed in both donor and recipient using the PCR-based replicon typing scheme (Caratolli et al., 2005). The FAB formula was determined for IncF plasmids (Villa et al., 2010). Plasmid size from donors and transformants was determined using S1 nuclease treatment followed by pulsed-field gel electrophoresis (S1-PFGE) (Barton et al., 1995). Plasmids linearized with S1 nuclease were further analyzed by Southern blot hybridization with DIG-labeled probes specific for the bla_{CTX-M} gene and for the replicon type of the plasmid carrying the ESBL gene. Detection was performed using the DIG DNA Labeling and Detection Kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions.

2.6. Clonality of the isolates

Pulsed-field gel electrophoresis (PFGE) analysis was done according to the manufacturer's instructions. Genomic DNA was digested with *Xba*I restriction endonuclease. A ladder was used as molecular weight marker. Computer analysis of the PFGE banding patterns was performed using the Fingerprinting Bionumerics software package. Dice similarity indices were used to generate a dendrogram describing the relationships among PFGE profiles. Interpretation of chromosomal DNA restriction patterns was based on the criteria of Tenover et al. (1995). *K. pneumoniae* strains were typed by multilocus sequence typing (MLST) through the amplification of internal fragments of 7 housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*), as described

Table 1
Prevalence of ESBL-producing *K. pneumoniae* isolates in hospital- and community-acquired infections in the region of Mahdia, Tunisia (July 2009 to December 2011).

	Total no. of Enterobacteriaceae isolates	No. of *K. pneumoniae* isolates	No. of ESBL-producing Enterobacteriaceae	No. of ESBL producing *K. pneumoniae* isolates
Ward	Hospital-associated isolates			
Pediatric unit	607	39	33	19
Medicine	468	27	15	13
Intensive care unit	94	31	19	2
Postoperative	149	16	11	5
Gynecology	291	15	4	4
Cardiology	19	4	2	2
Pneumology	32	10	1	1
Hemodialysis	24	2	2	2
Surgery	149	25	8	2
Orthopedics	123	16	0	0
Otorhinolaryngology	32	3	0	0
Total (nosocomial strains)	1988	188	95	50
	Community-acquired isolates			
	2882	150	45	15
	Total no. of isolates			
	4870	338	140	65

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