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Dynamics and molecular features of OXA-48-like-producing *Klebsiella pneumoniae* lineages in a Tunisian hospital

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ABSTRACT

Objectives: The aim of this study was to elucidate the molecular features of genes, plasmids and clones of OXA-48-like producing *Klebsiella pneumoniae* isolates recovered in Sahloul Hospital (Sousse, Tunisia) in the period 2012–2014.

Methods: In vitro antimicrobial susceptibility testing, S1 nuclease pulsed-field gel electrophoresis (S1-PFGE), Southern blotting and PCR-based replicon typing (PBRT) were performed. Extended-spectrum β -lactamase (ESBL) and carbapenemase genes were detected by PCR and sequencing. The clonality of isolates was assessed by PFGE and multilocus sequence typing (MLST).

Results: *Klebsiella pneumoniae* accounted for 26.8% (1095/4083) of clinical Enterobacterales isolates identified during 2012–2014, of which 21.9% (240/1095) were resistant to carbapenems, mostly harbouring *bla*_{OXA-48-like} genes (196/240; 81.7%). Plasmid analysis showed that *bla*_{OXA-204} and *bla*_{OXA-48} were mostly carried by IncA/C and IncL plasmids, respectively. The current data highlight the dominance of two ST101 and ST147 lineages spreading OXA-48 and OXA-204, respectively, through successive clonal spreads at this hospital. In addition, a large diversity of other *K. pneumoniae* lineages was also identified, such as ST15, ST36 and ST525 spreading OXA-48 as well as ST340, ST2032, ST301, ST199 and ST1561 spreading OXA-48 or OXA-204, constituting a reservoir of possible dominant clones in the future.

Conclusion: This study reports the full molecular characterisation of carbapenem resistance in *K. pneumoniae* and the predominance of a few clones responsible for the dissemination of OXA-48 and OXA-204 enzymes in a Tunisian hospital.

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1. Introduction

The Enterobacterales is a large family of opportunistic pathogens extensively involved in nosocomial infections. Due to the emergence and spread of multidrug-resistant (MDR) Enterobacterales in clinics, carbapenems have become one of the last-resort treatments for severe bacterial infections caused by these isolates in many healthcare settings. However, carbapenem-

resistant Enterobacterales (particularly *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* spp.) have been emerging for several years as a major public-health concern worldwide [1]. Resistance to carbapenems in Enterobacterales is related to two mechanisms, the first involving overexpression of cephalosporinases and/or extended-spectrum β -lactamase (ESBL)-type enzymes together with decreased permeability of the bacterial membrane owing to porin loss or alteration [2], and the second being related to the acquisition of one or more carbapenemase genes encoding enzymes capable of degrading these molecules.

According to the Ambler classification, the most common carbapenemases are KPC (class A), VIM, IMP and NDM (class B) and the OXA-48-type oxacillinases (class D) [3]. Among the different types of carbapenemases identified in Enterobacterales, OXA-48 is

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the most predominant in Mediterranean countries, especially France, Spain, Greece, Turkey, Tunisia and Morocco, as well as different countries in the Middle East such as Lebanon, the Sultanate of Oman and Kuwait [4–9]. In Europe, hospital outbreaks due to OXA-48-producing Enterobacteriales have been linked to transfer of patients from countries where OXA-48 distribution is considered endemic, such as Turkey and Morocco [10,11]. Since its discovery in 2001 from a carbapenem-resistant *K. pneumoniae* isolate in Istanbul, Turkey, 11 OXA-48-like variants with carbapenemase activity and differing by a few amino acid substitutions or deletions have been reported from different countries [12–17]. The *bla*_{OXA-48-like} genes are mostly plasmid-borne and associated with insertion sequence (IS) elements of the IS1999 type providing promoter sequences responsible for their expression [18,19].

In Tunisia, as in other North African countries, carbapenemase-producing Enterobacteriales have been increasing remarkably in recent years. In Tunisia, the first OXA-48-producer was reported in 2010 [20]. Since then, other studies have described the emergence not only of OXA-48 but also of its OXA-204 derivative [19,21–25]. In particular, two major *K. pneumoniae* lineages, namely of sequence type 101 (ST101) and ST147, have been shown to disseminate OXA-48 and OXA-204 enzymes, respectively. However, there are still limited data on the comparative dynamics of these two *K. pneumoniae* lineages with regard to the global epidemiology of OXA-48-like in clinics in Tunisia. This study provides a molecular characterisation of genes, plasmids and clones of OXA-48-like producing *K. pneumoniae* isolates recovered in Sahloul Hospital (Sousse, Tunisia) in the period 2012–2014.

2. Materials and methods

2.1. Bacterial isolates

From January 2012 to December 2014, a total of 240 carbapenem-resistant *K. pneumoniae* isolates were recovered from clinical specimens in the microbiology laboratory from patients admitted in different wards in Sahloul Hospital, a 629-bed university hospital with a surgical vocation. Isolates were selected on the basis of reduced susceptibility to at least one carbapenem (ertapenem and/or imipenem) as assessed by the disk diffusion method. Clinical isolates were identified using API20E test strips (bioMérieux, Marcy-l'Étoile, France).

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed 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 European Committee on Antimicrobial Susceptibility testing (EUCAST 2015; <http://www.eucast.org>). Susceptibility to colistin was tested by the microdilution method as recommended by EUCAST. *Escherichia coli* ATCC 25922 was used as a quality control strain.

2.3. Extended-spectrum β -lactamase and carbapenemase production

ESBL production was determined and confirmed by the double-disk synergy test. Phenotypic screening of carbapenemases was done using a Rosco KPC, MBL and OXA-48 Confirm Kit (Rosco Diagnostica A/S, Taastrup, Denmark) using meropenem disks alone and in combination with different inhibitors, i.e. dipicolinic acid against metallo- β -lactamases (MBLs), aminophenylboronic acid for Ambler class A β -lactamases and cloxacillin against AmpC β -lactamases, as recommended by the manufacturer. MBL production was tested by the Carba NP test and the ethylene diamine tetra-acetic acid (EDTA) double-disk synergy test [26]. Temocillin

was used for presumptive detection of Ambler class D oxacillinase-producers, which have high-level resistance to this molecule [27].

2.4. PCR and sequencing of β -lactamase-encoding genes

Whole-cell DNA was extracted using a NucleoSpin[®] Tissue Kit (Macherey–Nagel, Hoerdt, France) following the manufacturer's instructions. The presence of genes encoding resistance to carbapenems (*bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM} and *bla*_{OXA-48-like}) and broad-spectrum cephalosporins (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *bla*_{CMY}) was investigated by PCR [28–30]. The *bla*_{CTX-M-1} group gene was identified with an additional PCR using external primers ISEcp1 and P2D to detect the presence of ISEcp1 in association with the *bla*_{CTX-M} gene. All positive amplicons were sequenced by GENEWIZ (London, UK).

2.5. Genetic environment of carbapenemase genes

The genetic environment of carbapenemase genes was assessed by PCR to characterise the Tn1999 element in association with *bla*_{OXA-48} [8,18]. The presence of ISEcp1 upstream of the *bla*_{OXA-204} gene was screened to assess the presence of transposon Tn2016 [8].

2.6. Clonality of the isolates

Pulsed-field gel electrophoresis (PFGE) was performed on *Xba*I-digested genomic DNA. DNA fingerprints were analysed using the Dice correlation coefficient with tolerance and optimisation set at 0.5% and 1%, respectively (BioNumerics, Ghent, Belgium). Multi-locus sequence typing (MLST) was performed using the MLST scheme described by Diancourt et al. [31]. Allele sequences and STs were obtained from the MLST database at the Institut Pasteur (<http://bigsd.bpasteur.fr/klebsiella/klebsiella.html>).

2.7. Molecular characterisation of plasmids

Plasmids were typed using a commercial PCR-based replicon typing (PBRT) kit (Diatheva, Fano, Italy) designed according to the scheme developed by Carattoli et al. [32,33]. Plasmid size was determined using *S1* nuclease pulsed-field gel electrophoresis (S1-PFGE) [34]. S1-PFGE gels were further submitted to Southern blot hybridisation with appropriate probes specific for the *bla*_{OXA-48-like} gene and for the replicon type of the plasmid carrying the carbapenemases gene. Detection was performed using a DIG DNA Labeling and Detection Kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions.

3. Results

3.1. Epidemiology of carbapenem-resistant *Klebsiella pneumoniae* isolates

Between January 2012 and December 2014, 4083 clinical Enterobacteriales isolates were recovered in the microbiology laboratory of Sahloul Hospital, among which *K. pneumoniae* accounted for 26.8% (1095/4083). The number of *K. pneumoniae* isolates recovered per year was roughly equally distributed, with 341, 401 and 353 recovered in 2012, 2013 and 2014, respectively (Table 1).

Among the *K. pneumoniae* isolates, 21.9% (240/1095) were resistant to carbapenems (Table 1). Patients had been hospitalised in seven different wards, most commonly in urology (37.5%) and intensive care units (ICUs) (30.8%) (Table 1). Isolates were recovered from various specimens, mainly from urine (138/240; 57.5%) and blood (37/240; 15.4%). All isolates were MDR according to the definition of Magiorakos et al. [35] as they all presented a

Table 1

Prevalence and ward distribution of carbapenem-resistant *Klebsiella pneumoniae* in Sahloul Hospital, Sousse, Tunisia, during the period January 2012 to December 2014.

Year	No. of isolates	No. (%) of carbapenem-resistant isolates	No. (%) of carbapenem-resistant isolates per ward			
			Urology	Nephrology	ICUs	Other wards ^a
2012	341	83 (24.3)	42	0	35	6
2013	401	64 (16.0)	26	12	18	8
2014	353	93 (26.3)	22	30	21	20
Total	1095	240 (21.9)	90 (37.5)	42 (17.5)	74 (30.8)	34 (14.2)

ICU, intensive care unit.

^a Pediatric unit, burns ward and surgery.

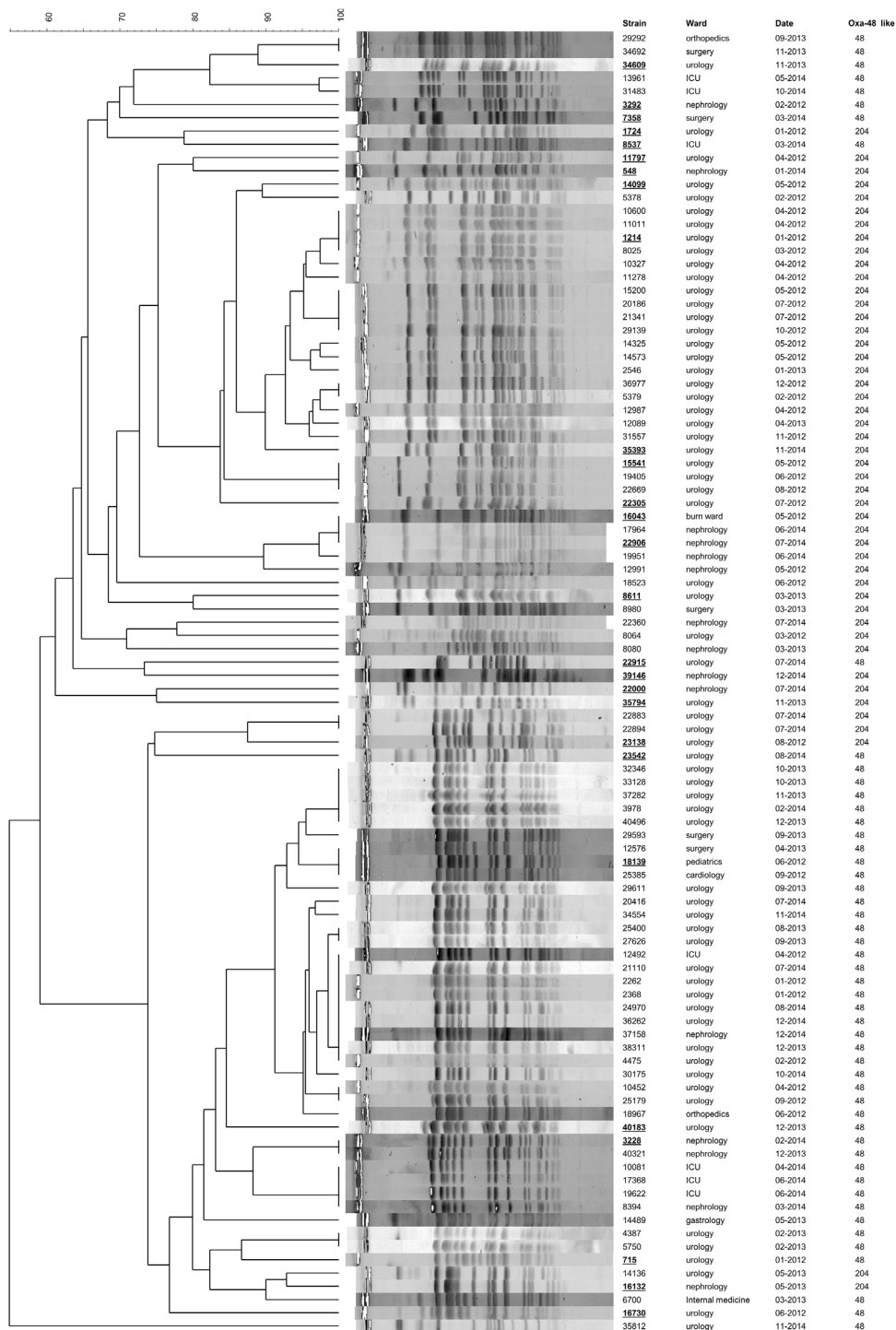


Fig. 1. Clonal relationship of carbapenemase-producing *Klebsiella pneumoniae* isolates from hospitalised patients.

resistant phenotype to at least three antibiotic families. All isolates were susceptible to colistin.

3.2. Phenotyping and genotyping of carbapenem-resistant *Klebsiella pneumoniae*

Among the 240 carbapenem-resistant *K. pneumoniae* isolates, 25 (10.4%) harboured a non-enzymatic resistance mechanism to carbapenems as they were not affected by any carbapenemase inhibitors tested but were susceptible to temocillin. Another 19 *K. pneumoniae* isolates (7.9%) were NDM-1-producers, as recently described [36]. The remaining carbapenem-resistant *K. pneumoniae* isolates (196/240; 81.7%) were resistant to temocillin but remained susceptible in the boronic acid, dipicolinic acid and EDTA assays, thereby excluding the expression of KPC-type or MBL enzymes.

PCR screening of these 196 *K. pneumoniae* isolates revealed the presence of *bla*_{OXA-48-like} in all of them. Most isolates also co-harboured *bla*_{CTX-M-type} genes (183/196; 93.4%), whereas 13 isolates (6.6%) harboured *bla*_{CMY-like} genes.

3.3. PFGE analysis

Among the 196 *bla*_{OXA-48}-positive *K. pneumoniae* isolates, a subset of 98 isolates was further subjected to PFGE analysis. Isolates were chosen in order to provide a representative picture of the different wards affected by OXA-48-like-producing *K. pneumoniae* according to the respective proportions of these isolates per ward. Indeed, a significant proportion of isolates was recovered from urology and ICUs (Table 1). In addition, particular attention was paid to exclude duplicate isolates so that only one *K. pneumoniae* isolate per patient and per year was included in this subset.

PFGE analysis highlighted different clusters of isolates as shown in Fig. 1. Two clusters of isolates sharing >85% similarity appeared dominantly represented, one comprising 18 OXA-204 isolates

mostly collected in 2012, and the second comprising 27 OXA-48 isolates mostly collected in 2013–2014. Numerous minor clones and singletons were also identified.

3.4. *bla*_{OXA-48 like} gene sequencing, plasmid determination and sequence type analysis

From the PFGE results, a subset of 27 *K. pneumoniae* isolates representative of the major clones was selected for further molecular investigations, including *bla*_{OXA-48 like} gene sequencing, plasmid determination and MLST analysis. Among these 27 *K. pneumoniae* isolates, 10 and 17 isolates harboured the *bla*_{OXA-48} and *bla*_{OXA-204} genes, respectively (Table 2). Among the 17 *bla*_{OXA-204}-positive isolates, 4 also harboured the *bla*_{CMY-4} gene. In addition, all but 3 isolates harboured the *bla*_{CTX-M-15} gene (Table 2). In all 10 *bla*_{OXA-48}-positive isolates, the *bla*_{OXA-48} gene was located on an IncL plasmid of 60 kb in size, as shown by Southern blot, and was part of the composite transposon Tn1999.2 variant presenting an IS1R disruption of the upstream IS1999 element, as shown by PCR mapping. In 15 of the 17 *bla*_{OXA-204}-positive isolates, the *bla*_{OXA-204} gene was located on an IncA/C plasmid. The two remaining *bla*_{OXA-204}-positive isolates harboured the *bla*_{OXA-204} gene on an IncHIB-M and an IncR plasmid, respectively (Table 2). The *bla*_{OXA-204}-bearing plasmid sizes ranged from 60–270 kb. The genetic environment of the *bla*_{OXA-204} gene had a backbone similar to the Tn2016 transposon with the presence of the *ISEcp1* element upstream of the *bla*_{OXA-204} gene.

Ten different STs were identified, with ST147 and ST101 being the most predominant. ST147 was identified in 11 isolates recovered from three wards, i.e. nephrology ($n=4$), urology ($n=6$) and ICUs ($n=1$). The second most predominant sequence type (ST101) was identified in six OXA-48-producing isolates and one OXA-204-producing isolate. ST36 was identified in two OXA-48-producing isolates. The seven remaining sequence types (ST340, ST2032, ST301, ST199, ST1561 for OXA-204-producers and ST525 and ST15 for OXA-48-producers) were singleton isolates

Table 2
Characteristics of 27 representative OXA-48-like-producing *Klebsiella pneumoniae* isolates.

Isolate	Ward	Date of isolation	Specimen	<i>bla</i> _{OXA-48-like} gene	Associated β-lactamase(s)	MLST	<i>bla</i> _{OXA-48} / <i>bla</i> _{OXA204} -carrying plasmid	
							Inc group	Size (kb)
715	Urology	06/01/2012	Urine	<i>bla</i> _{OXA-48}	CTX-M-15	ST101	L	60
1214	Urology	11/01/2012	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	200
18139	Paediatrics	16/06/2012	Urine	<i>bla</i> _{OXA-48}	CTX-M-15	ST101	L	60
1724	Urology	23/01/2012	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST340	A/C	200
11797	Urology	19/04/2012	Blood	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	200
14099	Urology	10/05/2012	Peritoneal fluid	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	270
15541	Urology	23/05/2012	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST2032	A/C	200
16043	Burns ward	28/05/2012	Pus	<i>bla</i> _{OXA-204}	CMY-4	ST301	A/C	200
16730	Urology	02/06/2012	Urine	<i>bla</i> _{OXA-48}	CTX-M-15	ST101	L	60
8611	Urology	19/03/2013	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	200
122305	Urology	30/07/2012	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	250
23138	Urology	14/08/2012	Peritoneal fluid	<i>bla</i> _{OXA-204}	CTX-M-15	ST199	HIB-M	240
16132	Nephrology	24/05/2013	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST101	R	60
34609	Urology	04/11/2013	Blood	<i>bla</i> _{OXA-48}	CTX-M-15	ST15	L	60
35794	Urology	14/11/2013	Urine	<i>bla</i> _{OXA-204}	CMY-4	ST1561	A/C	200
40183	Urology	23/12/2013	Urine	<i>bla</i> _{OXA-48}	CTX-M-15	ST101	L	60
548	Nephrology	07/01/2014	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	90
3292	Nephrology	04/02/2014	Urine	<i>bla</i> _{OXA-48}	CTX-M-15	ST36	L	60
22000	Nephrology	17/07/2014	Urine	<i>bla</i> _{OXA-204}	CMY-4	ST147	A/C	150
22906	Nephrology	21/07/2014	Urine	<i>bla</i> _{OXA-204}	CTX-M-15, CMY-4	ST147	A/C	200
22915	Urology	30/07/2014	Blood	<i>bla</i> _{OXA-48}	CTX-M-15	ST525	L	60
23542	Urology	04/08/2014	Urine	<i>bla</i> _{OXA-48}	CTX-M-15, CMY-4	ST101	L	60
35393	Urology	29/11/2014	Urine	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	200
39146	Nephrology	30/12/2014	Urine	<i>bla</i> _{OXA-204}	CTX-M-15, CMY-4	ST147	A/C	150
3228	Nephrology	03/02/2014	Blood	<i>bla</i> _{OXA-48}	CTX-M-15	ST101	L	60
7358	Surgery	08/03/2014	Blood	<i>bla</i> _{OXA-48}	CTX-M-15	ST36	L	60
8537	ICU	21/03/2014	Bronchial aspiration	<i>bla</i> _{OXA-204}	CTX-M-15	ST147	A/C	200

MLST, multilocus sequence typing; ICU, intensive care unit.

(Table 2). Of note, ST1561 is a single-locus variant of ST147, and ST2032 differs by two alleles, whilst all other STs were not related to ST101 or ST147.

4. Discussion

Klebsiella pneumoniae is a common cause of infections due to Enterobacteriales in Tunisian hospitals, contributing 26.8% of Enterobacteriales in Sahloul Hospital in this study in 2012–2014. These data again emphasise the major capability of *K. pneumoniae* to cause severe infections in humans and to readily spread between patients, thereby often leading to hospital outbreaks. Among these *K. pneumoniae* isolates, 21.8% were found to be resistant to carbapenems. Since the very first carbapenem-resistant *K. pneumoniae* isolate was reported in Tunisia in 2010, these data indicate a very rapid increase in the prevalence of these MDR bacteria at the hospital. The proportion is even higher than that reported from Tahar Sfar University Hospital in Mahdia (13.2%), in the same geographical area (70 km from Sousse) [37]. More globally, carbapenem-resistant *K. pneumoniae* have become a serious burden in North African countries since they have also been identified in Morocco [38] and Algeria [39]. No published data are available from Libya, but OXA-48-producers have been recovered from patients transferred from Libya to Tunisia [40]. Also, this enzyme has been extensively reported as a source of nosocomial and community outbreaks in many parts of the world such as France [41], Germany [42], Greece [43] and Russia [44].

Sahloul Hospital is a 629-bed university hospital with mainly surgical activities where carbapenems are heavily used. Resistance to carbapenems in this hospital has already been associated with non-fermentative bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [45–47]. One *K. pneumoniae* isolate resistant to carbapenems was also reported in this setting and produced a cephalosporinase combined with porin loss [48]. Recently, we also described the emergence of NDM-1-producing *K. pneumoniae* isolates harbouring the *bla*_{NDM-1} gene on an IncFIIk plasmid, with one isolate co-harboring *bla*_{NDM-1} and *bla*_{OXA-48} genes [36]. Here we describe a massive spread of OXA-48-like-producing *K. pneumoniae* over the period 2012–2014, and it was our objective to decipher the comparative dynamics of OXA-48- and OXA-204-producing *K. pneumoniae* over time, for which limited information is available in Northern African countries.

Since its first report in Tunisia, the epidemiology of OXA-48-producing *K. pneumoniae* has been dominated by wide spread of the ST101 lineage, also producing CTX-M-15 in most cases [49–51]. Recently, the *K. pneumoniae* ST147 lineage has also been identified as an emerging vector of the *bla*_{OXA-48} gene [52]. More globally, ST147 was repeatedly reported to spread ESBL or carbapenemase genes, such as in Greece where it has been responsible for the dissemination of VIM-1 and KPC-2 already since 2011 [53,54]. Finally, a limited number of reports also identified the *K. pneumoniae* ST147 lineage as a vector of the *bla*_{OXA-204} gene [21,23,37]. The data in the current study strongly prove that ST147 *K. pneumoniae* is mainly responsible for a surge in the *bla*_{OXA-204} gene in Sahloul Hospital, Tunisia.

In our setting, two major clonal groups have been identified over the period 2012–2014. A surge of OXA-204 was first identified in ST147 *K. pneumoniae* isolates, especially in 2012 in urology before emerging in other wards, whilst a second surge of OXA-48 was identified in 2013–2014. Plasmid analysis showed that the *bla*_{OXA-204} gene was carried on IncA/C plasmids, which might also have contributed to the spread of *bla*_{OXA-204} across different *K. pneumoniae* isolates. None the less, the success of ST147 *K. pneumoniae* in 2012 did not preclude the successful epidemiology

of ST101 *K. pneumoniae*, mostly harbouring the *bla*_{OXA-48} gene in 2013 and 2014.

Beside these two dominant *K. pneumoniae* lineage/OXA-48-type combinations (ST101/OXA-48 and ST147/OXA-204), OXA-48 was identified in other sequence types such as ST15, ST36 and ST525 in a few isolates. ST15 *K. pneumoniae* was previously reported as an NDM-1-producer in North African patients [55] and as an OXA-48-producer in Spain [56], whereas ST36 was identified in MBL-producing *K. pneumoniae* isolates from Greece [57]. Finally, the ST525 OXA-48-producing *K. pneumoniae* lineage is a new sequence type identified in this study. In addition, OXA-204 was identified for the first time in the ST2032, ST301, ST199 and ST1561 *K. pneumoniae* lineages. On the other hand, one OXA-204-producer was found in the ST101 lineage and in a few other sequence types, i.e. ST340, ST2032, ST301, ST199 and ST1561. Finally, ST340 *K. pneumoniae* had already been associated with the production of NDM and KPC carbapenemases, but not OXA-48, and was detected from clinical and environmental sources [58,59].

Finally, 14/17 OXA-204 isolates additionally carried the *bla*_{CTX-M-15} gene and 5 presented *bla*_{CMY-4}. In 2/17 cases, we noted the presence of the combination of three β -lactamases (CTX-M-15, OXA-204 and CMY-4), highlighting the common combination of several β -lactamases in a single isolate. Since oxacillinases produced alone possess weak activity against carbapenems, the co-existence of two or more β -lactamases has become a common bacterial strategy to increase antimicrobial resistance [60].

5. Conclusion

In this study, we have explored the dynamics of the global epidemiology of OXA-48-like-producing *K. pneumoniae* in Sahloul Hospital over the period 2012–2014 and identified the successive dominance of two *K. pneumoniae* lineage/OXA-48-type combinations (ST101/OXA-48 and ST147/OXA-204). Although the ST101 and ST147 lineages were both identified in 2012–2014, the respective proportion of isolates over this period indicated that OXA-204-producing ST147 *K. pneumoniae* has spread as an epidemic clone in 2012, followed by a second epidemic spread of the OXA-48-producing ST101 *K. pneumoniae* in 2013 and 2014. This suggests that OXA-204-producing ST147 *K. pneumoniae*, although reported as an emerging clone in Tunisia, should not be considered as replacing the OXA-48-producing ST101 *K. pneumoniae* lineage globally. On the contrary, ST101 and ST147 may rather equally co-exist in the global epidemiology of OXA-48-like-producing *K. pneumoniae* in Tunisia through successive clonal spreads at hospitals depending on local factors. In addition, we also highlighted a large diversity of minor *K. pneumoniae* lineages disseminating OXA-204 or OXA-48, which may constitute possible emerging dominant clones in the future. In all, these data underline the urgent need to control the diversification of OXA-48-like-producing *K. pneumoniae* lineages in clinics in Tunisia through reinforced preventive strategies in healthcare settings.

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Competing interests

None declared.

Ethical approval

Not required.

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References

- Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm!. *Trends Mol Med* 2012;18:263–72. doi:http://dx.doi.org/10.1016/j.molmed.2012.03.003.
- Poirel L, Héritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004;48:15–22.
- Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012;18:1503–7. doi:http://dx.doi.org/10.3201/eid1809.120355.
- Matar GM, Dandachel L, Carrer A, Khairallah MT, Nordmann P, Sabra A, et al. Spread of OXA-48-mediated resistance to carbapenems in Lebanese *Klebsiella pneumoniae* and *Escherichia coli* that produce extended spectrum β -lactamase. *Ann Trop Med Parasitol* 2010;104:271–4. doi:http://dx.doi.org/10.1179/136485910X12647085215651.
- Dortet L, Poirel L, Al Yaquobi F, Nordmann P. NDM-1, OXA-48 and OXA-181 carbapenemase-producing Enterobacteriaceae in Sultanate of Oman. *Clin Microbiol Infect* 2012;18:E144–8. doi:http://dx.doi.org/10.1111/j.1469-0691.2012.03796.x.
- Poirel L, Carbone E, Bernabeu S, Gutmann L, Rotimi V, Nordmann P. Importation of OXA-48-producing *Klebsiella pneumoniae* from Kuwait. *J Antimicrob Chemother* 2012;67:2051–2. doi:http://dx.doi.org/10.1093/jac/dks167.
- Dortet L, Oueslati S, Jeannot K, Tande D, Naas T, Nordmann P. Genetic and biochemical characterization of OXA-405, an OXA-48-type extended-spectrum β -lactamase without significant carbapenemase activity. *Antimicrob Agents Chemother* 2015;59:3823–8. doi:http://dx.doi.org/10.1128/AAC.05058-14.
- Potron A, Nordmann P, Poirel L. Characterization of OXA-204, a carbapenem-hydrolyzing class D β -lactamase from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2013;57:633–6. doi:http://dx.doi.org/10.1128/AAC.01034-12.
- Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect* 2014;20:821–30. doi:http://dx.doi.org/10.1111/1469-0691.12719.
- Dautzenberg MJ, Ossewaarde JM, de Kraker ME, van der Zee A, van Burgh S, de Greeff SC, et al. Successful control of a hospital-wide outbreak of OXA-48 producing Enterobacteriaceae in the Netherlands, 2009 to 2011. *Euro Surveill* 2014;19:20723.
- Gülmez D, Woodford N, Palepu MF, Mushtaq S, Metan G, Yakupogullari Y, et al. Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from Turkey with OXA-48-like carbapenemases and outer membrane protein loss. *Int J Antimicrob Agents* 2008;31:523–6. doi:http://dx.doi.org/10.1016/j.ijantimicag.2008.01.017.
- Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A. OXA-48-like carbapenemases producing Enterobacteriaceae in different niches. *Eur J Clin Microbiol Infect Dis* 2018;37:587–604. doi:http://dx.doi.org/10.1007/s10096-017-3112-7.
- Poirel L, Héritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004;48:15–22.
- Kasap M, Torol S, Kolayli F, Dundar D, Vahaboglu H. OXA-162, a novel variant of OXA-48 displays extended hydrolytic activity towards imipenem, meropenem and doripenem. *J Enzyme Inhib Med Chem* 2013;28:990–6. doi:http://dx.doi.org/10.3109/14756366.2012.702343.
- Lerner A, Solter E, Rachi E, Adler A, Reznitzer H, Miron D, et al. Detection and characterization of carbapenemase-producing Enterobacteriaceae in wounded Syrian patients admitted to hospitals in northern Israel. *Eur J Clin Microbiol Infect Dis* 2016;35:149–54. doi:http://dx.doi.org/10.1007/s10096-015-2520-9.
- Oteo J, Hernández JM, Espasa M, Fleites A, Sáez D, Bautista V, et al. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. *J Antimicrob Chemother* 2013;68:317–21. doi:http://dx.doi.org/10.1093/jac/dks383.
- Potron A, Pondinaud E, Poirel L, Belmonte O, Boyer S, Camiade S, et al. Genetic and biochemical characterization of OXA-232, a carbapenem-hydrolyzing class D β -lactamase from Enterobacteriaceae. *Int J Antimicrob Agents* 2013;41:325–9. doi:http://dx.doi.org/10.1016/j.ijantimicag.2012.11.007.
- Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemase: the phantom menace. *J Antimicrob Chemother* 2012;67:1597–606. doi:http://dx.doi.org/10.1093/jac/dks121.
- Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 β -lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. *Euro Surveill* 2013;18:20549.
- Ktari S, Mnif B, Louati F, Rekiq S, Mezghani S, Mahjoubi F, et al. Spread of *Klebsiella pneumoniae* isolates producing OXA-48 β -lactamase in a Tunisian university hospital. *J Antimicrob Chemother* 2011;66:1644–6. doi:http://dx.doi.org/10.1093/jac/dkr181.
- Charfi K, Mansour W, Ben Haj Khalifa A, Mastouri M, Aouni M, Mammeri H. Emergence of OXA-204 β -lactamase in Tunisia. *Diagn Microbiol Infect Dis* 2015;82:314–7. doi:http://dx.doi.org/10.1016/j.diagmicrobio.2015.04.003.
- Cuzon G, Naas T, Lesenne A, Benhamou M, Nordmann P. Plasmid-mediated carbapenem-hydrolyzing OXA-48 β -lactamase in *Klebsiella pneumoniae* from Tunisia. *Int J Antimicrob Agents* 2010;36:90–8. doi:http://dx.doi.org/10.1016/j.ijantimicag.2010.02.014.
- Grami R, Mansour W, Ben Haj Khalifa A, Dahmen S, Chatre P, Haenni M, et al. Emergence of ST147 *Klebsiella pneumoniae* producing OXA-204 carbapenemase in a university hospital, Tunisia. *Microb Drug Resist* 2016;22:137–40. doi:http://dx.doi.org/10.1089/mdr.2014.0278.
- Lahlaoui H, Poirel L, Barguelli F, Moussa MB, Nordmann P. Carbapenem-hydrolyzing class D β -lactamase OXA-48 in *Klebsiella pneumoniae* isolates from Tunisia. *Eur J Clin Microbiol Infect Dis* 2012;31:937–9. doi:http://dx.doi.org/10.1007/s10096-011-1389-5.
- Ouertani R, Ben Jomâa-Jemili M, Gharsa H, Limelette A, Guillard T, Brasme L, et al. Prevalence of a new variant OXA-204 and OXA-48 carbapenemases plasmids encoded in *Klebsiella pneumoniae* clinical isolates in Tunisia. *Microb Drug Resist* 2018;24:142–9. doi:http://dx.doi.org/10.1089/mdr.2016.0236.
- Bartolini A, Frasson I, Cavallaro A, Richter SN, Palù G. Comparison of phenotypic methods for the detection of carbapenem non-susceptible Enterobacteriaceae. *Gut Pathog* 2014;19:13. doi:http://dx.doi.org/10.1186/1757-4749-6-13.
- Hartl R, Widhalm S, Kerschner H, Alfalter P. Temocillin and meropenem to discriminate resistance mechanisms leading to decreased carbapenem susceptibility with focus on OXA-48 in Enterobacteriaceae. *Clin Microbiol Infect* 2013;19:230–2. doi:http://dx.doi.org/10.1111/1469-0691.12146.
- Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D. Increased detection of extended spectrum β -lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Vet Microbiol* 2010;145:273–8. doi:http://dx.doi.org/10.1016/j.vetmic.2010.03.019.
- Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011;70:119–23. doi:http://dx.doi.org/10.1016/j.diagmicrobio.2010.12.002.
- Shibata N, Kurokawa H, Doi Y, Yagi T, Yamane K, Wachino J, et al. PCR classification of CTX-M-type β -lactamase genes identified in clinically isolated Gram-negative bacilli in Japan. *Antimicrob Agents Chemother* 2006;50:791–5. doi:http://dx.doi.org/10.1128/AAC.50.2.791-795.2006.
- Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005;43:4178–82.
- Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005;63:219–28. doi:http://dx.doi.org/10.1016/j.mimet.2005.03.018.
- Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A. Differentiation of IncI and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. *PLoS One* 2015;10:e0123063. doi:http://dx.doi.org/10.1371/journal.pone.0123063.
- Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem* 1995;226:235–40. doi:http://dx.doi.org/10.1006/abio.1995.1220.
- Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–81. doi:http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x.
- Messaoudi A, Haenni M, Mansour W, Saras E, Bel Haj Khalifa A, Chaouch C, et al. ST147 NDM-1-producing *Klebsiella pneumoniae* spread in two Tunisian hospitals. *J Antimicrob Chemother* 2017;72:315–6. doi:http://dx.doi.org/10.1093/jac/dkw401.
- Mansour W, Haenni M, Saras E, Grami R, Mani Y, Ben Haj Khalifa A, et al. Outbreak of colistin-resistant carbapenemase-producing *Klebsiella pneumoniae* in Tunisia. *J Glob Antimicrob Resist* 2017;10:88–94. doi:http://dx.doi.org/10.1016/j.jgar.2017.03.017.
- Barguigua A, Zerouali K, Katfy K, El Otmani F, Timinouni M, Elmdaghri N. Occurrence of OXA-48 and NDM-1 carbapenemase-producing *Klebsiella pneumoniae* in a Moroccan university hospital in Casablanca, Morocco. *Infect Genet Evol* 2015;31:142–8. doi:http://dx.doi.org/10.1016/j.meegid.2015.01.010.
- Agabou A, Pantel A, Ouchenane Z, Lezzar N, Khemissi S, Satta D, et al. First description of OXA-48-producing *Escherichia coli* and the pandemic clone ST131 from patients hospitalized at a military hospital in Algeria. *Eur J Clin Microbiol Infect Dis* 2014;33:1641–6. doi:http://dx.doi.org/10.1007/s10096-014-2122-y.
- Ben Nasr A, Decré D, Compain F, Genel N, Barguelli F, Arlet G. Emergence of NDM-1 in association with OXA-48 in *Klebsiella pneumoniae* from Tunisia. *Antimicrob Agents Chemother* 2013;57:4089–90. doi:http://dx.doi.org/10.1128/AAC.00536-13.
- Poirel L, Bernabeu S, Fortineau N, Podglajen I, Lawrence C, Nordmann P. Emergence of OXA-48-producing *Escherichia coli* clone ST38 in France. *Antimicrob Agents Chemother* 2011;55:4937–8. doi:http://dx.doi.org/10.1128/AAC.00413-11.
- Kaase M, Schimanski S, Schiller R, Beyreiß B, Thürmer A, Steinmann J, et al. Multicentre investigation of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in German hospitals. *Int J Med Microbiol* 2016;306:415–20. doi:http://dx.doi.org/10.1016/j.ijmm.2016.05.009.

- [43] Galani I, Anagnostoulis G, Chatzikonstantinou M, Petrikkos G, Souli M. Emergence of *Klebsiella pneumoniae* co-producing OXA-48, CTX-M-15, and ArmA in Greece. *Clin Microbiol Infect* 2016;22:898–9, doi:http://dx.doi.org/10.1016/j.cmi.2016.08.002.
- [44] Ageevets VA, Partina IV, Lisitsyna ES, Ilina EN, Lobzin YV, Shlyapnikov SA, et al. Emergence of carbapenemase-producing Gram-negative bacteria in Saint Petersburg, Russia. *Int J Antimicrob Agents* 2014;44:152–5, doi:http://dx.doi.org/10.1016/j.ijantimicag.2014.05.004.
- [45] Mansour W, Dahmen S, Poirel L, Charfi K, Bettaieb D, Boujaafar N, et al. Emergence of SHV-2a extended-spectrum β -lactamases in clinical isolates of *Pseudomonas aeruginosa* in a university hospital in Tunisia. *Microb Drug Resist* 2009;15:295–301, doi:http://dx.doi.org/10.1089/mdr.2009.0012.
- [46] Mansour W, Poirel L, Bettaieb D, Bouallegue O, Boujaafar N, Nordmann P. Metallo- β -lactamase-producing *Pseudomonas aeruginosa* isolates in Tunisia. *Diagn Microbiol Infect Dis* 2009;64:458–61, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2009.04.003.
- [47] Poirel L, Mansour W, Bouallegue O, Nordmann P. Carbapenem-resistant *Acinetobacter baumannii* isolates from Tunisia producing the OXA-58-like carbapenem-hydrolyzing oxacillinase OXA-97. *Antimicrob Agents Chemother* 2008;52:1613–7, doi:http://dx.doi.org/10.1128/AAC.00978-07.
- [48] Dahmen S, Mansour W, Charfi K, Boujaafar N, Arlet G, Bouallègue O. Imipenem resistance in *Klebsiella pneumoniae* is associated to the combination of plasmid-mediated CMY-4 AmpC β -lactamase and loss of an outer membrane protein. *Microb Drug Resist* 2012;18:479–83, doi:http://dx.doi.org/10.1089/mdr.2011.0214.
- [49] Mammina C, Bonura C, Aleo A, Fasciana T, Brunelli T, Pesavento G, et al. Sequence type 101 (ST101) as the predominant carbapenem-non-susceptible *Klebsiella pneumoniae* clone in an acute general hospital in Italy. *Int J Antimicrob Agents* 2012;39:543–5, doi:http://dx.doi.org/10.1016/j.ijantimicag.2012.02.012.
- [50] Mezzatesta ML, Gona F, Caio C, Adembri C, Dell'utri P, Santagati M, et al. Emergence of an extensively drug-resistant ArmA- and KPC-2-producing ST101 *Klebsiella pneumoniae* clone in Italy. *J Antimicrob Chemother* 2013;68:1932–4, doi:http://dx.doi.org/10.1093/jac/dkt116.
- [51] Mansour W, Grami R, Ben Haj Khalifa A, Dahmen S, Châtre P, Haenni M, et al. Dissemination of multidrug-resistant bla_{CTX-M-15}/IncFIIK plasmids in *Klebsiella pneumoniae* isolates from hospital- and community-acquired human infections in Tunisia. *Diagn Microbiol Infect Dis* 2015;83:298–304, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2015.07.023.
- [52] Liapis E, Pantel A, Robert J, Nicolas-Chanoine MH, Cavalieri L, van der Meer-Marquet N, et al. Molecular epidemiology of OXA-48-producing *Klebsiella pneumoniae* in France. *Clin Microbiol Infect* 2014;20:1121–3, doi:http://dx.doi.org/10.1111/1469-0691.12727.
- [53] Giakkoupi P, Papagiannitsis CC, Miriagou V, Pappa O, Polemis M, Tryfinopoulou K, et al. An update of the evolving epidemic of bla_{KPC-2}-carrying *Klebsiella pneumoniae* in Greece (2009–10). *J Antimicrob Chemother* 2011;66:1510–3, doi:http://dx.doi.org/10.1093/jac/dkr166.
- [54] Karampatakis T, Antachopoulos C, Iosifidis E, Tsakris A, Roilides E. Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in Greece. *Future Microbiol* 2016;11:809–23, doi:http://dx.doi.org/10.2217/fmb-2016-0042.
- [55] Villa L, Poirel L, Nordmann P, Carta C, Carattoli A. Complete sequencing of an IncH plasmid carrying the bla_{NDM-1}, bla_{CTX-M-15} and qnrB1 genes. *J Antimicrob Chemother* 2012;67:1645–50, doi:http://dx.doi.org/10.1093/jac/dks114.
- [56] Madueño A, González García J, Fernández-Romero S, Oteo J, Lecuona M. Dissemination and clinical implications of multidrug-resistant *Klebsiella pneumoniae* isolates producing OXA-48 in a Spanish hospital. *J Hosp Infect* 2017;96:116–22, doi:http://dx.doi.org/10.1016/j.jhin.2017.02.024.
- [57] Papagiannitsis CC, Izdebski R, Baraniak A, Fiett J, Herda M, Hrabák J, et al. Survey of metallo- β -lactamase-producing Enterobacteriaceae colonizing patients in European ICUs and rehabilitation units, 2008–11. *J Antimicrob Chemother* 2015;70:1981–8, doi:http://dx.doi.org/10.1093/jac/dkv055.
- [58] Novović K, Trudić A, Brkić S, Vasiljević Z, Kojić M, Medić D, et al. Molecular epidemiology of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* in Serbia from 2013 to 2016. *Antimicrob Agents Chemother* 2017;61:e02550–16, doi:http://dx.doi.org/10.1128/AAC.02550-16.
- [59] Horna G, Velasquez J, Fernández N, Tamariz J, Ruiz J. Characterisation of the first KPC-2-producing *Klebsiella pneumoniae* ST340 from Peru. *J Glob Antimicrob Resist* 2017;9:36–40, doi:http://dx.doi.org/10.1016/j.jgar.2016.12.011.
- [60] Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: origin and diffusion. *Front Microbiol* 2012;3:110, doi:http://dx.doi.org/10.3389/fmicb.2012.00110.