Diagnostic Microbiology and Infectious Disease xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Clonal distribution of multidrug-resistant *Enterobacter cloacae*

Delphine Girlich [a], Laurent Poirel [a,b], Patrice Nordmann [a,b,c,*]

[a] *INSERM U914 "Emerging Resistance to Antibiotics", K.-Bicêtre, France*
[b] *Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland*
[c] *Hopital Fribourgeois-hôpital Cantonal, Fribourg, Switzerland*

ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form 15 December 2014
Accepted 9 January 2015
Available online xxxx

Keywords:
Multidrug-resistant bacteria
Prevalence
MLST
Enterobacter cloacae

ABSTRACT

A multilocus sequence typing (MLST) scheme including 7 housekeeping genes was used to evaluate whether the current spread of multidrug-resistant *Enterobacter cloacae* isolates worldwide might be associated to specific successful clones. Fifty *E. cloacae* clinical isolates of worldwide origin, with various β-lactamase content, and recovered at different periods of time were studied. Forty-four sequence types were identified, highlighting a high clonal diversity with 3 main lineages. This study revealed that a precise identification of the isolates by sequencing of the chromosomal *ampC* gene of *E. cloacae* would provide a significant added value to improve the reliability of the MLST scheme.

1. Introduction

Although *Enterobacter cloacae* is an enterobacterial species that possesses several naturally occurring resistance mechanisms, acquired multidrug resistance is increasingly observed. Resistance to β-lactams in that species may be related to overexpression of chromosomal *ampC* β-lactamase genes, acquisition of plasmid-mediated extended-spectrum β-lactamase (ESBL), or carbapenemase genes, those latter genes encoding KPC type; OXA-48 type; or metallo-β-lactamases of the VIM-, IMP-, and NDM-1 types (Girlich et al., 2014; Ikonomidis et al., 2007; Novak et al., 2014; Pasanen et al., 2014; Pestourie et al., 2014; Poirel et al., 2014). Identifying clonal spread of multidrug-resistant isolates recovered from geographically distant locations remains difficult, since data are being obtained mainly by using the pulsed-field gel electrophoresis technique, which provides heterogeneous and noncomparable data. By contrast, multilocus sequence typing (MLST) provides interlaboratory comparison of epidemiological data. We have used here a recently described MLST scheme including 7 housekeeping genes (Miyoshi-Akiyama et al., 2013) in order to perform an epidemiological comparison of 50 multidrug-resistant *E. cloacae*. This study focuses on multidrug-resistant isolates and, in particular, those producing the NDM-1 or OXA-48 carbapenemases and the ESBL CTX-M-15. The aim was to evaluate i) whether some specific sequence types (STs) with a peculiar β-lactamase content might be disseminated among various countries and ii) whether ESBL- and carbapenemase-producing isolates might correspond to the same strain backgrounds as those of the ESBL-positive but carbapenemase-negative isolates.

2. Methods

2.1. Clinical isolates

Fifty *E. cloacae* clinical isolates of worldwide origin and recovered at different periods of time (from 1994 to 2013) were included. They were randomly selected from our international collection, including isolates from India, Lebanon, France, Vietnam, Morocco, Turkey, and Algeria. They have been characterized for their β-lactamase content at the molecular level. The isolates were either wild-type strains (n = 3), AmpC overproducers (n = 10), ESBL producers of the CTX-M-15 type (n = 16), and carbapenemase producers (n = 24) (Table 1). Isolates were identified by the API20E biochemical test (bioMérieux, Marcy l'Etoile, France).

2.2. MLST and phylogenetic analysis

MLST primers targeted 7 housekeeping genes (*dnaA*, *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB*, and *rpoB*) with PCR conditions, as recommended (Miyoshi-Akiyama et al., 2013). Purified PCR products were sequenced using an ABI3130 apparatus (Applied Biosystems, Life Technologies SAS, Saint Aubin, France). Concatenated sequences of the 7 DNA fragments (3501 nucleotides in total) were obtained from the Web site (http://pubmlst.org/ecloacae/) and compared using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method. A phylogenic tree was inferred by bootstrap phylogenetic inference using Molecular Evolutionary Genetics Analysis Version 6.0. software (MEGA6) (Tamura et al., 2013). These sequences were compared with 3 concatenated reference sequences (ST2, ST3, and ST9), corresponding to the 3 main clades of ST for *E. cloacae* isolates, as reported by Miyoshi-Akiyama et al. (2013). Searches of the GenBank databases were carried out using the NCBI BLASTn option (www.ncbi.nlm.nih.

* Corresponding author. Tel.: +41-26-300-9581.
E-mail address: patrice.nordmann@unifr.ch (P. Nordmann).

http://dx.doi.org/10.1016/j.diagmicrobio.2015.01.003
0732-8893/

Table 1
E. cloacae isolates used in this study.

Isolate	ß-lactamase content	Year and country of isolation	ST	*dnaA*	*fusA*	*gyr*	*leuS*	*pyrG*	*rplB*	*rpoB*	lineage
5434	wild type	2011 France	237	4	6	4	6	37	36	25	1
7725	wild type	2011 France	238	58	40	81	9	79	37	38	3
7746	wild type	2011 France	247	58	61	99	102	97	6	55	7
KAU	overexpressed AmpC	2012 France	108	68	8	75	63	65	34	35	1
VIL	overexpressed AmpC	2012 France	50	4	4	4	6	37	4	25	1
BRE	overexpressed AmpC	2012 France	239	58	37	4	6	4	4	25	1
COUP	overexpressed AmpC + TEM-121	2012 France	248	92	60	100	99	9	42	56	-[a]
BALD	overexpressed AmpC + TEM-2	2012 France	240	8	9	6	9	9	4	8	3
CON	overexpressed AmpC	2012 France	241	4	4	37	6	81	4	25	1
AZA	overexpressed AmpC	2012 France	249	86	25	96	100	100	14	57	8
KHA	CTX-M-15	2008 France	198	68	8	75	63	65	34	6	1
FOU	CTX-M-15	2008 France	78	8	9	6	9	9	6	6	3
3	CTX-M-15 (ArmA)	2008 India	199	46	21	20	96	45	29	54	2
CAU	CTX-M-15 + TEM-1	2008 France	168	59	40	81	9	79	37	38	3
NAI	CTX-M-15 + SHV-12	2008 France	196	8	33	86	9	9	6	8	3
DAS	CTX-M-15 + TEM-1	2008 France	201	53	35	20	44	45	38	8	2
KER	CTX-M-15	2009 France	202	53	35	20	44	45	29	32	2
HOF	CTX-M-15	2009 France	195	53	35	20	44	45	4	53	2
RIV	CTX-M-15	2009 France	251	85	63	101	103	96	6	6	8
CON	CTX-M-15	2009 France	114[b]	53	35	20	44	45	4	6	2
ZER	CTX-M-15 + TEM-1	2010 France	114	53	35	20	44	45	4	6	2
BAL	CTX-M-15	2010 France	114	53	35	20	44	45	4	6	2
BAR	CTX-M-15 + TEM-1	2010 France	207	53	35	20	45	45	4	6	2
SAM	CTX-M-15 + TEM-1	2011 France	114	53	35	20	44	45	4	6	2
CAR	CTX-M-15	2011 France	236	59	64	81	9	79	37	6	8
BRA	CTX-M-15 + TEM-1 + SHV-28	2011 France	200	74	20	20	65	45	4	32	2
NOR	NMC-A	1994 France	250	84	62	95	98	94	43	52	6
KAR	VIM-1 + SHV-70	2011 France	229	87	16	25	97	22	9	15	6
KOW3	VIM-4 + CTX-M-15 + TEM-1 + SHV-31	2011 Kuwait	203	4	4	20	6	92	30	6	1
TWA	IMP-8	2011 Taiwan	194	11	6	4	13	39	4	9	1
TAW	IMP-8 + SHV-12	2011 Taiwan	204	4	4	4	6	95	4	6	1
RAZ	NDM-1	2012 Vietnam	193	49	20	7	44	90	24	32	2
PAY	NDM-1	2012 France	230	49	20	74	44	90	24	32	2
SEN	NDM-1	2012 France	205	4	6	4	61	39	4	25	1
BOQ	NDM-1	2012 France	231	46	20	20	96	45	29	54	2
ABA	NDM-1 + CTX-M-15 + TEM-1 + OXA-1	2013 France	200	74	20	20	65	45	4	32	2
GAT	NDM-1 + CTX-M-15 + TEM-1 + OXA-1	2013 France	206	67	20	20	44	45	4	32	5
IR38 C	NDM-1 + CTX-M-15	2012 India	235	49	21	19	44	94	12	32	4
LIB	NDM-1 + CTX-M-15	2012 Lebanon	32	3	24	3	35	3	16	17	3
MAR17	OXA-48 + TEM-1 + CTX-M-9	2009 Morocco	197	67	21	74	95	45	35	6	2
MAR18	OXA-48 + TEM-1 + SHV-12 + CTX-M-9	2009 Morocco	197	67	21	74	95	45	35	6	2
MAR19	OXA-48 + TEM-1 + CTX-M-15	2009 Morocco	192	46	20	74	44	45	24	42	2
BOU	OXA-48 + TEM-1 + CTX-M-15 + OXA-1	2010 Morocco	245	91	59	19	44	99	4	32	2
TUR	OXA-48 + SHV-5	2010 Turkey	120	46	20	20	44	45	29	6	2
MAR20	OXA-48 + TEM-1 + SHV-12	2011 Morocco	190	9	4	15	6	37	4	9	1
501	OXA-48 + TEM-1 + CTX-M-15 + OXA-1	2011 Morocco	244	90	20	19	44	45	4	6	2
BEU	OXA-48 + TEM-1 + SHV-12 + CTX-M-15 + DHA-1 + OXA-1	2011 France	182	49	20	19	44	90	24	32	2
ESS	OXA-48 + CTX-M-15	2011 Algeria	25	24	14	43	52	27	18	21	6
AZZ	OXA-48 + TEM-1 + CTX-M-15 + OXA-1	2011 Morocco	246	67	59	19	44	99	4	32	2
DOV	OXA-48 + TEM-1 + CTX-M-15 + OXA-1	2011 Morocco	114	53	35	20	44	45	4	6	2

[a] Isolates of ST 25, 229, 249, and 250 have been identified as *E. asburiae*; isolate of ST248 has been identified as *E. aerogenes* and should, therefore, be excluded from this MLST scheme if strictly restricted for *E. cloacae* isolates.
[b] *E. cloacae* with ST114 are indicated with a gray shadow.

Please cite this article as: Girlich D, et al, Clonal distribution of multidrug-resistant *Enterobacter cloacae*, Diagn Microbiol Infect Dis (2015), http://dx.doi.org/10.1016/j.diagmicrobio.2015.01.003