

OXA-48 Carbapenemase-Producing *Salmonella enterica* Serovar Kentucky Isolate of Sequence Type 198 in a Patient Transferred from Libya to Switzerland

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Here, we report a case of OXA-48-producing *Salmonella enterica* serovar Kentucky of sequence type 198 (ST198) from perianal screening cultures of a patient transferred from Libya to Switzerland. The *bla*_{OXA-48} gene was carried by Tn1999.2 and located on an ~60-kb IncL/M plasmid. This *Salmonella* strain also possessed the *bla*_{VEB-8}, *aac(6)-Ib*, *tet(A)*, *sull1*, and *mphA* resistance genes and substitutions in *GyrA* (Ser83Phe and Asp87Asn) and *ParC* (Ser80Ile). This finding emphasizes that prompt screening strategies are essential to prevent the dissemination of carbapenemase producers imported from countries where they are endemic.

The rapid emergence of OXA-48 carbapenemase-producing *Enterobacteriaceae* is currently a concern, mainly because the relatively low carbapenem MICs of these bacteria make their detection difficult (1–3). In some countries, these multidrug-resistant (MDR) isolates are now more frequently detected than those producing the classic (KPC, NDM, and VIM) carbapenemases (4–6). The *bla*_{OXA-48} gene is usually detected in *Klebsiella pneumoniae* and *Escherichia coli*, but it can also be found in other *Enterobacteriaceae* (2). However, to date, OXA-48-producing *Salmonella enterica* has been detected only in a French patient (7).

In March 2012, a polytraumatized male with severe brain injury due to a grenade detonation (September 2011) was transferred from Libya to a rehabilitation clinic located in northeastern Switzerland. During the hospitalization, different screening samples were analyzed for the presence of MDR Gram-negative pathogens (e.g., extended-spectrum β -lactamase [ESBL] producers) by implementation of selective chromID ESBL plates (bioMérieux). Colonies were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik), and antimicrobial susceptibility patterns were routinely obtained using the Vitek II system (bioMérieux). This strategy is part of the hospital hygiene policy of the clinic to prevent the spread of difficult-to-treat organisms (8–10).

At arrival, *E. coli* (*Ec*-38), *Citrobacter koseri* (*Ck*-39 and *Ck*-41), and *K. pneumoniae* (*Kp*-39-1, *Kp*-39-2, and *Kp*-41) isolates with antibiotic phenotypes suspicious for ESBL production were detected in samples from urine, nasopharynx, and perianal swabs (Table 1). Based on these results, the patient was kept in isolation in a single room and strict standard hygienic procedures were implemented (8, 10).

After 2 months (May 2012), a further perianal screening culture was again positive for an ESBL-producing *K. pneumoniae* isolate (*Kp*-43) and also for an MDR *Salmonella enterica* isolate of serotype Kentucky (*Sk*-1). This isolate was resistant to ciprofloxacin and last-generation cephalosporins and was flagged by the Vitek system as a possible carbapenemase producer (MICs for imipenem and ertapenem, 1 and 2 μ g/ml, respectively). The same

Salmonella Kentucky (*Sk*-2) strain was isolated from another sample obtained from a perianal screening swab performed 17 months after the admission (August 2013) (Table 1).

In September 2013, the patient was transferred to a long-term-care facility in Tunisia. During the 18-month period of residence at the Swiss rehabilitation clinic, he never developed diarrhea, and no clinical samples indicating possible ongoing infection(s) were positive for the above-described MDR *Enterobacteriaceae*. Moreover, no other patients hospitalized at the clinic and during the same time frame developed infection or colonization with MDR *Salmonella*.

All isolates were further characterized by microdilution ESB1F and GNX2F MIC plates (Trek Diagnostics) (11); CT-103 (Check-Points) and AMR-ve 0.5m (Alere) microarrays for detection of ESBL, plasmid-mediated AmpC (pAmpC), carbapenemase, and other resistance genes (12); PCR/DNA sequencing for *gyrA*, *parC*, *bla* genes, transposon Tn1999-like, and plasmid incompatibility groups (13–17); multilocus sequence typing (MLST) for *E. coli* (Environmental Research Institute [ERI] [see <http://mlst.ucc.ie>]), *K. pneumoniae* (Pasteur Institute [see <http://www.pasteur.fr>]), and *Salmonella* spp. (ERI); and analytical isoelectric focusing (aIEF) (14). Plasmids were extracted using alkaline extraction and electroporated into ElectroMAX *E. coli* DH10B (Invitrogen). Cells were selected on LB plates containing ampicillin (20 μ g/ml) or cefotaxime (1 μ g/ml) (Sigma) (16). Conjugation experiments were performed at both 37°C and 25°C using the rifampin-resistant *E. coli* strain JF33 and LB selective plates containing ampicillin (50 μ g/ml) plus rifampin (100 μ g/ml).

Ec-38 was of sequence type 131 (ST131) and produced the

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TABLE 1 Phenotypic and molecular characteristics of the MDR *Enterobacteriaceae* isolates detected in the screening cultures during the 18-month hospitalization of the patient

Antibiotic tested or isolate characteristic	MIC [$\mu\text{g/ml}$ (interpretation ^b)] or other characteristic(s) of indicated isolate from indicated sample							
	Urine (March 2012 ^b): <i>E. coli</i> (Ec-38)	Nasopharyngeal swab (March 2012)			Perianal swab (March 2012)		Perianal swab (May 2012 [August 2013]) ^a	
		<i>C. koseri</i> (Ck-39)	<i>K. pneumoniae</i> (Kp-39-1)	<i>K. pneumoniae</i> (Kp-39-2)	<i>C. koseri</i> (Ck-41)	<i>K. pneumoniae</i> (Kp-41)	<i>K. pneumoniae</i> (Kp-43)	S. Kentucky (Sk-1 [and Sk-2])
Piperacillin-tazobactam	32 (R)	64 (R)	≥ 128 (R)	16 (I)	32 (R)	16 (I)	32 (R)	≥ 128 (R)
Ticarcillin-clavulanate	128 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)
Cefoxitin	8 (NA)	8 (NA)	32 (NA)	8 (NA)	16 (NA)	8 (NA)	8 (NA)	8 (NA)
Ceftriaxone	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	≥ 256 (R)	64 (R)
Cefotaxime	≥ 128 (R)	≥ 128 (R)	≥ 128 (R)	≥ 128 (R)	≥ 128 (R)	64 (R)	≥ 128 (R)	64 (R)
Cefotaxime-clavulanate	≤ 0.064 (NA)	4 (NA)	8 (NA)	4 (NA)	8 (NA)	8 (NA)	4 (NA)	16 (NA)
Ceftazidime	16 (R)	32 (R)	128 (R)	64 (R)	64 (R)	64 (R)	128 (R)	≥ 256 (R)
Ceftazidime-clavulanate	0.25 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	≥ 256 (NA)
Cefepime	≥ 32 (R)	16 (R)	≥ 32 (R)	16 (R)	16 (R)	16 (R)	16 (R)	≥ 32 (R)
Aztreonam	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)
Imipenem	≤ 0.25 (S)	≤ 0.25 (S)	≤ 0.25 (S)	≤ 0.25 (S)	≤ 0.25 (S)	≤ 0.25 (S)	≤ 0.25 (S)	≤ 0.25 (S)
Meropenem	≤ 0.5 (S)	≤ 0.5 (S)	≤ 0.5 (S)	≤ 0.5 (S)	≤ 0.5 (S)	≤ 0.5 (S)	≤ 0.5 (S)	≤ 0.5 (S)
Ertapenem	≤ 0.125 (S)	0.5 (S)	2 (R)	≤ 0.125 (S)	≤ 0.125 (S)	≤ 0.125 (S)	≤ 0.125 (S)	1 (I)
Doripenem	≤ 0.064 (S)	≤ 0.064 (S)	0.25 (S)	≤ 0.064 (S)	≤ 0.064 (S)	≤ 0.064 (S)	≤ 0.064 (S)	0.25 (S)
Gentamicin	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≥ 32 (R)	≤ 0.5 (S)
Tobramycin	≥ 16 (R)	≥ 16 (R)	≥ 16 (R)	≥ 16 (R)	≥ 16 (R)	≥ 16 (R)	≥ 16 (R)	≥ 16 (R)
Amikacin	≥ 64 (R)	8 (S)	8 (S)	8 (S)	4 (S)	≤ 2 (S)	≤ 2 (S)	8 (S)
Ciprofloxacin	≥ 4 (R)	≥ 4 (R)	≥ 4 (R)	≥ 4 (R)	≥ 4 (R)	2 (R)	2 (R)	≥ 4 (R)
Trimethoprim-sulfamethoxazole	≥ 8 (R)	≥ 8 (R)	≥ 8 (R)	≥ 8 (R)	≥ 8 (R)	≥ 8 (R)	≥ 8 (R)	≤ 0.25 (S)
Colistin	≤ 0.125 (S)	≤ 0.125 (S)	≤ 0.125 (S)	≥ 8 (R) ^c	≤ 0.125 (S)	≤ 0.125 (S)	≤ 0.125 (S)	≤ 0.125 (S)
Polymyxin B	≤ 0.125 (NA)	≤ 0.125 (NA)	0.5 (NA)	≥ 8 (NA)	≤ 0.125 (NA)	0.5 (NA)	0.5 (NA)	0.5 (NA)
Doxycycline	≤ 1 (NA)	≥ 32 (NA)	≥ 32 (NA)	≥ 32 (NA)	≥ 32 (NA)	≥ 32 (NA)	≥ 32 (NA)	≤ 1 (NA)
Tigecycline	≤ 0.125 (S)	0.5 (S)	0.5 (S)	≤ 0.125 (S)	0.5 (S)	≤ 0.125 (S)	≤ 0.125 (S)	≤ 0.125 (S)
<i>bla</i> genes	CTX-M-15 TEM-1-like ^d	CTX-M-15 CMY-4 TEM-1-like	CTX-M-15 CMY-4 SHV-1-like ^d	CTX-M-15 CMY-4 SHV-1-like	CTX-M-15 CMY-4 TEM-1-like	CTX-M-15 CMY-4 SHV-1-like	CTX-M-15 CMY-4 SHV-1-like	OXA-48 VEB-8 ^e
Plasmid incompatibility group(s) ^f	F, FII, Nt-1	Nt-2	Nt-1, Nt-2	F, Nt-2	Nt-2	F, Nt-2	F, Nt-2	L/M
aIEF, isoelectric points ± 0.2 ^g	8.9, 7.6, 5.4	9.1, 8.9, 7.6, 5.4	8.9, 7.8, 7.6, 6.8, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.0, 7.6, 7.2, 6.3
MLST	ST131	NA	ST101	ST111	NA	ST111	ST111	ST198

^a Another perianal swab collected in August 2013 was positive for an MDR *Salmonella* Kentucky strain with the same phenotypic and molecular characteristics.

^b According to EUCAST criteria (11); R, resistant; I, intermediate; S, susceptible; NA, not applicable or not available. MICs were obtained by implementing the ESB1F and GNX2F plates (Trek Diagnostics).

^c The MIC for colistin was also elevated (4 $\mu\text{g/ml}$) when measured by Etest (bioMérieux).

^d No TEM- or SHV-type ESBLs were present. For *K. pneumoniae*, SHV-1-like indicates the natural β -lactamases.

^e *Salmonella* Kentucky was also positive for the following antibiotic resistance genes: *aac(6)-Ib*, *tet(A)*, *sul1*, and *mphA*. Amino acid substitutions in GyrA (Ser83Phe and Asp87Asn) and ParC (Ser80Ile) were also found.

^f Nt-1, nontypeable plasmid PCR positive only for replicon R; Nt-2, nontypeable plasmid PCR positive only for replicon FIB-M.

^g pI of ~ 8.9 , CTX-M-15; pI of ~ 9 , CMY-4; pI of ~ 5.4 , TEM-1-like; pI of ~ 7.6 , SHV-1-like; pI of ~ 7.4 , VEB-8; pI of ~ 7.2 , OXA-48 (<http://www.lahey.org/Studies/>).

^h Date of isolation.

CTX-M-15 ESBL, whereas the remaining *C. koseri* (Ck-39 and Ck-41) and *K. pneumoniae* (Kp-39-1, Kp-39-2, Kp-41, and Kp-43) isolates also coproduced the CMY-4 pAmpC. The *K. pneumoniae* isolates were of ST101 and ST111. Interestingly, Kp-39-2 was highly resistant to polymyxins (Table 1). Moreover, *C. koseri* and *K. pneumoniae* isolates were all PCR positive for a nontypeable plasmid of the FIB-M replicon.

Salmonella Kentucky was of ST198 and carried a plasmid of IncL/M. According to microdilution results, the isolate was resistant to ciprofloxacin and showed reduced susceptibility to ertapenem (MIC, 1 $\mu\text{g/ml}$) but not to the other carbapenems (all MICs, ≤ 0.25 $\mu\text{g/ml}$). This phenotype was due to amino acid substitutions in GyrA (Ser83Phe and Asp87Asn) and ParC (Ser80Ile) and to the production of OXA-48 carbapenemase. In particular, the *bla*_{OXA-48} gene was carried by Tn1999.2 and located on an ~ 60 -kb

IncL/M plasmid (13, 15). The *Salmonella* isolate was also highly resistant to last-generation cephalosporins (e.g., MIC for ceftazidime, ≥ 256 $\mu\text{g/ml}$) because of the production of the VEB-8 ESBL. According to microarray results, genes conferring resistance to aminoglycosides, tetracyclines, sulfonamides, and macrolides [*aac(6)-Ib*, *tet(A)*, *sul1*, and *mphA*, respectively] were also detected (Table 1). Only transconjugants and DH10B transformants carrying the *bla*_{OXA-48}-positive IncL/M plasmid were obtained. This indicates the possible chromosomal location of the *bla*_{VEB-8}, as usually observed for this class of genes (12).

Only two OXA-48-producing *Salmonella* Kentucky isolates of ST198 (also ciprofloxacin resistant) and one OXA-48-producing *Salmonella* Saintpaul isolate were previously reported in France. These strains were detected during 2009 to 2011 in the stools of a unique nonhospitalized female who traveled in Egypt (7). Like

wise, the OXA-48-producing *Salmonella* Kentucky isolate found in Switzerland was ciprofloxacin resistant, belonged to ST198, and was responsible for prolonged intestinal colonization of the patient transferred from Libya. The *bla*_{OXA-48} gene was located in a typical genetic environment (Tn1999-like and IncL/M plasmid) (3, 15). However, the isolate also coproduced the VEB-8, an ESBL previously reported only in one OXA-48-positive *E. coli* isolate originating from Libya (1). We also noted that the other isolates found in the patient produced the CMY-4, a pAmpC usually reported in North Africa (8, 18). Overall, these results indicate that in countries with endemic spread of OXA-48 producers and which have high frequencies of *Salmonella* diseases, there are high risks for the selection of very resistant clones (e.g., the ciprofloxacin-resistant hyperepidemic *Salmonella* Kentucky of ST198) that may further spread not only among people in the community but also in the environment and among food-producing animals (19–22).

In conclusion, this is the second report of OXA-48-producing *Salmonella enterica*. The isolate copossessed other resistance genes that rendered the isolate very resistant to standard antibiotic treatments. Reference laboratories for enteric pathogens should be aware that this MDR *Salmonella* may silently disseminate in the community with the potential to cause outbreaks, and it poses a serious threat to public health. Effective strategies to screen patients at risk of colonization (e.g., those transferred or recently traveling in specific areas) must be constantly implemented by all health care institutions to prevent the diffusion of difficult-to-treat pathogens (10, 22).

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