



HAL
open science

Spread of blaCTX-M-15-Producing Enterobacteriaceae and OXA-23-Producing Acinetobacter baumannii Sequence Type 2 in Tunisian Seafood

Yosra Mani, Wejdene Mansour, Agnese Lupo, Estelle Saras, Olfa Bouallègue,
Jean-Yves Madec, Marisa Haenni

► **To cite this version:**

Yosra Mani, Wejdene Mansour, Agnese Lupo, Estelle Saras, Olfa Bouallègue, et al.. Spread of blaCTX-M-15-Producing Enterobacteriaceae and OXA-23-Producing Acinetobacter baumannii Sequence Type 2 in Tunisian Seafood. Antimicrobial Agents and Chemotherapy, 2018, 62 (9), pp.e00727-18. 10.1128/AAC.00727-18.. anses-04018163

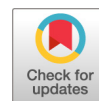
HAL Id: anses-04018163

<https://hal-anses.archives-ouvertes.fr/anses-04018163>

Submitted on 7 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Spread of *bla*_{CTX-M-15}-Producing *Enterobacteriaceae* and OXA-23-Producing *Acinetobacter baumannii* Sequence Type 2 in Tunisian Seafood

Yosra Mani,^a Wejdene Mansour,^{a,b} Agnese Lupo,^c Estelle Saras,^c Olfa Bouallègue,^{a,d} Jean-Yves Madec,^c Marisa Haenni^c

^aUnité de recherche: Résistances bactériennes émergentes et sécurité des soins "UR125P37," laboratoire de Microbiologie, Hôpital universitaire Sahloul, Sousse, Tunisia

^bInstitut Supérieur des Sciences Appliquées et de Technologie de Mahdia, Université de Monastir, Monastir, Tunisia

^cUnité Antibiorésistance et Virulence Bactériennes, Université Claude Bernard Lyon 1 – ANSES, Lyon, France

^dFaculté de Médecine de Sousse, Université de Sousse, Sousse, Tunisia

ABSTRACT Bivalves are filter-feeding animals and markers of bacterial pollution. We report a massive spread of *bla*_{CTX-M-15} through dominant *Escherichia coli* and *Klebsiella pneumoniae* lineages and/or plasmid subtypes (F31:A4:B1) as well as the presence of OXA-23-producing *Acinetobacter baumannii* sequence type 2 (ST2) in seafood, highlighting a direct risk for the consumer. These findings should urge authorities to consider hospital effluents, and also farm and urban effluents, as important sources of extended-spectrum-beta-lactamase (ESBL)/carbapenemase producers that filter-feeding animals can concentrate and further spread to humans.

KEYWORDS seafood, ESBL, carbapenemase, *E. coli*, *Acinetobacter*, OXA-23, CTX-M-15, IncF, plasmid

Bivalves are filter-feeding animals that host high concentrations of microbial communities that may promote horizontal gene transfer (1), and their low trophic level as primary consumers enables them to further disseminate microorganisms along the food chain. Therefore, they are relevant markers of biological pollution, and human waste was accused as the source of detected multidrug resistant (MDR) *Escherichia coli* in bivalves in Norway and KPC-3-producing *E. coli* sequence type 167 (ST167) in mussels in Tunisia (2, 3). The goal of this study was to clarify the burden caused by bacteria resistant to broad-spectrum cephalosporins from wastewater effluents (including hospital, urban, and farm effluents) on shellfish intended for human consumption.

Three categories of bivalves were sampled in unrelated markets over three different time periods in Tunisia. First, 400 specimens of depurated mussels (*Mytilus galloprovincialis*) collected in the Bizerte lagoon were purchased in five different batches (containing 40, 50, 95, 105, and 110 pieces, respectively) between January and March 2015. Second, 36 oysters (*Crassostrea gigas*) from Bizerte were purchased on a single day in March 2016. Third, three batches (30, 68, and 83 pieces) of scallops (*Placopecten magellanicus*) from the Monastir lagoon (several hundred kilometers south from Bizerte) were purchased between April and May 2016. All bivalves were aseptically transported at 4°C to the laboratory and were immediately processed. After removal of shell debris and algae under tap water, bivalves were dried, disinfected (70% ethanol), opened using a sterilized scalpel, and disposed in tubes containing peptone salt broth for 24 h at 37°C. Each tube contained either

Received 11 April 2018 Returned for modification 7 May 2018 Accepted 27 June 2018

Accepted manuscript posted online 2 July 2018

Citation Mani Y, Mansour W, Lupo A, Saras E, Bouallègue O, Madec J-Y, Haenni M. 2018.

Spread of *bla*_{CTX-M-15}-producing *Enterobacteriaceae* and OXA-23-producing *Acinetobacter baumannii* sequence type 2 in Tunisian seafood. *Antimicrob Agents Chemother* 62:e00727-18. <https://doi.org/10.1128/AAC.00727-18>.

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to Marisa Haenni, marisa.haenni@anses.fr.

one (for oysters and scallops) or a pool of five (for mussels) bivalve sample(s). Overnight cultures were streaked on selective MacConkey agar plates containing 4 mg/liter cefotaxime, and one colony per morphology per plate was picked up. Extended-spectrum cephalosporin-resistant (ESC-R) bacteria were identified in all batches of mussels (52 positive tubes out of the 80 tested), 3/36 oysters (8.3%), and 26/181 scallops (14.4%). Identification (Vitek-2; bioMérieux, Marcy l'Etoile, France) and typing (XbaI-pulsed-field gel electrophoresis [PFGE]) were performed on all isolates. For one representative of each PFGE profile per sampling date, resistance genes, clones, and plasmids were characterized more deeply by PCR and sequencing, phylogrouping (for *E. coli*), S1-PFGE, Southern blot, multilocus sequence typing (MLST) (according to the following websites: <http://bigsdbs.pasteur.fr/klebsiella/klebsiella.html> for *K. pneumoniae*; https://pubmlst.org/bigsdbs?db=pubmlst_mlst_seqdef for *E. coli*; and <https://pubmlst.org/abaumannii/> for *A. baumannii*) and plasmid typing (using plasmid MLST [pMLST] and the IncF formula according to the following website: <https://pubmlst.org/plasmid/>) (Table 1).

Two *A. baumannii* isolates were incidentally isolated (since our study design was first intended to identify extended spectrum beta-lactamase [ESBL]/pAmpC producers) in one mussel (2015) and one oyster (2016) from the same city, Bizerte. Both isolates belonged to ST2 and produced the OXA-23 carbapenemase. OXA-23-producing *A. baumannii* strains have been widely reported in North African hospitals (Algeria, Tunisia, and Libya), and OXA-23-producing *A. baumannii* ST2 was reported in Tunis, which is close to Bizerte (4). Several hospitals in Bizerte have their effluents discharged into the Bizerte Lagoon, where mussel and oyster farms are located, which makes seafood contamination by hospitals activities very likely. The same polluted situation is observed in the Monastir Lagoon.

A dominant CTX-M-15 epidemiology was also observed in different species of *Enterobacteriaceae* (Table 1). *bla*_{CTX-M-15} was identified in one *Citrobacter freundii* isolate and in one ST394 and two ST101 *K. pneumoniae* isolates; the last lineage widely spreads *bla*_{CTX-M-15} in North Africa (5). CTX-M-15 was also produced by 80% ($n = 53/66$) of the ESBL-producing *E. coli* heavily contaminating mussels (46/80 ESBL-positive tubes), scallops (18/181 ESBL-positive animals), and oysters (1/36). Interestingly, ST617, ST410, and ST131 *E. coli* strains, all three widely recognized in humans, were dominant. As an example, ST617 was found in 22/53 ESBL-positive samples in mussels and in 9/18 in scallops. Only two other STs (ST88 and ST155) were sporadically identified. These data again suggest a human origin of the seafood contamination and a massive ESBL load not even diluted by the aquatic environment. Moreover, ST617 and ST410 were recovered at different periods and places in Tunisia, suggesting a wide prevalence in the environment (6–11).

Surprisingly, hybridizations on S1-PFGE gels revealed a unique *bla*_{CTX-M-15}-bearing IncF plasmid of the F31:A4:B1 formula in all ST617 and ST410 isolates and in one ST131 isolate. This very same *bla*_{CTX-M-15} IncF plasmid has regularly been identified in ST617 and ST410 in numerous animal species, foodstuffs, and countries worldwide (12–16), and in two ST44 and ST131 clinical isolates in the United States (17), thereby suggesting that this plasmid is a major genetic support for the dissemination of *bla*_{CTX-M-15}.

Thus, we first report OXA-23-producing *A. baumannii* ST2 in seafood, highlighting a direct risk for the consumer. We also show a massive spread of *bla*_{CTX-M-15} in seafood, particularly through dominant *E. coli* and *K. pneumoniae* lineages and/or plasmid subtypes (F31:A4:B1). These data strongly suggest a contamination of human origin, even though other sources cannot be excluded. These findings should urge authorities to set up monitoring programs for the surveillance of MDR bacteria (including those resistant to carbapenems) and to consider hospital effluents, but also farm and urban effluents, as sources of ESBL/carbapenemase producers that filter-feeding animals can concentrate and further spread to humans through the food chain.

TABLE 1 Characteristics of *Enterobacteriaceae* (one representative per lineage and sampling date) recovered from bivalve mollusks

Animal host	Sampling date	Batch no. (no. of pieces)	No. of positive tubes/total no. of tubes ^a	Isolate no.	No. of clonal isolates	Bacterial species	Phylogroup	Sequence type ^b	Phenotype	ESBL/carbapenemase gene	ESBL-carrying plasmid	Plasmid subtype
Mussel	15 January 2015	1 (105)	21/21	40610	21	<i>E. coli</i>	A	ST617	ESBL	<i>bla</i> _{CTX-M-15}	IncF	F31:A4:B1
	27 January 2015	2 (50)	2/10	40631	2	<i>E. coli</i>	A	ST617	ESBL	<i>bla</i> _{CTX-M-15}	IncF	F31:A4:B1
	04 February 2015	3 (40)	2/8	40633	2	<i>E. coli</i>	B2	ST131	ESBL	<i>bla</i> _{CTX-M-27}	IncF	F31:A4:B1
	07 March 2015	4 (110)	22/22	40637	20	<i>E. coli</i>	A	ST410	ESBL	<i>bla</i> _{CTX-M-15}	IncF	F31:A4:B1
				40639	1	<i>E. coli</i>	A	ST188	ESBL	<i>bla</i> _{CTX-M-1}	IncF	F18:A--:B1
				40656	1	<i>K. pneumoniae</i>		ST394	ESBL	<i>bla</i> _{CTX-M-15}	IncF	FK7:A--:B--
	25 March 2015	5 (95)	5/19	40660	2	<i>K. pneumoniae</i>		ST101	ESBL	<i>bla</i> _{CTX-M-15}	IncF	FK1:A--:B--
				40662	1	<i>E. coli</i>	A	ST110	ESBL	<i>bla</i> _{CTX-M-15}	IncY	
				40663	2	<i>A. baumannii</i>		ST2	Carba			
				43728	1	<i>A. baumannii</i>		ST2	Carba			
Oyster	12 March 2016	1 (36)	3/36	43729	1	<i>E. coli</i>	A	ST410	ESBL	<i>bla</i> _{OXA-23}	IncF	F31:A4:B1
				43731	8	<i>E. coli</i>	B2	ST131	ESBL	<i>bla</i> _{CTX-M-14}	IncF	F29:A--:B10
Scallop	17 May 2016	2 (68)	8/68	43734	8	<i>E. coli</i>	A	ST617	ESBL	<i>bla</i> _{CTX-M-15}	IncF	F31:A4:B1
	25 May 2016	3 (83)	10/83	43735	8	<i>C. freundii</i>		ND	ESBL		IncH11	
				43736	1	<i>E. coli</i>	A	ST617	ESBL	<i>bla</i> _{CTX-M-15}	IncF	F31:A4:B1
				43737	1	<i>E. coli</i>	B1	ST155	ESBL	<i>bla</i> _{CTX-M-1}	IncF	F18:A--:B1

^aFor mussels, each tube was a pool of five individuals. For oysters and scallops, each piece was analyzed individually.

^bST, sequence type; ND, not determined.

ACKNOWLEDGMENTS

This work was supported by the French agency for food, environmental and occupational health safety (ANSES). Y.M. has a fellowship from the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique in Tunisia.

We have no conflicts of interest to declare.

REFERENCES

- Taylor NG, Verner-Jeffreys DW, Baker-Austin C. 2011. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends Ecol Evol* 26:278–284. <https://doi.org/10.1016/j.tree.2011.03.004>.
- Grevskott DH, Svanevik CS, Sundel M, Wester AL, Lunestad BT. 2017. Marine bivalve mollusks as possible indicators of multidrug-resistant *Escherichia coli* and other species of the Enterobacteriaceae family. *Front Microbiol* 8:24. <https://doi.org/10.3389/fmicb.2017.00024>.
- Mani Y, Mansour W, Mammeri H, Denamur E, Saras E, Boujaafar N, Bouallegue O, Madec JY, Haenni M. 2017. KPC-3-producing ST167 *Escherichia coli* from mussels bought at a retail market in Tunisia. *J Antimicrob Chemother* 72:2403–2404. <https://doi.org/10.1093/jac/dkx124>.
- Mathlouthi N, Ben Lamine Y, Somai R, Bouhalila-Besbes S, Bakour S, Rolain JM, Chouchani C. 2018. Incidence of OXA-23 and OXA-58 carbapenemases coexpressed in clinical isolates of *Acinetobacter baumannii* in Tunisia. *Microb Drug Resist* 24:136–141. <https://doi.org/10.1089/mdr.2016.0306>.
- Mansour W, Grami R, Ben Haj Khalifa A, Dahmen S, Chatre P, Haenni M, Aouni M, Madec JY. 2015. 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* 83:298–304. <https://doi.org/10.1016/j.diagmicrobio.2015.07.023>.
- Rocha-Gracia RC, Cortes-Cortes G, Lozano-Zarain P, Bello F, Martinez-Laguna Y, Torres C. 2015. Faecal *Escherichia coli* isolates from healthy dogs harbour CTX-M-15 and CMY-2 beta-lactamases. *Vet J* 203:315–319. <https://doi.org/10.1016/j.tvjl.2014.12.026>.
- Bachiri T, Bakour S, Ladjouzi R, Thongpan L, Rolain JM, Touati A. 2017. High rates of CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* in wild boars and Barbary macaques in Algeria. *J Glob Antimicrob Resist* 8:35–40. <https://doi.org/10.1016/j.jgar.2016.10.005>.
- Schaufler K, Semmler T, Wieler LH, Wohrmann M, Baddam R, Ahmed N, Muller K, Kola A, Fruth A, Ewers C, Guenther S. 2016. Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410—another successful pandemic clone? *FEMS Microbiol Ecol* 92:fiv155. <https://doi.org/10.1093/femsec/fiv155>.
- Falgenhauer L, Imirzalioglu C, Ghosh H, Gwozdziński K, Schmiedel J, Gentil K, Bauerfeind R, Kampfer P, Seifert H, Michael GB, Schwarz S, Pfeifer Y, Werner G, Pietsch M, Roesler U, Guerra B, Fischer J, Sharp H, Kasbohrer A, Goesmann A, Hille K, Kreienbrock L, Chakraborty T. 2016. Circulation of clonal populations of fluoroquinolone-resistant CTX-M-15-producing *Escherichia coli* ST410 in humans and animals in Germany. *Int J Antimicrob Agents* 47:457–465. <https://doi.org/10.1016/j.ijantimicag.2016.03.019>.
- Lopez-Cerero L, Egea P, Serrano L, Navarro D, Mora A, Blanco J, Doi Y, Paterson DL, Rodriguez-Bano J, Pascual A. 2011. Characterisation of clinical and food animal *Escherichia coli* isolates producing CTX-M-15 extended-spectrum beta-lactamase belonging to ST410 phylogroup A. *Int J Antimicrob Agents* 37:365–367. <https://doi.org/10.1016/j.ijantimicag.2011.01.001>.
- Timofte D, Maciucă IE, Williams NJ, Wattret A, Schmidt V. 2016. Veterinary Hospital Dissemination of CTX-M-15 Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* ST410 in the United Kingdom. *Microb Drug Resist* 22:609–615. <https://doi.org/10.1089/mdr.2016.0036>.
- Dahmen S, Haenni M, Chatre P, Madec JY. 2013. Characterization of *bla*_{CTX-M} IncFII plasmids and clones of *Escherichia coli* from pets in France. *J Antimicrob Chemother* 68:2797–2801. <https://doi.org/10.1093/jac/dkt291>.
- Madec JY, Poirer L, Saras E, Gourguechon A, Girlich D, Nordmann P, Haenni M. 2012. Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*_{CTX-M-15}-carrying plasmids. *J Antimicrob Chemother* 67:578–581. <https://doi.org/10.1093/jac/dkr542>.
- Fischer J, Rodriguez I, Baumann B, Guiral E, Beutin L, Schroeter A, Kaesbohrer A, Pfeifer Y, Helmuth R, Guerra B. 2014. *bla*_{CTX-M-15}-carrying *Escherichia coli* and *Salmonella* isolates from livestock and food in Germany. *J Antimicrob Chemother* 69:2951–2958. <https://doi.org/10.1093/jac/dku270>.
- Seni J, Falgenhauer L, Simeo N, Mirambo MM, Imirzalioglu C, Matee M, Rweyemamu M, Chakraborty T, Mshana SE. 2016. Multiple ESBL-producing *Escherichia coli* sequence types carrying quinolone and aminoglycoside resistance genes circulating in companion and domestic farm animals in Mwanza, Tanzania, harbor commonly occurring plasmids. *Front Microbiol* 7:142. <https://doi.org/10.3389/fmicb.2016.00142>.
- Irrgang A, Falgenhauer L, Fischer J, Ghosh H, Guiral E, Guerra B, Schmoger S, Imirzalioglu C, Chakraborty T, Hammerl JA, Kasbohrer A. 2017. CTX-M-15-producing *E. coli* isolates from food products in Germany are mainly associated with an IncF-type plasmid and belong to two predominant clonal *E. coli* lineages. *Front Microbiol* 8:2318. <https://doi.org/10.3389/fmicb.2017.02318>.
- Li JJ, Spychala CN, Hu F, Sheng JF, Doi Y. 2015. Complete nucleotide sequences of *bla*_{CTX-M}-harboring IncF plasmids from community-associated *Escherichia coli* strains in the United States. *Antimicrob Agents Chemother* 59:3002–3007. <https://doi.org/10.1128/AAC.04772-14>.