Characterization of Environmental CTX-M-15-Producing *Stenotrophomonas maltophilia*

Ana Maravić,^a Mirjana Skočibušić,^a Željana Fredotović,^a Svjetlana Cvjetan,^{b,c} Ivica Šamanić,^a Jasna Puizina^a

Department of Biology, Faculty of Science, University of Split, Split, Croatia^a; Department of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia^b; Mediterranean Institute for Life Sciences, Split, Croatia^c

Stenotrophomonas maltophilia is a widespread environmental microorganism (1) that has emerged as a significant opportunistic pathogen (2) due to its intrinsic resistance to almost all available antibiotics. Apart from native β -lactamases L1 and L2 (1), acquired extended-spectrum β -lactamases (ESBLs) were also identified (3, 4), pointing to *S. maltophilia* as a potential reservoir. Furthermore, resistance to trimethoprim-sulfamethoxazole (SXT), a therapy of choice for *S. maltophilia*, is being increasingly reported (5–8).

Forty-one *S. maltophilia* isolates were recovered from retail shellfish (*Mytilus galloprovincialis*; 8 isolates) purchased at fish markets in Split, Croatia, and from coastal marine waters near Split (33 isolates) in 2012. Strains were isolated on imipenem (16 μ g/ml)-containing tryptic soy agar at 30°C and identified using API 20NE.

Whole-cell DNA was extracted and used for PCR detection of ESBL genes (9). Nineteen isolates carried *bla*_{CTX-M-15}, six of which additionally harbored *bla*_{TEM-116}, and one carried *bla*_{TEM-127} (Table 1). Metallo- β -lactamase genes were not detected (10). TEM-116 is usually associated with environmental *Enterobacteriaceae* (11) and *Pseudomonas* spp. (12) and has never before been identified in *S. maltophilia*. More importantly, CTX-M-15 was previously identified only in an *S. maltophilia* clinical strain from France (4). S1 nuclease pulsed-field gel electrophoresis (PFGE) of plasmid DNA followed by Southern blotting (9) showed that *bla*_{CTX-M-15} was located on large plasmids of various sizes (Table 1).

Conjugation transfer of *bla*_{CTX-M-15} using *Escherichia coli* J53 (13) at 37°C and 27°C and using azide (100 μ g/ml) and cefotaxime (8 μ g/ml)-containing Luria-Bertani agar failed even after repeated attempts. Only a 160-kb plasmid (isolate 248) was successfully transferred into *E. coli* JM109 using heat shock transformation. For PCR-based replicon typing (14), plasmid DNA from the transformant was used, while for other isolates, each plasmid band was cut from the gel and, after confirmation as *bla*_{CTX-M-15} positive by PCR, was used as a template. Interestingly, all plasmids belonged to the IncFIB incompatibility group. IncFII, IncFIA, and IncFIB *bla*_{CTX-M-15}-bearing plasmids have been previously reported in Croatia (9, 15). A possible explanation for the unusual lack of IncF plasmid replication in *E. coli* recipients could be that plasmids adapted to *S. maltophilia*, altering their host range and specificity of replication traits (16). Further studies are needed to better characterize these resistance plasmids.

PFGE of XbaI-digested genomic DNA (17, 18) showed heterogeneity among CTX-M-15-producing isolates (data not shown). Isolates were further investigated for class 1, 2, and 3 integrase,

Published ahead of print 4 August 2014

Address correspondence to Mirjana Skočibušić, Mirjana.Skocibusic@pmfst.hr.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.03601-14

TABLE 1 Isolation data and resistance determinants of ESBL-producing and SXT-resistant *S. maltophilia* isolates

Isolate	Origin	Isolation date ^a	β -Lactamase(s) identified	Size of CTX-M-15-bearing plasmid (kb)	Size(s) of other plasmid(s) (kb)	MIC of SXT (μ g/ml)	<i>int</i> gene	<i>sul</i> gene(s)	Gene cassette
122	Mussel	06/01/2012	CTX-M-15	200	50	>64		<i>sul2</i>	
123	Mussel	05/15/2012	CTX-M-15	200	50	>64		<i>sul2</i>	
124	Mussel	07/04/2012	CTX-M-15	160	40	0.5			
125	Mussel	09/05/2012	CTX-M-15	160	100	0.5			
126	Mussel	09/26/2012	CTX-M-15	200		>64	<i>int1</i>	<i>sul1</i>	<i>dfrA17-aadA5</i>
138	Seawater	07/04/2012	CTX-M-15	160	7	0.25			
159	Seawater	07/04/2012	CTX-M-15	160		>64	<i>int1</i>	<i>sul1</i>	
165	Seawater	12/05/2012	CTX-M-15	200	7, 30	>64	<i>int1</i>	<i>sul1</i>	
166	Seawater	11/14/2012	CTX-M-15	160	40	0.5	<i>int1</i>		<i>aacA4</i>
167	Seawater	09/05/2012	CTX-M-15	160	50	>64	<i>int1</i>	<i>sul1, sul2</i>	<i>dfrA17-aadA5</i>
168	Seawater	06/15/2012	CTX-M-15	80	7	>64	<i>int1</i>	<i>sul2</i>	<i>dfrA17-aadA5</i>
203	Seawater	06/15/2012	CTX-M-15, TEM-116	100		>64	<i>int1</i>		<i>dfrA17-aadA5</i>
204	Seawater	06/01/2012	CTX-M-15, TEM-116	160	7, 70	>64	<i>int1</i>	<i>sul1</i>	<i>cmlA1</i>
208	Seawater	05/16/2012	CTX-M-15, TEM-116	160	7	>64		<i>sul2</i>	
209	Seawater	06/01/2012	CTX-M-15, TEM-116	160	7	1	<i>int1</i>		<i>cmlA1</i>
210	Seawater	01/20/2012	CTX-M-15, TEM-116	160	7	1	<i>int1</i>		<i>cmlA1</i>
211	Seawater	03/01/2012	TEM-127			0.5	<i>int1</i>		<i>aacA4</i>
218	Seawater	09/05/2012	CTX-M-15, TEM-116	250	30	0.5	<i>int1</i>		<i>catB2</i>
247	Mussel	06/15/2012	CTX-M-15	160	7	>64	<i>int1</i>	<i>sul1</i>	
248	Seawater	11/14/2012	CTX-M-15	160	50	>64	<i>int1</i>	<i>sul1, sul2</i>	<i>dfrA17-aadA5</i>

^a Isolation dates are shown as month/day/year.

gene cassettes, and the *sul1* and *sul2* genes by PCR (7, 8). Class 1 integron gene cassettes were amplified using primers 5'CS and 3'CS (7) and then sequenced and analyzed using BLAST. SXT MICs, assessed by Etest (19), ranged from 0.25 to >64 µg/ml. In combination with *dfrA* and *sul2*, *sul1* may lead to high resistance to SXT in *S. maltophilia* (8). Of 12 SXT-resistant isolates, 9 were class 1 integrase positive and 7 possessed *sul1*; 2 isolates carried *sul1* and *sul2* (Table 1). Previously, *sul1* was identified in clinical isolates from China, Taiwan, Europe, and the Americas (5–8). It is noteworthy that the *dfrA17-aadA5* gene cassette has previously been detected only in clinical isolates from China (8).

This report presents the first description of IncF::CTX-M-15 in *S. maltophilia* isolates of environmental and clinical origins and of SXT resistance traits previously found only in clinical isolates, emphasizing the possibility that *S. maltophilia* isolates are hidden reservoirs for these multidrug resistance determinants.

ACKNOWLEDGMENTS

We thank António Correia (University of Aveiro, Portugal) for providing *E. coli* J53.

This work was supported by the Ministry of Science, Education and Sports, Croatia (grants 177-0000000-3182 and 177-1191196-0829).

REFERENCES

- Brooke JS. 2012. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clin. Microbiol. Rev. 25:2–41. <http://dx.doi.org/10.1128/CMR.00019-11>.
- Falagas ME, Kastoris AC, Vouloumanou EK, Rafailidis PI, Kapaskelis AM, Dimopoulos G. 2009. Attributable mortality of *Stenotrophomonas maltophilia* infections: a systematic review of the literature. Future Microbiol. 4:1103–1109. <http://dx.doi.org/10.2217/fmb.09.84>.
- al Naiemi N, Duim B, Bart A. 2006. A CTX-M extended-spectrum beta-lactamase in *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. J. Med. Microbiol. 55:1607–1608. <http://dx.doi.org/10.1099/jmm.0.46704-0>.
- Lavigne J-P, Gaillard J-B, Bourg G, Tichit C, Lecaillon E, Sotto A. 2008. Étude de souches de *Stenotrophomonas maltophilia* sécrétrices de BLSE: détection de CTX-M et étude de la virulence. Pathol. Biol. 56:447–453. <http://dx.doi.org/10.1016/j.patbio.2008.07.013>.
- Barbolla R, Catalano M, Orman BE, Famiglietti A, Vay C, Smayevsky J, Centrón D, Piñero SA. 2004. Class I integrons increase trimethoprim-sulfamethoxazole MICs against epidemiologically unrelated *Stenotrophomonas maltophilia* isolates. Antimicrob. Agents Chemother. 48:666–669. <http://dx.doi.org/10.1128/AAC.48.2.666-669.2004>.
- Toleman MA, Bennett PM, Bennett DMC, Jones RN, Walsh TR. 2007. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of *sul* genes. Emerg. Infect. Dis. 13:559–565. <http://dx.doi.org/10.3201/eid1304.061378>.
- Liaw S-J, Lee Y-L, Hsueh P-R. 2010. Multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*: roles of integrons, efflux pumps, phosphoglucosyltransferase (SpgM), and melanin and biofilm formation. Int. J. Antimicrob. Agents 35:126–130. <http://dx.doi.org/10.1016/j.ijantimicag.2009.09.015>.
- Hu L-F, Chang X, Ye Y, Wang Z-X, Shao Y-B, Shi W, Li X, Li J-B. 2011. *Stenotrophomonas maltophilia* resistance to trimethoprim/sulfamethoxazole mediated by acquisition of *sul* and *dfrA* genes in a plasmid-mediated class 1 integron. Int. J. Antimicrob. Agents 37:230–234. <http://dx.doi.org/10.1016/j.ijantimicag.2010.10.025>.
- Maravić A, Skočibušić M, Šamanić I, Fredotović Ž, Cvjetan S, Jutrović M, Puizina J. 2013. *Aeromonas* spp. simultaneously harbouring *bla*_{CTX-M-15}, *bla*_{SHV-12}, *bla*_{PER-1} and *bla*_{FOX-2} in wild-growing Mediterranean mussel (*Mytilus galloprovincialis*) from Adriatic Sea, Croatia. Int. J. Food Microbiol. 166:301–308. <http://dx.doi.org/10.1016/j.jfoodmicro.2013.07.010>.
- Poirer L, Walsh TR, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn. Microbiol. Infect. Dis. 70:119–123. <http://dx.doi.org/10.1016/j.diagmicrobio.2010.12.002>.
- Machado E, Coque TM, Canton R, Sousa JC, Silva D, Ramos M, Rocha J, Ferreira H, Peixe L. 2009. Leakage into Portuguese aquatic environments of extended-spectrum-β-lactamase-producing *Enterobacteriaceae*. J. Antimicrob. Chemother. 63:616–618. <http://dx.doi.org/10.1093/jac/dkn510>.
- Maravić A, Skočibušić M, Šamanić I, Puizina J. 2012. Antibiotic susceptibility profiles and first report of TEM extended-spectrum β-lactamase in *Pseudomonas fluorescens* from coastal waters of the Kaštela Bay, Croatia. World J. Microb. Biotechnol. 28:2039–2045. <http://dx.doi.org/10.1007/s11274-012-1006-5>.
- Tacão M, Moura A, Correia A, Henriques I. 2014. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. Water Res. 48:100–107. <http://dx.doi.org/10.1016/j.watres.2013.09.021>.
- Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods 63:219–228. <http://dx.doi.org/10.1016/j.mimet.2005.03.018>.
- Literacka E, Bedenic B, Baraniak A, Fiett J, Tonkic M, Majic-Bencic I, Gniadkowski M. 2009. *bla*_{CTX-M} genes in *Escherichia coli* strains from Croatian hospitals are located in new (*bla*_{CTX-M-3a}) and widely spread (*bla*_{CTX-M-3a} and *bla*_{CTX-M-15}) genetic structures. Antimicrob. Agents Chemother. 53:1630–1635. <http://dx.doi.org/10.1128/AAC.01431-08>.
- Carattoli A. 2009. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob. Agents Chemother. 53:2227–2238. <http://dx.doi.org/10.1128/AAC.01707-08>.
- Berg G, Roskot N, Smalla K. 1999. Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. J. Clin. Microbiol. 37:3594–3600.
- Tenover FC, Arbeit RD, Goering VR, Mickelsen PA, Murray BE, Pershing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233–2239.
- Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial susceptibility testing. CLSI M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.