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

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Stenotrophomonas maltophilia is a widespread environmental microorganism (1) that has emerged as significant opportunistic pathogen (2) due to intrinsic resistance to almost all available antibiotics. Apart from native β-lactamases L1 and L2 (1), acquired ESBLs were also identified (3, 4), pointing S. maltophilia as 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 (16µg/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 blaCTX-M-15, six additionally harboured blaTEM-116 and one carried blaTEM-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 was never before identified in S. maltophilia. More importantly, CTX-M-15 was previously identified only in S. maltophilia clinical strain from France (4). S1-PFGE of plasmid DNA followed by Southern blotting (9), showed that blaCTX-M-15 was located on large plasmids of various sizes (Table 1). Conjugation transfer of blaCTX-M-15 using E. coli J53 (13) at 37ºC and 27 ºC, and using azide (100 µg/ml) and cefotaxime (8 µg/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 transformant was used, while for other isolates each plasmid band was cut from the gel, and after confirmed as blaCTX-M-15-positive by PCR, was used as template. Interestingly, all plasmids belonged to IncFIB incompatibility group. IncFII, IncFIA and IncFIB blaCTX-M-15-bearing plasmids were previously reported in Croatia (9, 15). 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 integrases, gene cassettes, and *sul1* and *sul2* genes, by PCR (7, 8). Class 1 integron gene cassettes were amplified using primers 5’CS and 3’CS (7), 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 of them possessed *sul1*; 2 isolates carried *sul1* and *sul2* (Table 1). Previously, *sul1* was identified in clinical isolates from China, Taiwan, Europe, and Americas (5-8). Noteworthy, *dfrA17-aadA5* gene cassette was previously detected only in clinical isolates from China (8).

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

**ACKNOWLEDGMENTS**

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<tr>
<th>Isolate no.</th>
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<th>Isolation dates</th>
<th>β-lactamases identified</th>
<th>CTX-M-15-bearing plasmids (kb)</th>
<th>Other plasmids (kb)</th>
<th>MIC of SXT (µg/ml)</th>
<th>Genes</th>
<th>Gene cassettes</th>
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Isolation dates are shown as month/day/year.