A CTX-M extended-spectrum β -lactamase in *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*

Extended-spectrum β -lactamases (ESBLs) are an important cause of bacterial resistance throughout the world (Paterson & Bonomo, 2005). CTX-M ESBLs were first reported in 1986 (Matsumoto *et al.*, 1988) and their dissemination among bacterial species and in various parts of the world during the past decade is of growing concern (Bonnet, 2004). Recent studies indicate that the CTX-M enzymes predominate among the ESBLs of community strains (Pitout *et al.*, 2005). CTX-M ESBLs probably originated from *Kluyvera* species and these enzymes are mostly found in members of the *Enterobacteriaceae*. Recently, the emergence of these enzymes has been reported in *Acinetobacter baumannii* (Nagano *et al.*, 2004).

Pseudomonas aeruginosa and *Stenotrophomonas maltophilia* are opportunistic pathogens responsible for nosocomial infections. Phenotypic detection of ESBLs in these species is complex for various reasons. In *P. aeruginosa*, false-negative results may occur due to chromosome-encoded β -lactamases, such as the AmpC enzymes, which may be overexpressed, due to the simultaneous presence of metallo-enzymes with carbapenem-hydrolysing activities (the IMP and VIM series), or due to combined mechanisms of resistance, such as impermeability and efflux (Weldhagen *et al.*, 2003). In *S. maltophilia*, clavulanate tests may produce false-positive results via inhibition of the L2 chromosome-encoded β -lactamase (Walsh *et al.*, 1997).

In this study, we describe the presence of CTX-M-1 β -lactamase in two clinical bacterial strains of *P. aeruginosa* and *S. maltophilia*.

P. aeruginosa and *S. maltophilia* strains were isolated during a prevalence study on ESBLs in May 2004 in Amsterdam at the microbiological laboratory of the Academic Medical Center (AMC). The *P. aeruginosa*

strain was isolated from the sputum of a 21-year-old male cystic fibrosis outpatient. The *S. maltophilia* strain was isolated from the sputum of a hospitalized, male neonate at the Department of Neonatology at AMC. Neither patient showed any signs of invasive infection due to the presence of these strains. Antimicrobial regimens before admission were not documented and the patients were not receiving any antibiotic treatment at the time of isolation of these strains.

The strains were identified as *P. aeruginosa* and *S. maltophilia* using VITEK-2 (bioMérieux) and classical biochemical determination (routine determination at the microbiology laboratory). Species determination was confirmed by PCR and sequence analysis of the 16S rRNA gene, using the generic primers p515F (5'-TGCCAGCAGCCGCGGTAA-3') and p13b (5'-AGGCCCGGGAACGTATTCAC-3') (Relman *et al.*, 1992).

Antibiotic susceptibilities were determined according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) with the disc diffusion method (NCCLS, 2005). ESBL production was detected using a combination of the double disc test and the combined disc test (DCDT) (N. al Naiemi, B. Duim, V. van der Veen, M. D. de Jong & A. Bart, unpublished data), which included discs of ceftazidime, cefotaxime, cefpodoxime and cefepime placed around a disc containing amoxycillin plus clavulanate. The test was done at two distances between the discs: 30 and 20 mm (centre to centre). The DCDT also included a disc containing ceftazidime plus clavulanate and a disc containing cefoxitin for detection of AmpC production.

The presence of the ESBL genes *bla*_{SHV}, *bla*_{TEM} and *bla*_{CTX-M} was determined by PCR and sequence analysis using *bla*_{SHV}- and *bla*_{TEM}-specific primers as described previously (al Naiemi *et al.*, 2005) and primers CTX-MA-F1

(5'-CGYTTTSCRATGTGCAG-3') and CTX-MB-F2 (5'-ACCGCRATATCRTTGGT-3'), slightly modified from primers used by Bonnet *et al.* (2001). To prevent carry-over contamination, the Uracil system (Roche) was applied and ultrahigh-purity water was used as a negative control. The PCR conditions were identical to those described previously (al Naiemi *et al.*, 2005). Both DNA strands were sequenced with the amplification primers and two additional internal *bla*_{SHV} and *bla*_{TEM} primers as described previously (al Naiemi *et al.*, 2005).

The ESBL phenotype of the *P. aeruginosa* and *S. maltophilia* strains is shown in Fig. 1. As the *P. aeruginosa* isolate was sensitive to inhibition by clavulanate (Fig. 1), the action of OXA β -lactamases, which are frequently present in *P. aeruginosa*, is unlikely (Bradford, 2001). The susceptibility of this *P. aeruginosa* strain for cefoxitin is noteworthy. *P. aeruginosa* is usually resistant to cefoxitin, as it contains an inducible AmpC enzyme (Livermore, 1991). However, failure of cefoxitin to induce AmpC production in *P. aeruginosa* has been reported previously (Ramadan *et al.*, 1995).

PCR and sequence analysis revealed the presence of the *bla*_{CTX-M-1}, *bla*_{SHV-1} and *bla*_{TEM-116} genes in the *P. aeruginosa* strain and *bla*_{CTX-M-1} and *bla*_{SHV-1} in the *S. maltophilia* strain. Of these β -lactamases, only CTX-M-1 has been shown to produce a transferable ESBL phenotype (Sirot *et al.*, 1987).

To our knowledge, this is the first description of the presence of CTX-M ESBLs in *P. aeruginosa* and *S. maltophilia*. The CTX-M ESBLs provide these pathogens with an additional powerful resistance mechanism with potential serious clinical implications, including limitation of the therapeutic options.

It remains to be determined how the CTX-M-1 genes have disseminated to these

Fig. 1. Phenotypic detection of ESBL. (a) DCDT for *S. maltophilia* with CTX-M-1, at 30 mm distance between the discs (centre to centre). Production of ESBL is indicated by the synergy between the CAZ, CTX and CFP discs and the AM/CL disc (arrows). In addition, the CAZ/CL zone was ≥ 5 mm larger than that of CAZ, indicating the production of ESBL. (b) DCDT for *P. aeruginosa* with CTX-M-1, at 20 mm distance between the discs (centre to centre). Synergy is evident between the CFP and CAZ discs and the AM/CL disc, indicating the production of ESBL. The CAZ/CL zone was ≥ 5 mm larger than that of CAZ, indicating the production of ESBL. CFP, Cefepime; CPD, cefpodoxime; AM/CL, amoxycillin/clavulanate; CTX, cefotaxime; CAZ, ceftazidime; FOX, ceftioxit; CAZ/CL, ceftazidime/clavulanate.

P. aeruginosa and *S. maltophilia* strains. The association of CTX-M β -lactamase-encoding genes with mobile elements such as the ISEcp1 insertion element and integrons (Eckert *et al.*, 2006) may facilitate the spread of *bla*_{CTX-M} genes among bacteria.

As phenotypic detection of ESBLs in non-fermenters is complicated and many clinical microbiological laboratories have no routine surveillance of ESBLs in non-fermenters, ESBLs may be underestimated and underreported in these strains. Therefore, *P. aeruginosa* and *S. maltophilia* may become hidden reservoirs for such ESBLs, as is already the case for OXA β -lactamases in *P. aeruginosa*.

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