Double-Disk Synergy Test Positivity in *Stenotrophomonas maltophilia* Clinical Strains

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**ABSTRACT.** The double-disk synergy test (DDST) using Mueller–Hinton agar and antibiotic disks with centrally positioned disks of amoxicillin–clavulanate, ampicillin–sulbactam, and piperacillin–tazobactam and, at a center-to-center distance of 25–30 mm, 2–4 disks with 10 various β-lactam antibiotics per one plate was performed in 58 clinical isolates of *Stenotrophomonas maltophilia* to determine the effectivity of 3 β-lactamase inhibitors. When tested with clavulanate as the central β-lactamase inhibitor synergic action on tested strains was the most frequent with aztreonam (81.0 % of strains), cefoperazone (63.8 %), and cefepime (60.3 %). With sulbactam the synergic action, i.e. DDST positivity, was high in the case of cefoperazone (15.5 %), ampicillin, aztreonam and piperacillin (8.6 % each); with tazobactam it was the most frequent with aztreonam (53.4 %), cefoperazone (44.8 %) and cefepime (37.9 %). No synergy was demonstrated after application of meropenem regardless of the kind of β-lactamase inhibitor used. In 58 strains of *S. maltophilia*, 55 different profiles of DDST positivity were found. The results confirm that clavulanate is the most effective inhibitor of *S. maltophilia* β-lactamases. The utilization of DDST (performed in the recommended way) for the typization of strains *Stenotrophomonas* species and for the estimation of potential effectiveness combinations of β-lactams with β-lactamase inhibitors for the therapy of stenotrophomonade infections was suggested.

**Abbreviations**

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<th>DDST</th>
<th>ESBLE</th>
<th>β-lactamase inhibitors</th>
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<tr>
<td>Amp</td>
<td>Cfm</td>
<td>Mer meropenem</td>
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<tr>
<td>Amx</td>
<td>Cfp</td>
<td>Pip piperacillin</td>
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<td>Cef</td>
<td>Cfx</td>
<td>Taz tazobactam</td>
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<td>Cla</td>
<td>Clavulanate</td>
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*S. maltophilia* has recently attracted the attention of many bacteriologists. The frequency of occurrence of *S. maltophilia* infections in compromised patients is increasing; at present, this originally environmental species has become a serious nosocomial pathogen. Especially endangered have been seriously weakened, immunosuppressed and neutropenic patients in whom these Gram-negative nonfermentative rods are able to cause various infections of tissues and organs (Denton and Kerr 1998). In addition, many *S. maltophilia* strains exhibit resistance against many commonly used broad-spectrum antibiotics, including carbapenems, which complicates the therapy of infections (Pankuch et al. 1994; Denton and Kerr 1998; Valdezate et al. 2001).

Several studies have been published reporting positivity in DDST in a number of *S. maltophilia* clinical strains after application of some cephalosporins, Azt, and Cla as β-lac inhibitor (Blahová et al. 1998; Cantón et al. 1999). DDST is a routinely used method of ESBL detection in *Enterobacteriaceae* (Jarlier et al. 1988). Blahová et al. (1998) considered positive ESBL reactions in DDST in *S. maltophilia* as an evidence of ESBL presence in this bacterial species. However, their conclusions were not accepted by Spanish microbiologists who explained DDST positivity by specific properties of *S. maltophilia* β-lacs (Muñoz Bellido and García-Rodriguez 1998). Paton et al. (1994) described that the properties of some serine β-lacs of this bacterium correspond to ESBL characteristics.

The aim of our study was to contribute to the elucidation of possible occurrence of DDST positivity in *S. maltophilia* even after application of penicillins and Mer as a representative of carbapenems; the effectiveness of Sul and Taz in DDST was compared with that of Cla, which is considered for the best inhibitor of β-lacs of *S. maltophilia*.
MATERIALS AND METHODS

Bacterial isolates. In the years 1993–98, 58 S. maltophilia strains were isolated from the patients of the Teaching Hospital (54 strains) and the Military Hospital (4) in Olomouc (1993: 4 strains; 1994: 4; 1995: 3; 1996: 11; 1997: 23; 1998: 13). These isolates were detected in blood (n = 17 strains), pus (11), urine (9), mouth, throat or nose smears (8), sputum (5), endotracheal secretion and bronchoalveolar lavage (4), smears from cannula (3) and ear swab (1). The whole collection of isolated stenotrophomonads was stored in glycerol broth at –20 ºC until tested. All strains were identified by standard microbiological methods of the NEFERM test (Lachema, Czechia); complementary tests (Denton and Kerr 1998) were also used.

Correctness of species identification was confirmed by the analysis of cellular fatty acids according to Hejnar et al. (2002). Fatty acids iso-15:0, anteiso-15:0, 16:0, and 16:1 (typical for S. maltophilia species) were dominating (Denton and Kerr 1998; Finkmann et al. 2000; Hejnar et al. 2002).

Antimicrobial agents. The following antibiotic disks were used for testing (in μg): Amp 10 + Sul 10, Azt 30, Cef 30, Cfx 30, Cfa 30, Mer 10 (Oxoid, UK); Amp 10, Cfp 30, Cfm 30, Pip 100 + Taz 10 (Bio-Rad, France), and Amx 20 + Cla 10, Cft 30, Pip 100 (Sanofi Diagnostics Pasteur, France).

Double-disk synergy test was performed on Mueller–Hinton agar according to Jarlier et al. (1988). An antibiotic disk containing an inhibitor of β-lacs, i.e. Amx–Cla, Amp–Sul, or Pip–Taz was placed to the center of Petri dish. Two to four antibiotic disks, containing appropriate β-lactam antibiotics (total number 10) were located at a distance of 25 mm (center-to-center). In the case of a highly sensitive strain forming wide inhibition zones the distance between the central disk and peripheral ones had to be increased up to 30 mm. After 18 h of incubation at 37 ºC, the presence of clear-cut extension of the edge of β-lactam inhibition zone toward the disk containing β-lac inhibitor was considered as a positive result of DDST (Fig. 1). All tests were done in duplicate.

RESULTS

After application of Cla, the synergic effect on S. maltophilia strains was recorded most often with Azt (47 strains; 81.0 %), Cfp (37; 63.8 %), and Cef (35; 60.3 %). For Sul the synergy (DDST positivity) was the highest with Cfp (9; 15.5 %), Amp, Azt and Pip (5 strains each; 8.6 %). Taz manifested the most frequent synergy with Azt (31; 53.4 %), Cfp (26; 44.8 %) and Cef (22; 37.9 %). The only β-lactam that did not manifest synergy with any of β-lac inhibitors was Mer (Table I).

The DDST positivity after application of Taz corresponded with that of Cla in 88.6 % of cases. The remaining 11.4 %, presenting synergy with Taz only, were the following β-lactams – Cef (3.6 %), Cfm (2.9 %), Cft (2.1 %), Cfp, Cfx, Cla, and Pip (0.7 % each).