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In vitro synergistic/additive activity of levofloxacin with meropenem against *Stenotrophomonas maltophilia*☆

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Abstract

Synergy testing of levofloxacin and meropenem by Etest and time–kill assay (TKA) was performed against 30 genetically unique clinical *Stenotrophomonas maltophilia* isolates. Synergy was demonstrated in 18/30 (60%) isolates by Etest and in 13/30 (43%) by TKA; the remaining isolates were indifferent. Methods showed agreement for 25/30 (83%) of isolates.

Keywords: Levofloxacin; Meropenem; *Stenotrophomonas*; Synergy

Stenotrophomonas maltophilia is a multidrug-resistant nosocomial cause of pneumonia and indwelling venous catheter-related bacteremia. In a recently published extensive literature review of *S. maltophilia* infections, Falagas et al. (2009) found that a mortality rate of up to 37.5% could be attributed to *S. maltophilia* infections. In addition, patients who received "inappropriate" antibiotic treatment were found to have greater mortality attributed to *S. maltophilia* infection, compared with those who received "appropriate" antibiotic treatment. *S. maltophilia* exhibits high-level intrinsic resistance to a variety of structurally unrelated antibiotics, including β-lactams, quinolones, aminoglycosides, tetracycline, disinfectants, and heavy metals (Alonso and Martinez, 1997; Looney et al., 2009; Zhang et al., 2000). There is no ideal standard treatment, but trimethoprim/sulfamethoxazole is typically used when *S. maltophilia* is considered a pathogen (Falagas et al., 2008; Looney et al., 2009; Mendoza et al., 2007; Sader and Jones, 2005). However, it may be contraindicated because of allergy or other issues. In addition, global emergence of trimethoprim/sulfamethoxazole resistance in *S. maltophilia* mediated by the acquisition of *sul* genes has been reported (Toleman et al., 2007).

Levofloxacin may represent an alternative drug in the treatment of infections caused by *S. maltophilia* (% susceptible range, 78–87%) (Bonfiglio et al., 2000; Galles et al., 2008; Passerini de Rossi et al., 2009; Sader and Jones, 2005). However, *S. maltophilia* can quickly develop resistance to fluoroquinolones by mutations in outer membrane proteins and overproduction of efflux pumps (Mendoza et al., 2007; Zhang et al., 2000). An in vitro study with biofilms and planktonic cells of *S. maltophilia* by Passerini de Rossi et al. (2009) suggested that fluoroquinolones, particularly levofloxacin due to its better pharmacokinetic parameters, are candidates for synergy studies. Many suggest that the probability for the emergence of resistance during treatment of *S. maltophilia* infections warrants the consideration of administering antibiotic combinations (Falagas et al., 2008).

We chose to evaluate levofloxacin in combination with meropenem for possible synergy against *S. maltophilia* because (1) our pilot synergy study with 7 isolates showed 57% synergy with this combination, (2) this combination is used as empiric treatment in infected hospitalized patients, and (3) we found few similar in vitro synergy studies in the literature. The aim of this present study was to test for synergistic activity of levofloxacin and meropenem against *S. maltophilia* and to compare results from the Etest method to time–kill assay (TKA).

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A total of 30 genetically unique clinical *S. maltophilia* isolates were collected from May 2007 to June 2009 from different patients in the New Orleans metropolitan area. Identification was performed using the Vitek® system (bioMérieux, Durham, NC). DNA fingerprinting of the isolates was performed with the Diversilab® System (bioMérieux) using rep-polymerase chain reaction technology. Mueller–Hinton broth and Mueller–Hinton II agar plates (Becton-Dickinson Microbiology Systems, Sparks, MD) were used for the Etest MIC determination and the Etest synergy method. TKA was performed using Mueller–Hinton broth for growth medium and trypticase soy agar with 5% sheep blood plates (Becton-Dickinson) for colony counts. Standard laboratory powders—levofloxacin (Sigma Aldrich, St. Louis, MO) and meropenem (AstraZeneca Pharmaceuticals LP, Wilmington, DE)—and Etest strips (AB Biodisk, Solna, Sweden) were used.

MICs were performed by Etest in triplicate following manufacturer's guidelines, with mean values used. Etest MICs (μg/mL) for levofloxacin were 0.38 to 2 for 27/30 isolates (90% susceptible) and 4, 8, and 24 (10% resistant) for the remaining 3 isolates; for meropenem, MICs were >32 for 28/30 isolates (93%) and 0.5 and 1 (7%) for the remaining 2 isolates. Interpretive standards (μg/mL) for *S. maltophilia* are levofloxacin ≤2 = susceptible (Clinical and Laboratory Standards Institute [CLSI], 2009). There are no CLSI (formerly the National Committee for Clinical Laboratory Standards) interpretive guidelines for testing *S. maltophilia* with meropenem. The percentage susceptibility of additional antibiotics tested (by Etest) using CLSI interpretive standards included ceftazidime (37%), chloramphenicol (17%), minocycline (97%), ticarcillin/clavulanate (33%), and trimethoprim/sulfamethoxazole (100%).

The Etest synergy method (Pankey and Ashcraft, 2005) was performed in triplicate, the summation fractional inhibitory concentration (∑FIC) calculated for each set of MICs, and the mean ∑FIC used for comparison with the TKA (Pillai et al., 2005). Meropenem and levofloxacin Etest strips were placed on different sections of a Mueller–Hinton agar plate. The agar was marked adjacent to the previously determined MIC value on each strip. For the meropenem MICs that were >32 μg/mL and exceeded the concentration on the Etest strip, the highest concentration (32 μg/mL) was marked on the agar. The strips were removed after 1 h of incubation at room temperature. A new levofloxacin strip was placed on the area of the previously removed meropenem strip so that the levofloxacin MIC corresponded with the mark of the meropenem MIC. The meropenem Etest strip was applied in reciprocate fashion to the area of the previous levofloxacin strip. The resulting combination ellipses were read after 24 h of incubation at 35°C. To evaluate the effect of the combination, we calculated the FIC for each antibiotic in each combination. The following formulas were used to calculate the ΣFIC: (1) FIC of levofloxacin = MIC of levofloxacin in combination/MIC of levofloxacin alone; (2) FIC of meropenem = MIC of meropenem in combination/MIC of meropenem alone; (3) ΣFIC = FIC of levofloxacin + FIC of meropenem. Synergy was defined by a ΣFIC of ≤0.5 (indifference, >0.5 but ≤4).

TKAs were performed according to CLSI guidelines (National Committee for Clinical Laboratory Standards, 1999). An inoculum of approximately 10^5 CFU/mL was verified after plating in duplicate using a spiral plater and scanner (Spiral Biotech, Bethesda, MD). Each isolate was tested against levofloxacin and meropenem alone and in combination at a concentration equal to the mean Etest MIC. Colony counts on all isolates were performed at 0 and 24 h. Performing serial dilutions and plating with a spiral plater, which further dilutes and plates the sample, reduced the possibility of antibiotic carryover. *Synergy* was defined as a ≥2 $\log_{10}$ *decrease* in colony count after 24 h by the combination compared with the most active single agent; the number of surviving organisms in the presence of the combination had to be ≥2 $\log_{10}$ CFU/mL below the starting inoculum. *Indifference* was defined as a <2 $\log_{10}$ *increase* or *decrease* in colony count at 24 h by the combination compared with that by the most active drug alone. *Antagonism* was defined as a ≥2 $\log_{10}$ *increase* in colony count after 24 h by the combination compared with the most active drug alone (Pillai et al., 2005).

Synergy with levofloxacin and meropenem was demonstrated in 18/30 (60%) of *S. maltophilia* isolates by the Etest method (∑FICs, 0.1–0.5), with 12/30 (40%) showing indifference (∑FICs, 0.7–1.2). Of the 12 isolates that showed indifference, 10 isolates had ∑FICs ≤1, suggestive of a possible *additive* effect with the combination (Pillai et al., 2005). Therefore, the combination demonstrated either a synergistic or an additive effect on 28/30 (93%) of isolates. TKA showed synergy in 13/30 (43%) of isolates ($\log_{10}$ change, −2.0 to −4.4), with 17/30 (57%) showing indifference ($\log_{10}$ change, −1.9 to +1.6). Of the 17 isolates showing indifference by TKA, 11 isolates had $\log_{10}$ changes of −0.1 to −1.9, suggesting a possible *additive* effect. The combination showed either a synergistic or an additive effect on 24/30 (80%) of isolates by TKA. Two of 3 nonsusceptible levofloxacin isolates (MICs, 8 and 24 μg/mL) showed synergy by Etest and TKA. Concordance between Etest and TKA was high: 25/30 (83%). No antagonism was found with either method (Table 1).

A similar in vitro synergy study with levofloxacin and meropenem against *S. maltophilia*—but using the checkerboard method—demonstrated 2/20 (10%) synergy and 18/20 (90%) indifference (Isenberg et al., 1999).

Synergy testing methods are not standardized for reproducibility and interpretation, making a comparison of results from different methods (TKA, checkerboard, and Etest) in the same study or in different studies extremely difficult. We evaluated 2 methods in our study. Checkerboard is an inhibitory method and does not compare well with TKA (a bactericidal method). Even though the Etest synergy method may be classified as an inhibitory method, it has been found to be a better predictor of bactericidal activity