In vitro combination of tigecycline with other antibiotics in Stenotrophomonas maltophilia isolates

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Background/aim: The aim of this study was to determine the usefulness of tigecycline in combination treatment of Stenotrophomonas maltophilia infections by evaluating the in vitro synergistic effects of tigecycline with various antibiotics using the E-test method.

Materials and methods: Synergy testing by E-test was performed with various antibiotic combinations in 10 S. maltophilia isolates identified as a cause of infection. The antibiotics used in the study included tigecycline (TGC), cefoperazone-sulbactam (CPS), ceftazidime (TZ), levofloxacin (LEV), and trimethoprim-sulfamethoxazole (cotrimoxazole) (TS). Four different combinations (TGC-CPS, TGC-TZ, TGC-LEV, TGC-TS) were studied with the E-test synergy method.

Results: S. maltophilia isolates were found to have the highest level of susceptibility to trimethoprim-sulfamethoxazole, tigecycline, and levofloxacin. The fractional inhibitory concentration (FIC) index was calculated as FIC = MICAB/MICA + MICBA/MICB. The FIC index values were calculated and classified as synergistic (FIC < 0.5), additive (FIC = 0.5–1), indifferent (FIC = 1–4), and antagonistic (FIC > 4). According to FIC index values, synergy was found with the highest rate with TGC-CPS and TGC-LEV combinations (20%). Antagonistic activity was not found in any combination.

Conclusion: When trimethoprim-sulfamethoxazole cannot be used because of resistance or allergy, tigecycline alone or in combination may be included as an alternative option. Although in vitro results are promising, clinical data are required.

Key words: Combination, Stenotrophomonas maltophilia, synergy, tigecycline
2. Materials and methods
The E-test synergy method was performed using various antibiotic combinations and 10 *S. maltophilia* isolates identified as causative agents. The isolates used in this study were selected from *S. maltophilia* isolates causing nosocomial infection in various services and intensive care units at the Gazi University Hospital between January 2011 and June 2015. The distribution of the clinical specimens of the isolates were as follows: peripheral blood culture 4 (40%), catheter blood culture 2 (20%), ETA (endotracheal aspirate) culture 2 (20%), pleural fluid culture 1 (10%), and bile fluid culture 1 (10%).

2.1. In vitro E-test synergy method
The isolates obtained in our study were stored at –80 °C until the study was performed. The isolates were identified using a BBL Crystal Enteric/Nonfermenter ID Kit (Becton Dickinson, USA).

The minimum inhibitory concentration (MIC) values of tigecycline, cotrimoxazole, levofloxacin, ceftazidime, and cefoperazone-sulbactam were determined using the E-test method. For the E-test, suspensions equivalent to 0.5 McFarland standard were obtained from pure bacterial colonies and inoculated onto Mueller–Hinton medium (Becton Dickinson). E-test strips were prepared for each antibiotic. After incubation for 24 h at 35 °C, MICs were read and interpreted according to the manufacturer's instructions. To prevent misinterpretation of tigecycline MICs, the agar plates were used within 12 h after preparation. The susceptibility breakpoints for cotrimoxazole, levofloxacin, and ceftazidime were interpreted using the Clinical Laboratory Standard Institute's criteria for *S. maltophilia*. The cefoperazone-sulbactam susceptibility was determined using the CLSI criteria for Enterobacteriaceae (14). Tigecycline susceptibility was determined using the Food and Drug Administration's breakpoints for Enterobacteriaceae (15).

For the synergy method, the E-test strip of drug A was applied to the surface of agar plates and left for 1 h at room temperature. Subsequently, the strip was removed and a strip of drug B was applied onto the imprint of strip A. The plates were incubated for 24 h at 35 °C and then the MIC levels of each drug and combination were read. The fractional inhibitor concentration (FIC) index was calculated using the formula FIC = MICAB/MICA + MICBA/MICB. The FIC index was interpreted as follows: synergistic, ≤0.5, additive, >0.5 to <1, indifferent, >1 to ≤4, and antagonistic, >4.

*Pseudomonas aeruginosa* ATCC (American Type Culture Collection) 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains.

3. Results
The MIC values of the antibiotics among *S. maltophilia* isolates are shown in Table 1. The susceptibilities of the antibiotics were as follows: cotrimoxazole (100%), tigecycline (80%), levofloxacin (80%), ceftazidime (70%), and cefoperazone-sulbactam (50%).

In vitro interactions (synergic, additive, indifferent, and antagonistic) of the 4 combinations studied (TGC-CPS, TGC-TZ, TGC-LEV, TGC-TS) according to FIC results are shown in Table 2. Synergy was found with the highest rate in TGC-CPS and TGC-LEV combinations (20%) and antagonistic activity was not found in any combination.

4. Discussion
*S. maltophilia* has a high level of intrinsic resistance to β-lactams, quinolones, aminoglycosides, tetracyclines, disinfectants, and heavy metals. Management of these infections is difficult because of resistance to many antimicrobial agents. The therapeutic agent recommended for *S. maltophilia* is cotrimoxazole. Some recent studies have instead recommended antimicrobial combination

|     | TGC (D: ≤2 µm/mL) | CPS (D: ≤16 µm/mL) | TS (D: ≤2/38 µm/mL) | TZ (D: ≤8 µm/mL) | LEV (D: ≤2 µm/mL) |
|-----|-------------------|-------------------|---------------------|------------------|------------------|
| 1st isolate | 0.75 | 64 | 0.25 | 256 | 0.5 |
| 2nd isolate | 0.38 | 64 | 0.064 | 256 | 0.25 |
| 3rd isolate | 0.5 | 48 | 0.047 | 4 | 0.5 |
| 4th isolate | 1 | 16 | 0.038 | 8 | 0.5 |
| 5th isolate | 4 | 6 | 0.047 | 1 | 4 |
| 6th isolate | 0.38 | 16 | 0.125 | 8 | 0.5 |
| 7th isolate | 4 | 96 | 0.064 | 256 | 8 |
| 8th isolate | 0.5 | 4 | 0.032 | 3 | 0.5 |
| 9th isolate | 0.25 | 48 | 0.032 | 3 | 0.25 |
| 10th isolate | 2 | 3 | 0.125 | 0.75 | 0.25 |

TGC: Tigecycline, TS: Trimethoprim-sulfamethoxazole, LEV: levofloxacin CPS: cefoperazone-sulbactam, TZ: ceftazidime.
therapies, especially for patients with septic shock or neutropenia, immunocompromised patients, and patients intolerant of cotrimoxazole, but only a few studies have been focused on these antibiotic combinations (16).

Zelenitsky et al. conducted a study comparing cotrimoxazole monotherapy and its combinations with various antibiotics (ciprofloxacin, ceftazidime, gentamycin, and tobramycin) in 4 clinical isolates in an in vitro pharmacodynamic infection model. They found that cotrimoxazole worked as a bacteriostatic agent against all isolates when given alone, and all combinations of cotrimoxazole were more active than monotherapy as determined by bacterial reductions at both 24 and 48 h. They concluded that their preclinical data supported further investigation of antibiotic combinations in the treatment of serious S. maltophilia infections (17).

In clinical S. maltophilia isolates, tetracycline derivatives minocycline, doxycycline, and tigecycline have been shown to have high in vitro efficacy. There is a very little evidence of their use in treatment, however (6). Tigecycline, a wide-spectrum glycylcycline derivative, may overcome tetracycline resistance related to efflux pumps and ribosomal target modification. Studies have found that tigecycline is effective for strains resistant to cotrimoxazole (18,19). In a global study evaluating 1586 isolates, the susceptibility rates were 96% for cotrimoxazole and 95.5% for tigecycline (13). In a study conducted by Church et al., 17% of the S. maltophilia isolates were resistant to cotrimoxazole. Minocycline, tigecycline, and colistin had the highest efficacy. Colistin and tigecycline combination produced the best results (20). In another study conducted by Wei et al., synergism and antagonism were not detected in tigecycline + cotrimoxazole and tigecycline + ceftazidine combinations. All of the isolates showed indifferent activity. While synergy was found in a few isolates in the tigecycline and moxifloxacin combination, antagonistic action was not detected in any combination (21).

According to the results of in vitro studies, tigecycline could be considered an alternative option in the treatment of S. maltophilia infections, especially in combination therapy (22). However, the choice between monotherapy and combination therapy remains controversial. In a study performed by Tekce et al., the efficacy of tigecycline treatment was compared with cotrimoxazole in nosocomial S. maltophilia infections over a 3-year period. Clinical improvement was similar in the two groups: 69.2% in the cotrimoxazole group and 68.4% in the tigecycline group. The authors concluded that tigecycline can be considered as an alternative option in the treatment of S. maltophilia infections (23). Apart from this study, anecdotal evidence about the use of tigecycline in treatment has been reported in some studies. There are no data about the use of this agent in combination in clinical practice (24–26).

In our study, cotrimoxazole showed the lowest MIC levels against S. maltophilia isolates, followed by levofloxacin and tigecycline. We also evaluated tigecycline in combination with 4 different antibiotics. The best results were obtained with TGC + CPS and TGC + LEV combinations. Additive interaction was detected mainly in the TGC + CPS combination. In vitro synergy studies do not show the effects of antibiotic pharmacodynamics and host immune response, but synergistic combinations (TGC + CPS and TGC + LEV) may still be a therapeutic option in certain S. maltophilia infections. Our results should be supported with clinical studies.

Table 2. The results of in vitro interactions of 4 antibiotic combinations.

| Antibiotic combinations | Synergic number (%) | Additive number (%) | Indifferent number (%) | Antagonist number (%) |
|-------------------------|---------------------|---------------------|------------------------|----------------------|
| TGC-CPS                 | 2 (20)              | 4 (40)              | 4 (40)                 | 0 (0)                |
| TGC-TZ                  | 1 (10)              | 4 (40)              | 5 (50)                 | 0 (0)                |
| TGC-LEV                 | 2 (20)              | 1 (10)              | 7 (70)                 | 0 (0)                |
| TGC-TS                  | 0 (0)               | 2 (20)              | 8 (80)                 | 0 (0)                |

TGC: Tigecycline, TS: trimethoprim-sulfamethoxazole, LEV: levofloxacin, CPS: cefoperazone-sulbactam, TZ: ceftazidime.

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