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PINAR ŞAHİNTÜRK

MURAT CENGİZ

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Letter to the Editor

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Promising synergistic interactions among antimicrobials against multidrug-resistant *Escherichia coli* isolated from animals

Pınar ŞAHİNTÜRK[1,2], Murat CENGİZ[1,*]

[1]Laboratory of Molecular Pharmacology, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Uludağ University, Bursa, Turkey

[2]Institute of Health Science, Uludağ University, Bursa, Turkey

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To the Editor,

Multidrug resistance (MDR) is defined as an acquired nonsusceptibility to at least one agent in three or more antimicrobial classes (1). Overexpression of the multidrug efflux pump AcrAB-TolC has been shown to directly contribute to MDR in *Escherichia coli* and controls the susceptibility of *E. coli* strains to many structurally unrelated antibiotics, including β-lactams, fluoroquinolones (FQs), and aminoglycosides (2). *E. coli*, characterized by extensive antibiotic resistance, is an important public health issue, and a high risk for the treatment of infectious diseases can arise from recommended available dosage regimens. This is one of the most challenging problems in the study of infectious diseases. In cases involving a lack of effective agents, a combination of two or more antibiotics is often used with the expectation of achieving a synergistic effect (3). Therefore, interactions among FQ, β-lactams, aminoglycosides, and polymyxin were examined as a part of the approach to the resistance problem. Drug combination studies were performed using the checkerboard and time-kill methods. The fractional inhibitory concentration index (FICi) was interpreted as follows: FICi ≤ 0.5 = synergy; FICi > 4.0 = antagonism; and FICi > 0.5–4 = no interaction. Clinical *E. coli* isolates from various animals were collected from the Animal Hospital of the Faculty of Veterinary Medicine (Bursa, Turkey). Five representative *E. coli* isolates were chosen from 24 different random amplified polymorphic DNA (RAPD) patterns based on MDR profile and genotype. Broth microdilution testing was performed to determine the MICs of nalidixic acid (NAL), enrofloxacin (ENR), danofloxacin (DAN), ciprofloxacin (CIP), orbifloxacin (ORB), gatifloxacin (GAT), ampicillin (AMP), ceftiofur (CEF), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP), sulfamethoxazole (SMX), trimethoprim (TMP), gentamicin (GEN), tetracycline (TET), oxytetracycline (OTC), erythromycin (ERY), chloramphenicol (CHL), and colistin (CST) according to the guidelines of the Clinical Laboratory Standards Institute (4). Antimicrobial dilutions were prepared in CAMHB, and inocula with a density equivalent to a 0.5 McFarland standard were added to wells containing the antimicrobials. PCR and qRT-PCR were used to characterize the molecular mechanisms of resistance.

The sum of the MIC values ranged from 1133.58 to 1474.96 μg/mL. *E. coli* isolates had two single (Se83→Leu, Ser83→Thr) and two double (Ser83→Leu+Ser80→Ile, Ser83→Leu+Asp87→Glu) topoisomerase mutations. In addition, one isolate (*E. coli* E306) contained the *oqxB* gene. An increased expression of *marA*, *acrB*, and *soxS* was detected in two, two, and five isolates, respectively; a decreased expression of *ompF* was detected in one isolate (*E. coli* E246). The FICi values of the β-lactam/FQ, β-lactam/aminoglycosides, β-lactam/polymyxin, and FQ/polymyxin combinations for multidrug-resistant *E. coli* isolates ranged from 0.09 to 2.03, 0.06 to 1.50, 0.01 to 2.03, and 0.15 to 1.25, respectively. The incidence of synergy and no interaction of 16 different combinations was 68% and 32%, respectively. CEF/ENR, CEF/DAN, CEF/GEN, CEF/CST, CST/CIP, and CST/DAN exhibited synergistic activity against all *E. coli* isolates tested.

The in vitro activity of the combinations against multidrug-resistant *E. coli* isolates was verified by time-kill assay. Synergy was defined as a ≥2 $\log_{10}$ decrease in the colony count at 6 or 24 h, with the combination treatment compared with the initial inoculum. The incidence of synergy for the combinations at 6 and 24 h was 51.5% and 37.5%, respectively. At the 6-h incubation time point, FEP/ENR, FEP/DAN, CEF/CIP, CEF/DAN, FEP/CST, CRO/CST, CIP/CST, ENR/CST, and DAN/CST therapies resulted in a ≥2 $\log_{10}$ reduction in viable counts against at least three of the five *E. coli* isolates. At the 24-h incubation

* Correspondence: cengizm@uludag.edu.tr

time point, FEP/ENR, FEP/DAN, CEF/DAN, FEP/CST, and ENR/CST therapies resulted in a ≥2 $\log_{10}$ reduction in viable counts against at least three of the 5 *E. coli* isolates. These results showed that for *E. coli*, the synergy incidence detected by the checkerboard method is higher than that by the time-kill method, and the CEF/DAN combination exhibited synergistic activity in the checkerboard method and also at 6 h and 24 h of the time-kill assays. These data strongly support that antimicrobial combinations between different groups can be considered for effective inhibition of multidrug-resistant *E. coli* containing various resistance determinants.

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