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Impact of pH on the activity of co-used antimicrobials against resistant *Escherichia coli* strains of animal origin

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Abstract: In this study, combination antimicrobial therapy, due to its higher potential against resistant bacteria, was evaluated for the inhibition of multidrug-resistant *E. coli* strains. The influence of pH as an environmental variable on the activity of antimicrobial combinations was evaluated by calculating the factional concentration indexes at pH values 5.0, 6.0, 7.3, and 8.0.

The highest synergistic activity rates of ceftriaxone + colistin, danofloxacin + colistin, danofloxacin + ceftiofur, and ceftiofur + gentamicin combinations were 50%, 33%, 100%, and 50%, respectively measured at ≥7.3 pH. The lowest synergistic activity rates for all combinations were observed at the acidic pH values of 5.0 and 6.0. The results of this study clearly demonstrated that acidic pH of the medium impaired the activity of the antimicrobial combinations. Although ceftriaxone and ceftiofur exert optimal activity at acidic pH values, the synergistic activity of the co-used drugs reached its highest level at pH ≥7.3.

Key words: pH, antimicrobial combination, *E. coli*, resistance, animals

1. Introduction

Multidrug-resistant (MDR) bacteria are significant health problems globally. MDR is a commonly observed phenomenon in *Escherichia coli* (*E. coli*) strains and there are various genes encoding resistance against many compounds from various classes of antimicrobials. Mechanisms of resistance include enzymatic degradation of the compound, modification of the target, discharge of the antimicrobial from the bacteria, and prevention of the entrance of antimicrobial into the bacterial cell. The co-use of antimicrobials reduces the possibility of resistance, increases efficacy, and provides broad-spectrum activity [1–4]. The activity of antimicrobials such as fluoroquinolones, aminoglycosides, and macrolides is influenced by the pH of the bacterial medium or body fluids [5,6]. The ionized fraction of a compound is not able to efficiently penetrate the bacterial membrane; hence, minimum inhibitory concentrations (MICs) are elevated. At physiological pH, most fluoroquinolones are in their least ionized state, allowing a major fraction of the molecules to freely enter bacterial cells [7]. Low pH values markedly decrease activity of aminoglycosides. In contrast to this, acidic pH values almost linearly increase the activity of β-lactam antibiotics [6]. There is very limited data on the activity of colistin (CST) at various pH values. Burian et al. [8] showed that the activity of CST was gradually reduced by lowering the pH. Current data, although limited, are at a satisfying enough level to estimate the possible effects of pH on the activity of some antimicrobials alone. In addition, there are only a few scientific reports on the pH–antimicrobial activity relationship for combinations used in human medicine [5,9]. In contrast, there are no scientific studies on the activity of co-used antimicrobials being pH-dependent in the veterinary field. Therefore, the objective of this study was to investigate impact of pH on the synergistic activity of co-used antimicrobials: ceftriaxone (CRO) + CST, danofloxacin (DAN) + CST, DAN + ceftiofur (CEF), CEF + gentamicin (GEN). Six *E. coli* strains having different resistance profiles were utilized as subjects in this study.

2. Materials and methods

For isolation of *E. coli*, the swabs were directly inoculated onto specific agars and incubated in aerobic conditions. Candidate *E. coli* colonies were identified by API 20 E, and results evaluated by the API-Web system. Broth microdilution testing was performed to determine the MICs of the antimicrobials according to the guidelines of

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Clinical Laboratory Standards Institute [10]. MICs were defined as the minimum concentration of antibiotic that inhibited growth of the organism. Random amplified polymorphic DNA (RAPD) analysis was used to determine the genetic relatedness of *E. coli* isolates from animals. Six representative *E. coli* isolates from cattle were chosen from different RAPD patterns based on their resistance profiles. The activities of CRO + CST, DAN + CST, DAN + CEF, and CEF + GEN were tested with the fractional inhibitory concentration (FIC) method at pH values of 5.0, 6.0, 7.3, and 8.0. Mueller–Hinton broth medium was used for FIC, and the pH values were adjusted using a pH meter by the addition of sterile 1N HCl and 1N NaOH solutions [7,11,12]. The inoculum was 10^5 CFU per well and the microplates were incubated at 37 °C. FICs were evaluated after 16–18 h for *E. coli* isolates. FICs (MIC of antibiotic in combination)/(MIC antibiotic alone) of the combinations were determined using the checkerboard method and summed to give åFIC indices, which were interpreted as follows: FICI ≤ 0.5 = synergy; FICI > 4.0 = antagonism; FICI > 0.5–4 = indifference.

3. Results

The 6 *E. coli* isolates chosen for the study were from 5 different epidemiological origins according to their RAPD patterns. RAPD patterns and the antimicrobial resistance profiles of the *E. coli* strains are given in the Table. The FICIs of the CRO + CST combination were calculated at different pH values (Figure 1). FICIs ranged from 0.28 to 12 for pH 5.0; from 0.5 to 12 for pH 6.0; from 0.37 to 12 for pH 7.3; from 0.37 to 12 for pH 8.0. Synergistic activity at pH values of 5.0, 6.0, 7.3, and 8.0 was observed for 2/6, 2/6, 2/6, and 3/6 of the *E. coli* isolates, respectively. Antagonism was detected for 3 or 4 of the *E. coli* isolates at each pH value tested. FICIs of the DAN + CST combination ranged from 0.63 to 8 for pH 5.0; from 4.03 to 8 for pH 7.3; from 0.28 to 8 for pH 8.0 (Figure 2). FICI was 8 for all *E. coli* isolates at pH 6. Synergistic activity was observed for 2/6 of the *E. coli* isolates at pH 8. FICIs of the DAN + CEF combination ranged from 0.38 to 8 for pH 5.0; from 0.16 to 8 for pH 6.0; from 0.03 to 1.03 for pH 7.3; from 0.03 to 0.3 for pH 8.0 (Figure 3). Synergistic activity at pH values of 5.0, 6.0, 7.3, and 8.0 was observed for 2/6, 4/6, 5/6, and 6/6 of *E. coli* isolates, respectively. Antagonism was detected for 3/6 of *E. coli* isolates at pH 5.0 and for 1/6 for pH 6.0. FICIs of the CEF + GEN combination ranged from 0.03 to 8 for pH 5.0; from 0.03 to 8 for pH 6.0; from 0.03 to 1.13 for pH 7.3; from 0.03 to 1.5 for pH 8.0 (Figure 4). Synergistic activity at pH levels of 5.0, 6.0, 7.3, and 8.0 was observed for 2/6, 2/6, 4/6, and 3/6 of *E. coli* isolates, respectively. The highest synergism rate was observed at pH 7.3.

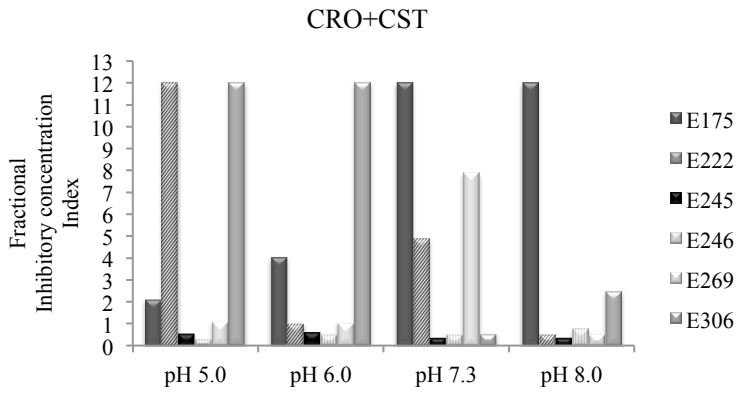
4. Discussion

Although there are a sufficient number of reports about the influence of pH on the activity of antimicrobials used alone, there is no data on pH-dependent activity of co-used antimicrobials against resistant strains in the veterinary field. The pH at the site of infection is reduced to below the neutral value due to bacterial activity and host immune response [13]. pH-dependent activity is a consequence of the changes in known pharmacokinetics/pharmacodynamics of the compound arising from environmental drivers. The activity of fluoroquinolones is affected by acidification of the medium [11], as the high concentrations of H^+ ions at low pH values change the electric charge of the fluoroquinolone molecule and thus reduce the penetration of the drug to the bacterial cell [8]. However, finafloxacin exerts stronger activity at slightly acidic pH values while

Table. RAPD patterns and resistance profiles of *E. coli* strains.

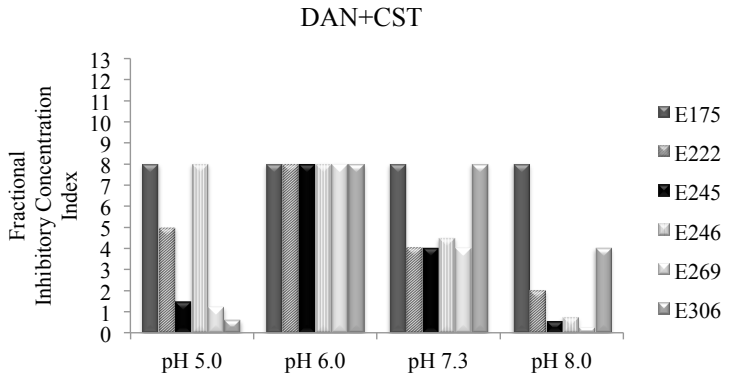
Isolate ID	RAPD Patterns	Resistance Profiles
E175	L	sulfamethoxazole
E222	T	nalidixic acid, ciprofloxacin, sulfamethoxazole, trimethoprim, tetracycline, oxytetracycline, chloramphenicol
E245	S	nalidixic acid, ciprofloxacin, orbifloxacin, gatifloxacin, ampicillin, ceftiofur, tetracycline, oxytetracycline, erythromycin, chloramphenicol
E246	S	nalidixic acid, gatifloxacin, ampicillin, trimethoprim, tetracycline, oxytetracycline, chloramphenicol, colistin
E269	E	nalidixic acid, sulfamethoxazole, trimethoprim, tetracycline, oxytetracycline, colistin
E306	C	nalidixic acid, ciprofloxacin, orbifloxacin, ampicillin, trimethoprim, tetracycline, oxytetracycline, erythromycin, chloramphenicol

RAPD: Random amplified polymorphic DNA.
L, T, S, E, and C: Each RAPD pattern.



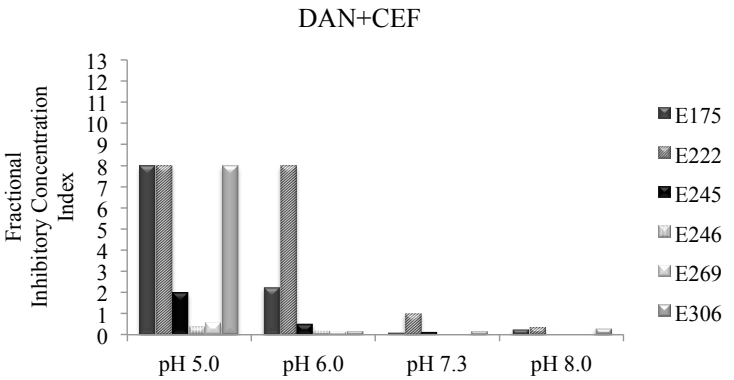
CRO: Ceftriaxone, CST: Colistin

Figure 1. Fractional inhibitory indexes of the ceftriaxone + colistin combination against resistant *E. coli* strains.



DAN: Danofloxacin, CST: Colistin

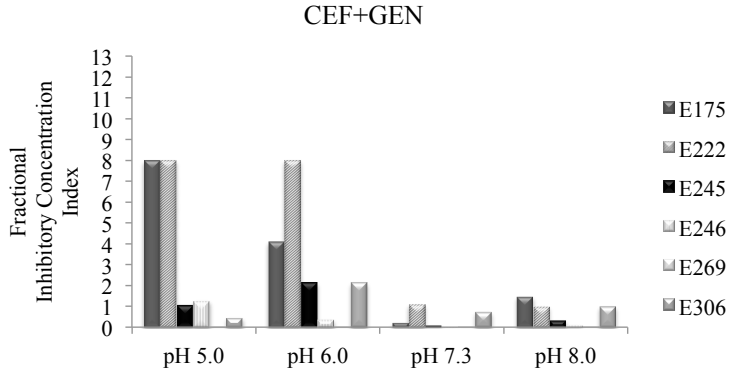
Figure 2. Fractional inhibitory indexes of the danofloxacin + colistin combination against resistant *E. coli* strains.



DAN: Danofloxacin, CEF: Ceftiofur

Figure 3. Fractional inhibitory indexes of the danofloxacin + ceftiofur combination against resistant *E. coli* strains.

MICs of moxifloxacin, levofloxacin, and ciprofloxacin have 2- to 8-fold increases at pH 5.8 and 6.2 [12]. Lowering the pH of the medium from pH 7.4 to pH 5.5 decreases the MICs up to 512-fold for widely used cephalosporins such cephalexin, cefuroxime, cefoxitin, ceftriaxone, and ceftobiprole. The activity of trimethoprim alone and the trimethoprim–sulfamethoxazole combination significantly increases at higher pH values. However, this combination



CEF: Ceftiofur, GEN: Gentamicin

Figure 4. Fractional inhibitory indexes of the ceftiofur + gentamicin combination against resistant *E. coli* strains.

results in different activity against various strains [14]. Some bacterial strains are more susceptible to gentamicin at pH values above 7, while the same strains are resistant to this antibiotic at pH values below 6 [14]. In this study, a remarkable increase in the antimicrobial activity was observed for DAN + CEF. Synergistic activity increased at high pH values (7.3, 8.0) against all strains except for *E. coli* E222 at pH 7.3. As has been reported previously, the activity of DAN and CEF alone increases at high pH and low pH values, respectively. The pH levels at which DAN + CEF exerted maximum synergism were pH 7.3 and 8.0. Similarly, the activity of the CEF + GEN combination was increased at pH levels 7.3 and 8.0. Higher pH values (7.3 and 8.0) decreased FICIs up to 10-fold, and synergistic activity increased 2-fold. Acidic pH values markedly reduced the activity of gentamicin and increased the activity of β-lactam antibiotics [6,14]. In this study, CEF did not cause a decrease in the synergistic potential of either combination, while β-lactam antibiotics exerted optimum activity at acidic pH values. CRO + CST and DAN + CST combinations showed different activity at low and high pH values. The CRO + CST combination exerted more synergistic activity against *E. coli* strains than DAN + CST. Synergistic activity was observed against 3/6 *E. coli* isolates at pH values 7.3 and 8.0. Burian et al. found that CST exerted the highest activity against *E. coli* at pH values 7.0 and 8.0 [8]. The lowest synergistic activity was observed for the DAN + CST combination against only 2/6 *E. coli* isolates at pH 8.0. It can be concluded that the pH of the medium impairs the activity of the antimicrobial combinations. Although CRO and CEF are β-lactam antibiotics exerting optimal activity at acidic pH values, the synergistic activity of the co-used drugs reaches its highest level at pH values ≥7.3.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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