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EFFECT OF EDTA-TROMETHAMINE ON THE SUSCEPTIBILITY OF CAMPYLOBACTER JEJUNİ AND CAMPYLOBACTER COLİ TO ANTIMICROBIAL AGENTS

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Campylobacter jejuni ve Campylobacter coli'nin antibiyotiklere duyarlılığına EDTA-tromethamin'in etkisi

Özet: *Altı antimikrobiyal ajan (ampisilin, tetrasiklin, eritromisin, kloramfenikol, nalidiksik asit ve kolistin sülfat) ve EDTA-tromethamin ile kombinasyonlarının Campylobacter jejuni ve C. coli üzerindeki sinerjistik etkileri incelendi. EDTA-ampisilin, EDTA-tetrasiklin, EDTA-eritromisin ve EDTA-kolistin sülfat bileşimlerinin campylobacterler üzerinde belirgin sinerjistik etkileri görüldü. Kloramfenikol, EDTA-tromethamin ile birlikte kullanıldığında zayıf bir ortak etki belirlendi. EDTA-tromethamin ve nalidiksik asit kombinasyonunda belirgin bir etki artışı bulunmadı. C. jejuni ve C. coli'nin, EDTA-tromethamin ve antibiyotiklere duyarlılıkları arasında bir fark belirlenmedi.*

Summary: *Combinations of EDTA-tromethamine and 6 antimicrobial agents (ampicillin, tetracycline, erythromycin, chloramphenicol, nalidixic acid and colistin sulfate) were tested for synergistic activities against Campylobacter jejuni and C. coli. A marked synergistic action was seen when campylobacters were exposed to combinations of ampicillin-EDTA, tetracycline-EDTA, erythromycin-EDTA and colistin sulfate-EDTA. When chloramphenicol was mixed with EDTA-tromethamine, a slight synergistic effect was noticed. There was no synergistic effect recorded with combination of EDTA-tromethamine and nalidixic acid. There was no difference between the susceptibility of C. jejuni and C. coli to combinations of EDTA-tromethamine and antimicrobial agents.*

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Introduction

Cell surfaces of Gram-negative bacteria are demaged by exposure of cells to EDTA (ethylenediaminetetraacetic acid) (8). Tromethamine (tris-hydroxymethyl-aminomethane) enhances the effect of EDTA (5). Gram-negative bacteria exposed to EDTA-tromethamine have increased permeability to extracellular solutes and leakage of intracellular solutes (7). Such bacteria become more sensitive to the effect of lysosyme and antimicrobial agents (3). Combinations of certain antibiotics and EDTA-tromethamine cause a decrease in the MIC (minimal inhibitory concentration) for *Escherichia coli* (11), *Pseudomonas aeruginosa* (12) and *Proteus vulgaris* (14). Cilinically, combinations of EDTA-tromethamine and antibiotics have been used effectively in the treatment of pseudomonas rhinitis (10) and multiple fistulas (2) in dogs and metritis in mare (15). Cell surfaces of Gram-positive bacteria are more resistant to the effect of EDTA-tromethamine (13).

The purpose of the present study was to determine the inhibitory effect of combinations of 6 antimicrobial agents and EDTA-tromethamine on *C. jejuni* and *C. coli*.

Materials and Methods

Three *C. jejuni* and 6 *C. coli* strains isolated from animal origins by selective method were used in this study. All of *C. jejuni* and 4 of 6 *C. coli* were isolated from chickens, one *C. coli* came from a cow with mastitis and one *C. coli* came from an aborted sheep fetus. *Campylobacter spp.* were identified according to the established criteria (9), and stored at – 70 °C until used. Cephalothin resistant, nalidixic acid sensitive organisms that hydrolysed hippurate were identified as *C. jejuni* and those did not as *C. coli*.

The antimicrobial agents tested were ampicillin, colistin sulfate, nalidixic acid, tetracycline, erythromycin and chloramphenicol.

Minimal inhibitory concentrations (MIC) were determined by standard methods for agar dilution tests on Mueller-Hinton agar containing 5% defibrinated sheep blood. All strains stored frozen were subcultured on this medium under microaerophilic atmosphere. These bacteria were suspended to a concentration of 10^8 CFU/ml (colony forming unit per ml) in brucella broth. A 0.01 ml calibrated loop was used to spot the suspension onto plates of Mueller-Hinton

agar containing two-fold concentrations of antibiotics. This inoculum corresponded to 10^6 CFU per spot. The plates were incubated at 37 °C in microaerophilic atmosphere for 36 hours. The MIC was defined as the lowest concentration of drug allowing no visible growth. The MICs for the 6 antimicrobial agents were also tested on Mueller-Hinton agar supplemented with 1mM EDTA and 50 mM tromethamine in the same procedure. Appropriate controls for bacteria, antibiotics and EDTA-tromethamine were included in each test. Each experiment was performed twicely.

Results

The MIC for the 6 antimicrobial agents and antimicrobial agents plus EDTA-tromethamine are shown in Table 1. The addition of

Table 1. In vitro susceptibility of 3 *C. jejuni* and 6. *C. coli* strains to antimicrobial agents with or without EDTA-tromethamine.

ANTIBIOTIC	Number of strains		MIC (μg/ml)	
			Without EDTA	With EDTA-trom[a].
Ampicillin	C. jejuni	3	8	2
	C. coli	2	4	1
		2	2	0.5
		1	8	2
		1	32	8
Colistin	C. jejuni	1	512	512
		2	512	128
	C. coli	6	512	128
Erythromycin	C. jejuni	3	1	0.25
	C. coli	6	1	0.25
Chloramphenicol	C. jejuni	3	4	2
	C. coli	5	4	2
		1	64	32
Tetracycline	C. jejuni	1	2	2
		2	2	0.5
	C. coli	6	2	0.5
Nalidixic acid	C. jejuni	3	4	4
	C. coli	6	4	4

[a]1 mM EDTA plus 50 mM tromethamine

EDTA-tromethamine to medium with nalidixic acid showed no decrease in MIC. Marked decreases (4–fold) in the MIC were seen with combinations of erythromycin plus EDTA-tromethamine and ampicillin plus EDTA-tromethamine. A significant decrease (4–fold) in the MIC also occured with combinations of tetracycline plus EDTA-tromethamine and colistin sulfate plus EDTA-tromethamine except one *C. jejuni* strain. Chloramphenicol, when combined with EDTA-tromethamine had a two-fold decrease (not significant) in MIC for *C. jejuni* and *C. coli*. There was no difference between the susceptibility of *C. jejuni* and *C. coli* to the additive effect of EDTA-tromethamine. The origin of the organisms had also no effect on the results. In control group, 1 mM EDTA plus 50 mM tromethamine did not inhibit the growth of *C. jejuni* and *C. coli*.

Discussion and Conclusion

Sublethal injury to bacteria has significant practical consequences. Injury can include distruption of mebrans, DNA or RNA molecules and the loss of a number of metabolic functions (6). The principal action of EDTA-tromethamine on Gram-negative bacteria appears to be the extraction of functional divalent cations (Ca^{+2}, Mg^{+2},Zn^{2+}) from the cell wall, which in turn causes the release of phospholipid and a protein lipopolysaccharide complex (8). The loss of these substances affects the structural integrity of the cell wall and causes the formation of an osmotically fragile cell.

Antimicrobial agents are divided into groups based on their mode of action on bacterial cell (1). These sites of antimicrobial activity include; the bacterial cell wall (ampicillin); the cytoplasmic membrane (colistin sulfate); inhibition of protein synthesis at the ribosome level (chloramphenicol, tetracycline, erythromycin) and DNA inhibitors (nalidixic acid). In the present study, at least one antibiotic was chosen from each group, to observe whether the mode of action would affect the activity or not.

A significant decrease in the MIC occured with combinations of ampicillin and EDTA-tromethamine, in all strains of *C. jejuni* and *C. coli* studied. In other studies performed by using penicillin which acts on cell wall like ampicillin, it has been found that penicillin plus EDTA-tromethamine had decreased MIC for *E. coli* (11), *Ps. aeruginosa* (12) and *Pr. vulgaris* (14), but no effect on MIC for Gram-positive bacteria (13). The activity of EDTA-tromethamine plus ampi-

cillin on *C. jejuni* and *C. coli* may be explained by the site of action these agents on bacterial cell wall synthesis. Ampicillin inhibits the bacterial cell wall formation preventing cross linking of peptidogluycan strands (1). The action of EDTA may occur in the first step of the peptidoglycan biosynthesis where divalent cations (Mg^{+2} or Mn^{+2}) are required. The chelation of these cations by EDTA plus the action of ampicillin may explain their synergistic action on *C jejuni* and *C coli*.

No synergistic effect on campylobacters was recorded with combinations of nalidixic acid and EDTA-tromethamine. This has been supported by the findings of other studies in which it has been determined that combination of nalidixic acid with EDTA-tromethamine did not alter the MIC for *Ps. aeruginosa* (12) and *Pr. vulgaris* (14). But, a slight decrease in MIC for *E. coli* has been observed when the concentration of EDTA was increased (11).

The synergistic action, observed in the present study, with combinations of EDTA-tromethamine and colistin sulfate was not unexpected since both agents act on the cell wall. Variable results have been obtained in other studies (11, 12, 14), when EDTA-tromethamine was combined with polymyxin-B, another agent of which primary site of action is cytoplasmic membrane (4).

In the present study, the synergistic actions seen with tetracycline plus EDTA or erythromycin plus EDTA were also not unexpected. Tetracycline, erythromycin and chloramphenicol act at the ribosome level as inhibiting protein synthesis (1). This action, combined with the effect of EDTA which enhances the cell wall permeability and degrades ribosomes, may explain their synergistic action. It has been reported that synergistic effect occurs between combinations of EDTA-tromethamine and oxytetracycline or chloramphenicol on *E. coli* (11).

When the findings were compared, no significant difference was observed between two species, *C. jejuni* and *C. coli*, tested. This may show that there is no major structural difference between the cell wall composition of *C. jejuni* and *C. coli*.

In summary, the enhancement of antimicrobial activity in the presence of EDTA-tromethamine appears to be a function of increased permeability of bacterial cell, allowing greater penetration of antimicrobial agent. From a clinical view, this combined activity may have limited use because local infections due to *C. jejuni* and *C. coli* are rather scarce. But, it must be kept in mind that the addition of

EDTA and tromethamine to selective media which contain several antibiotics, may inhibit the growth of campylobacters.

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