ANTIMICROBIAL RESISTANCE AMONG *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM VARIOUS SOURCES IN THAILAND

Unchalee Tansuphasiri[1], Wiriya Matra[2] and Leelaowadee Sangsuk[3]

[1]Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok; [2]Department of Pharmacy, Ang Thong Hospital, Ang Thong; [3]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Abstract. Antimicrobial resistance among *Clostridium perfringens* isolated from feces of humans and pigs, food and other environmental sources was examined by testing of 201 PCR-confirmed strains for resistance to 7 antimicrobial agents. The minimal inhibitory concentrations (MICs) were determined by the agar dilution method. Overall, *C. perfringens* showed the highest resistance to tetracycline (56.2%), followed by imipenem (24.9%), metronidazole (9.5%), penicillin G (9%), vancomycin (4.5%), chloramphenicol (3%) and ceftriaxone (1%). The majority of the isolated strains from pig feces (77.8%), environment (72.7%), human feces (44.9%) and food (28%) showed resistance to tetracycline. Strains isolated from human feces only showed low resistance to ceftriaxone (2.5%) and vancomycin (10.1%). Penicillin G had high activity, with overall MIC_{50} and MIC_{90} of 0.06 and 1.0 μg/ml, respectively, and low rate of resistance (10-12% for strains isolated from humans, animals and food). Among 62.7% of antimicrobial resistant strains, 39.3% were resistant to a single drug and 23.4% were multiple-drug resistant (MDR). Of overall 47 MDR strains, 63.8% were derived from human feces and were resistant to two to six drugs.

INTRODUCTION

Clostridium perfringens, a spore-forming anaerobic bacillus, is widely distributed in the environment (soil, sewage, food, dust) and in the intestinal tracts of human and domestic animals. It has been recognized as one of the most important and common organism causing a broad spectrum of human and veterinary diseases. In human, it is responsible for a number of clinical conditions ranging from relatively mild food poisoning to the potentially life-threatening gas gangrene (Rood and Cole, 1991); in domestic livestock, it causes a wide range of enteric diseases (Songer, 1996). The role of *C. perfringens* and its enterotoxin in foodborne diarrhea as well as non-food related diarrhea, sporadic diarrhea, infectious diarrhea and antibiotic-associated diarrhea (AAD) are already well documented (Borriello *et al*, 1984; Mpamugo *et al*, 1995; Carman, 1997).

Correspondence: Unchalee Tansuphasiri, Department of Microbiology, Faculty of Public Health, Mahidol University, 420/1 Rajvithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2354-8528; Fax: 66 (0) 2354-8538
E-mail: unchalee@loxley.co.th

Recent attention has focused on the role of widespread use of antimicrobials in growth promotion and therapy of infections in food-producing animals as a potential transfer route of antimicrobial-resistant bacteria or the genes encoding antimicrobial resistance into the human food chain (Piddock *et al*, 2000). As a consequence, resistance is most common where there is heavy use of antimicrobials and appreciable host-to-host contact. Humans and animals live in close association with large numbers of bacteria, most of which are found in the large intestine, where they are exposed to antimicrobials, exchange genetic material with other bacteria and, on excretion, contaminate the environment or colonize other animals and humans (van den Bogaard *et al*, 2000). Since *C. perfringens* is an indicator bacterium that constitutes a natural part of the intestinal flora of both humans and animals, using this bacterial species makes it feasible to compare the levels of resistance between populations.

In Thailand, there are no data on antimicrobial resistance of *C. perfringens* isolated from various sources. The aim of the present study was to determine and compare the prevalence

and degree of resistance to 7 antimicrobials commonly used in human medicine in *C. perfringens* isolated from fecal samples of human and pig, food and other environmental sources by conventional culture and by a duplex-PCR assay.

MATERIALS AND METHODS

Samples

A total of 269 samples for isolation of *C. perfringens* were obtained from various sources including fecal samples from human (n = 74) and animals (n = 175), and food samples (n = 20). Human feces were from patients who attended hospitals in Bangkok and the vicinity during the period 2003-2004, and animal feces were collected from pigs at abattoirs in Nakhon Pathom Province, Thailand. The food samples were "Somtum Poo" (papaya salad with prickled rice-field crab) obtained from street vendors around the Victory Monument, Bangkok. Some *C. perfringens*, available as stock strains (n = 43) were originally isolated from other environmental sources, *ie* cosmetics (n = 4), herbs (n = 17), and food, drinking water, ice and used gloves (n = 22) at the National Institute of Health (NIH), Thailand.

Bacterial isolation and identification

Fecal samples. Specimens of feces were subjected to bacteriologic culture and identification as described previously (Tansuphasiri *et al*, 2002). Briefly, after heat treatment of samples at 80ºC for 20 minutes to kill vegetative cells, the samples were then streaked onto a tryptose-sulfite-cycloserine (TSC) agar (Merck) supplemented with 5% (w/v) egg yolk and incubated in an anaerobic jar at 37ºC for 24 hours. Approximately 3-5 black colonies surrounded by a zone of precipitate were subcultured onto Columbia blood agar (Difco). Isolates were presumptively identified by Gram staining and lecithinase production, and confirmed biochemically as *C. perfringens* based on the following tests: lactose and inositol fermentation, stormy fermentation in litmus milk, nitrate reduction, gelatinase production, and motility tests (FDA, 1998).

Food samples. "Somtum Poo" food samples were transported to the laboratory and processed immediately. A large piece of crab in the food sample was cut into small pieces using sterilized scissors, then 25 g portions were aseptically weighed into sterile stomacher bags, and homogenized for 1 minute in 250 ml of 0.1% peptone water (Difco) using stomacher (Stomacher 400, London, UK). One ml aliquots of the suspension were mixed with 9 ml of Reinforced clostridium medium (Scharlau, Spain). After incubation at 37ºC for 24 hours, a few drops of suspension were streaked onto TSC-egg yolk agar and the same procedure as described for fecal specimens for isolation and identification of *C. perfringens* were performed.

Duplex PCR

Three to five colonies from each primary isolation plate were used for PCR analysis. Template DNAs from all sample isolates were extracted by the boiling method. Positive and negative control DNA for PCR analysis were extracted from *C. perfringens* ATCC 12916 and ATCC 3624 reference strains using a home-made silica membrane-based spin column method as previously described (Tansuphasiri *et al*, 2004). PCR was performed using dual primers as described previously by Tansuphasiri (2001) for detection of *plc* and *cpe* encoding phospholipase C (PLC) and *C. perfringens* enterotoxin (CPE).

Antimicrobial susceptibility

The minimal inhibitory concentrations (MICs) were determined by the agar dilution procedure recommended by NCCLS (2001) using Brucella agar (Difco) supplemented with 5 μg of hemin and 1 μg vitamin K1 per milliliter. A total of 7 antimicrobials obtained from the indicated sources were used: ceftriazone (Roche), chloramphenicol (Sigma), penicillin G (Sigma), imipenem (Merck Sharp & Dohme), metronidazole (Biolab), tetracycline (Sigma), and vancomycin (Eli Lilly). Serial 2-fold dilutions of the antimicrobials were prepared and added to molten Brucella agar supplemented with hemin and vitamin K1, to obtain the following final concentrations: ceftriazone, 0.50-256 μg/ml; chloramphenicol, 1.0-128 μg/ml; penicillin G, 0.03-128 μg/ml; imipenem, 0.03-64 μg/ml; metronidazole, 0.06-256 μg/ml; tetracycline, 0.06-256 μg/ml; and vancomycin, 0.12-256 μg/ml.

Prior to antimicrobial susceptibility test, isolates were subcultured twice on Brucella blood agar plate (BBA). Colonies from 48-hour BBA

cultures were suspended in enriched thioglycollate medium without indicator (Difco), to a turbidity equivalent to that of a 0.5 McFarland standard. The inocula were applied to the antimicrobial-containing plates with a Steers-type replicator (Mast Lab, England) that delivered 1-2 μl per spot or a final concentration of approximately 10^5 CFU per spot. Media without antimicrobial were used as control and were inoculated before and after each antimicrobial-containing series of plates. Plates were incubated at 37ºC in an anaerobic jar for 48 hours. One control plate of each set was incubated aerobically to detect possible aerobic contamination and the other anaerobically to determine the viability of the organisms. The growth on the plates was interpreted. The MIC was defined as the lowest concentration of antimicrobial that yielded no growth as compared to control plates containing no antimicrobial. MIC_{50} and MIC_{90} was the concentration at which 50% and 90%, respectively, of strains was inhibited.

C. perfringens strains ATCC 13124 and *Bacteroides fragilis* ATCC 25285 were used as control organisms. The tests with these control organisms followed the procedures described above. MIC values obtained from control strains in parallel with *C. perfringens* tested strains fell within the acceptable range. Interpretation of susceptible (S) and resistance (R) of all isolates to each antimicrobial tested followed the recommendation of NCCLS (2001).

RESULTS

Detection of *C. perfringens*

Of the 269 samples examined, 80 samples (29.7%) could be identified as *C. perfringens* by PCR (data not shown). These 80 positive samples were derived from the following sources: human feces, 35 of 74 samples (47.3%); pig feces, 37 of 175 samples (21.1%), and food samples (Somtum Poo), 8 of 20 samples (40%).

Some *C. perfringens*, available as stock strains (n = 45), had been isolated at the NIH, Thailand, or received for verification from other laboratories. They were subcultured on blood agar and re-identified by both conventional biochemical tests and PCR analysis. Results showed that two strains were not *C. perfringens*, as they were gram-negative and negative for *plc* detection by PCR analysis, while the other 43 strains were positive for *plc*.

For detection of enterotoxigenic strains, 3-5 colonies from each primary isolation culture on TSC-egg yolk agar plate that showed the colony characteristic of *C. perfringens* (black colony surrounded by opalescence zone) were subjected for detection of *plc* and *cpe* by duplex PCR. A total of 201 colonies from primary culture were positive for *plc*, and all of these colonies after secondary growth on Columbia blood agar and biochemical identification indicated these colonies were *C. perfringens*, *ie* positive tests for stormy fermentation in litmus milk media, nitrate reduction, fermentation of lactose and inositol, gelatinase production, and non-motility. Only 17 out of 201 colonies (8.5%) were positive for both *plc* and *cpe* (the presence of 2 bands of 280-bp and 420-bp). All these enterotoxin gene-positive strains were isolated from 4 samples of human feces, and none from the other sources. However, one stock strain that had been isolated from canned fish at the NIH, Thailand, was also positive for *cpe*.

Antimicrobial susceptibility testing

A total of 201 strains of *C. perfringens*, *ie* 89 from human feces, 54 from pig feces, 25 from food (16 strains from "Somtum Poo" samples and 9 from stock cultures) and 33 from other environmental samples (*ie* herbs, cosmetics, drinking water, ice and gloves) were tested for their susceptibility to 7 antimicrobials. The breakpoints of each antimicrobial for susceptible, intermediate and resistant were according to NCCLS (2001), except in the case of vancomycin we used the breakpoint of vancomycin recommended by EUCAST (2004) for aerobic bacteria, and used *S. aureus* ATCC 29213 as a quality control strain in this study. The test with reference quality control strains for all antimicrobials tested were in the acceptable ranges (data not shown).

Tables 1 and 2 show the MIC values and percentage of resistance of *C. perfringens* isolated from various sources, and these results are summarized in Table 3. According to sources, strains isolated from pig feces showed the highest MIC_{50} and MIC_{90} of tetracycline (32 and 64 μg/ml, respectively). These MIC values of tetracycline were also high in strains isolated from

Table 1
MIC values (MIC_{50} and MIC_{90}) of *C. perfringens* strains isolated from various sources.

Source and antimicrobial agent	MIC (μg/ml)			Geometric mean (GM)
	Range	MIC_{50}	MIC_{90}	
Human feces (89)[a]				
Ceftriaxone	≤0.5-256	≤0.5	1	0.12
Chloramphenicol	1-128	2	8	2.86
Imipenem	0.12-32	8	32	8.18
Metronidazole	0.12-256	0.5	8	0.88
Penicillin G	0.03-128	0.06	1	0.11
Tetracycline	≤0.06- >128	8	64	8.27
Vancomycin	0.25-128	0.5	1	0.76
Pig feces (54)				
Cetriaxone	≤0.5-16	≤0.5	1	0.65
Chloramphenicol	1-64	2	16	3.38
Imipenem	0.03-32	2	8	1.18
Metronidazole	0.12-64	0.25	4	0.53
Penicillin G	≤0.03-4	0.06	1	0.11
Tetracycline	2- >128	32	64	23.22
Vancomycin	0.25-1	0.25	0.5	0.41
Food (25)				
Cetriaxone	≤0.5-32	≤0.5	2	0.28
Chloramphenicol	1-4	1	2	1.52
Imipenem	0.25-16	4	16	0.65
Metronidazole	0.12-64	0.25	0.5	0.48
Penicillin G	≤0.03-2	0.06	0.12	0.07
Tetracycline	≤0.06-64	4	16	1.87
Vancomycin	0.25-4	0.25	0.5	0.49
Other environmental sources (33)[b]				
Cetriaxone	≤0.5-2	0.5	1	0.90
Chloramphenicol	2-4	2	4	2.47
Imipenem	2-32	4	16	4.54
Metronidazole	0.12-1	0.5	0.5	0.42
Penicillin G	≤0.03-0.12	0.03	0.06	0.04
Tetracycline	≥0.06-64	16	32	6.72
Vancomycin	0.25-1	0.25	0.5	0.47

[a]Number of isolated strains.

[b]Stock strains isolated from herbs (n = 17), cosmetics (n = 4), and from drinking water, ice and used gloves (n = 12).

human feces (MIC_{50} and MIC_{90} of 8 and 64 μg/ml, respectively), food (4 and 16 μg/ml), and other environmental sources (16 and 32 μg/ml). MICs of imipenem showed the second highest values for all four group sources, with MIC_{50} and MIC_{90} of 8 and 32 μg/ml for human feces strains, 2 and 8 μg/ml for pig feces strains, 4 and 16 μg/ml for both food and environmental isolated strains. Isolated strains from human and pig feces had higher MIC_{90} of penicillin G, chloramphenicol, metronidazole and tetracycline when compared to food and environmental isolated strains. MICs of vancomycin and ceftriaxone showed low values with slight differences when comparing between each group of isolation.

Overall, penicillin G was the most active drug (Table 3), with MIC_{50} and MIC_{90} of 0.06 and 1 μg/ml, respectively, and geometric mean MIC was 0.09 μg/ml. Ceftriaxone and vancomycin were also highly active, with MIC_{50} and MIC_{90} of ≤0.5 and 1.0 μg/ml for ceftriaxone, and 0.25 and 0.5 μg/ml for vancomycin. Imipenem and tetracycline were the least active drugs, with MIC_{50} and MIC_{90} of 4 and 16 μg/ml for imipenem, and 16 and 64 μg/ml for tetracycline. Chloramphenicol and metronidazole were also highly active,

Table 2
Percentage of resistance of *C. perfringens* strains isolated from various sources.

Source	Number of strains	Resistance (%)						
		CF	CH	IM	ME	PE	TE	VA
Human feces	89	2.5	5.6	42.7	13.5	10.1	44.9	10.1
Pig feces	54	0.0	1.9	9.3	5.6	11.0	77.8	0.0
Food	25	0.0	0.0	12.0	16.0	12.0	28.0	0.0
Others	33	0.0	0.0	12.1	0.0	0.0	72.7	0.0
Total	201	1.0	3.0	24.9	9.5	9.0	56.2	4.5

Breakpoint : Ceftriaxone (CF), 32 μg/ml; Chloramphenicol (CH), 16 μg/ml; Imipenem (IM), 8 μg/ml; Metronidazole (ME), 16 μg/ml; Penicillin G (PE), 1 μg/ml, Tetracycline (TE), 8 μg/ml; Vancomycin (VA), 8 μg/ml.

Table 3
MIC values and percentage of resistance of *C. perfringens* strains tested against 7 antimicrobial agents.

Antimicrobial agent	MIC (μg/ml)			Geometric mean (GM) of MIC	Resistance (%)
	Range	MIC_{50}	MIC_{90}		
Ceftriaxone	≤0.5-256	≤0.5	1	0.29	1.0
Chloramphenicol	1-128	2	4	2.70	3.0
Imipenem	0.03-32	4	16	3.22	24.9
Metronidazole	0.12-256	0.25	8	0.63	9.5
Penicillin G	≤0.03-128	0.06	1	0.09	9.0
Tetracycline	≤0.06->128	16	64	8.77	56.2
Vancomycin	0.25-128	0.25	0.5	0.56	4.5

with MIC_{50} and MIC_{90} of 0.25 and 8.0 μg/ml for metronidazole, and 2 and 4 μg/ml for chloramphenicol. The MIC_{90} of chloramphenicol and metronidazole were less than the breakpoints.

C. perfringens showed the highest rate of resistance to tetracycline (56.2%), followed by imipenem (24.9%), metronidazole (9.5%), penicillin G (9%), vancomycin (4.5%), chloramphenicol (3%) and ceftriaxone (1%) (Table 2). The majority of the isolated strains from pig feces (77.8%), environmental sources (72.7%), human feces (44.9%) and food (28%) showed resistance to tetracycline. Isolated strains from human feces showed only 2.5% resistance to ceftriaxone. Interestingly, all vancomycin resistant strains (10.1%) were isolated from human feces only. None of the food strains was resistant to ceftriaxone, vancomycin and chloramphenicol, and none of the environmental strains was resistant to ceftriaxone, chloramphenicol, metronidazole, penicillin G and vancomycin.

The frequency distribution of resistance determinants (resistance pattern) of *C. perfringens* from the various sources is shown in Table 4; 62.7% of all strains tested showed resistance to one or more drugs whereas 37.3% of all isolates were not resistant. Among the antibiotic resistant strains, 39.3% were resistant to a single drug and 23.4% were multiple-drug resistant (MDR) with 13.9, 3.0, 3.0, 1.0 and 2.5% resistant to 2, 3, 4, 5 and 6 drugs, respectively. Isolated strains from pig feces and environmental sources showed high resistant determinant rate of 79.6% and 72.7% compared to strains from human feces (56.2%) and food (36%). Of 47 MDR strains, 30 (63.8%) were derived from human feces, 8 (17%) from pig feces, 5 (10.6%) from food, and 4 (8.5%) from environment sources.

Susceptibilities of 17 *C. perfringens* enterotoxigenic strains tested against 7 drugs are presented in Table 5. All of these strains were isolated from human feces. The data revealed

Table 4
Antimicrobial resistance pattern of *C. perfringens* strains isolated from various sources.

Antimicrobial resistance pattern	Total (n = 201)		Human feces (n = 89)		Pig feces (n = 54)		Food (n = 25)		Others (n = 33)	
	n	%	n	%	n	%	n	%	n	%
No resistance	75	37.3	39	43.8	11	20.4	16	64.0	9	27.3
Single resistance	79	39.3	20	22.5	35	64.8	4	16.0	20	60.6
Multiple resistance	47	23.4	30	33.7	8	14.8	5	20.0	4	12.1
2	28	13.9	18	20.2	3	5.5	3	12.0	4	12.1
3	6	3.0	1	1.2	4	7.4	1	4.0	0	0.0
4	6	3.0	4	4.5	1	1.9	1	4.0	0	0.0
5	2	1.0	2	2.2	0	0.0	0	0.0	0	0.0
6	5	2.5	5	5.6	0	0.0	0	0.0	0	0.0

Table 5
MIC values and percentage of resistance of enterotoxigenic *C. perfringens* strains isolated from human feces.

Antimicrobial agent	Range (μg/ml)	MIC_{90}	GM of MIC	Resistance (%)
Ceftriaxone	≤0.5-2	1	0.59	0.0
Chloramphenicol	2-4	4	2.17	0.0
Imipenem	4-16	8	5.10	5.9
Metronidazole	0.25-0.50	0.50	0.38	0.0
Penicillin G	0.03-0.12	0.06	0.05	0.0
Tetracycline	≤0.06-64	16	1.06	23.5
Vancomycin	0.50	0.50	0.50	0.0

n = 17

that tetracycline was the least active agent to *C. perfringens* enterotoxigenic strains with the rate of resistance 23.5% followed by imipenem (5.9%). None was resistant to ceftriaxone, chloramphenicol, penicillin G and vancomycin. Among these drugs, penicillin G was the most active drug, with MIC_{90} of 0.06 μg/ml (GM = 0.05 μg/ml) followed by vancomycin with MIC_{90} of 0.5 μg/ml (GM = 0.50 μg/ml). Antimicrobial susceptibility of enterotoxigenic *C. perfringens* strains could not be compared with non-enterotoxigenic strains because of the small number of samples.

DISCUSSION

This study focused on *C. perfringens* as this anaerobic, spore-forming bacterium is widely distributed in the environment, and is commonly found as a member of the normal intestinal flora of man and animals used as fecal indicator. It causes a broad spectrum of human and veterinary diseases due to the toxins produced, with at least 14 different protein-toxins found to be produced by this organism (Rood and Cole, 1991). Enterotoxin-positive strains of *C. perfringens* are recognized as the cause of foodborne diarrhea as well as several non-food related diarrhea, including sporadic diarrhea and antibiotic-associated diarrhea (Carman, 1997).

In Thailand, few data are available on the detection of this organism. Analysis of foodborne anaerobic bacteria are not routinely performed due to difficulties in isolation and identification. The procedures require special techniques that may be expensive, time-consuming and labor intensive. For this reason, outbreaks of *C. perfringens* often are not recognized. Also, knowledge of antimicrobial susceptibility of this organism isolated from human, animals, foods and environment is limited. Most reports on antimicrobial resistance are for facultative anaero-

bic bacteria isolated from patients or from sick and dying animals.

In this study, the enterotoxigenic and non-enterotoxigenic *C. perfringens* strains isolated from human and animal feces, food and environmental samples were identified by using biochemical tests and a duplex-PCR procedure that enables *C. perfringens* species identification and differentiation between enterotoxigenic and non-enterotoxigenic strains (Tansuphasiri, 2001). In addition, the MICs of 7 antimicrobial agents commonly used in human medicine for all *C. perfringens* isolated strains were determined using the reference agar dilution method.

For food samples, we purposely focused on isolating *C. perfringens* from "Somtum Poo", a tropical food from the north-eastern area of Thailand. The "Somtum Poo" is normally prepared freshly and contains a small pickled crab. The crab from the rice field is not cooked and may be contaminated with soil and water containing *C. perfringens*. This food is very popular among Thai people and generally is sold by street vendors in the crowded areas which may also be a cause of dust contamination. A number of *C. perfringens* available as stock strains, isolated from several Thai food and canned food sources, was also re-identified by the duplex PCR and one strain from canned fish was positive for *cpe*. As expected, 8 of 20 samples (40%) of "Somtum Poo" were contaminated with *C. perfringens*, but they were non-enterotoxigenic strains when tested by the duplex PCR.

For fecal specimens, we examined both human and pig feces, because human and animal live in close association with large numbers of bacteria, of which the majority are found in the large intestine, where the intestinal flora of healthy animals and humans is considered to be the most important reservoir of resistant bacteria and resistant genes (Murray, 1997). As contamination of carcasses with fecal flora during slaughtering inevitably occurs, food of animal origin may serve as a vehicle to transport resistant bacteria and resistant genes between animal and man (van den Bogaard *et al*, 2000). A risk to humans exists when the antimicrobial used in animals is also used in human medicine.

In this study, tetracycline was the least active agent to *C. perfringens* with overall rate of resistance 56%. Most of these tetracycline resistant strains (77.8%) were isolated from pig feces. This drug has been used widely in the treatment of animal infections and also used inappropriately in animals as growth-promoting and prophylactic agents rather than to treat infection. Studies of the occurrence of antimicrobial resistance in *C. perfringens* from pigs in South Western Australia have shown that the percentage of isolated *C. perfringens* resistant to tetracycline is significantly higher in piggeries using a number of antimicrobials than from the one piggery that did not use antimicrobials (Khachatourians, 1998). The antimicrobials to which food animals are exposed provide selective pressure leading to the appearance and persistence of drug-resistant strains. These antimicrobial resistant bacteria arising from agricultural practices may enter human environment and move about with people and goods. Also the increased use of antimicrobials in farming, together with the practice of raw sewage discharge into receiving waters, has resulted in a significant increase in the number of antimicrobial resistant bacteria present in aquatic environments (Young, 1993).

In this study, *C. perfringens* isolated from human feces also showed high tetracycline MIC values with the rate of resistance of 45%. Tetracycline resistance in *C. perfringens* is primarily due to the presence of TetA(P) which is an integral inner-membrane protein that mediates the active efflux of tetracycline from the cell (Bannam and Rood, 1999).

Of the three antimicrobials that were highly active against *C. perfringens* with the low MIC values, ceftriaxone could inhibit the most of *C. perfringens* strains tested with overall rate of ceftriaxone resistance of 1%. Penicillin G is still an effective drug based upon its low MIC values and low rate of resistance (9% overall or 10% among strains isolated from human feces) and may be considered when choosing an antimicrobial agent for prophylaxis or treatment of *C. perfringens* in both humans and animals.

This study also revealed a high prevalence of antimicrobial-resistant *C. perfringens* strains. Among 62.7% of antimicrobial resistant strains, 39.3% were single drug resistance and 23.4% were MDR. Most of MDR strains (63.8%) were derived from human feces (33.7% among human feces strains) and were resistant to two to

six drugs. Most of human feces strains (20.2%) showed resistance to two drugs and 5.6% were resistant to six drugs tested, whereas most of the strains from other sources showed resistance to two or three drugs. This may reflect the widespread or inappropriate use of antimicrobials in both human medicine and in animals resulting in the emergence of MDR strains. High prevalence and degree of antimicrobial resistance found in indicator bacteria in the fecal flora of humans and animals are considered to be indicators of antimicrobial usage. They correlate with the amounts and types of antimicrobials consumed by these populations and changes in resistance can be considered as an early warning system for resistance to be expected in potentially pathogenic bacteria (van den Bogaard *et al*, 2000). In food animals a low prevalence and low degree of antimicrobial resistance in the intestinal flora should be considered a distinguishing quality and safety mark.

We found the presence of enterotoxigenic strains not to be significant, since only 4 samples from 74 fecal specimens of human were positive for enterotoxigenic *C. perfringens*. These fecal samples were obtained from hospitalized patients with nondiarrheal diseases, so they might harbor a small number of these enterotoxigenic strains in their intestines. However, it suggests the need of more studies to evaluate the role of enterotoxigenic *C. perfringens* in patients with foodborne diarrhea as well as non-food related diarrhea, sporadic diarrhea, infectious diarrhea and antibiotic-associated diarrhea. These organisms must be looked for routinely, and a periodic evaluation of antimicrobial susceptibility should be performed. In this study, enteroxigenic strains were resistant only to tetracycline (23.5%) and imipenem (5.9%).

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