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Antimicrobial susceptibility of canine *Clostridium perfringens* strains from Switzerland

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Abstract

Fifty *Clostridium perfringens* strains were isolated from individual dogs with acute diarrhoea that were not given antibiotics. Toxin types and minimal inhibitory concentrations of 15 antibiotics were determined for each of them. All strains harboured the α-toxin gene, 12 of them had both the α- and entero-toxin gene and 5 had both the α- and β_2-toxin gene. Eighteen percent of the isolates showed resistance to tetracycline and 54 % showed decreased susceptibility to metronidazole which is one of the most frequently used antibiotics in the treatment of canine diarrhoea. Apart from that, all isolates were susceptible to the remaining antibiotics tested. These findings lead to the conclusion that despite a general susceptibility to antibiotics in *C. perfringens*, resistance is developing in isolates from dogs. Therefore, careful identification of the pathogenic agent and antibiotic susceptibility testing should be performed prior to therapy in order to minimise further selection of antibiotic resistance.

Keywords: anaerobes, antibiotic resistance, dog, diarrhoea, MIC

Antibiotika-Empfindlichkeit von *Clostridium perfringens* bei Hunden aus der Schweiz

Fünfzig *Clostridium perfringens* Stämme wurden aus Kot von Hunden mit akutem Durchfall isoliert. Die Hunde erhielten vorhergehend keine Antibiotika. Für jedes Isolat wurden die Toxingene und die minimale Hemmkonzentration von 15 Antibiotika bestimmt. Alle Isolate enthielten das α-Toxin-Gen, 12 enthielten sowohl das α- als auch das entero-Toxin-Gen und 5 sowohl das α- als auch das β_2-Toxin-Gen. Fast zwanzig Prozent der Isolate waren resistent gegen Tetracyclin und 54 % zeigten verminderte Empfindlichkeit gegenüber Metronidazol, eines der am häufigsten eingesetzten Antibiotika für die Behandlung von Durchfall bei Hunden. Gegen die restlichen getesteten Antibiotika wurden keine Resistenzen gefunden. Diese Resultate zeigen, dass trotz generell unproblematischer Resistenzlage bei *C. perfringens*, sich Resistenzen bei Isolaten von Hunden entwickeln. Deshalb ist eine sorgfältige Identifikation des verursachenden Erregers und Empfindlichkeitstestung desselben vorgängig einer Therapie wichtig.

Schlüsselwörter: Anaerobe Erreger, Antibiotikaresistenz, Hund, Diarrhoe, MHK

Introduction

Clostridium perfringens is a gram positive, rod-shaped, and endospore forming bacterium which grows under strict anaerobic conditions. *C. perfringens* is ubiquitous in nature but is mostly found in soil, where it is able to persist due to its ability to form highly resistant spores (Hatheway et al., 1998). In animals and humans, it is mostly isolated from the intestinal tract and, to a smaller extent, from the urogenital and respiratory tract (Allen et al., 2003). Although *C. perfringens* is a normal inhabitant of these areas (Allen et al., 2003), it may often cause diarrhoea and other infections in various animal species including dogs (Kather et al., 2006; Songer et al., 1996) and is therefore considered a pathogen in human and veterinary medicine. Intestinal infections in dogs are mainly caused by *C. perfringens* producing the major toxin α, sometimes associated with an additional β_2- or entero-toxin (Weese et al., 2001; Sasaki et al., 1999). Although the significance of *C. perfringens* as a cause of canine diarrhoea is controversial (McKenzie et al. 2010; Sasaki et al., 1999), intestinal infections with *C. perfringens* are routinely treated with antibiotics (German et al., 2010), thus imposing selection pressure on the bacteria and selecting for resistant strains. In *C. perfringens*, several resistance mechanisms have already been described so far,

e.g. those of the tetracycline, chloramphenicol and macrolide-lincosamide-streptogramin resistance (Park et al., 2010; Abraham et al., 1987; Dutta et al., 1981). However, only few studies on the antimicrobial resistance profile of *C. perfringens* in human and animals were conducted so far (Tansupharisi et al., 2005; Marks et al., 2003), and the resistance situation of strains found in Switzerland is unknown. The objective of this study was to obtain an overview on antibiotic susceptibility among *C. perfringens* isolated from dogs with acute diarrhoea in Switzerland.

Animals, Material and Methods

Isolation and typing of strains

C. perfringens were isolated from 50 fecal swabs taken from individual dogs with symptoms of acute diarrhoea presented to a small animal practice in the canton of Vaud, Switzerland. The dogs were not premedicated with any antibiotics. Swabs were streaked onto membrane *Clostridium perfringens* agar plates (mCP; Oxoid, Basel, Switzerland) and the plates were incubated under anaerobic conditions at 37 °C for 24 hours. Colonies on mCP agar plates displaying typical colony morphology were subcultivated onto tryptone soy agar containing 5 % sheep blood (TSA-SB; Becton Dickinson, Basel, Switzerland). Colonies displaying a double hemolysis on TSA-SB agar were tested by multiplex PCR for the presence of the *C.perfringens*-specific α-, β-, β_2-, ε-, entero- and ι-toxin genes as described previously (Albini et al., 2008).

Antibiotic susceptibility testing

Minimal inhibitory concentrations (MICs) were determined in Brucella broth supplemented with lysed horse blood (TREK Diagnostic Systems, East Grinstead, West Sussex, United Kingdom) by using customized Sensititre susceptibility plate ANO2B (TREK Diagnostic Systems) according to the guideline M11-A7 of the Clinical and Laboratory Standards Institute (CLSI, 2007). MIC values were defined as the lowest concentration of antibiotic exhibiting no visible growth of bacteria for the following antibiotics: ampicillin/sulbactam, amoxicillin/clavulanic acid, ampicillin, cefotetan, cefoxitin, chloramphenicol, clindamycin, imipenem, meropenem, metronidazole, mezlocillin, penicillin, piperacillin, piperacillin/tazobactam, and tetracycline. Breakpoints used in this study were those for gram positive anaerobes as defined by the European Committee of Antimicrobial Susceptibility Testing (EUCAST; online: www.eucast.org) except for cefotetan, cefoxitin, mezlocillin and tetracycline, for which CLSI breakpoints for anaerobes according to the informational supplement M100-S21 were used (CLSI, 2011). *Bacteroides fragilis* (ATCC 25285) and *Bacteroides thetaiotaomicron* (ATCC 29741) were used for quality control and MIC values were within acceptable ranges.

Results

All the 50 *C. perfringens* isolates were found to harbour the α-toxin gene. Among them, 12 (24 %) had both the α- and entero-toxin gene and 5 (10 %) contained both the α- and β_2-toxin gene. Minimal inhibitory concentrations and the MIC_{50} and MIC_{90} values of all antibiotics tested for all *C. perfringens* isolates are given in Table 1. All isolates were susceptible to ampicillin/sulbactam, amoxicillin/clavulanic acid, ampicillin, cefoxitin, cefotetan, chloramphenicol, clindamycin, meropenem, mezlocillin, imipenem, penicillin, piperacillin, and piperacillin/tazobactam with MICs situated below the resistance breakpoint. Nine (18 %) isolates were resistant to tetracycline with an MIC of ≥ 16 µg/ml. Although all isolates were susceptible to metronidazole, 27 (54 %) isolates showed a decreased susceptibility with an MIC of 4 µg/ml, which is just one two-fold dilution below the EUCAST breakpoint (MIC > 4 µg/ml) for this drug (Tab. 1).

Discussion

The results of the above described MIC assessment showed that canine *C. perfringens* isolates display a generally high susceptibility for antibiotics commonly used against anaerobic pathogens. Similar investigations conducted in other countries than Switzerland showed comparable results with only few strains showing resistance to antibiotics routinely used to treat *C. perfringens* infections (Marks et al., 2003). For instance, isolates analysed in this study still displayed low MICs to beta-lactam antibiotics, which are among the most commonly used antibiotics in therapy of canine diarrhoea (German et al., 2010). However, resistance to beta-lactam antibiotics has been found in *C. perfingens* indicating that *C. perfringens* is able to also acquire resistance to this class of drugs (Williamson, 1983). Of note, more than 50 % of the tested isolates showed a decreased susceptibility to metronidazole (4 µg/ml; MIC_{50} and MIC_{90} = 8 µg/ml) which is also a first choice drug for therapy of diarrhoea in dogs (German et al., 2010). Other studies reported lower MICs to this antibiotic (MIC_{50} of 0.25–1 µg/ml and MIC_{90} of 0.5–8 µg/ml) (Tansuphasiri et al., 2005; Marks et al., 2003) indicating that emergence of resistance to this drug in isolates from Switzerland is possible. Almost one fifth (18 %) of the *C. perfringens* isolates were resistant to tetracycline with MICs above 8 µg/ml (MIC_{50} = 8 µg/ml, MIC_{90} > 8 µg/ml). Tetracycline resistance is frequently found in *C. perfringens* with studies reporting 21 % to 54 % of resistance to this drug (Park et al., 2010; Tansuphasiri et al. 2005; Marks et al., 2003) due to the acquisition of tetracycline resistance genes (Kather et al., 2006; Lyras et al., 1996).
All *C. perfringens* isolates contained toxin-genes known to cause enteritis and diarrhoea in dogs. Other studies in other countries also reported the presence of similar toxin-gene profiles with α- and entero-toxin being the most

Table 1: Minimal inhibitory concentration (MIC) of 15 antibiotics for 50 *Clostridium perfringens* isolates from dogs.

	Number of strains with MIC (µg/ml) of													
	≤ 0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	MIC50	MIC90
Antimicrobial														
Ampicillin/sulbactam		50											≤ 0.25	≤ 0.12
Amoxicillin/clavulanic acid		50											≤ 0.12	≤ 0.12
Ampicillin		50											≤ 0.12	≤ 0.12
Cefotetan						50							≤ 2	≤ 2
Cefoxitin				25		21	4						≤ 1	4
Chloramphenicol							50						4	4
Clindamycin		27		3	7	10	3						≤ 0.12	4
Imipenem	34		9	7									≤ 0.12	0.5
Meropenem			50										≤ 0.25	≤ 0.25
Metronidazole					1	22	27						8	8
Mezlocillin						50							≤ 2	≤ 2
Penicillin	19	22	8	1									0.25	0.5
Piperacillin						50							≤ 2	≤ 2
Piperacillin/tazobactam		50											≤ 0.12	≤ 0.12
Tetracycline		6		1			6	28	9				8	> 8

The dilution ranges tested for each antibiotic are those contained within the white area. Values situated above or below this range indicate MIC values higher than the highest concentration tested and values smaller than or equal to the lowest concentration tested respectively. Resistance breakpoints for anaerobes (vertical lines) were obtained from the European Committee of Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) (see Animals, Material and Methods).

frequent toxins found in *C. perfringens* from dogs (Sasaki et al., 1999; Weese et al., 2001).

Based on the findings in this study and considering that *C. perfringens* is one of the most often isolated bacteria from the intestinal tract of animals and humans, it can be stated, that canine *C. perfringens* still appears to be susceptible to the major antibiotic classes. Yet, resistance to tetracycline has been found and decreased susceptibility to metronidazole has been observed in the isolates from this study which may suggest slow adaption of *C. perfringens* to this first choice drug for treatment of canine diarrhoea. Consequently, a correct identification of the causative agent and accurate antibiotic susceptibility testing prior to therapy of diarrhoeic dogs is recommended for an adequate therapy of *C. perfringens* infections.

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Sensibilité aux antibiotiques des Clostridium perfringens chez les chiens en Suisse

Cinquante souches de *Clostridium perfringens* ont été isolées de chiens atteints de diarrhée aiguë. Ces chiens n'avaient précédemment pas reçu d'antibiotiques. Pour chaque isolat on a cherché les gènes de toxines et déterminé la concentration inhibitrice minimale de 15 antibiotiques. Tous les isolats contenaient le gène de l'α-toxine, 12 d'entre eux contenaient les gènes de l'α- et de l'entero-toxine et 5 les gènes de l'α- et de la β_2-toxine. Près de 20 % des isolats étaient résistants

Sensibilità agli antibiotici del Clostridium perfringens nei cani in Svizzera

Dalle feci di cani che presentavano diarrea acuta, sono stati isolati 50 ceppi di *Clostridium perfringens*. In precedenza questi cani non avevano assunto antibiotici. Per ogni isolato sono stati determinati i geni delle tossine e la concentrazione minima inibente di 15 antibiotici. Tutti gli isolati contenevano il gene dell'α-tossina, 12 contenevano i geni dell'α- e dell'entero-tossina e 5 i geni dell'α- e della β_2-tossina. Quasi il venti percento degli isolati risultavano resistenti alla

à la tetracycline et 54 % présentaient une sensibilité diminuée au métronidazole, l'un des antibiotiques les plus fréquemment utilisés pour le traitement des diarrhées chez le chien. On n'a pas trouvé de résistance aux autres antibiotiques testés. Ces résultats montrent que, malgré un taux de résistances peu élevé chez les souches de *C. perfringens*, certaines résistances peuvent apparaître chez des souches isolées de chiens. C'est pourquoi une identification précise de l'agent causal et un test de sensibilité de celui-ci devraient être effectués avant un traitement.

tetraciclina e il 54 % mostravano una ridotta sensibilità al metronidazolo, uno degli antibiotici più comunemente usato per il trattamento della diarrea nei cani. Agli antibiotici rimanenti non si è riscontrata alcuna resistenza. Questi risultati dimostrano che nonostante i ceppi di *C. perfringens* sono generalmente suscettibili agli antibiotici, negli isolati provenienti dai cani si sviluppano resistenze. Perciò è importante effettuare prima di iniziare una terapia, un'accurata identificazione dell'agente patogeno e un test di sensibilità.

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