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 β₂-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

**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 β₂ 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.

### 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-toxin gene. Minimal inhibitory concentrations and the MIC₅₀ and MIC₉₀ values of all antibiotics tested for all *C. perfringens* isolates are given in Table 1. All isolates were susceptible to ampicillin/sublactam, 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. perfringens* 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; MIC₅₀ and MIC₉₀ = 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 (MIC₅₀ of 0.25–1 μg/ml and MIC₉₀ of 0.5–8 μg/ml) (Tansupharisi 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 (MIC₅₀ = 8 μg/ml, MIC₉₀ > 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...
Antimicrobial susceptibility of canine Clostridium perfringens strains

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).

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

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>≤ 0.06</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
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<th>32</th>
<th>64</th>
<th>128</th>
<th>MIC50</th>
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<tr>
<td>Ampicillin/sulbactam</td>
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<td>Amoxicillin/clavulanic acid</td>
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<td>Cefotetan</td>
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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 *Clostridium 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.

### References


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