

ANTIMICROBIAL SUSCEPTIBILITY OF *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM PIGLETS WITH OR WITHOUT DIARRHEA IN BRAZIL

Felipe Masiero Salvarani¹, Rodrigo Otávio Silveira Silva¹, Prhiscylla Sadanã Pires¹, Eduardo Coulaud da Costa Cruz Júnior², Isabella Silva Albefaro¹, Roberto Maurício de Carvalho Guedes², Francisco Carlos Faria Lobato^{1*}

¹Universidade Federal de Minas Gerais, Departamento de Medicina Veterinária Preventiva, Laboratório de Bacteriose e Pesquisa, Belo Horizonte, MG, Brasil; ²Universidade Federal de Minas Gerais, Departamento de Clínica e Cirurgia Veterinárias, Laboratório de Patologia Animal, Belo Horizonte, MG, Brasil.

Submitted: May 04, 2011; Returned to authors for corrections: October 30, 2011; Approved: June 07, 2012.

ABSTRACT

The minimum inhibitory concentration (MIC) was determined for 13 antibiotics against *Clostridium perfringens* isolated from Brazilian piglets. The collection of isolates was performed in June to October 2010. All isolates were susceptible to amoxicillin and ceftiofur, whereas most were resistant to tetracycline and lincomycin. Avilamycin and narasin were more effective against isolates from non-diarrheic than from diarrheic piglets. The other antimicrobials were less active in need of high concentrations to inhibit the growth of the *C. perfringens* type A. These results suggest the need for further studies evaluating molecular factors related to the antimicrobial resistance of *C. perfringens*.

Key words: *Clostridium perfringens*, piglet, diarrhea, antibiotic.

Clostridium perfringens type A is an anaerobe commonly isolated from the guts of piglets and is considered a normal inhabitant of piglet intestines. Current evidence suggests that this microorganism is involved in diarrhea in nursing piglets, especially those less than four days of age (17). The main source of immediate post-farrowing infection in piglets is spores in the environment and in the feces of sows (8). Data concerning the prevalence of this pathogen in piglets are rare in the scientific literature. A retrospective study conducted at the Veterinary Diagnostic Laboratory at Iowa State University, USA identified *C. perfringens* type A as the main causative agent of neonatal diarrhea in the years 2005 and 2006, composing 48% of the 273 strains isolated from diarrheic piglets (22).

Previously, only alpha toxin and enterotoxin were thought to play a role in the pathogenicity of *C. perfringens* type A-related diarrhea in piglets. However, Songer and Glock (17) suggested that these toxins are not the ones responsible for lesions and liquid accumulation in the intestinal tract of diseased animals. Rather, a novel toxin encoded by the *cpb2* gene, called beta-2 toxin, is speculated to be the main factor driving the development of clinical diarrhea by *C. perfringens* type A in neonatal piglets (19). The adoption of a high pig density in intensive systems and other recent changes in Brazilian pig farming have increased the infection pressure and the stress levels to which animals are subjected. Therefore, conditions facilitate the emergence of pathogenic agents that had once coexisted in equilibrium with the

*Corresponding Author. Mailing address: Escola de Veterinária da UFMG, Departamento de Medicina Veterinária Preventiva, Laboratório de Bacteriose e Pesquisa.; E-mail: flobato@vet.ufmg.br

host and its microbiota. Antibiotics have become an essential tool for pig farmers as a means of the therapy, prophylaxis, and growth promotion (1).

In contrast, the World Health Organization has questioned the use of antibiotics as growth promoters. Therefore, a number of countries, such as members of the European Union, have reduced the use of antibiotics. The use of medicated feed in swine production was restricted due to the risk of antibiotic residues in the meat and in the selection of resistant strains, which could lead to human infections with resistant bacteria (2,20). In Brazil, antimicrobials are still widely used in swine production, and little is known about *C. perfringens* antibiotic susceptibility. The objective of this work was to evaluate the minimum inhibitory concentration (MIC) of 13 antibiotics against 50 *C. perfringens* type A isolates isolated from neonatal piglets with or without diarrhea.

The *C. perfringens* type A isolates studied were isolated from one- to seven-day-old piglets with or without diarrhea. Samples were taken from 15 different pig farms located at Triângulo Mineiro and Alto Paranaíba, Minas Gerais, an important swine-producing region of Brazil. The collection of isolates was performed in June to October 2010. All pig farms sampled had a history of diarrhea outbreaks in the farrowing house, especially in the pigs' first week of life. The pig farms not using antibiotic growth promoters in the diet and fecal samples were collected from piglets which had not been treated at birth with any antibiotics and coccidiostats. Fecal samples were collected directly from the rectal ampulla into sterile containers and subjected to a bacteriologic culture on *C. perfringens* selective agar (DIFCO™

SPS agar, Difco Laboratories, Detroit, USA). Plates were incubated at 37°C in an anaerobic atmosphere with 10% H₂, 10% CO₂, 80% N₂ and were examined after 24 hours (15). Presumptive identification of *C. perfringens* was determined by colonial and microscopical morphology and confirmed by biochemical tests and multiplex-PCR (21). All isolated were classified as *C. perfringens* type A, and 31 (62%) were positive for the *cpb2* gene.

The MIC was determined by the agar dilution method, as recommended by the CLSI (5). The following 13 antibiotics used in the Brazilian swine industry were evaluated: amoxicillin, avilamycin, ceftiofur, florfenicol, josamycin, leucomycin, lincomycin, narasin, neomycin, norfloxacin, streptomycin, tetracycline, and tylosin (16). For each antimicrobial tested, the MIC₅₀ and MIC₉₀, i.e., the minimum concentration that inhibited growth of 50% and 90% of the isolates, respectively, were calculated. *Bacteroides fragilis* (ATCC 25285) was used as a control strain.

The MIC of the 13 antimicrobials tested against 50 *C. perfringens* type A isolates are summarized in Table 1. The antibiotics that showed the best MIC₅₀ and MIC₉₀ values were amoxicillin, ceftiofur and narasin. These results are in agreement with those reported by Tansuphasiri *et al.* (18) and Rood (13). Both described a high sensitivity of *C. perfringens* isolated from pigs to beta-lactams. In broiler chickens and dogs, the results for beta-lactams were similar to those described in this study (10,15). However, one report demonstrated that isolates of *C. perfringens* from cattle exhibited decreased sensitivity to beta-lactams antibiotics (14).

Table 1. Distribution of the minimum inhibitory concentration ($\mu\text{g/mL}$) of 13 antimicrobials for 50 *C. perfringens* type A isolates isolated from one- to seven-day-old piglets, with and without diarrhea.

Antibiotic	Number of <i>C. perfringens</i> isolates with MIC values ($\mu\text{g/mL}$)												MIC ₅₀	MIC ₉₀
	0.25	0.5	1	2	4	8	16	32	64	128	256	>256		
Amoxicillin	5	31	9	2	2	1	0	0	0	0	0	0	0.5	1
Avilamycin	0	0	15	2	16	9	0	0	2	1	0	5	4	128
Ceftiofur	21	9	2	4	6	5	0	0	0	0	3	0	0.5	8
Florfenicol	0	1	2	24	9	2	2	2	0	7	1	0	2	128
Josamycin	0	0	0	1	3	17	6	0	2	0	5	16	16	>256
Leucomycin	0	0	7	14	13	0	2	0	0	0	7	7	4	>256
Lincomycin	0	0	0	6	8	8	1	1	0	0	7	19	16	256
Narasin	0	2	1	41	0	2	0	0	0	0	0	4	2	8
Neomycin	0	0	1	0	0	0	0	0	0	1	4	44	>256	>256
Norfloxacin	0	0	3	0	0	2	1	3	9	23	9	0	128	256
Streptomycin	0	0	0	0	0	0	0	0	0	0	8	42	>256	>256
Tetracycline	0	0	0	4	0	4	21	2	0	5	14	0	16	256
Tylosin	13	23	5	2	1	0	3	3	0	0	0	0	0.5	16

In this study, tylosin was highly effective against piglet *C. perfringens* isolates. Our results contrast with those of Devriese *et al.* (6), who reported that a high percentage of piglet *C. perfringens* isolates were resistant to this antibiotic. It is interesting to note that while tylosin is an antibiotic commonly used for porcine proliferative enteropathy, our results suggest that it can also be effective for the treatment and control of *C. perfringens* in piglets.

Florfenicol and leucomycin showed a low MIC₅₀ but a high MIC₉₀. According to Post and Songer (12), these results suggest that *in vivo* resistance exists in a proportion of the isolates. Reports evaluating the efficacy of these antibiotics against isolates from pigs are rare; however, studies with other domestic species indicate a high degree of susceptibility of *C. perfringens* isolates to both antibiotics (6,11).

Neomycin, norfloxacin, streptomycin, josamycin, tetracycline, and lincomycin had high MICs, suggesting poor efficacy of these antibiotics against isolates of *C. perfringens* isolated from piglets. Genes responsible for resistance to tetracycline and lincomycin have previously been described in *C. perfringens* isolates isolated from humans and domestic animals (23,4,14,11,9,3,7,15). This finding may explain the high resistance ratio found for these two drugs in the present study. As for neomycin, norfloxacin, streptomycin, and josamycin, to our knowledge, no studies regarding the genetic mechanisms of resistance to bacteria of the genus *Clostridium* have been published. In this work, *C. perfringens* demonstrated a decreased inherent sensitivity to these drugs, instead of resistance mediated by acquired genes or mutations. It should be emphasized that this study is the first evaluating the MIC for josamycin against *C. perfringens* isolated from pigs.

When the inhibition profile of isolates from piglets with diarrhea was compared to isolates from animals without diarrhea, the most notable differences included sensitivity toward avilamycin and narasin. The MIC₉₀ of these two drugs was higher in the isolates obtained from diarrheic piglets. For avilamycin and narasin, concentrations of 2 and 8 µg/mL,

respectively, could inhibit the growth of isolates not associated with diarrhea, whereas a concentration greater than 256 µg/mL was required to inhibit the growth of isolates from piglets with diarrhea, a 128- and 32-fold increase in concentration, respectively. These results of avilamycin and narasin might suggest the hypothesis of a possible gene-mediated resistance or mutations present in isolates from diarrheic animals. The inhibition profile of the other antibiotics was not influenced by the clinical status of the piglets. These results suggest that some antibiotics, particularly ionophores, cannot be used for the treatment of diarrhea caused by *C. perfringens* type A and that the utilization of these drugs should be restricted to growth promotion. When the inhibition profiles of *cpb2*-positive and *cpb2*-negative isolates were compared, no significant differences in the MICs were observed.

Antimicrobial sensitivity tests represent an *in vitro* estimate of the sensitivity or resistance of certain biological agents to a set of drugs. The results of these tests are generally well correlated with therapeutic results. However, there is no guarantee that *in vivo* treatment will be effective because many factors other than chemotherapy interact with the host organism. This study is the first that demonstrates a striking difference in antimicrobial susceptibility between *C. perfringens* isolates from piglets with and without diarrhea. These results suggest the need for further studies evaluating molecular factors related to the antimicrobial resistance of *C. perfringens*.

ACKNOWLEDGEMENTS

This work was supported by Pró-Reitoria de Pesquisa da Universidade Federal de Minas Gerais (PRPq/UFMG), CNPq, FAPEMIG, INCT/IGSPB–CNPq and FEPMVZ. We also thank the companies Hertape Calier Animal Health, Elanco Animal Health and Pfizer for the antibiotics used in this experiment. We thank Dr. Luiz Macedo (UFMG) for providing the reference strain and the veterinarians at the company Integral,

who aided in the selection of farms and the collection of samples. FCFL and RMCG were supported with fellowships from CNPq.

REFERENCES

- Barcellos, D.E.S.N.; Marques, B.M.F.P.P.; Mores, T.J.; Coelho, C.F.; Borowski, S.M. (2009). Aspectos práticos sobre o uso de antimicrobianos em suinocultura. *Acta Scien. Vet.* 37 (1), 151-155.
- Burch, D. (2005) Problems of antibiotics resistance in pig in the UK. *In Practice.* 27, 37-43.
- Chalmers, G.; Martin, S.W.; Hunter, D.B.; Prescott, J.F.; Weber, L.J.; Boerling, P. (2008) Genetic diversity of *Clostridium perfringens* isolated from healthy broiler chicken at a commercial farm. *Vet. Microbiol.* 127, 116-127.
- Chopra, I.; Roberts, M. (2001) Tetracycline antibiotics: mode of action, application, molecular biology and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.* 65, 232-260.
- CLSI. (2009). Methods for antimicrobial susceptibility testing of anaerobic bacteria. Seventh edition. Approved Standart M11-07. *Clinical and Laboratory Standards Institute*, Wayne, PA.
- Devriese, L.A.; Daube, G.; Hommez, J.; Haesebrouck, F. (1993). In vitro susceptibility of *Clostridium perfringens* isolated from farm animals to growth-enhancing antibiotics. *J. Appl. Microbiol.* 75 (1), 55-57.
- Gholamiandehkordi, A.; Eeckhaut, V.; Lanckriet, A.; Timbermont, L.; Bjerrum, L.; Ducatelle, R.; Haesebrouck, F.; Van Immerseel, F. (2009). Antimicrobial resistance in *Clostridium perfringens* isolates from broilers in Belgium. *Vet. Res. Commun.* 33, 1031-1037.
- Holmgren, N.; Baverud, A.; Lindberg, A.; Linder, A. (2006) *Clostridium perfringens* type A from indoor and outdoor sows and piglets. 19th International Pig Veterinary Society Congress, Copenhagen, Denmark, p.322.
- Johansson, A.; Greko, C.; Engström, B.E.; Karlsson, M. (2004). Antimicrobial susceptibility of Swedish, Norwedian and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance genes. *Vet. Microbiol.* 99, 251-257.
- Kather, E.J.; Marks, S.L.; Foley, J.E. (2006). Determination of the prevalence of antimicrobial resistance genes in canine *Clostridium perfringens* isolates. *Vet. Microbiol.* 113 (1-2), 97-101.
- Marks, S.L.; Kather, E.J. (2003). Antimicrobial susceptibilities of canine *Clostridium difficile* and *Clostridium perfringens* isolates to commonly utilized antimicrobial drugs. *Vet. Microbiol.* 94, 39-45.
- Post, K.W.; Songer, J.G. (2004). Antimicrobial susceptibility of *Clostridium difficile* isolated from neonatal pigs with enteritis. *Anaerobe.* 10, 47-50.
- Rood, J.I.; Budle, J.R.; Wales, A.J.; Sidhu, R. (1985). The occurrence of antibiotic resistance in *Clostridium perfringens* from pigs. *Aust. Vet. J.* 62 (8), 276-279.
- Sasaki, Y.; Yamamoto, K.; Tamura, Y.; Takahashi, T. (2001). Tetracycline-resistance genes of *Clostridium perfringens*, *Clostridium septicum* and *Clostridium sordellii* isolated from cattle affected with malignant edema. *Vet. Microbiol.* 81 (1), 61-69.
- Silva, R.O.S.; Salvarani, F.M.; Assis, R.A.; Martins, N.R.S.; Pires, P.S.; Lobato, F.C.F. (2009). Antimicrobial susceptibility of *Clostridium perfringens* strains isolated from broiler chickens. *Braz. J. Microbiol.* 40, 261-263.
- SINDAN, Sindicato Nacional da Indústria de produtos para Saúde Animal. Compêndio de produtos veterinários, 2010. Disponível em: <<http://www.cpv.com.br/cpv/index.html>>. Acesso em: 01 de março de 2010.
- Songer, J.G.; Glock, R.D. (1998). Enteric infection of swine with *Clostridium perfringens* types A and C. *J. Swine Health Prod.* 6, 223-225.
- Tansuphasiri, U.T.; Matra, W.; Sangsuk, L. (2005). Antimicrobial resistance among *Clostridium perfringens* isolated from various sources in Thailand. *Southeast Asian J Trop Med Public Health.* 36 (4), 954-961.
- Van Asten, A.J.A.M.; Nikolau, G.N.; Gröne, A. (2010), The occurrence of *cpb2*-toxigenic *Clostridium perfringens* and the possible role of the β 2-toxin in enteric disease of domestic animals, wild animals and humans. *Vet. J.* 183, 135-140.
- Vaz, E.K. (2009). Resistência antimicrobiana: como surge e o que representa para a suinocultura. *Acta Scien. Vet.* 37 (1), 147-150.
- Vieira, A.A.S.; Guedes, R.M.C.; Salvarani, F.M.; Silva, R.O.S.; Assis, R.A.; Lobato, F.C.F. (2008). Genotipagem de *Clostridium perfringens* isolados de leitões diarreicos. *Arq. Inst. Biol.* 75, 513-516.
- Yaeger, M.Y. (2007). Prospective and retrospective studies on *Clostridium perfringens* type A enteritis in neonatal swine. American Association of Swine Veterinarian 38th Annual Meeting Proceedings. p.101-103.
- Watkins, K.L.; Shryock, T.R.; Dearth, R.N.; Saif, Y.M. (1997). In vitro antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. *Vet. Microb.* 54, 195-200.



All the content of the journal, except where otherwise noted, is licensed under a [Creative Commons License](https://creativecommons.org/licenses/by-nc/4.0/)