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ANTIMICROBIAL SUSCEPTIBILITY OF *CLOSTRIDIUM PERFRINGENS* STRAINS ISOLATED FROM BROILER CHICKENS

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SHORT COMMUNICATION

ABSTRACT

Clostridium perfringens is a normal inhabitant of the intestinal tract of chickens as well as a potential pathogen that causes necrotic enteritis and colangio hepatitis. The minimum inhibitory concentration (MIC) of seven different compounds used for therapy, growth promotion or prevention of coccidiosis was determined by agar dilution method for 55 *C. perfringens* strains isolated from the intestines of broiler chickens. All strains showed high susceptibility to penicillin, avilamycin, monensin and narasin. Only 7.3% of the strains showed an intermediated sensitivity to lincomycin, and 49 (89.1%) were considered susceptible. For tetracycline and bacitracin, 41.8% and 47.3% of strains, respectively, were considered resistant.

Key words: Necrotic enteritis, growth promoters, anticoccidials, antibiotics

Clostridium perfringens is a common environmental bacterium and is readily isolated from the intestine of birds and mammals (14). In broiler chicken, it is responsible for necrotic enteritis, an acute enteric disease, and for a subclinical disease with focal necrosis in the intestine or *C. perfringens*-associated hepatitis, with cholangio hepatitis or fibrinoid necrosis in the liver (7). Besides the economic importance of *C. perfringens* in poultry, it also constitutes a risk to the public health through the food chain (8), being one of the most frequently isolated bacterial pathogens in foodborne disease outbreaks in humans (20). It is thought that antibacterial substances used for growth promotion or anticcocidials may prevent pathogenic effects of *C. perfringens* (13), but there are few data about this microorganism susceptibility to those substances in Brazil. The objetive of this study was to determine the *in vitro* susceptibility of *C. perfringens* to some antimicrobials of relevance for poultry production.

A total of 55 strains were isolated from broiler chicken intestines in a slaughterhouse in Patos de Minas, Minas Gerais, Brazil. Specimens of duodenal contents were subjected to a bacteriologic culture on *C. perfringens* selective agar (SPS, Difco, USA). Plates were incubated at 37ºC in an anaerobic atmosphere with 10% H_2, 10% CO_2, 80% N_2 and were examined after 48 hours. Presumptive identification of *C. perfringens* was determined by colonial and microscopical morphology and confirmed by biochemical tests and multiplex-PCR (15). The following antibiotics manufactured for analytical pourpose were tested: lincomycin, penicilin, tetracyclin, bacitracin (Sigma, USA), avilamycin, narasin and monensin (Elanco Animal Health, Brazil).

Minimum inhibitory concentrations (MICs) were determined on Brucella agar (Difco, USA) plates containing doubling diluitions of the antibiotics, from 0,25 to 256,0 mg/L, and suplemmented with 5% of sheep blood (16). Prior to antimicrobial susceptibility testing, isolates were subcultured on a tioglicolate broth (Difco, USA). After incubation in anaerobic atmosphere for 18 hours at 37ºC, the cultures were suspended in a 0,85% NaCl to an optical density equivalent to

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that of a McFarland 0,5 standart. Strains were inoculated with a Steers inoculum replicator. All strain were tested twice and, for negative control, *Bacteroides fragillis* (ATCC25285) was included in every test plates.

The results are summarize at Table 1. *C. perfringens* isolates examined was highly susceptible to the two ionophore antibiotics, narasin and monensin, and to penicillin and avilamycin. Lincomycin demonstrated a low MIC for 49 (89.1%) isolates, while 23 (41.8%) and 26 (47.3%) strains were considered resistant to tetracycline and bacitracin, respectivelly.

Despite the decreased sensitivity of *C. perfringens* isolates from cattle to beta-lactamic antibiotics has been described (17), penicillin caused inhibition of all strains at lowest concentration tested (0,25 mg/L) in this study, in agreement to previous works with *C. perfringens* poultry isolates (4,9,13,21). Avilamycin showed a good effect too, similar to reported by Martel *et al.* (13). Higher MICs to avilamycin have also shown by others investigations (6,9,21), but those results were interpreted as inherent lower susceptibility to these drugs, and not as resistance mediated by acquired genes or mutation.

For lincomycin, 49 (89,1%) and four (7.3%) showed a low and a moderate MIC, respectivelly. Only two (3.6%) isolates were considered resistant. Similar results were reported by Martel *et al.* (13), whereas Watkins *et al.* (21) described a higher number of lincomycin-resistant strains in broiler chicken and turkey C. perfringens isolates. Resistance genes studies for lincomycin are poorly reported and resistance may be due to as yet unknown genes (13). For bacitracin, 26 (47.3%) strains were considered resistant, while five (9.1%) and 24 (43.6%) strains showed a low and a moderate MIC, respectivelly. Although Watkins *et al.* (21) and Chalmers *et al.* (4) reported 88% and 95% resistant C. perfringens strains, other investigations have shown the effectiveness of bacitracin in the control of necrotic enteritis in broiler chicken (2,18). However, Johansson *et al.* (9) found a low rate of resistance in Swedish and Danish isolates, countries in which bacitracin was no longer in use.

High MICs for tetracycline have already been described for poultry, cattle, dogs and human *C. perfringens* isolates (4,9,10,12,13,17,21). It was previously been reported that *Clostridium* species can carry tetracycline resistance genes witch encode a ribosome protecting cytoplasmatic protein (5).

In Brazil, clostridiosis is controlled by a routine combination of antimicrobial growth promoters (AGPs) and ionophorous anticoccidials, both of which have an antibiotic effect on *C. perfringens*. Increasingly, the use of antibiotics in animal production is linked to the emergence of antibiotic-resistant bacterial strains in humans (3). As a result, the World Health Organization has recommended that AGPs should be replaced by alternative strategies (22). In 2006, the European Union banned all AGPs in animal feed (1). Since then, a growing pressure for AGP withdrawal in Brazil has been put forward by poultry meat importers, recommending the control of necrotic enteritis by anticoccidials, good hygiene management and modified diet. In that case, anticoccidials susceptibility to *C. perfringens* will be essential. The present study showed that narasin and monensin have a good effect against *C. perfringens* strains, similar to previously described (4,21). Martel *et al.* (13) and Johansson *et al.* (9) reported similar results for Northern Europe farms *C. perfringens* isolates, where narasin has been used in almost all broiler flocks only up to 1998. These authors suggested that the development of resistance to these ionophore antimicrobial is slow.

Hughes *et al.* (8), studying the reasons for prescription of antibiotic use in broiler farms, reported that the major indications for therapeutic purposes in the United Kingdom were enteric diseases, particularly necrotic enteritis. Considering the world tendency to ban AGPs from animal production, manufacturers should consider a management that prevents known predisposing factors, including the control of coccidiosis, composition and form of the feed, good hygiene of environment. But, recently the disease re-emerged in countries which banned AGPs (20), confirming that alternatives to prevention and control

Table 1. Distribution of minimal inhibitory concentration for fifty-five broiler chicken *Clostridium perfringens* isolates and their classification.

Antimicrobial	Distribution (%) of MICs											Classification (%)		
(mg.L^{-1})	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**S**	**I**	**R**
Penicillin	100											100		
Narasin	89.1	10.9										100		
Monensin	61.8	34.5	3.6									100		
Avilamycin	61.8	38.2										100		
Lincomycin	12.7	9.1	45.5	21.8	7.3	1.8			1.8			89.1	7.3	3.6
Tetracycline	10.9		1.8	7.3	20	18.2	30.9	5.4	1.8	3.6		40	18.2	41.8
Bacitracin		1.8	7.3		18.2	25.5	3.6	40		3.6		7.3	43.6	49.1

S, susceptible; I; Intermediate or Moderate MIC; R, Resistant.

of *C. perfringens* infections in poultry needs to be more investigated. Recent studies showed that vaccination of poultry against *C. perfringens* could be helpful. Lovland *et al.* (11) reported a vaccine based on *C. perfringens* type A and C toxoids for the prevention of subclinical lesions, while a live attenuated vaccine was recently reported by Thompson *et al.* (19). Prebiotics, probiotics and competitive exclusion products are on research too, but more efforts should be done in this complex area (20).

This is the first study of antibotics and anticoccidials suceptibility of *Clostridium perfringens* isolated from broiler chicken in Brazil.

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RESUMO

Sensibilidade antimicrobiana de estirpes de *Clostridium perfringens* isoladas de aves de corte

Clostridium perfringens é um habitante normal da microbiota intestinal de frangos, sendo um agente potencialmente patogênico, causador de enterite necrótica e colangio-hepatite. A concentração inibitória mínima (CIM) de sete drogas utilizadas na terapêutica, como agentes promotores de crescimento ou na prevenção de coccidiose foi determinada pelo método de diluição em ágar para 55 estirpes de *C. perfringens* isoladas do intestino de frangos de corte. Todas as estirpes revelaram alta sensibilidade à penicilina, avilamicina, narasin e monensina, apenas 7,3% demonstraram CIM intermediário para lincomicina e 89.1% foram consideradas sensíveis. Para tetraciclina e bacitracina, 41,8% e 47.3% das amostras, respectivamente, foram consideradas resistentes.

Palavras-chave: Enterite necrótica, promotores de crescimento, coccidiostáticos, antimicrobianos.

REFERENCES

1. Bedford, M. (2000). Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. *World Poult. Sci. J*. 56, 347-365.
2. Brennan, J.; Skinner, J.; Barnum, D.A.; Wilson, J. (2003). The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. *Poult. Sci*. 82 (3), 360-363.
3. Bywater, R.J. (2005). Identification and surveillance of antimicrobial resistance dissemination in animal production. *Poult. Sci*. 84 (4), 644-648.
4. 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.
5 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.
6. 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.
7. Engstrom, B.E.; Fermer, C.; Lindberg, A.; Saarinen, E.; Baverud; Gunnarsson, A. (2003). Molecular typing of isolates of *Clostridium perfringens* from healthy and diseased poultry. *Vet. Microbiol*. 94, 225-235.
8. Hughes, L.; Hermans, P.; Morgan, K. (2008). Risk factors for the use of prescription antibiotics on UK broiler farms. *J.Antimicrob. Chemother*. 1-6.
9. Johansson, A.; Greko, C.; Engstrom, B.E.; Karlsson, M. (2004). Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance gentes. *Vet. Microbiol*. 99, 251-257.
10. 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.
11. Lovland, A.; Kaldhusdal, M.; Redhead, K.; Skejerve, E.; Lillehaung, A. (2004). Maternal vaccination against subclinical necrotic enteritis in broilers. *Avian Pathol*. 33 (1), 83-92.
12. 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.
13. Martel, A.; Devriese, L.A.; Cauwerts, K.; De Gussem, K.; Decostere, A.; Haesebrouck, F. (2004). Susceptibility of *Clostridium perfringens* strains from broiler chicken to anbiotics and anticoccidials. *Avian Pathol*. 33, 3-7.
14. Mccourt, M.T.; Finlay, D.A.; Laird, C.; Smyth, J.A.; Bell, C.; Ball, H.J. (2005). Sandwich ELISA detection of *Clostridium perfringens* cells and a-toxin from field cases of necrotic enteritis of poultry. *Vet. Microbiol*. 106, 259-264.
15. Meer, R.R.; Songer, J.G. (1997). Multiplex polimerase chain reaction assay for genotyping of *Clostridium perfringens*. *Am. J. Vet. Res*. 58, 702-705.
16. NCCLS. (2004). Methods for antimicrobial susceptibility testing of anaerobic bacteria**.** Fifht edition. Approved Standart M11-A6. *National Committee for Clinical Laboratory Standards*, Wayne, P.A.
17. 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.
18. Si, J.; Gong, J.; Han, Y.; Yu, H.; Brennan, J.; Zhou, H.; Chen, S. (2007). Quantification of cell proliferation and alpha-toxin gene expression of *Clostridium perfringens* in the development of necrotic enteritis in broiler chickens. A*ppl. Environ. Microbiol*. 73 (21), 7110-7113.
19. Thompson, D.R.; Parreira, V.R.; Kulkarni, R.R.; Prescott, J.F. (2006). Live attenuated vaccine-based control of necrotic enteritis of broiler chickens. *Vet. Microbiol*. 113, 25-34.
20. Van Immerseel, F.; De Buck, J.; Pasmans, F.; Huyghebaert, G.; Haesebrouck, F.; Ducatelle, R. (2004). *Clostridium perfringens* in poultry: an emerging threat for animal and public health. *Avian Pathol*. 33 (6), 537-549.
21. 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. Microbiol*. 54, 195-200.
22. World Health Organization. (1997). The medical impact of the use of antimicrobial in food animals. Publication WHO/EMZ/ZOO/97.4. World Health Organisation, Geneva, Switzerland.