ANTIMICROBIAL SUSCEPTIBILITY OF CLOSTRIDIUM PERFRINGENS STRAINS ISOLATED FROM BROILER CHICKENS

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Submitted: June 06, 2008; Returned to authors for corrections: August 08, 2008; Approved: April 16, 2009.

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 intermediate 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 anticoccidials may prevent pathogenic effects of C. perfringens (13), but there are few data about this microorganism susceptibility to those substances in Brazil. The objective 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% H2, 10% CO2, 80% N2 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 purpose were tested: lincomycin, penicillin, tetracycline, 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 dilutions of the antibiotics, from 0.25 to 256.0 mg/L, and supplemented 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
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 fragilis (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, respectively.

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, respectively. 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, respectively. 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 which encode a ribosome protecting cytoplasmatic protein (5).

In Brazil, clostridiosis is controlled by a routine combination of antimicrobial growth promotors (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

| 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 | 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.

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Table 1. Distribution of minimal inhibitory concentration for fifty-five broiler chicken *Clostridium perfringens* isolates and their classification.
of \textit{C. perfringens} infections in poultry needs to be more investigated. Recent studies showed that vaccination of poultry against \textit{C. perfringens} could be helpful. Lovland et al. (11) reported a vaccine based on \textit{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 antibiotics and anticoccidials susceptibility of \textit{Clostridium perfringens} isolated from broiler chicken in Brazil.

ACKNOWLEDGMENTS

This work was supported by funds from Fapemig and CNPq. We thank Elanco Animal Health for the antibiotics and Maria Auxiliadora Roque de Carvalho (ICB/UFMG) for the reference strain.

RESUMO

Sensibilidade antimicrobiana de estirpes de \textit{Clostridium perfringens} isoladas de aves de corte

\textit{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 \textit{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. Para lincomicina e gentamicina e 89,1\% foram consideradas resistentes. Para tetraciclina e bacitracina, 41,8\% e 47,3\% das amostras, respectivamente, foram consideradas resistentes. Para tetraciclina e bacitracina, 41,8\% e 47,3\% das amostras, respectivamente, foram consideradas resistentes.

Palavras-chave: Enterite necrótica, promotores de crescimento, anticoccidiais, antibióticos.

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