Abstract

Colistin sulfate (CS) is used in Canada for the treatment of post weaning diarrhea (PWD), to overcome conventional therapeutic antibiotics failures. The aim of the present study was to determine the effect of a conventional oral regimen of CS for the treatment of PWD, on the development of *E. coli* resistance and to evaluate the effect of ETEC: F4 infection on CS intestinal absorption.

A total of 48 pigs were used, challenge was carried out by oral administration of $10^9$CFU of a hemolytic ETEC: F4 strain resistant to nalidixic acid. CS was administered at a dose of 50,000 UI/kg twice a day for 5 days. Feces were examined clinically and bacteriologically before and after challenge to evaluate presence of diarrhea and *E. coli* fecal excretion. ETEC: F4 virulence factors were monitored and CS plasma concentrations were quantified by an HPLC-MS/MS. From one until six days after CS administration, a significant reduction in the fecal excretion of ETEC: F4, total *E. coli*, ETEC: F4 virulence factors and in diarrhea scores was observed in the challenged treated group compared to the challenged untreated group ($p<0.0001$). No significant difference in growth performances was observed in treated compared to non-treated pigs ($p>0.71$). A significant selection pressure on *E. coli* total population was observed following CS treatment ($p<0.0001$). Challenge with ETEC: F4 resulted in an increase in intestinal absorption of CS. Our study is the first to demonstrate in an experimental model of PWD, that CS at a dose of 50,000 IU/kg is effective in reducing fecal excretion of *E. coli*. However, this regimen was associated with a selection pressure on *E. coli* CS resistance, and did not improve growth performance in challenged pigs. Thus, the use of this antibiotic in pig should be revised.

Introduction

Post-weaning diarrhea (PWD) is an important enteric disease in pig production in Canada, this disease usually occurs shortly after weaning and is characterised by watery diarrhea, dehydration, loss of body weight and mortality of infected piglets (Fairbrother et al., 2005). ETEC is the most common cause of PWD in pigs, this pathotype is characterized by the production of enterotoxins and adhesins, both essential for disease development (Nagy and Fekete, 2005). Enterotoxins produced by ETEC may be heat stable or heat labile. The predominant ETEC isolates from Canadian pigs farms with PWD showed a high frequency of resistance to multiple antibiotics (Amezgua et al., 2002). This phenotypic antibiotic resistance has caused several therapeutic failures, which initiated veterinarians to look for others molecules to counteract this digestive disorder. Colistin sulfate (CS), a cationic antimicrobial peptide, is among the alternatives that are used to treat PWD. However, CS is “off-label” used in Canada for the treatment of PWD in pig by transposition of application data from countries where CS is approved.

The first objective of the present study was to determine the effect of an oral regimen of CS at 50,000 IU/kg twice a day for 5 consecutive days, on the level of fecal shedding of ETEC: F4, total *E. coli* population, CS resistant *E. coli*, ETEC: F4 virulence factors, fecal consistency, and the growth rate of weaned pigs after an ETEC: F4 challenge. The second objective was to determine the effect of ETEC: F4 in an experimental infection on piglets, on CS intestinal absorption by measure of blood concentrations.
Material and Methods

A total of 48 Yorkshire-Landrace piglets were used in this study, after weaning (21 d old), piglets were fed a standard non-medicated ration for post-weaning pigs and they had unlimited access to feed and water throughout the 7 wk of study.

The challenge strain for experimental infection of pigs was a nalidixic acid-resistant (NaA) variant of ETEC: F4 strain ECL8559 (O149: LT: STa: STb: East1: paa: hemβ: F4) and was hemolytic when grown on blood agar. After 1 wk of acclimatization (28 d old), each pig in challenged groups, was orally challenged with 5 mL of trypticase soy broth containing 10^7 CFU of the freshly grown ETEC: F4 following the administration of 10 mL CaCO3. CS administration was conducted one day after piglet’s challenge, at a dose of 50,000 IU/kg twice a day for 5 successive days.

Fresh fecal samples were obtained from pigs using sterile rectal swabs and bacteriological examination of fecal samples was performed before and at 1, 2, 3, 5, 6, 7, 8, 10, 13, 20, 27, 36 days after piglet’s inoculation to evaluate fecal excretion of the challenged ETEC: F4 strain and total E. coli population.

ETEC: F4 virulence factors were monitored by multiplex PCR.

After the challenge infection, piglets were daily observed for signs of anorexia, lethargy and diarrhea. The severity of diarrhea was assessed visually by using a fecal consistency scoring.

In order to follow the growth rate, pigs were weighed individually using an electric scale prior to inoculation and at d7, d20, d27 and d36 post ETEC: F4 challenge.

After the last administration of CS (after 5 days of treatment), blood samples (3 mL) were collected from the jugular vein at 0.5, 12, 24, and 48 hours post CS treatment using potassium EDTA tubes. These samples were used to determine CS plasma concentrations by a liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).

Results

The excretion of ETEC: F4 recovered from the feces throughout the experimental period for the challenged treated group compared with the challenged untreated group was expressed in log_10 CFU/g and shown in Figure 1. CS treatment at a dose of 50,000 IU/kg was induced a significant reduction in fecal total E. coli shedding between d1 and d6 in the challenged treated compared to the challenged untreated group (P < 0.0007).

The ratio of log CS E. coli resistant / log total E. coli showed that before challenge period and before exposure to the antibiotic, the CS E. coli resistant fecal shedding between the challenged treated and untreated group was very similar. In the other hand, starting from d2 post CS treatment, the challenged treated pigs exhibited a higher percentage of CS resistant E. coli in the total E. coli population compared with the challenged untreated pigs, this selective pressure was maintained throughout the CS administration, with a significant effect between d3 and d5 (P < 0.0005).

After 3 days of CS treatment, prevalence of STa virulence gene was reduced in both treated pigs, with a significant reduction in the unchallenged treated groups compared to others groups (P < 0.0001). STb prevalence was significantly reduced in the unchallenged treated compared to the control group (P < 0.0002). In the other hand, a significant reduction in the prevalence of LT and not of F4 prevalence in the challenged treated group compared to the challenged untreated group (P < 0.0001) was observed. After 2 and 7 days after the end of the CS treatment (d7, d12), we did not detect a statistically significant difference in the prevalence of STa, STb, LT and F4 virulence gene between the 4 groups.

After 2 days of CS administration (d2), fecal consistency scores were significantly decreased in the challenged treated compared to the challenged untreated groups trials (P < 0.0001). The same finding was observed at d3 and d4. Until d5 (6 days post challenge), no differences were observed in the diarrhea scores between challenged untreated and treated groups.

Following oral challenge with ETEC: F4 and the end of the CS treatment (d6), no difference was detected in the body weight of all piglets. After 2 weeks of CS treatment discontinuation (d19), the unchallenged untreated (control) presented a higher body weight compared the challenged untreated group (P = 0.0005) and the unchallenged treated group presented a higher mean weight compared to the challenged untreated group (P = 0.0008). After 30 days of CS treatment ending (d35), the unchallenged treated group and the control group presented a higher mean weight compared to the challenged treated and untreated group.

Following 5 days of CS treatment, the CS concentration at different sampling time in plasma of piglets was showed in figure 2. Thereby, ETEC: F4 oral challenge has exacerbated the intestinal absorption of CS in challenged compared to the unchallenged weaned piglets.
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The challenge strain for experimental infection of pigs was a nalidixic acid-resistant (Nalr) variant of ETEC: F4 strain ECL8559 (0149: LT: STa: STb: East1: paa: hemB: F4) and was hemolytic when grown on blood agar. After 1 wk of acclimatization (28 d old), each pig in challenged groups, was orally challenged with 5 mL of trypticase soy broth containing 10^9 CFU of the freshly grown ETEC: F4 following the administration of 10 mL CaCO3. CS administration was conducted one day after piglet’s challenge, at a dose of 50,000 UI/kg twice a day for 5 successive days.

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In the other hand, a significant reduction in the prevalence of LT and not of F4 prevalence in the challenged treated group compared to the challenged untreated group (P < 0.0001) was observed. After 2 and 7 days after the end of the CS treatment (d7, d12), we did not detect a statistically significant difference in the prevalence of STa, STb, LT and F4 virulence gene between the 4 groups.

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Following 5 days of CS treatment, the CS concentration at different sampling time in plasma of piglets was showed in figure 2. Thereby, ETEC: F4 oral challenge has exacerbated the intestinal absorption of CS in challenged compared to the unchallenged weaned piglets.

Figure 1: Evolution of fecal ETEC: F4 counts (means ± standard deviation [SD]). Challenge was performed at d-2 and treatment with colistin sulfate (CS) at the dose of 50,000 IU/kg was started at d0 (36 hours post challenge) with two administrations per day for a period of 5 days. CS treatment resulted in a significant reduction in fecal ETEC: F4 shedding between d1 and d6 (P < 0.0001). *: P < 0.0001. **: P<0.007

Figure 2: Evolution of plasma CS concentrations over time (means ± standard deviation [SD]). CS concentrations were obtained by HPLC-MS/MS after 0.5, 2, 24 and 48 hours of CS treatment ending at a therapy regimen of 50,000 IU/kg. At 0.5 hours, CS concentration was statistically higher in the challenged treated compared to the unchallenged treated group with P = 0.0002.
Discussion

In the current study, maximum ETEC: F4 shedding and diarrhea score were observed one-day post challenge. This result is consistent with other experimental studies in which higher frequency of watery diarrhea was observed after the first day of the oral challenge with ETEC: F4 (Wellock et al., 2008).

In our study, the use of CS in the treatment of experimental PWD was associated with a rapid reduction of ETEC: F4 fecal shedding and fecal consistency scores. However, this CS therapeutic regimen was unable to catch up piglets’ growth retardation caused by ETEC: F4 challenge. In the other hand, CS treatment was associated with the emergence of E. coli colistin resistance.

The potential for the emergence of resistance to colistin among E. coli has been found to be important in countries where colistin is widely used in pig production (Mateu and Martin, 2000). Furthermore, in human medicine, the increasing of the systemic use of colistin against multidrug-resistant pathogens was associated with the increase of colistin-resistant aerobic Gram-negative strains during the treatment of nosocomial infections with this antibiotic (Halaby et al., 2013).

In our study, we have demonstrated that ETEC: F4 oral challenge had exacerbated the intestinal absorption of CS in challenged compared to the unchallenged weaned piglets. This finding is probably due to the rapid release of large amounts of lipopolysaccharide (LPS) by the colonizing ETEC in the challenged piglets. Further studies are needed to better understand the CS intestinal absorption mechanism such as the impact of LPS in the release of proinflammatory cytokines. Indeed, several studies have shown that administration of bacterial LPS results in the production and release of TNF-α and IL-1, these proinflammatory cytokines increased epithelial tight junction permeability in vitro in Caco-2 cells. In the other hand, it was demonstrated that IL-1 activated endothelial cells (EC) to induce vascular leakage via loss of vascular endothelial cadherin (Dagvadorj et al., 2015).

Conclusion

This is the first report on the use of CS for the treatment of experimental E. coli-induced diarrhea in weaned pigs. In our study we showed that under controlled breeding conditions of piglets, CS had an effect on bacteria shedding only during treatment period. However, CS did not enhance the growth performance of infected animals and it has exerted a selection pressure in pig’s E. coli commensal population resistant to colistin. In the other hand, the use of CS in other countries was accompanied by an increase in CS E. coli resistance in pig production, and it should be remembered that colistin is classified in the category of very high importance in human medicine by Health Canada, so we consider that the use of this antibiotic as first therapeutic choice in pig production is not a judicious practice.

Acknowledgements

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