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Faecal shedding of CTX-M-producing *Escherichia coli* in horses receiving broad-spectrum antimicrobial prophylaxis after hospital admission

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ABSTRACT

The objective of this longitudinal study was to investigate the occurrence and genetic background of faecal *Escherichia coli* resistant to cefotaxime (CTX) in horses receiving broad-spectrum antimicrobial prophylaxis after admission to a veterinary teaching hospital. The ten horses enrolled in the study were treated with cefquinome either alone ($n = 4$) or in combination with metronidazole ($n = 3$) or other antimicrobial agents ($n = 3$). CTX-resistant coliforms in faeces collected before, during and after treatment were quantified on selective MacConkey agar supplemented with CTX, and a colony isolated randomly from each positive sample was characterized by pulsed-field gel electrophoresis, and by PCR detection and sequencing of bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{CMY}. All horses were negative for CTX-resistant coliforms at admission but became positive within the first three days of treatment. The average faecal densities of CTX-resistant coliforms increased significantly following antimicrobial prophylaxis ($P < 0.001$). Genetic characterization of 29 faecal isolates revealed that this effect was due to proliferation of *E. coli* producing either CTX-M-1 ($n = 28$) or CTX-M-14 ($n = 1$). Five CTX-M-1 isolates produced additional β-lactamases (TEM-1, CMY-34 and the novel variant CMY-53). Shedding of CTX-M-producing *E. coli* appeared intermittent in four horses and persisted two weeks after antimicrobial treatments in five of six patients tested after discharge from hospital. Nosocomial transmission was suggested by finding five identical CTX-M-1-producing *E. coli* pulsotypes in multiple horses. The originality of the study lies in the unanticipated high frequency and genetic diversity of CTX-M-producing *E. coli* observed in the faecal flora of hospitalized patients receiving broad-spectrum antimicrobial prophylaxis.

1. Introduction

Various studies have shown that the use of β-lactam antibiotics, especially cephalosporins, is a risk factor for carriage of *Enterobacteriaceae* producing extended-spectrum β-lactamase (ESBL) in both humans (Paterson and Bonomo, 2005; Urbánek et al., 2007) and animals (Jørgensen et al., 2007; Cavaco et al., 2008). ESBL-producing *E. coli* are reported increasingly in food animals, and there is increasing evidence that animal reservoirs may contribute to the recent spread of these bacteria in humans (Carattoli, 2008; Moodley and Guardabassi, 2009; Leverstein-van Hall et al., 2011). The ESBL-type reported most frequently in food animals in Europe is CTX-M-1 (Meunier et al., 2006; Jørgensen et al., 2007; Madec et al., 2008; Bortolaia et al., 2010). Although the distribution of ESBL types in human clinical isolates of *E. coli* varies between countries and continents (Cantón et al., 2008; Carattoli et al., 2008; Coque et al., 2008), a generalized increase in the occurrence of the CTX-M-type has been observed worldwide. During the last decade, these enzymes have disseminated rapidly not only within healthcare settings but also in the community, and in some geographical areas have become the most prevalent

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ESBL among *Enterobacteriaceae* (Livermore and Hawkey, 2005; Rossolini et al., 2008).

In recent years, ESBL genes have been reported in bacteria from various animal species (Carattoli, 2008) but little is known about the frequency and distribution of ESBL types in horses. Vo et al. (2007) documented the occurrence of CTX-M-1 in five equine clinical isolates of *E. coli* and *Klebsiella pneumoniae* in the Netherlands. Ewers et al. (2010) described the occurrence of human pandemic CTX-M-15-producing *E. coli* O25:H4 ST131 in a single clinical isolate from a horse in Germany. The objective of the present study was to investigate the occurrence and genetic background of cephalosporin-resistant *E. coli* in the faeces of horses receiving broad-spectrum antimicrobial prophylaxis after admission to a veterinary teaching hospital. A longitudinal study was conducted on ten patients treated prophylactically with cefquinome, a fourth generation cephalosporin used commonly in equine medicine, which was administered alone or in combination with other antimicrobial drugs.

2. Materials and methods

2.1. Study population and treatment protocol

The study was performed at the large animal referral hospital of the Faculty of Life Sciences, University of Copenhagen. The first ten consecutive cases of adult (≥2 years) equine patients admitted to the surgery unit and treated prophylactically with cefquinome were enrolled in the study from October 1st to December 21st 2009 according to the following exclusion criteria: poor prognosis of survival, and nasogastric intubation for administration of water, salts, oil or feed. Cefquinome (Cobactan® 1 mg/kg IV q12h) was administered to all these patients after hospital admission. Six horses received additional antimicrobial treatment during or prior to hospitalization (Table 1). All horses were kept in individual boxes.

In the months after completion of the study (March 2010–June 2010), seven cases of clinical infections with ESBL-producing *E. coli* were confirmed among horses admitted to the same hospital and also included in the study for comparison.

2.2. Counts of CTX-resistant coliforms

From each horse, faeces were collected by the hospital staff on days 0 (immediately before cefquinome treatment), 1, 3, 5 and 7 with a couple of exceptions (i.e. samples on days 5 and 7 could not be obtained from one and two horses, respectively). Fresh faeces were collected from the floor using sterile gloves before daily cleaning. All samples were refrigerated and analysed in the laboratory within 48 h. One gram of faeces, taken from the core (centre) of each sample, was used to prepare 10-fold dilutions in physiological saline, and 10 μl of each dilution was transferred onto MacConkey agar (Oxoid, Basingstoke, UK) supplemented with 2 μg/ml CTX (Sigma–Aldrich, St Louis, USA). Lactose-positive colonies were counted following the recommendations by the Nordic Committee on Food Analysis (Niemela, 1983) after 24 h of incubation at 37 °C to determine mean bacterial densities (CFU/g). A follow-up study was conducted to examine shedding of CTX-resistant coliforms 14 days after the end of treatment, which corresponded to 17–22 days after the start of

Table 1
List of the ten horses included in the study.

Horse ID	Breed	Age	Sex	Indication for prophylaxis	Cefquinome treatment	Other antimicrobial treatment
C1	DW	16	M	Olecranon fracture	IV bid for 7 days	Trimethoprim-sulfadiazine (Norodine Equine Paste©) bid, po for 5 days prior to hospital admission
C2	DW	8	F	Traumatic wound – left frontleg	IV bid for 5 days	None
C3	Icelandic	9	F	Large colon torsion	IV bid for 5 days	Metronidazole IV for 3 d in combination with cefquinome
C4	Oldenborger	2	F	Traumatic right eye surgery – periorbital eye surgery	IV bid for 5 days	None
C5	DW	3	F	Tramatic wound – left frontleg	IV bid for 4 days	Procaine penicillin (Penovet©) IM and ceftiofur (Excenel©) IA after hospital admission and before treatment with cefquinome
C6	Icelandic	12	F	Peritonitis	IV bid for 4 days	Metronidazole IV for 3 d in combination with cefquinome
C7	Haflinger	14	F	Unspecific colic	IV bid for 6 days	Metronidazole IV for 4 d in combination with cefquinome
C8	DW	7	F	Post-operative colic	IV bid for 5 days	None
C9	Icelandic	2	F	Septic tenosynovitis – left hindleg	IV bid for 5 days	Benzylpenicillin and streptomycin (Norostrep©) IM prior to hospital admission
C10	DW	14	M	Traumatic wound – left hindleg	IV bid for 7 days	None

Abbreviations: DW, Danish Warmblood; M, male; F, female; IV, intravenous; IM, intramuscular; IA, intra-articular; bid, twice daily treatment; po, oral treatment.