Emergence of CTX-M-2-producing *Escherichia coli* in diseased horses: evidence of genetic exchanges of $\text{bla}_{\text{CTX-M-2}}$ linked to ISCR1

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Sir,

The impact of CTX-M-producing bacteria on animal health remains unclear. The CTX-M-2 enzyme was first identified in a *Salmonella* Mbandaka strain isolated from faeces of a 4-month old child. Later on, CTX-M-2-producing *Enterobacteriaceae* have also been reported in faeces from healthy poultry and horses. In this study, we describe the molecular characterisation of different *bla*\textsubscript{CTX-M-2}-carrying plasmids in multidrug-resistant *E. coli* isolates recovered from cases of diseased horses. From November 2008 through June 2010, 5 cephalosporin-resistant *E. coli* isolates were obtained from diseased horses hospitalised at the Faculty of Veterinary Medicine, Ghent University, Belgium. The isolates were recovered from horses with an abdominal fistula (*E. coli* 2657), abdominal wound infection (*E. coli* 3744), peritonitis (*E. coli* 1443), funiculitis (*E. coli* 1454) and arthritis (*E. coli* 4410) (Supplementary Table 1). Most horses received a prolonged empirical therapy with several antimicrobial agents, such as β-lactams, aminoglycosides and fluoroquinolones.

Antimicrobial susceptibility testing of the *E. coli* isolates was assessed by the disc diffusion method and interpreted according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (Supplementary Table 1). PCR to screen for extended-spectrum β-lactamase genes were performed. All isolates harboured the plasmid-encoded *bla*\textsubscript{CTX-M-2} gene. *E. coli* 4410 and 3744 also carried a narrow-spectrum *bla*\textsubscript{TEM-1} gene. The epidemiologic relationship among the isolates was studied by pulsed field gel electrophoresis (PFGE). XbaI PFGE revealed no genetic relatedness between the isolates (data not shown). Plasmid transfer experiments were then carried out. Briefly, *E. coli* J5, resistant to rifampicin, was used as the recipient strain. Conjugation experiments were performed overnight in Luria-Bertani broth at 37°C with a donor/recipient ratio of 1:5. Transconjugants were selected on MacConkey agar plates (Oxoid LTD, Basingstoke, Hampshire, England) supplemented with ceftiofur (8 mg/L) and rifampicin (250 mg/L).
antimicrobial susceptibility of the transconjugants was also determined\(^3\) to identify the co-transferred non-β-lactam resistance (Table 1). The \(bla_{\text{CTX-M-2}}\)-carrying plasmids were extracted from the transconjugants and were further characterized by EcoRI RFLP (Restriction Fragment Length Polymorphism) analysis and Southern blot hybridisation.\(^3\) The plasmids pB4-25\(^5\), p1684Sa02\(^3\) and p04-9275\(^1\) were included in this analysis. The incompatibility (Inc) group of each \(bla_{\text{CTX-M-2}}\)-carrying plasmid was defined by the PCR-based replicon typing method.\(^7\) The genetic environment of \(bla_{\text{CTX-M-2}}\) was investigated as described.\(^8\) The different genetic regions (integron cassette array, Intl1 to ISCR1, ISCR1 to \(bla_{\text{CTX-M-2}}\), and \(bla_{\text{CTX-M-2}}\) to 3′-CS) were defined by PCR and the sizes were compared with known genetic regions present on the \(bla_{\text{CTX-M-2}}\)-carrying plasmids pB4-25\(^5\), p1684Sa02\(^3\) (accession number EF592570)\(^3\) and p04-9275 (accession number EF592571)\(^1\). Integron cassette arrays were also further characterized by sequencing (Table 1).\(^8\) All isolates contained a high-molecular-weight conjugative \(bla_{\text{CTX-M-2}}\)-carrying plasmid (>100 kb). Plasmids p1443, p1454, p2657, and p3744 belonged to IncHI1 group and only plasmid p4410 belonged to IncFIB (Table 1). To our knowledge, this is the first time that \(bla_{\text{CTX-M-2}}\) is identified on IncHI1 and IncFIB plasmids. RFLP analysis of plasmid DNA revealed the same fingerprint pattern for 3 out of 4 IncHI1 plasmids (supplementary figure 1). The differences seen in RFLP patterns for the IncHI1 plasmids may possibly reflect the rapid evolution of these plasmids. Southern blot hybridisation with a \(bla_{\text{CTX-M-2}}\) probe showed a > 10kb EcoRI fragment for all plasmids (supplementary figure 1). Characterisation of the genetic environment showed that \(bla_{\text{CTX-M-2}}\) was part of novel complex class 1 integrons (Table 1). The gene cassette array of the complex class 1 integron located on the IncHI1 plasmids differed in size with known gene cassette arrays of \(bla_{\text{CTX-M-2}}\)-carrying complex class 1 integrons (Table 1). Sequencing of the 1664-bp cassette array revealed the association of the
trimethoprim resistance gene cassette \textit{dfrA17} and the streptomycin/spectinomycin resistance gene cassette \textit{aadA5}. The IncFIB plasmid p4410 showed the same gene cassette array, \textit{dfrA1}--\textit{aadA1}, as pB4-25 and p1684Sa02 but the size of the \textit{IntI1-ISCR1} region of plasmid p4410 was approximately 15-20 kb in comparison to the known size of 5000 bp (Table 1). The common region 1 (\textit{ISCR1}) was found to be linked to the \textit{bla}_{\text{CTX-M-2}} gene, as reported.\textsuperscript{1} All these results may indicate that \textit{ISCR1} mobilized the \textit{bla}_{\text{CTX-M-2}} gene from one integron to another through rolling circle transposition, a phenomenon that has been reported.\textsuperscript{1}

Together, these findings show the recent emergence of \textit{bla}_{\text{CTX-M-2}}-producing \textit{E. coli} in diseased horses. Previous reports have shown that \textit{bla}_{\text{CTX-M-2}} is consistently linked with \textit{ISCR1} and located on IncHI2 plasmids from bacteria of human and animal origin.\textsuperscript{1,3-5} The fact that \textit{bla}_{\text{CTX-M-2}} was now found on IncHI1 and IncFIB plasmids suggests the mobility of \textit{bla}_{\text{CTX-M-2}} via \textit{ISCR1} between plasmids of different incompatibility groups. The potential for transposition of \textit{bla}_{\text{CTX-M-2}} has health implications since extended-spectrum cephalosporins are extensively used in human and veterinary medicine. Indeed, the horses from this study received a prolonged treatment with different antimicrobial agents to recover from an infection with an ESBL (Extended-Spectrum β-Lactamase)-producing bacterium.

The flexibility of \textit{bla}_{\text{CTX-M-2}} also highlights the need to develop appropriate means to control dissemination of this gene and associated resistance genes. Therefore, comparative genomic analysis of different plasmids carrying \textit{bla}_{\text{CTX-M-2}} might be useful to fully understand their evolution, plasticity and spread. While \textit{bla}_{\text{CTX-M-2}} has frequently been detected in human pathogens,\textsuperscript{1} this is the first report of \textit{bla}_{\text{CTX-M-2}}-producing \textit{E. coli} isolated from diseased animals and more specifically horses. The emergence of \textit{bla}_{\text{CTX-M-2}} is therefore a clear cause for concern.
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Transparency declarations

None to declare

References


5. Smet A, Martel A, Persoons D et al. Comparative analysis of extended-spectrum β-lactamase-carrying plasmids from different members of Enterobacteriaceae isolated


<table>
<thead>
<tr>
<th>plasmid nr.</th>
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<th>Inc group</th>
<th>Complex class 1 integron</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>cassette array</td>
</tr>
<tr>
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<td>HI2</td>
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<td>FIB</td>
<td><em>dfra1</em>-<em>aadA1</em>(^{a})  (1586 bp)</td>
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\(^{a}\)pB4-25: *bla*\(_{CTX-M-2}\)-carrying plasmid isolated from *E. coli*, poultry, Belgium, 2008\(^{3}\)

\(^{b}\)p1684Sa02: *bla*\(_{CTX-M-2}\)-carrying plasmid isolated from *Salmonella enterica* serovar Virchow (genbank accession number EF592570), poultry, Belgium, 2002\(^{3}\)
p04-9275: blaCTX-M-2-carrying plasmid isolated from Salmonella enterica serovar Typhimurium (genbank accession number EF592571), human gastroenteritis, French Guiana, 2004

Antimicrobial susceptibility testing of the E. coli isolates was assessed by the disc diffusion method (Kirby-Bauer) and interpreted according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM). Antimicrobial drugs used were the following: tetracycline (TET), sulphonamides (SUL), trimethoprim (TMP), streptomycin (STR), kanamycin (KAN) and fourth-generation cephalosporins (4GC).

dfrA: gene encoding resistance to trimethoprim

aadA: gene encoding resistance to streptomycin