OXA-23-producing *Acinetobacter* species from horses: a public health hazard?

Annemieke Smet1*, Filip Boyen1, Frank Pasmans1, Patrick Butaye1,2, Ann Martens3, Alexandr Nemec4, Pieter Deschaght5, Mario Vaneechoutte5 and Freddy Haesebrouck1

1Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; 2Department of Bacteriology and Immunology, CODA-CERVA-VAR, Groeselenberg 99, 1180 Brussels, Belgium; 3Department of Surgery and Anaesthesiology of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; 4Laboratory of Bacterial Genetics, National Institute of Public Health, Srobarova 48, 100 42 Prague, Czech Republic; 5Department of Clinical Chemistry, Microbiology and Immunology, Faculty of Medicine & Health Sciences, Ghent University, De Pintelaan 185, Ghent, Belgium

*Corresponding author. Tel: +003292647435; Fax: +003292647494; E-mail: annemieke.smet@ugent.be

Keywords: carbapenemases, animals, insertion sequences

Sir,

The prevalence of carbapenem-resistant *Acinetobacter* spp. in human patients is rising. Acquired carbapenem resistance among *Acinetobacter* spp. is mostly due to class D (OXA) β-lactamases.1 Several OXA-type carbapenemases have been described, including OXA-23. Since then, OXA-23 has been increasingly encountered among *Acinetobacter* spp. from humans worldwide1 and recently it has also been found in an *Acinetobacter* species from cattle.1 However, knowledge about carbapenemase-producing *Acinetobacter* spp. of animal origin remains very limited, making it difficult to assess its impact on public health.

In this study, we describe the presence of OXA-23-producing strains belonging to an undescribed *Acinetobacter* species in the faeces of hospitalized horses. In February 2012, faecal samples were collected from 20 hospitalized horses at the Faculty of Veterinary Medicine, Ghent University, Belgium. A total of 2 g of sample was suspended in 18 mL of buffered peptone water and incubated aerobically overnight at 37°C. Thereafter, a loopful of this enrichment medium was inoculated on MacConkey agar plates (Oxoid Ltd, Basingstoke, UK) containing 1 mg/L imipenem and on a selective *Acinetobacter* medium without carbapenems.2 Both media were incubated overnight aerobically at 37°C. Two *Acinetobacter* isolates (H60437 and H60467) were obtained from the MacConkey agar plates containing imipenem and two (H60716 and H60730) from the selective *Acinetobacter* medium. All isolates were from different horses hospitalized in different stables and were not associated with the illness for which the horses were hospitalized.

The horse from which isolate H60437 was obtained had been treated for a wound infection with penicillin [30 mL of procaine penicillin intramuscularly (im) for 5 days], the horse from which isolate H60716 was obtained had been treated with cefquinome (450 mg im) for 4 days at home and with penicillin (21 mL of procaine penicillin im) for 5 days after surgical drainage of a metacarpal abscess. The two other horses did not receive antimicrobials during their hospitalization.

The 16S rRNA gene of these isolates was sequenced and phylogenetic analysis revealed that they all belonged to a separate entity, a not yet formally described *Acinetobacter* species (Figure S1, available as Supplementary data at JAC Online), showing >98% sequence similarity with each other.4 Genotypic comparison of the four equine isolates was performed by PFGE.5,6 H60437 and H60467 had the same fingerprint pattern, indicating that they were identical, whereas the two other isolates had fingerprint patterns that differed from each other and from the pattern of H60437 and H60467 (data not shown).

The MIC of imipenem was determined using the broth microdilution method according to the CLSI guidelines (Table 1).6 The two isolates (H60437 and H60467) obtained from the MacConkey agar plates containing imipenem had an MIC of 16 mg/L, indicating clinical resistance to this antimicrobial agent.6 The MICs of imipenem for the two other isolates, H60716 and H60730, were 0.12 and 0.03 mg/L, respectively, indicating that they were susceptible to imipenem.6 Since colistin is considered to be a last-choice drug for treatment of human patients infected with multidrug-resistant Gram-negative bacteria, the MIC of this antibiotic was also determined using the microdilution method. All four isolates had an MIC of 2 mg/L, indicating clinical susceptibility.6 Results of susceptibility testing for other antimicrobial agents, as determined by the Kirby–Bauer disc diffusion test, are shown in Table 1.6

The presence of *bla*OXA-like carbapenemase genes in the isolates was studied by PCR.2 A *bla*OXA-like gene was found in isolates H60437 and H60467, but was absent in isolates H60716 and H60730. Sequence analysis of the amplicon obtained from isolates H60437 and H60467 confirmed the presence of *bla*OXA-23 (Table 1). The genetic environment of *bla*OXA-23 was investigated by PCR mapping and sequencing.2 Upstream of this gene, the insertion sequence IS*Acba*1 was detected in both H60437 and H60467. This insertion sequence, which belongs to the IS4 family,2 contained the promoter sequences [a −35 sequence (TTAGAA) separated by 16 bp from a −10 sequence (TTATT)] that up-regulate the expression of *bla*OXA-23, resulting in high-level hydrolysis of carbapenems.2
Acinetobacter pittii was indeed already reported among sequences responsible for up-regulation of bla<sub>OXA-23</sub> carbapenems because it lacks IS<sub>AbAI</sub> on its chromosome and the fact that this species is susceptible to meropenem (MEM), tetracycline (TET), sulphonamides (SUL), trimethoprim (TMP), gentamicin (GEN), neomycin, amikacin, enrofloxacin and colistin.

This work was supported by internal funding.

**Acknowledgements**

We thank Nathalie Van Ryselberghie, Arlette Van de Kerckhove, Leentje Van Simaey and Bart Saerens for their skilled technical assistance.

**Funding**

This work was supported by internal funding.

---

**Table 1.** Characteristics of the *Acinetobacter* isolates obtained from faeces of hospitalized horses

<table>
<thead>
<tr>
<th>Isolate number(s)</th>
<th>MIC of imipenem (mg/L)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antimicrobial resistance pattern&lt;sup&gt;b&lt;/sup&gt;</th>
<th>bla&lt;sub&gt;OXA-23&lt;/sub&gt;</th>
<th>IS&lt;sub&gt;AbAI&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H60437 and H60467</td>
<td>16</td>
<td>AMP, AMC, CFT, CFQ, IPM, MEM, TET, SUL, TMP, GEN</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H60716</td>
<td>0.12</td>
<td>TMP</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>H60730</td>
<td>0.03</td>
<td>TET, TMP</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>The control strains used were *Escherichia coli* ATCC 25922 (0.06 – 0.25 mg/L) and *Staphylococcus aureus* ATCC 29213 (0.015 – 0.06 mg/L).

<sup>b</sup>Antimicrobial drugs tested were the following: ampicillin (AMP), amoxicillin/clavulanic acid (AMC), ceftiofur (CFT), cefquinome (CFQ), imipenem (IPM), meropenem (MEM), tetracycline (TET), sulphonamides (SUL), trimethoprim (TMP), gentamicin (GEN), neomycin, amikacin, enrofloxacin and colistin.

**Transparency declarations**

None to declare.

**Supplementary data**

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**