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Letter to the Editor

First report of tetracycline resistance mediated by the *tet*(O) gene in *Haemophilus parasuis*

Sir,

Beyond asymptomatic carriage, *Haemophilus parasuis* is the causative agent of Glässer's disease in swine. Piglets are most likely to develop clinical disease and can suffer from symptoms such as pneumonia, meningoencephalitis, polyserositis or polyarthritis, leading to major economic losses in the swine industry worldwide. To minimise losses due to porcine respiratory infections, a range of antibiotics, including tetracyclines, are frequently used for treatment. The tetracycline resistance gene *tet*(O) has been described in bacterial species of the respiratory tract such as *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* [1,2]. It encodes a ribosomal protection protein that prevents tetracycline binding to the ribosome. To the best of our knowledge, the *tet*(O) gene has not been detected in *H. parasuis* so far. Thus, this is the first report of a *tet*(O)-carrying plasmid found in this species.

Two recently described *H. parasuis* isolates with elevated minimum inhibitory concentrations (MICs) to tetracycline (8 μg/mL and 16 μg/mL, respectively) [3] were screened by PCR for tetracycline resistance genes of classes A–E, G, H, M and O, which are frequently found in Gram-negative bacteria [4]. Both isolates (nos. 41 and 46) were identified as carriers of the *tet*(O) gene. Southern blotting was performed to determine the location of the *tet*(O) gene within the bacterial genome. In both isolates, the gene was detected on a plasmid of ca. 6 kb in size. The plasmid was extracted using a Plasmid Miniprep Kit (QIAGEN, Hilden, Germany) and electrotransformation experiments were performed using a Gene Pulser Xcell™ Electroporation System (Bio-Rad, Munich, Germany) with the following settings: 2.5 kV; 25 μF; and 800 Σ. However, repeated plasmid electrotransformation experiments using *Escherichia coli* recipient strains (AS19, JM109 and JM110) yielded negative results, indicating the non-transferability of the plasmids into the *E. coli* recipient strains.

To determine the complete nucleotide sequence of the *tet*(O)-carrying plasmids, DNA was amplified using divergent primers starting from the *tet*(O) gene (fw: 5′-GGTCAATCTGAACTATGCGG-3′; and rv: 5′-CGGGAAATGAAAGCAAAGC-3′) and the amplicons were subsequently sequenced. The plasmids, designated pHPSO1 (GenBank accession no. MK251542) and pHPSO2 (MK251543), were 6033 bp and 6034 bp in size, respectively, and showed 99.97% nucleotide sequence identity to each other. Sequence analyses revealed that besides the *tet*(O) gene, three plasmid mobilisation genes (*mobA*, *mobB* and *mobC*) were located on the plasmids (Fig. 1). The two plasmids differed from each other only in a silent mutation in the *mobA* gene and a single nucleotide insertion in the *mobA*/*mobB* overlapping region on plasmid pHPSO2 compared with pHPSO1. The deduced amino acid (aa) sequences of Tet(O) and MobC had a typical size of 639 aa and 101 aa, respectively, and the deduced MobA (468 aa) and MobB (160 aa) sequences of plasmid pHPSO1 were also of the expected size. In contrast, insertion of a nucleotide detected in the sequence of plasmid pHPS02 resulted in an exchange of 6 aa and a premature stop codon in the *mobA* reading frame. This insertion also extended the *mobB* reading frame. This led to altered deduced amino acid sequences of MobA and MobB, which were 395 aa and 239 aa in size, respectively. Presumably, this frameshift mutation leads to a functionally inactive mobilisation region of pHPSO2. Comparison with the sequences deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) using the BLASTn algorithm (https://blast.ncbi.nlm.nih.gov) revealed that the insertion detected in *mobA*/*mobB* was not present in any of the other published sequences. However, the insertion was located in a homopolymer hexanucleotide (AAAAAA) that has previously been reported to facilitate frameshift mutations [5]. In other bacterial species, e.g. *Mannheimia haemolytica* (AJ966516.1) and *Aggregatibacter actinomycetemcomitans* (CP003496.2), a substitution was observed at the final A (A → G) of the homopolymer run. Smith and Thomas investigated the effects of premature termination of the *mobA* gene in small staphylococcal plasmids and showed that disruption of *mobA* after 251 aa, which also truncated *mobB*, did not prevent relaxation complex formation [6]. None the less, it should be noted that the nucleotide sequences of the *mob* genes found in Gram-positive bacterial species differ considerably from the *mob* sequences of pHPS01 and pHPS02.

Comparison of plasmids pHPSO1 and pHPSO2 with nucleotide sequences in the databases revealed that they were 99.72% and 99.69% identical with plasmid pB1006 from *P. multocida* (FJ234438.1) isolated from diseased pigs in Spain [2]. Compared with plasmid pB1006, a total of 16 mutations were present in the non-coding regions of plasmids pHPSO1 and pHPSO2 as well as 1 silent mutation inside the *tet*(O) gene. In addition, the aforementioned silent mutation in *mobA* and the 1-bp insertion leading to a frameshift mutation in the *mobA*/*mobB* overlapping region were exclusively detected in pHPSO2. However, due to the high similarity of plasmids pHPSO1 and pHPSO2 with plasmid pB1006 from *P. multocida*, it would be interesting to know whether they are capable of replicating in this host.

Comparison of the plasmids found in this study with other plasmids from *H. parasuis* revealed a high degree of similarity to the mobilisation regions of plasmids pHS-Tet (AY862435.1) and pB1000 from *H. parasuis* strains NU5-3 (KP164832.1) and BB1050 (GU080066.1). Nevertheless, differences were seen in the resistance gene regions and surrounding areas (Fig. 1).

In conclusion, the sequence data and analyses lead to the assumption that members of the family Pasteurellaceae share plasmids with identical or very similar plasmid backbones and variable resistance gene regions. These results support the

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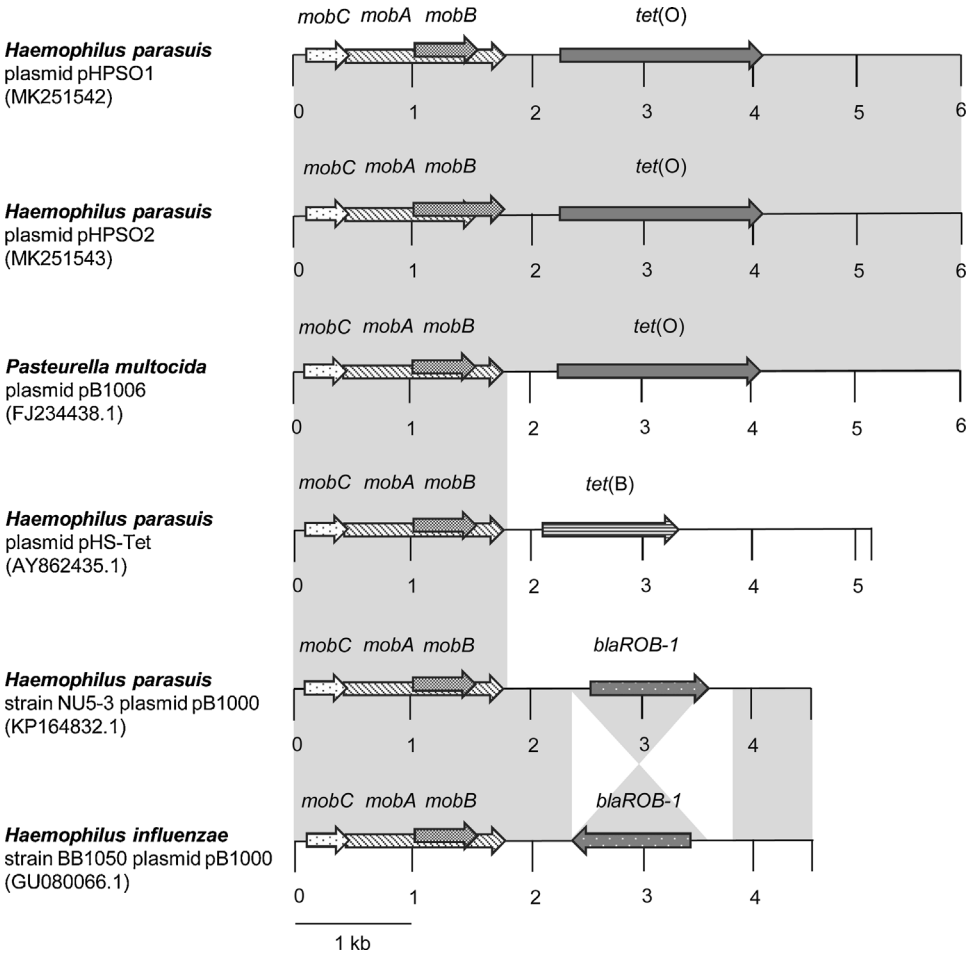

Fig. 1. Schematic comparison of plasmids isolated from *Haemophilus parasuis* isolates 46 and 41 with previously published sequences. GenBank accession numbers are given in parenthesis next to the plasmids. Shaded areas indicate >95% nucleotide sequence identity. A distance scale (in kb) is presented below each map.

suggestion that intergenus transfer of small plasmids occurs between pathogens of the respiratory tract of swine.

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Competing interests

None declared.

Ethical approval

Not required.

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