Increased MICs of gamithromycin and tildipirosin in the presence of the genes erm(42) and msr(E)-mph(E) for bovine Pasteurella multocida and Mannheimia haemolytica

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Keywords: bovine respiratory disease, ICE, horizontal gene transfer, susceptibility testing

Sir,

Bovine respiratory disease (BRD) is one of the economically most important diseases in animal production, with global losses of the feedlot industry due to BRD being estimated to be over US$ 3 billion per year.1 Antimicrobial agents, particularly macrolides, are commonly used to combat bacteria involved in BRD, such as Mannheimia haemolytica, Pasteurella multocida and Histophilus somni.2 After the approval of the 16-membered macrolide tilmicosin (Micotil®) in 1992 and the 15-membered trimilide tulathromycin (Draxxin®) in 2005 for use in BRD, two new macrolides were approved during 2011 for the treatment of BRD. These are the 15-membered macrolide gamithromycin (Zactran®) and the 16-membered macrolide tildipirosin (Zuprevo®). Little is known about the mechanisms of resistance to these two new veterinary antimicrobial agents.3

Recently, three genes involved in macrolide resistance of P. multocida and M. haemolytica were identified: erm(42), coding for a novel rRNA methylase;4 msr(E), coding for an ABC transporter;3 and mph(E), coding for a macrolide phosphotransferase.4 The last two genes were present in an operon structure.4 To determine the role of erm(42) and msr(E)-mph(E) in macrolide and lincosamide resistance, these genes were cloned and expressed in P. multocida B130.4 A closer analysis of these clones for their MICs of selected macrolides and lincosamides revealed that erm(42) conferred resistance to erythromycin, tilmicosin and clindamycin, but not to the triamilide tulathromycin. Although erm(42)-carrying P. multocida B130 showed an 8-fold increase in the tulathromycin MIC to 16 mg/L (Table S1, available as Supplementary data at JAC Online), this MIC classified the P. multocida isolate as susceptible.6 In contrast, msr(E)-mph(E) conferred resistance to erythromycin, tilmicosin and tulathromycin, but not to the lincosamide clindamycin (Table S1).6 When the new macrolides became commercially available, we tested the aforementioned P. multocida B130 clones for their MICs of gamithromycin and tildipirosin by broth macrolidification according to CLSI recommendations.6 The recipient strain P. multocida B130 showed 8-fold lower MICs of 0.25 mg/L for both gamithromycin and tildipirosin, compared with tulathromycin (2 mg/L) (Table S1). In the presence of erm(42), the MIC of tildipirosin increased 128-fold to 32 mg/L, while that of gamithromycin increased only 16-fold to 4 mg/L. In the presence of msr(E)-mph(E), an opposite observation was made: the MIC of tildipirosin increased only 8-fold to 2 mg/L, while that of gamithromycin increased 256-fold to 64 mg/L. Based on these increases in the MICs, it appears that erm(42) has mainly an effect on the tildipirosin MIC, whereas msr(E)-mph(E) increases the gamithromycin MIC for P. multocida B130.

To see whether naturally occurring P. multocida and M. haemolytica isolates from BRD cases that carry the genes erm(42) and/or msr(E)-mph(E) show similar MICs of gamithromycin and tildipirosin, a total of 40 P. multocida and 29 M. haemolytica isolates (Table 1), whose macrolide resistance gene status was determined by previously described PCR assays,6 were tested. These isolates were collected in the Pfizer Animal Health Susceptibility Surveillance Program for BRD between 1999 and 2007 from various states in the USA. The macrolide-susceptible P. multocida isolates (n=8) showed low MICs of 0.25 – 0.5 mg/L for both gamithromycin and tildipirosin. Slightly higher MICs of 0.5 – 1 and 0.5 – 2 mg/L for gamithromycin and tildipirosin, respectively, were detected for the macrolide-susceptible M. haemolytica isolates (n=7). These MICs are in agreement with the gamithromycin MIC50 and MIC90 values for 144 P. multocida and 142 M. haemolytica isolates obtained from animals enrolled in field studies in the USA.7 Moreover, the observed tildipirosin MICs are in the same range as determined for 105 P. multocida and 88 M. haemolytica isolates.8

References
The data obtained in this study for the *P. multocida* and *M. haemolytica* isolates that carried any of the genes *erm* (42) or *msr* (E)-mph(E) showed that, if all three genes were present, the *P. multocida* isolates showed MICs of 16–64 mg/L for gamithromycin and 16–32 mg/L for tildipirosin, whereas similar MICs of 32–64 mg/L for both macrolides were seen for the corresponding *M. haemolytica* isolates. The 10 *P. multocida* isolates that carried only *erm* (42) exhibited low MICs of 2–4 mg/L for gamithromycin, but had higher MICs of 16–32 mg/L for tildipirosin. The single *M. haemolytica* isolate that harboured only *erm* (42) showed MICs of 4 and 32 mg/L for gamithromycin and tildipirosin, respectively. Finally, the two *P. multocida* isolates that carried only the *msr* (E)-mph(E) operon exhibited a high MIC of 32 mg/L for gamithromycin and a low MIC of 2 mg/L for tildipirosin. Unfortunately, no *M. haemolytica* isolates that carried *msr* (E)-mph(E) but lacked *erm* (42) were available for this study. The genes *erm* (42) and *msr* (E)-mph(E) are part of the resistance gene regions of the recently identified integrative and conjugative element ICE*Pmu*1, which has been shown to move across genus boundaries and express its resistance genes in different hosts, such as *P. multocida* and *M. haemolytica*. Consequently, the three macrolide resistance genes usually occur together. Isolates that carry only *erm* (42) or *msr* (E)-mph(E) may harbour deletions in the resistance gene regions of ICE*Pmu*1 and have been observed at a distinctly lower frequency in our studies.

In conclusion, the analysis of *P. multocida* and *M. haemolytica* field isolates from BRD cases confirmed the results obtained with the cloned *erm* (42) and *msr* (E)-mph(E) amplicons. Pronounced increases in the gamithromycin MICs were seen in the presence of *msr* (E)-mph(E), whereas distinct increases in the tildipirosin MICs were detected in the presence of *erm* (42). Isolates that carry all three genes showed elevated MICs of both new macrolides.

### Funding

This study was financially supported by grant SCHW382/10-1 of the German Research Foundation (DFG). G. B. M. received a scholarship of the Gesellschaft der Freunde der Tierärztlichen Hochschule Hannover e.V.

### References

Susceptibility testing of *Escherichia coli* isolates from urines: are we at risk of reporting false antibiotic resistance to co-amoxiclav?

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**Keywords:** urinary tract infections, UTIs, susceptibility testing, laboratory methods

Sir,

The BSAC recently published a change to the recommended inhibition zone diameter breakpoint for testing susceptibility of Enterobacteriaceae to amoxicillin/clavulanate (co-amoxiclav),\(^1\) whereby the maximum zone diameter for inferring resistance was increased from 14 to 20 mm. There was no change to the MIC breakpoint, which remains at 8 mg/L. This change resulted in a significant increase in the proportion of clinical isolates reported as resistant in our laboratory, which necessitated the release of additional antibiotic susceptibilities to clinicians.

The purpose of this study was to investigate the co-amoxiclav MICs for a group of clinical isolates of Enterobacteriaceae that were inferred to be resistant according to the current BSAC criteria and compare these with two commercially available testing systems for determining MICs.

Isolates of *Escherichia coli* from urine samples submitted to South Devon Healthcare NHS Foundation Trust for culture between 7 and 21 June 2011 were tested for susceptibility to co-amoxiclav by disc diffusion in accordance with BSAC methodology. The growth medium was Iso-Sensitest P07794 agar (Oxoid, Basingstoke, UK) and amoxicillin/clavulanate 30 μg discs (20 μg of amoxicillin/10 μg of clavulanate; Oxoid, Basingstoke, UK) were used. Zones of inhibition were measured after an incubation period of 18–20 h at 35°C. *E. coli* isolates with a zone diameter of 14–20 mm were selected for determination of MIC, performed by Vitek 2 (bioMérieux) according to the manufacturer’s instructions. Etest (bioMérieux) was also performed according to the manufacturer’s instructions (2:1 amoxicillin/clavulanate ratio), with the exception of using Iso-Sensitest agar as outlined in BSAC recommendations.\(^2\) Identification of *E. coli* was confirmed by API 20E (bioMérieux). The validity of all three testing methods was assured by inclusion of the control strain *E. coli* NCTC 10418.

A total of 91 isolates had an inhibition zone diameter of 14–20 mm by the disc diffusion method during the 2-week period and would have been interpreted as resistant according to the modified BSAC guidelines. Eighty-eight of the isolates (96.7%) were susceptible (MIC ≤8 mg/L) when tested by Vitek 2 (see Figure 1a). The three resistant strains had MICs of

![Figure 1](image-url)

**Figure 1.** Disc diffusion inhibition zone diameters for *E. coli* urine isolates (*n=91*) compared with MICs as determined by (a) Vitek 2 and (b) Etest.