Rare occurrence of vancomycin-resistant *Enterococcus faecium* among livestock animals in China

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Running title: Vancomycin-resistant *Enterococcus faecium* in swine

Keywords: Swines; multilocus sequence typing; glycopeptides

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Sir,

In China, there is a huge burden of antimicrobial resistant bacteria in the livestock animals.[1,2] Nonetheless, vancomycin-resistant enterococci (VRE) have never been found in the country's farms, livestock animals and meats, with the exception of a single report of vanA-positive *Enterococcus faecalis* from chicken exported to Japan.[3] In the country, glycopeptide antibiotics including avoparcin have never been approved for use in animal industry. Recent reports of vancomycin-resistant *E. faecium* (VREm) in Michigan swine demonstrated that this type of antimicrobial resistance could emerge and persist in the absence of avoparcin use.[4,5]

Here, we investigated the occurrence of VRE among livestock animals in Hong Kong, China. From September 2008 to March 2013, cloacal or intestinal swabs were obtained from animals in a central slaughterhouse (cattle and pigs) and wet markets (chickens). On each date of sampling, the following numbers of animals were tested at random: chicken (20 animals per batch), cattle (10 animals per batch) and pigs (2-7 animals per batch). For each animal species, samples collected on the same day were pooled into a bile-esculin-azide broth supplemented with 6 mg/L vancomycin, followed by subcultured onto a ChromID VRE agar (BioMerieux Vitek, Hazelwood, France).[6] The Vitek 2 (BioMérieux. Mercy l'Etoile, France) and a species-specific PCR assay were used for bacterial identification.[7]

In total, 1889 faecal specimens from 460 cattle (46 batches), 469 pigs (137 batches) and

960 chickens (48 batches) were cultured. One of the batches collected from pigs in January 2013 was culture positive for a vancomycin-resistant *E. faecium*. No VRE was recovered from all the other animal batches. Disk diffusion test and Etest showed that it was resistant to vancomycin (≥256 mg/L), teicoplanin (≥256 mg/L), ampicillin, chloramphenicol, erythromycin, nitrofurantoin and tetracycline but was susceptible to fosfomycin, levofloxacin, rifampicin, and high level gentamicin and streptomycin.[8] PCR experiments showed that it was positive for *vanA*, *ermB* and *tetM*. [4,7] Multilocus sequence type (MLST) identified the strain as sequence type (ST) 6, which is a member of the swine-adapted clonal complex (CC) 5 lineage.[4,5] PCR mapping of Tn*1546* carrying *vanA* was carried out as previously described.[9] An IS*1216V*-IS*3*-like combined element was detected in the left end of Tn*1546*. The remaining part of the Tn*1546* structure was otherwise identical to the reference Tn*1546* from strain BM4147 (GenBank accession M97297.1).

As far as we are aware, this study provides the first description of *vanA* positive *E. faecium* isolated from food animals in China. Since animals from different farm sources were already mixed at the slaughterhouse, the exact farm origin of the swine with the VREm cannot be determined. In China including Hong Kong, most of the human VREm strains were of CC17 and no ST6/CC5 strains had been recovered from human hosts.[6,10] In Europe, ST6/CC5 VREm with similar genotypic features have been reported to occur among swines from Denmark, Portugal, Spain and Switzerland over extended periods of time (1995-2006)

and to cause colonization in humans.[11] Therefore, the detection of this ST6/CC5 VREm clone from Chinese swine is of concern. Since widespread use of antibiotics in Chinese swine farms will likely provide selection pressure for the multidrug-resistant VREm,[2] further surveillance is required to track the epidemiology of CC5 VREm among livestocks in China. Additionaly, stricter regulation and monitoring of antibiotic use in animal husbandry is required, especially for enforcing the avoparcin ban.

Funding

This work was supported by a grant from the Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Disease for the HKSAR Department of Health.

Transparency declaration

Authors have nothing to declare.

References

1. Ho PL, Chow KH, Lai EL *et al*. Extensive dissemination of CTX-M-producing Escherichia coli with multidrug resistance to 'critically important' antibiotics among food animals in Hong Kong, 2008-10. *J Antimicrob Chemother* 2011; **66,** 765-8.

2. Zhu YG, Johnson TA, Su JQ *et al*. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc Natl Acad Sci U S A* 2013; **110,** 3435-40.

3. Tanimoto K, Nomura T, Hamatani H *et al*. A vancomycin-dependent VanA-type *Enterococcus faecalis* strain isolated in Japan from chicken imported from China. *Lett Appl Microbiol* 2005; **41,** 157-62.

4. Donabedian SM, Perri MB, Abdujamilova N *et al*. Characterization of vancomycin-resistant *Enterococcus faecium* isolated from swine in three Michigan counties. *J Clin Microbiol* 2010; **48,** 4156-60.

5. Gordoncillo MJ, Donabedian S, Bartlett PC *et al*. Isolation and Molecular Characterization of Vancomycin-Resistant *Enterococcus faecium* from Swine in Michigan, USA. *Zoonoses Public Health* 2012; electronically published ahead of print on 7 September 2012.

6. Ho PL. Carriage of methicillin-resistant *Staphylococcus aureus*, ceftazidime-resistant Gram-negative bacilli, and vancomycin-resistant enterococci before and after intensive

care unit admission. *Crit Care Med* 2003; **31,** 1175-82.

7. Depardieu F, Perichon B, & Courvalin P. Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. *J Clin Microbiol* 2004; **42,** 5857-60.

8. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement M100-S22*. Wayne, PA, USA, 2012.

9. Jensen LB, Ahrens P, Dons L *et al*. Molecular analysis of Tn*1546* in *Enterococcus faecium* isolated from animals and humans. *J Clin Microbiol* 1998; **36,** 437-42.

10. Sun H, Wang H, Xu Y *et al*. Molecular characterization of vancomycin-resistant Enterococcus spp. clinical isolates recovered from hospitalized patients among several medical institutions in China. *Diagn Microbiol Infect Dis* 2012; **74,** 399-403.

11. Freitas AR, Coque TM, Novais C *et al*. Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn1546 on indistinguishable plasmids. *J Clin Microbiol* 2011; **49,** 925-31.