Transfer of vanA from an Enterococcus faecium isolate of chicken origin to a CC17 E. faecium isolate in the intestine of cephalosporin-treated mice

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Sir,

Vancomycin-resistant Enterococcus faecium isolates can be detected in both human patients and animals but seem in general to separate into different clonal groups. E. faecium strains acquired during hospitalization and associated with nosocomial outbreaks and infections belong to clonal complex 17 (CC17), whereas poultry isolates group into CC9. The vanA gene encoding vancomycin is located on transposon Tn1546. Even though several studies have shown variations in Tn1546, similar Tn1546 transposons have been detected in E. faecium isolates of both human and animal origin.1 This indicates a horizontal transfer of Tn1546 between E. faecium belonging to different clonal groups.

The aim of the present study was to investigate whether transfer of the vanA gene was possible from an isolate of animal origin to a CC17 E. faecium isolate of human clinical origin in the intestine of cephalosporin-treated mice.

An E. faecium isolate of chicken origin harbouring the vanA gene was used as the donor strain.2 The isolate had multilocus sequence type 245 (ST245) and belongs to CC9. The recipient was a human E. faecium isolate from a bloodstream infection and belongs to CC17. The isolate was resistant to ampicillin and susceptible to vancomycin.

Six female NMRI mice weighing 25–30 g were caged in pairs. From day 0 to day 4 the mice were treated subcutaneously twice a day with cefuroxime (3.0 mg/day). On day 1 the mice were fed orally using a steel feeding tube with a suspension of the donor strain (1.4×10^9 cfu in 200 µL of 0.9% saline). The mice were fed the recipient strain on day 2 (3.75×10^9 cfu in 200 µL of saline).

The number of donors, recipients and transconjugants in faecal samples was measured 24 h after the first treatment of cefuroxime and on days 2, 3, 4, 5, 6, 7, 8, 9 and 11. The samples were collected from each cage; each sample contained faeces from both mice in the particular cage. The faecal samples were homogenized in saline and plated on Enterococcus agar plates (BD Diagnostic Systems, Sparks, MD, USA) supplemented with 32 mg/L vancomycin and 32 mg/L erythromycin, 32 mg/L vancomycin and 16 mg/L ampicillin or 32 mg/L erythromycin and 16 mg/L ampicillin. The experimental protocol was approved by the Ethics Committee for Animal Experimentation (animal inspectorate approval no. 2007/561-1430).

None of the mice was colonized with vancomycin-resistant E. faecium prior to inoculation of the donor strain. There was a large variation in the colonization of the mice among the three cages. This could be explained by differences in the colonization resistance among the mice.

Transfers of the vanA gene from the donor strain of animal origin to the CC17 recipient occurred in the intestine of the mice in two of the three cages (Figure 1). Antimicrobial susceptibility testing showed that all in vivo transconjugants were resistant to vancomycin. PCR confirmed the presence of vanA in all transconjugants. Transconjugants were recovered between day 3 and day 8 with a maximum of 250 cfu/g of faeces in both cages (Figure 1b).

The conjugation occurred in the two cages where the colonization of the mice was the highest; the mice in the last cage were poorly colonized. This indicates that in order for transfer to occur high numbers of both donor and recipient must be present; this is in accordance with previous in vivo conjugation studies.2 Higher transfer rates have been obtained in gnotobiotic animals.3,4 Gnotobiotic animals have no colonization resistance whereas mice treated with antimicrobial agents still have some colonization resistance, even though it is decreased compared with untreated animals.

We previously demonstrated transfer of the vanA gene cluster from an E. faecium isolate of animal origin to an E. faecium isolate of human origin in the intestine of humans.2 However, this study was performed without any antimicrobial treatment and the recipient strain BM4105-RF was ST172, which is not part of the hospital-acquired CC17.

A high level of gastrointestinal colonization by enterococci is a frequent predecessor of serious enterococcal infection and this is promoted by the use of systemic or local antimicrobial agents. In this study the mice were treated subcutaneously with the second-generation cephalosporin cefuroxime. Other studies have shown that intestinal colonization of vancomycin-resistant enterococci is promoted by exposure to broad-spectrum antimicrobial agents that have poor anti-enterococcal activity.5 Cefuroxime has poor anti-enterococcal activity and is the cephalosporin of choice in Denmark. A large increase in the consumption of second-generation cephalosporins for systemic use in hospitals has been recorded in Denmark over the past 10 years.6

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In conclusion, the present study showed that vanA of animal origin can be transferred to a CC17 recipient obtained from a patient with a bloodstream infection. To our knowledge, this is the first in vivo study showing a zoonotic transfer of the vanA gene to a CC17 E. faecium isolate.

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Transparency declarations
None to declare.

References
Distinct two-component systems in methicillin-resistant Staphylococcus aureus can change the susceptibility to antimicrobial agents

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Sir,

Staphylococcus aureus produces many virulence factor toxins and exoenzymes, including exfoliative toxin, enterotoxin, toxic shock syndrome toxin (TSST), haemolysins, proteases, lipases and nuclease. Current advances in the study of S. aureus have shown that many regulatory elements for bacterial signal transduction and gene expression, as represented by two-component systems (TCSSs) and the Sar family, respectively, control the expression of virulence factors.1 Genome-wide analysis of several S. aureus strains revealed that 16 types of TCSSs are encoded in chromosomal DNA. Besides the influence of TCSSs on the expression of virulence factors, some TCSSs, including vra and aps/gra, have recently been demonstrated to be associated with susceptibility to antibacterial agents.2,3 However, the effects of these TCSSs on susceptibility to antibacterial agents has never been compared in order to establish which TCSSs play a role in susceptibility to antibacterial agents. Therefore, in this study, a battery of TCSS-inactivated mutant strains of S. aureus MW2 were analysed to evaluate their possible involvement in the regulation of susceptibility to several antibacterial agents.

We attempted to construct insertionally inactivated mutants for 16 different TCSSs in the community-acquired methicillin-resistant S. aureus MW2 strain by pCL52.1 (a thermosensitive E. coli shuttle vector) integration as described previously.4 The target gene was selected as a forward open reading frame (ORF) in an operon that includes two ORFs encoding sensor and regulatory elements. S. aureus was grown in trypticase soy broth (TSB) (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Tetracycline (10 mg/L) was added when necessary.

The MICs of various antibacterial agents were determined using the microdilution method described previously.5 Oxacillin, bacitracin and vancomycin (Sigma Chemical Co., Ltd, St Louis, MO, USA) and teicoplanin (Sigma–Aldrich, Tokyo, Japan), as well as gentamicin, erythromycin, ofloxacin, benzalkonium chloride and chlorhexidine gluconate (Wako Pure Chemical Industries, Ltd, Osaka, Japan), were used. The MIC, which gives a killing rate >99.9%, was determined to evaluate the antibacterial activity of the antimicrobial peptides human β-defensin-3 (hBD3) and LL-37, following the protocols described previously.5

We were able to construct all but one TCSS mutant, termed VicRK (Table 1), because VicRK was reported as an essential gene in cell wall metabolism in S. aureus. Table 1 shows the MICs of five different classes of antibacterial agents, including: (i) cell wall inhibitors (β-lactams; oxacillin, vancomycin, teicoplanin and bacitracin); (ii) protein synthesis inhibitors (erythromycin and gentamicin); (iii) a DNA synthesis inhibitor (ofloxacin); (iv) disinfectants (chlorhexidine gluconate and benzalkonium chloride) (data not shown); and (v) antimicrobial peptides (hBD3 and LL-37) against 15 TCSS mutants of the S. aureus MW2 strain.

The MICs of oxacillin and gentamicin and the MBCs of hBD3 and LL-37 against aps/gra (FK64) and arl (FK67) mutants were decreased, although the MIC and MBC values were different between the two strains. The MICs of oxacillin, vancomycin, teicoplanin and gentamicin for the vra mutant (FK72) were decreased. The mutation of one uncharacterized TCS (FK77) decreased the MIC of bacitracin, while the MICs of other antibiotic agents tested were not altered. The MIC of oxacillin for the srr mutant (FK68) was increased by 2-fold, suggesting that srr may play a down-regulatory role in bacterial resistance to oxacillin. No mutations altered the MICs of the two disinfectants.

Among the TCSSs tested in this study, vra and aps/gra have already been reported to affect the susceptibilities to some antibacterial agents, while arl, srr and one uncharacterized TCSS were not reported previously. Interestingly, several TCSSs affected susceptibility to oxacillin. It is well known that clinically isolated methicillin-resistant S. aureus (MRSA) strains show a variety of β-lactam resistance levels, from low to high. Many factors are known to affect the β-lactam resistance level in MRSA strains, but the precise underlying mechanisms are still not clearly elucidated.6 Our study shows that multiple TCSSs might be associated with the variety of β-lactam resistance levels observed in clinical strains of MRSA.