In summary, molecular study of vancomycin-resistant *E. faecalis* 605 describes the mechanisms of resistance and the power of this hospital isolate to disseminate resistance markers including van genes. Analysis such as this is important for the creation of antibiotic policy and our understanding of the epidemiology of resistance markers in hospital wards.

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**References**


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Sir,

A proteome comparison of the vancomycin-intermediate *Staphylococcus aureus* (VISA), Mu50 and the vancomycin-susceptible *S. aureus* (VSSA), N315, concluded that 17 ORFs: *murk*, *metB*, *yihB*, *atl*, *opuD*, *lysP*, *mutS*, *uhpT*, *modA*, *glpT*, *odhA*, *sdhB*, *rrn*, *prfC*, *mrp*-homologue, SA2486 and *ribH*, appeared to be disrupted in the published Mu50 genome (NC 002758) and were therefore potentially implicated in the VISA phenotype.1 The aim of this study was to determine whether mutations in one or more of these 17 genes were consistently found in VISA isolates, so providing evidence for the molecular basis of the VISA phenotype. We studied the 17 genes in 10 clinical VISA, including Mu50, and 11 heteroVISA, including Mu3; 11 VSSA strains were sequenced. These sequences were analysed comparatively against the published genomes of Mu50 and the VSSAs N315 and MW2. The strains included related strain sets (PC1-heteroVISA/PC3-VISA;2 LIM1-heteroVISA/LIM3-VISA;3 LLA-VSSA/ILLE-heteroVISA), which may indicate whether the heteroVISA phenotype is a genetic precursor to VISA. The vancomycin resistance status of the strains were confirmed by MIC determination (BSAC methodology) and the population analysis profile–area under the curve4 method. DNA was extracted and used to perform PCR with sets of specifically designed primers and then sequencing was performed on each PCR product. The gene sequences were manipulated using a reverse-complement tool (http://www.r9corporation.fsnet.co.uk/bioinformatics_tools/reversecomplement.htm), and aligned alongside the N315/MW2 sequence for the corresponding gene, using the CLUSTALW alignment tool (http://www.ebi.ac.uk/clustalw). Any observed base substitutions, deletions and insertions were confirmed using the corresponding sequence chromatogram, and then compared with the equivalent sequences in the published Mu50 sequences.

Only the sequences of *glpT*, *uhpT*, SA2486 and the *mrp*-homologue were confirmed to be disrupted in Mu50 and Mu3. The genes *glpT* and *uhpT* were disrupted by a base substitution at G1064A and C718T, respectively, both resulting in the formation of a stop codon. The genes SA2486 and *mrp*-homologue were disrupted by deletion at A814 and A5917, respectively. For the remaining 13 genes, sequences from Mu50 and Mu3 were identical, or encoded predicted...
products that were identical to those of N315 or MW2. These findings call into question the accuracy of the published Mu50 genome and its use as a comparator in vancomycin resistance studies. Although these four disrupted genes could be responsible, at least in part, for the vancomycin-intermediate resistant phenotype of Mu50, as suggested by Avison et al., these changes are not essential for the vancomycin-resistant phenotype because the genes were not disrupted in any of the other clinical VISA and heteroVISA investigated. Furthermore, in the clinical VISA and heteroVISA, the sequences of all the genes studied, except the mrp-homologue, were predicted to encode the same products as those in the VSSA strains N315 and MW2, indicating that vancomycin resistance cannot be attributed to loss of these functions. In five of the VISA and six of the heteroVISA tested, the predicted products of the mrp-homologue sequences differed from those produced by N315, MW2 and any clinical VSSA tested by the same four amino acid substitutions (T142A, N147T, D172N and A211V); the remaining VISA and heteroVISA strains produced an Mrp product identical to N315. These findings suggest some degree of clonality between VISA/heteroVISA isolates.

Mu50 and other clinical VISA and heteroVISA strains from around the world share common phenotypic characteristics (e.g. thickened cell walls and reduced cross linking of glycan chains). However, the disrupted genes identified in Mu50 and Mu3 appear to be functional in many VISA, indicating that the VISA phenotype can be effected by various means. A study of the expression of genes involved in the biosynthesis and turnover of peptidoglycan in these isolates would be expected to provide a better understanding of their vancomycin resistance.

Nucleotide sequence accession numbers

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Detection of TEM-52 in Salmonella enterica serovar Enteritidis isolated in Scotland
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Sir,
Non-typhoidal salmonellae are one of the principal pathogens implicated in cases of food poisoning worldwide. In the UK, Europe and USA, Salmonella enterica serovar Enteritidis (S. Enteritidis) is one of the most commonly isolated serotypes, and is thought to be spread to humans through the food chain from reservoirs in food-producing animals. Antibiotic resistance is relatively uncommon in S. Enteritidis. During 1996–2000, the overall incidence of multidrug resistance (resistance to four or more antibiotics) in this serovar was less than 1% in England and Wales. Although antibiotics are rarely required in cases of salmonella enterocolitis, they are crucial if the infection spreads from the intestine. In the treatment of extra-intestinal salmonella infections, the antibiotics of choice are extended-spectrum cephalosporins and fluoroquinolones. Recently, Salmonella isolates harbouring extended-spectrum β-lactamases (ESBLs) capable of hydrolysing third-generation cephalosporins have been reported. This is of particular concern for the treatment of salmonellosis in children, because fluoroquinolones cannot be used in this age group.

Here we report the presence of the ESBL TEM-52 in an S. Enteritidis strain isolated during a hospital outbreak in Scotland. Previously, TEM-52 has only been reported in salmonellae isolated in Hungary and Korea. An outbreak of salmonellosis was identified in a general hospital in Glasgow, Scotland, in the period 20 December 2001–21 January 2002. During this outbreak, nine isolates were obtained from five patients with salmonella gastroenteritis, and two asymptomatic members of staff. Isolates were identified as S. enterica serovar

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