Variant esp gene as a marker of a distinct genetic lineage of vancomycin-resistant Enterococcus faecium spreading in hospitals

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In the USA, vancomycin-resistant Enterococcus faecium (VREF) is endemic in hospitals, despite lack of carriage among healthy individuals. In Europe, however, hospital outbreaks are rare, but VREF carriage among healthy individuals and livestock is common. We used amplified fragment-length polymorphism analysis to genotype 120 VREF isolates associated with hospital outbreaks and 45 non-epidemic isolates from the USA, Europe, and Australia. We also looked for the esp virulence gene in these isolates and in 98 VREF from animals. A specific E faecium subpopulation genetically distinct from non-epidemic VREF isolates was found to be the cause of the hospital epidemics in all three continents. This subpopulation contained a variant of the esp gene that was absent in all non-epidemic and animal isolates. Identification of the variant esp gene will be important in guiding infection-control strategies, and the Esp protein could be a new target for antibacterial therapy. The emergence of vancomycin-resistant Enterococcus faecium (VREF) is of great concern. In the USA, the proportion of VREF among enterococci isolated from blood cultures increased from 0% in 1989 to 25·9% in 1999.1 In contrast, European hospital outbreaks occur infrequently,2 although VREF carriage among healthy individuals and livestock is common. Greater use of glycopeptides such as vancomycin in US hospitals, and use of avoparcin as a growth promoter by the veterinary industry in Europe are assumed to be the cause of these epidemiologic differences. However, the predominant role of antibiotic pressure in the epidemiology of VREF was challenged recently by the occurrence of three outbreaks of VREF in the Netherlands, which has one of the lowest antibiotic uses in the developed world. We postulated that hospital outbreaks are caused by a specific E faecium subpopulation.

We genotyped VREF isolates from two hospital outbreaks in the USA, one in the UK, three in the Netherlands, and one in Australia, by use of amplified fragment-length polymorphism analysis.3 Strains were regarded as epidemic if they were isolated from the same hospital, if the patients had been in contact during the outbreak period, and if the amplified fragment-length polymorphism patterns showed at least 90% similarity. We also genotyped 16 isolates from healthy individuals. The banding patterns were analysed with BioNumerics software (version 1.5, Applied Maths, Kortrijk, Belgium). The Pearson product moment correlation coefficient was calculated, and the unweighted pair group method with arithmetic averages was used for cluster analysis. The primers 5′-GCGTCAACACTTGCATTGCCGAA-3′ and 5′-TGCAGTTGGGACAGTAAAGG-3′ were used to sequence a 492 bp PCR fragment of the purK housekeeping gene from representative epidemic clones from Europe, the USA, and Australia (table), from 20 non-epidemic hospital strains, four strains isolated from healthy individuals, and 17 strains from Dutch farm animals. Additionally, we searched the human isolates and 98 isolates from farm animals for the esp gene, which has been associated with increased virulence in E faecalis,4 but which has so far not been detected in E faecium. The presence of the esp gene was determined by use of a DNA probe, based on the E faecalis esp gene, generated by primers 5′-TTGCTAATGGCAGTGCTACCGGCA-3′ and 5′-GCGTCAACACTTGGATCCTTGC-3′. The probe was used to screen chromosomal digests of VREF by Southern blotting, and an 890 bp internal esp fragment from 12 of the VREF hospital isolates was sequenced and compared with the previously described E faecalis esp gene.4

On the basis of typing and epidemiologic linkage, 120 hospital isolates were regarded as epidemic and 45 as non-epidemic (table). The result (figure) revealed a genetically related epidemic VREF genogroup associated with hospital outbreaks in the three different continents, and which was distinct from non-epidemic hospital isolates and from isolates recovered from healthy European individuals. Additional analysis of purK confirmed the existence of a distinct epidemic VREF genogroup. 36 of the 38 epidemic isolates investigated
had an identical purK sequence (allele 1), which differed in sequence by 1–30 bp from 23 of the 24 non-epidemic human isolates investigated (p<0.0001, Fisher’s exact test; table) and from 17 selected isolates from various species of farm animal (data not shown).

The esp gene was present in 15 of 16 epidemic clones tested, but absent from all 29 non-epidemic hospital isolates tested, and from all 16 isolates from healthy individuals (p<0·0001, Fisher’s exact test; table). The gene was also absent from all 98 VREF isolated from Dutch animals. Sequence analysis of an 890 bp internal esp fragment from representative hospital isolates were identical and contained 25 bp mutations resulting in ten aminoacid substitutions compared with the E faecalis esp gene. One clone contained 24 of these 25 mutations. Furthermore, the E faecium esp gene was detected in all ten blood isolates from one US hospital, one Dutch hospital, the UK hospital, and the Australian hospital, and in none of 16 stool isolates recovered from healthy individuals.

Although only a small part of the E faecium esp gene was sequenced, these findings show that the E faecium esp gene is highly conserved in isolates that caused hospital outbreaks across three continents, and is distinct from the esp gene in E faecalis. These preliminary data also suggest that in E faecium, like in E faecalis, the presence of esp is associated with virulence. The large number of mutational differences between the E faecium and E faecalis esp gene suggests that the epidemic subpopulation of E faecium did not acquire this gene recently. Therefore, we postulate that this subpopulation of E faecium has existed for some time, but that it only became apparent after acquisition of vancomycin resistance. This hypothesis is consistent with the isolation of ampicillin-resistant, vancomycin-sensitive E faecalis strains before the emergence and epidemic spread of vancomycin-resistant isolates of the same DNA type in a Finnish hospital.

The existence of an epidemic VREF subpopulation with specific genetic characteristics has important consequences for clinical practice. Screening for the presence of the variant esp gene or the specific purK allele could provide a useful marker in infection control. The presence of the variant esp gene in E faecium seems to be associated with in-hospital spread and possibly with increased virulence. Since Esp is a cell-surface protein in E faecalis, and can be recognised by antibodies, it could be a potential target for eradication of the epidemic VREF subpopulation from the gastrointestinal tract of carriers.

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Enterococcal vanB resistance locus in anaerobic bacteria in human faeces

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While developing a rapid method to detect carriers of vancomycin-resistant enterococci (VRE), we found the vanB gene by PCR in 13 of 50 human faecal specimens that did not contain cultivable VRE. Passingage under antibiotic selection allowed us to isolate two species of anaerobic bacteria that were vanB PCR positive, vancomycin resistant, and teicoplanin sensitive. Sequence analysis of the 16S rRNA genes showed that one isolate resembled Eggerthella lenta (98% identity), and the other Clostridium innocuum (92% identity). Southern hybridisation and nucleotide sequencing showed a vanB locus homologous to that in VRE. We propose that vanB resistance in enterococci might arise from gene transfer in the human bowel.

The emergence of vancomycin-resistant enterococci (VRE) has been linked to the use of various antimicrobials, which suggests either that genetic mutations are arising or that gene transfer from vancomycin-resistant species is occurring. The vanA and vanB loci, both of which are located on mobile DNA elements, confer glycopeptide resistance to enterococci. In Australia, VanB VRE is predominant, and ever since VRE was identified at our hospital high-risk patients have been screened for carriage of VRE. Those patients found to be carriers are nursed in special isolation facilities. To improve the screening process a 500 ml volume of this overnight broth culture with a glass-bead cell homogenisation technique in the presence of 1% Triton-X 100 (Oxoid, Basingstoke, UK) to amplify any enterococci that were not readily cultivated faecal swabs in 10 mL of Enterococcosel (EC) broth. All 150 specimens were tested with the culture-enrichment PCR method described above. From this experiment, 13 out of 50 faecal samples from patients were vanB-PCR positive, and all 100 negative controls were negative. We had previously sequenced the vanB gene from locally obtained screening isolates of VRE, which showed two different vanB 2 sequence types which we designated vanB 2.1 and vanB 2.2. These sequences were identical to Genbank Accession numbers U95426 and U95428, which were first described by Patel et al. Among the 13 positives described above, seven had the sequence type vanB 2.1, five were sequence type vanB 2.2, and one was vanB 1.

To check for VRE in these vanB-PCR-positive faecal specimens, a sample from each was inoculated into EC broth, incubated overnight at 35°C, and plated on to EC agar containing 6 µg/mL vancomycin. No VRE were identified. A further aliquot from each specimen was plated directly onto EC agar with and without 6 µg/mL vancomycin or Slanetz and Bartley agar (Oxoid, Basingstoke, UK). Ten of 12 specimens (one was lost) yielded one or more enterococcal species (five Enterococcus faecalis, five E faecium; five other species). All of these tested negative by PCR for vanB. To isolate the organism harbouring vanB, the 12 remaining vanB-PCR-positive faecal samples were cultured under selective growth conditions. First, a sample from each specimen was incubated aerobically for 22 h at 35°C in Brain Heart Infusion Broth (BHI, Oxoid, Basingstoke, UK). Then a 10 µL aliquot of this primary enrichment broth was sequentially passaged through three replicates of either EC broth alone, BHI alone, BHI with 50 µg/mL of aztreonam, BHI with a colistin sulphate and nalidixic acid supplement (Oxoid), or BHI with 50 µg/mL metronidazole. After each step of the sequential passaging process a 500 µL sample was withdrawn from each broth and tested by PCR for vanB. The PCR signal could be maintained only through sequential passaging for two faecal specimens (designated 043 and 055). However, the PCR signal from both these specimens became negative in BHI with metronidazole, which suggests that the vanB gene was in an anaerobe. Sample 055 but not sample 043 also became negative under aztreonam selection. A sample from the 3rd passage of faecal sample 043 in aztreonam was used to inoculate another BHI that contained 50 µg/mL vancomycin. The broth was incubated anaerobically for 22 h, then subcultured onto brain heart infusion agar and horse-blood (HBA) agar and incubated anaerobically for 48 h. Of four colony types present, one contained thin gram-positive rods and was positive by PCR for vanB. The PCR product was sequenced and confirmed as vanB 2.2, which matched the vanB sequence obtained from the faecal specimen from which the isolate was obtained. From the nucleotide sequence of the 16S rRNA gene, the organism was identified as being closely related to Eggerthella lenta strain JCM9970 (98% sequence identity). The organism was designated ML01043 and the 16S rRNA gene sequence was deposited in Genbank under accession number AF304434.

Initially the vanB PCR signal for sample 055 could be maintained only through to the third passage in BHI without any antibiotics. Further passaging of this sample in BHI under anaerobic conditions with penicillin, vancomycin, trimethoprim, and cefazolin, and then screening for metronidazole-sensitive colonies on HBA gave a colony type that contained thin, gram-negative, spore-containing rods that were vanB PCR positive. The vanB sequence from this organism was vanB 2.2, which matched the sequence from the original 055 faecal specimen. Nucleotide sequencing of the...