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Hepatitis B Virus Resistance to Adefovir in a Nucleotide Naive Patient with Chronic Hepatitis B Virus Infection

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showed < 70% similarity (Figure 1) and were considered unrelated. Multilocus sequence typing (MLST) analysis showed that isolates of the outbreak strain had an identical allelic profile, ST17 belonging to the clonal complex 17 (CC-17). Four *E. faecium* isolates were used as controls in the MLST analysis, two vancomycin-susceptible isolates from HGS had the *ddl2* allele, instead of the *ddl1* of the *vanB2* isolates, and belonged to ST16; and two other *vanB* producers from other hospitals were ST18 and ST132.

Infections caused by VanB vancomycin-resistant *E. faecium* belonging to CC-17 are still relatively uncommon in Europe. In two hospitals in Zaragoza (Spain), a clonal relationship was demonstrated between seven *vanB2*-containing *E. faecium* isolates.⁴ The first VanB *E. faecium* isolated in a Spanish hospital was described in 2004.⁵ In Sweden, a nosocomial outbreak of VanB2 *E. faecium* with the *purK* allele 1 has been reported.⁶

An enhanced infection control programme was introduced in the HGS during 2006. Patients infected by VanB enterococci were isolated in single rooms whenever possible, and these rooms were mechanically cleaned each day. The outbreak of VanB *E. faecium* finished in August 2006, with no further cases detected.

In conclusion, to the best of our knowledge, we describe the first outbreak in Spain of a *vanB2 E. faecium* strain belonging to CC-17 and which was multiply resistant to vancomycin, ampicillin and fluorquinolones.

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Transparency declarations

None to declare.

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Hepatitis B virus resistance to adefovir in a nucleotide naive patient with chronic hepatitis B virus infection

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Sir,
Antiviral therapy of chronic hepatitis B (CHB) remains a major clinical challenge. The new orally active nucleos(t)ide analogues, such as lamivudine, adefovir and entecavir, have been shown to be efficacious in controlling viral replication, decreasing inflammatory activity within the liver and preventing the progression of CHB infection to liver cirrhosis and hepatocellular carcinoma.¹ However, due to the spontaneous error rate of the hepatitis B virus (HBV) polymerase gene and persistence of HBV in infected individuals, long-term antiviral treatment is needed in most individuals and therefore increases the risk of selecting drug-resistant mutants.

Drug resistance is associated with the development of polymerase gene mutations, followed by a rise in HBV viral load and a subsequent increase in alanine transaminase levels with exacerbation of liver disease. Genotypic resistance occurs in ~20% of individuals per year, but at a lower rate in those treated with adefovir (3% at year 2 and 29% at year 5 of treatment).² The major lamivudine resistance mutations in HBV are located in the highly conserved reverse transcriptase (rt) domain of the polymerase gene, specifically in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the C domain, at position rtM204I/V. Additional compensatory mutations are selected downstream in the B domain at positions rtL180M and rtV173L, which augment drug resistance and enhance viral replication efficiency *in vitro*.³ Adefovir resistance

Correspondence

Table 1. Amino acid mutations in the HBV polymerase gene detected by direct nucleic acid sequencing

Associated resistance	Mutation sites	Wild-type	Patient sera	
			July 2004	June 2006
LAM	rt173	V	V	L
LAM	rt180	L	M	M
ADV	rt181	A	V	V
LAM	rt204	M	V	V
ADV	rt233	I	I	I
ADV	rt236	N	N	N

Note: mutations are shown in bold.
LAM, lamivudine; ADV, adefovir.

is associated with mutations located in the B and D domains, at positions rtA181T/V and rtN236T, respectively.² Adefovir is effective in suppressing lamivudine-resistant HBV and, therefore, is most commonly used in patients with lamivudine-resistant HBV infection.² While acquired resistance to adefovir is important, primary resistance to this nucleotide analogue was reported recently.⁴ A variant of HBV was detected in three non-responders even before they commenced treatment with adefovir. The associated mutation rtI233V significantly increased resistance to adefovir *in vitro*. This variant occurs in ~ 2% of individuals with CHB.⁴

In this report, molecular evidence is presented of the emergence of primary adefovir resistance in a patient on prolonged lamivudine treatment for chronic HBV infection.

A 40-year-old lady presented to St James's Hospital, Dublin, with hepatitis Be antigen (HBeAg)-positive chronic HBV infection in April 1999. A liver biopsy performed in July 1999 showed bridging fibrosis consistent with early cirrhosis. She was given interferon monotherapy 5 MU daily but this was stopped after 16 weeks because of severe side effects. She remained HBeAg-positive at the end of interferon treatment. No HBV DNA levels were determined before or after interferon treatment. In July 2000 she was started on lamivudine monotherapy (100 mg/day). She became HBeAg-negative in April 2003. HBV DNA was undetectable at this time by hybridization assay (Digene HClI) (limit of detection 1.4×10^3 IU/mL). She continued on lamivudine monotherapy and remained stable until July 2006 when alanine transaminase levels were noted to be raised at 46 IU/L (normal range < 35 IU/L). HBV DNA levels measured by realtime PCR were $5.2E + 02$ IU/mL (HBV PCR kit, Artus, Hamburg, Germany) and $3.8E + 03$ IU/mL (Cobas Ampliprep/Cobas Taqman HBV test, Roche Diagnostics, Mannheim, Germany) in July 2004 and June 2006, respectively. Resistance to lamivudine was suspected. Nucleic acid sequencing of the HBV polymerase gene from sera collected in 2004 and June 2006 was performed as described previously.⁵ Mutations associated with lamivudine resistance were detected at positions rtL180M and rtM204V in both samples, with the rtV173L mutation appearing in serum collected from 2006 (Table 1). The latter mutation did not appear to affect the replicative capacity of HBV since there was no biologically significant difference in HBV DNA levels in July 2004 and June 2006. Surprisingly, a mutation associated with adefovir resistance was identified at position rtA181V in both samples,

although the patient has never been treated with adefovir. She has now started treatment with tenofovir (300 mg/day).

Genotypic primary resistance to adefovir can occur without prior adefovir treatment. In this report, the patient received lamivudine monotherapy for several years and developed the rtA181V mutation which confers resistance to adefovir.² This finding supports a previous report of the emergence of the rtA181T mutation in 4 of 23 patients on long-term lamivudine therapy. Similar to the current case, the amino acid substitution at position 181 appeared concomitantly with the appearance of YMDD motif mutations.⁶ The results suggest that amino acid substitutions at 181 of the HBV polymerase gene may confer resistance to the licensed nucleoside and nucleotide antiviral analogues, lamivudine and adefovir, respectively. Furthermore, while the rtI233V mutation has been shown to cause primary adefovir resistance,⁴ there was no evidence of the mutation in this case. The emergence of the rtA181V mutation in a patient with no prior adefovir treatment confirms the need to test for the presence of adefovir resistance mutations in patients who develop lamivudine resistance.

This report showed that development of primary adefovir resistance can be associated with long-term lamivudine monotherapy. Furthermore, HBV polymerase gene sequencing should become part of the treatment monitoring algorithm to improve treatment efficacy. The amino acid substitution at position 181 may be important in conferring cross-resistance to anti-HBV drugs and therefore should be investigated further.

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Direct detection of *vanB2* using the Roche LightCycler *vanA/B* detection assay to indicate vancomycin-resistant enterococcal carriage – sensitive but not specific

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Sir,

Vancomycin-resistant enterococci (VRE) are an infection control issue in many hospitals, such that rapid, accurate detection has important clinical consequences. There are five genotypes responsible for acquired vancomycin resistance (*vanA*, *vanB*, *vanD*, *vanE*, *vanG*), with *vanB* the most prevalent in Australia.^{1,2} Detection of VRE is generally by culture-based methods, with confirmation of species and *van* genotype based on molecular methods.²

The Roche LightCycler VRE Detection Kit (Roche Diagnostics, Mannheim, Germany) has been developed to rapidly assess for VRE colonization.³ This kit provides the primers and the hybridization probes for the amplification and sequence-specific detection of *vanA*, *vanB1* and *vanB2/3*, along with an internal control for detection of sample inhibition. It has a processing time of approximately 1.5 h. Using this kit with DNA directly extracted from clinical specimens, detection of VRE can be reduced to less than 24 h. However, when used in

this manner, it is important to note that the system detects *van* genes rather than VRE *per se*. Thus, the presence of *vanB* in non-enterococcal species may influence the sensitivity and specificity of this approach for detection of VRE.^{4–7}

We assessed the usefulness and accuracy of the LightCycler VRE detection system in the rapid identification of *vanB* VRE colonization in 59 faecal samples previously obtained from hospital inpatients (53 haemodialysis, 6 non-haemodialysis patients) and stored at -80°C . Twelve of these faecal specimens (from 6 haemodialysis and 6 non-haemodialysis patients) were shown previously to contain *vanB2 Enterococcus faecium*, while 42 specimens were repeatedly shown to be culture-negative for VRE.⁴ As we have previously reported, four specimens were shown to be *vanB2* anaerobe culture-positive, but were VRE culture-negative (one specimen contained *Clostridium boltea* and *Ruminococcus* sp., two specimens contained *Clostridium hathewayi* and one specimen contained an un-speciated *Clostridium*), while a further specimen contained a *vanB2* anaerobe (*C. hathewayi*) and *vanB2 E. faecium*.⁴ Faecal specimens were defrosted and DNA was extracted directly from 200–220 mg of faeces using the QIAamp[®] DNA Stool Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's recommendations. In addition, 17 h enrichment cultures in antibiotic-free Enterococcosel broth (BBL, Cockeysville, MD, USA) of these faecal samples were prepared and DNA extracted as described previously.⁴ Control isolates were *Enterococcus faecalis* ATCC 29212 (*van A/B* negative), *E. faecalis* ATCC 51299 (*vanB* positive) and a clinical *vanA E. faecium* isolate obtained from our institution. All DNA was stored at -20°C then tested for the presence of *vanA* or *vanB* using the LightCycler VRE Detection Kit (Roche Diagnostics, Catalogue no. 3 334 996) and the LightCycler instrument (Roche Diagnostics) with software version 3.5, according to the manufacturer's instructions. The limit of detection was determined by dilution of the plasmid DNA positive control (1000 copies per $5\ \mu\text{L}$) provided in the kit. Dilutions were performed in triplicate with the resulting standard curve imported into the data analysis mode. The sensitivity and specificity for detection of VRE was calculated. Statistical differences in the rates of accurate VRE identification and PCR inhibition were compared using χ^2 /Fisher's exact test.

All 12 *vanB2 E. faecium* culture-positive samples were positive for *vanB* by LightCycler on both the direct faecal specimen and the 17 h enrichment culture (100% sensitivity; Table 1). However, specificity for detecting VRE with LightCycler was 36% and 30% using direct DNA extraction and 17 h enrichment broth culture, respectively. Of the four specimens known to contain *vanB* anaerobes, three of these were positive by LightCycler by both methods (Table 1). The fourth specimen was most likely below the limit of detection as this was only positive following 36 h enrichment broth culture (data not shown). In addition, the specimen that contained both a *vanB2* anaerobe (*C. hathewayi*) and *vanB2 E. faecium* was also positive for *vanB* by LightCycler. Overall, the LightCycler demonstrated a lower detection limit of 40 genome equivalents per reaction (data not shown). The rate of assay inhibition using the LightCycler procedure was 0% for 17 enrichment broth cultures and 3% for direct detection, similar to that observed previously for the Roche LightCycler VRE kit.³

The Roche LightCycler VRE detection assay demonstrates high sensitivity (100%), but limited specificity (30–36%) for the detection of *vanB* VRE in surveillance specimens of faeces. This