

## The epidemiology of glycopeptide-resistant enterococci on a haematology unit – analysis by pulsed-field gel electrophoresis

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### SUMMARY

As part of an interventional study to determine glycopeptide-resistant enterococci (GRE) acquisition on a three-ward haematology unit, rectal swabs were taken weekly from 293 patients recruited to the study between June 1995 and December 1996. The GRE isolates obtained from the first positive rectal swab from 120 colonized patients, the isolates from 7 patients with clinical infection and 43 isolates obtained from the ward environment were compared by pulsed-field gel electrophoresis (PFGE). Sixty-three of 120 patients were colonized by one of strains A–H, while 49 were colonized by unique strains. The first 18 weeks were associated with the highest prevalence of GRE by rectal swab, with a single strain A responsible for 52% of acquisitions on ward 2, 22% on ward 3 and 36% on ward 4. Other smaller ward associated clusters were evident. Environmental sampling of ward 2 during this time showed that all but 2 of 30 isolates were indistinguishable from strain A. As the GRE prevalence fell, rectal swab and environmental isolates became more heterogeneous, and strain A disappeared after week 55. GRE prevalence rose again in the final 15 weeks of the study, and a new predominant strain B emerged on ward 2 responsible for 50% of new acquisitions. In the seven patients with clinical infection with GRE, the clinical isolates were compared with the contemporaneous rectal swab isolate, and were found to be the same in only two cases. An analysis of five long-term carriers colonized for a median of 19 weeks (range 11–34) showed colonization with at least two and in one case six distinct strains, raising the question of how many strains may be colonizing a patient at any one time, and suggesting that multiple colonies should be analysed. These data suggest that cross-infection was an important factor in the spread of GRE when the colonization rate was high.

### INTRODUCTION

Since their emergence in 1987 [1, 2], glycopeptide-resistant enterococci (GRE) have become important nosocomial pathogens, and are ideally suited to survival in haematology units where patients are admitted for prolonged periods and antibiotics are used intensively. Enterococci are able to survive in the

environment, such that contaminated medical equipment [3], ward surfaces [4] and staff hands [5] may act as vectors to facilitate cross infection on the ward. Alternatively some have postulated that low-level GRE carriage may occur in the community via the food chain [6], and that bowel overgrowth of GRE may occur on exposure to antibiotics that select for their growth [7].

After GRE first emerged on the haematology unit at University College London Hospital in December 1993, three point prevalence studies had demonstrated

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that 30–40% of patients carried GRE by rectal swab. Between June 1995 and December 1996 we undertook a prospective interventional study [8] which showed that the rate of GRE acquisition measured by weekly rectal swab could be significantly reduced by a change in antibiotic policy and heightened infection control measures. In the current study, all first rectal swab isolates, clinical isolates and isolates obtained from environmental sampling during this 18 month period were compared by pulsed-field gel electrophoresis (PFGE) – generally regarded as the optimum method for genotypic and epidemiological analysis of enterococci [9], to determine how GRE may have spread around the unit. In addition, GRE obtained by weekly rectal swab from five long-term carriers were compared to determine whether patients were colonized by more than one strain.

## MATERIAL AND METHODS

### Patients

The haematology unit at UCLH consists of approximately 35 designated beds in 3 adjacent wards, with most patients being nursed in single rooms. Only a small proportion of rooms had en-suite bathroom and toilet facilities (ward 4 and three rooms on ward 2). Two hundred and ninety-three patients were screened between June 1995 and December 1996, the majority of whom were undergoing bone marrow or peripheral blood stem cell transplants or were receiving induction or consolidation chemotherapy for haematological malignancy.

### Isolation of GRE

Patients were screened for GRE colonization by weekly rectal swab. Swabs were enriched in brain heart infusion broth (Oxoid, Basingstoke, UK) containing tryptose 10 g/l, NaCl 5 g/l, nalidixic acid 7.5 mg/l, colistin 5 mg/l and horse serum 10%, and incubated for 24 h at 42 °C. The broth was subcultured to selective medium containing bile aesculin agar (Difco, Detroit, MI, USA), nalidixic acid 15 mg/l, colistin 10 mg/l, vancomycin 8 mg/l and horse serum 10%, and incubated aerobically at 42 °C for 24 h. Aesculin-positive colonies were subcultured overnight on to 5% horse blood agar, and enterococci were identified to species level by 'API Strep' (Bio-Mérieux, Hazelwood, MO, USA). High- and low-

level vancomycin resistance was confirmed by growth of the isolate up to a 5 µg and 30 µg disc respectively on Isosensitest agar (Oxoid). Environmental samples were taken with swabs moistened in brain heart infusion broth and processed as above.

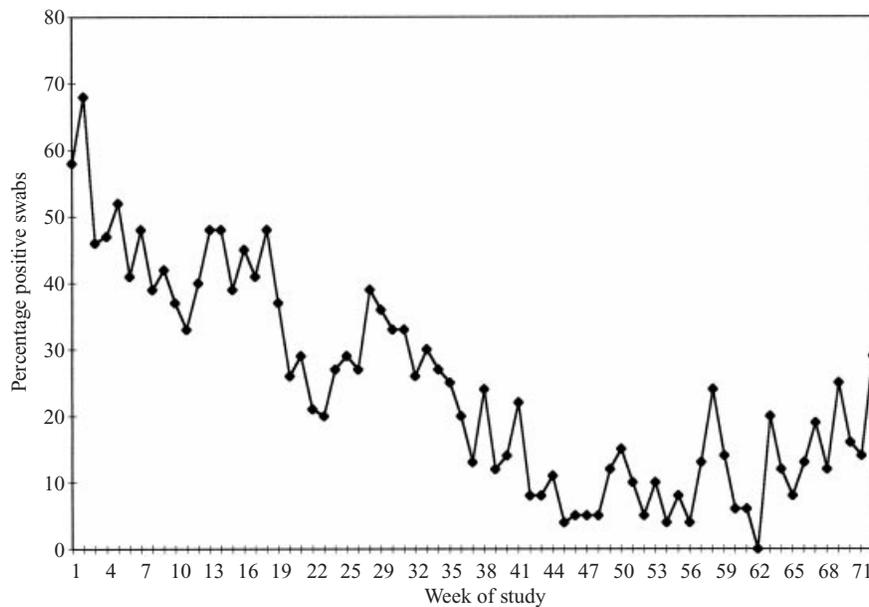
### PFGE

Isolates were compared by pulsed field gel electrophoresis (PFGE). DNA preparation was by the method of Bannerman [10], and restricted with *Sma*I (Boehringer Mannheim) following the manufacturers instructions. Fragments were separated through 1.2% agarose (Mol Grade Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) for 30 h in 0.5% TBE (44.5 mM Trizma base, 44.5 mM boric acid, 1 mM EDTA), with pulses increasing from 1–35 sec in an electric field of 6 V/cm in a CHEF DRII apparatus. Ethidium bromide stained gels were analysed with the aid of Gel Compar 4.1 (Applied Maths, Katrijk, Belgium). Dendrograms of percentage similarity were calculated by Pearson's correlation coefficient and represented by the unweighted pair group method with mathematical averages algorithm. The criteria used for assessing similarity corresponded to that suggested by Morrison et al. [11].

## RESULTS

### Bowel colonization by GRE

Over the 18 month period, 120 of the 293 patients recruited were colonized as detected by rectal swab. Thirty-one were positive either at the start of the study or on admission to the ward, but the remaining 89 GRE carriers had had at least one negative rectal swab, suggesting that they had acquired GRE whilst on the unit. The initial 18 weeks were associated with a high prevalence rate of GRE (Fig. 1), with 43 out of 75 (57%) patients admitted during that time acquiring GRE. Following the introduction of a new antibiotic policy [8] and heightened infection control measures, the middle 38 weeks of the study were associated with a fall in GRE prevalence and new acquisitions – 25 out of 129 patients (19%). For the final 15 weeks, the original antibiotic policy was reinstated, but heightened infection control measures were maintained, and this was associated with a rise in GRE prevalence and new acquisitions – 21 out of 58 patients (36%) [8].



**Fig. 1.** Graph to show the prevalence of GRE by rectal swab from an average of 30 swabs per week. Week 19 saw the introduction of a new antibiotic policy and heightened infection control measures. At week 58 the original antibiotic policy was reinstated.

**Table 1.** *PFGE analysis of the first rectal swab isolates of GRE from 120 colonized patients*

Strain	Number of patients colonized	Location (ward)
A	35	2, 3 and 4
B	11	2
C	1	2
D	2	4
E	6	3
F	3	3
G	2	3
H	3	2 and 3
Uni	49	
NtI	8	

Uni, unique; NtI, non-typable isolates.

DNA fingerprinting of the first positive rectal swab isolates from all 120 colonized patients showed that 63 patients harboured 1 of 8 distinct strains A–H, 49 were colonized by unique strains, and 8 isolates failed to produce banding patterns (Table 1). Strain A was responsible for most GRE carriage, but this was found only in the first 33 weeks of the study, where it was responsible for 24/46 (52%) of GRE acquisitions on ward 2, 5/23 (22%) on ward 3 and 5/14 (36%) on ward 4. Strain E was unique to ward 3 at this time, where it was the predominant strain responsible for 6/20 (30%) of GRE acquisitions. After week 55 when the original antibiotic policy was reinstated, a new

strain emerged on ward 2, and was responsible for 11/22 (50%) of new acquisitions, but it was not recovered on the other two wards where the isolates had become more heterogeneous (Fig. 2).

### Clinical isolates

There were seven patients with clinically significant isolates of GRE during the study – three each from urine and from blood and one from a line tip. These were compared with the isolates obtained by rectal swab at the time of the infection, and the first rectal swab isolate (Table 2). The clinical isolates and contemporaneous rectal swab isolates were the same in only 2 out of 6 cases. GRE was isolated from a line tip in a patient who died suddenly – no rectal swab was obtained antemortem, but the clinical isolate was indistinguishable from the predominant strain A.

### Environmental isolates

The first 30 of 43 environmental isolates (EI–30) were taken from ward 2 in the first 3 months of the study – 19 from the side rooms of 5 colonized patients (typically from mattresses, light switches, TV remote controls, telephones and some areas touched primarily by healthcare staff, e.g. patient observation charts), and the remainder from communal bathrooms and commodes. Twenty-eight of the first 30 isolates were

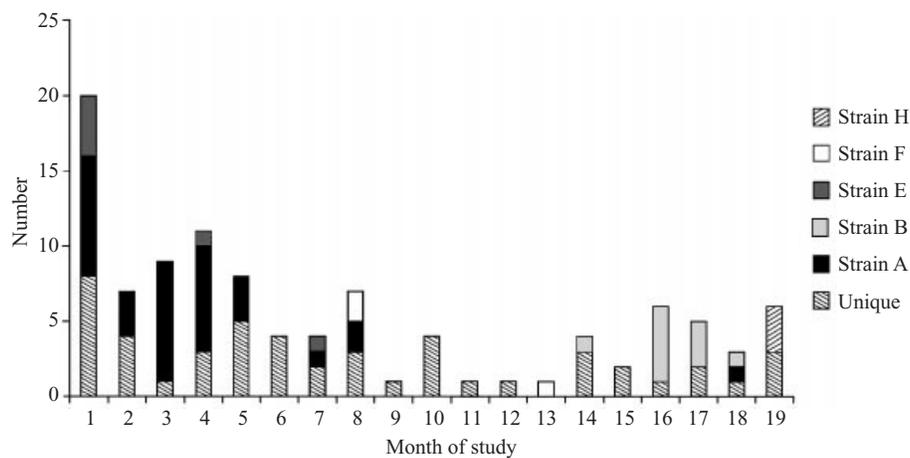


Fig. 2. New GRE acquisitions by month and strain type.

Table 2. Comparison of PFGE banding patterns from the seven clinical isolates and the rectal swab isolates from the same patients

Patient	Clinical isolate	Contemporaneous rectal swab	First rectal swab
7	Strain E	Strain E	Strain E
75	Strain A	Different	Strain A
32	Strain A	Different	Strain A
9	Strain C	Strain C	Different
147	Unique	Different	<i>E. avium</i>
NS1	Strain B	Different	Different

Patient 34, strain A from line tip post mortem. No rectal swab done.

indistinguishable from each other (Fig. 3). When these were compared with the first rectal swab isolates from the five patients occupying the rooms at the time of sampling, all appeared indistinguishable from strain A. The environmental isolates E13 and E15 were recovered from a toilet, and represented a single strain of *E. faecalis*. Only two patients were colonized with glycopeptide-resistant *E. faecalis* on that ward at the time of sampling, one of which failed to produce a banding pattern, and the other appeared dissimilar to the above environmental strain. Isolates E32–34 were indistinguishable from each other, and were cultured from a room recently vacated by a patient colonized with the same strain. The room had been cleaned by domestic staff, yet these isolates were obtained from the mattress, cupboard door and fridge door. Three further isolates E41–43, were cultured from a commode, nurse call buzzer and mattress respectively following the apparent thorough cleaning of vacated rooms. Isolates E35–38 were cultured from two nurse's uniforms on ward 2, one of which was

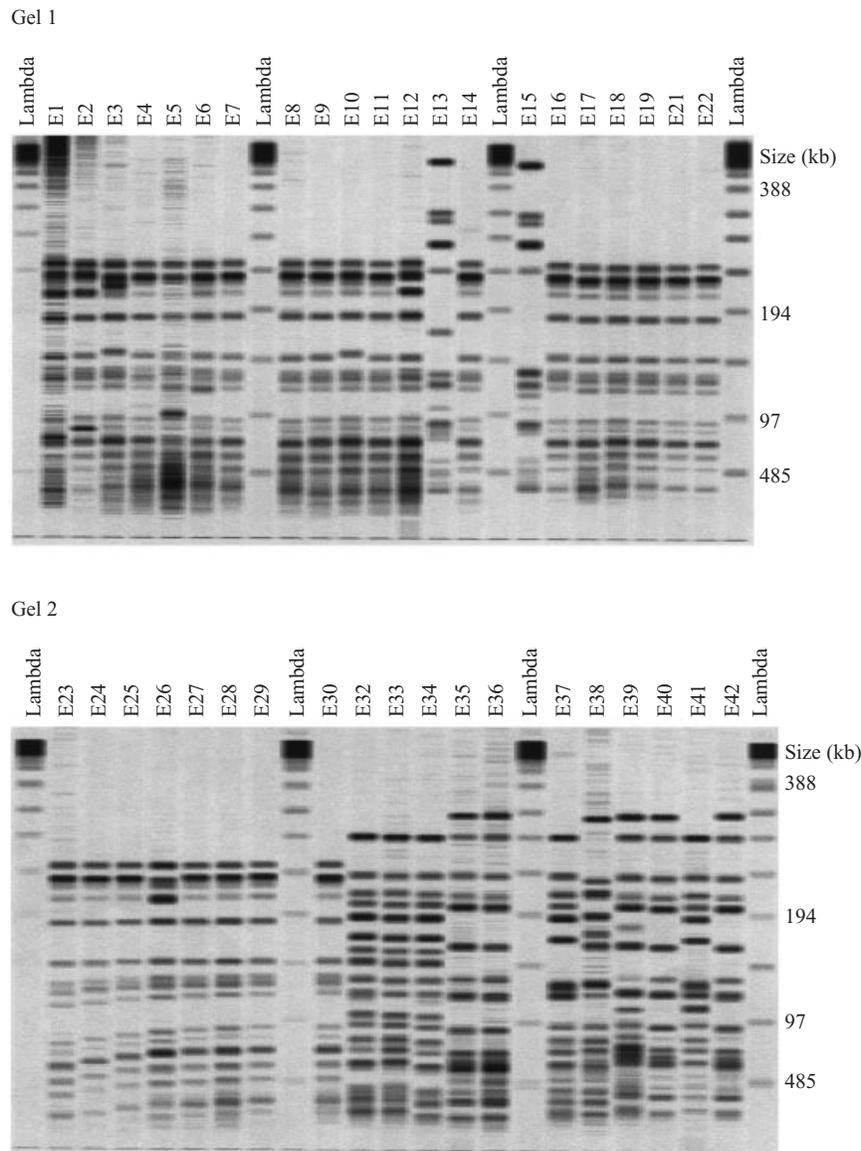
indistinguishable from that of a colonized patient on the same ward.

### Serial patient isolates

Some patients were hospitalized for prolonged periods, and the weekly rectal swab isolates from five such long-term carriers were compared to ascertain whether patients were colonized by more than one strain. Table 3 shows that all 5 patients were colonized by between 2 and 6 strains – some of which had been detected in other patients (strains A, B, F and G), while the remainder were unique. Patient 103 was colonized over a long period (34 weeks) with three distinct strains, which appeared to arise sequentially, and similarly in patient 266 who was colonized by two strains. Whether this represents a true phenomenon, or merely reflects the relative predominance of a strain at any one time when only a single colony is analysed each week is debatable. In the remaining three patients, strains waxed and waned over time. Strain F occurred in three patients – patients 3 and 76 were at opposite ends of ward 2 for a period of 9 weeks, while patient 103 was on ward 3 at a later time. This strain was subsequently seen in two further patients on ward 3 – both of whose admissions overlapped with that of patient 103.

### DISCUSSION

GRE had been endemic on the haematology unit at UCLH for at least 2 years before the study, with approximately 50% of patients colonized by rectal swab. DNA fingerprinting of 120 first rectal swab isolates demonstrated that 53% of strains appeared more than once. Whilst previous studies have demon-



**Fig. 3.** PFGE of *SmaI* digested DNA from 43 GRE isolates recovered from the ward environment.

strated that some GRE outbreaks are due to the dissemination of a single strain [12, 13], this study and others have shown that many different strains may be present, especially in circumstances where GRE are endemic [14]. The predominance of some strains during an outbreak has been noted previously [15]. In this study, strain A was particularly prevalent, and was found on all three wards during the first 8 months of the study accounting for 41% of all new GRE acquisitions at that time. By the end of the study, strain B had become the predominant strain on ward 2 only, accounting for 50% of new acquisitions. These data together with the presence of smaller ward-associated clusters, suggested that cross-infection played an important part in GRE acquisition on this

unit – especially when the rate of GRE acquisition was high. Bonten et al. [16] examined the concept of ‘colonization pressure’ on the spread of GRE on a medical intensive care unit. They found that the proportion of patients colonized with GRE was the most important variable affecting the acquisition of GRE in new patients, being more important than antibiotic exposure and compliance with infection control measures. This effect was most pronounced when the GRE colonization rate was high. Though no genotypic analysis was performed, they postulated that this was due to an increased incidence of cross-infection, since any lapses in infection control practice would be expected to be associated with a greater risk of GRE transmission when GRE prevalence was high.

Table 3. *The PFGE profiles of serial GRE isolates from weekly rectal swabs from five long-term carriers*

Patient no ...	3	46	76	103	266
Period of observation (weeks) ...	19	23	17	34	11
PFGE profile in sequence					
Isolate 1	G	A	P	F	B
Isolate 2	I	L	Q	F	B
Isolate 3	I	A	P	F	B
Isolate 4	F	L	Q	V	B
Isolate 5	J	N	Q	F	B
Isolate 6	F	L	Q	F	Y
Isolate 7	F	L	Q	F	Y
Isolate 8	F	O	F	W	Y
Isolate 9		L	P	W	
Isolate 10		L	P	W	
Isolate 11			R	W	
Isolate 12			R	W	
Isolate 13			S		
Isolate 14			T		
No. of strains	4	4	6	3	2

Several environmental surveys were carried out at various times during the study. All but 2 of the first 30 GRE isolates obtained from the environment of ward 2 during the first 3 months of observation, were of strain A, despite the isolates coming from varied sites around the ward, including communal bathroom facilities. A previous study of the thermotolerance of enterococci and susceptibility to hypochlorite suggested that there is variability between strains [17]. It may be that some enterococci are more suited to survival in the ward environment than others, but as yet there are no data to support this. Previous studies have shown that the survival of GRE in the environment seems to vary from a few days [18] to several months [19]. There is some controversy in the literature as to the importance of environmental contamination in the spread of GRE. In the setting of a children's hospital, Gray and George [20] found that colonization of the environment with GRE was common, and that there appeared to be a relationship between the incidence of GRE acquisition, standards of cleanliness and environmental contamination. In contrast, Bonten et al. [21] found that in an intensive care unit, environmental contamination tended to be transient, and that the rate of bacterial contamination on surfaces was low. Urine containers were the only site that showed persistent colonization. They postulated that the environment was less important in cross-infection than the presence of colonized patients. Whether the predominance of strain A in our ward

environment was merely a reflection of the majority of patients being colonized with that strain in the early part of the study, or whether the ward environment represented a potent source of cross-colonizing GRE is not clear. Certainly, after the introduction of heightened infection control measures including ward cleaning procedures, surveys showed a much reduced rate of environmental contamination, and the isolates obtained became more heterogeneous as did those isolates obtained from rectal swabs.

A significant number of patients (41%) appeared to be colonized with unique strains of GRE, suggesting that not all GRE were acquired via cross-infection on the ward. The source of GRE in these patients is unknown, but the possibility exists that the patients own endogenous enterococci may acquire glycopeptide resistance from mobile genetic elements whilst on the ward. Alternatively, the finding that bowel colonization with GRE emerges after the administration of glycopeptides to normal volunteers [7] suggests that there may be low-level colonization with GRE in the community. The food chain has been implicated as a source of GRE in Europe, consequent on the previous widespread use of glycopeptides in farming [22], and the finding of GRE in farm effluent and uncooked meats [6]. However, glycopeptides have never been used in this way in the United States where nosocomial infection with GRE is common. Clinical use of vancomycin is particularly widespread in the United States, and may be a factor in the continuing high prevalence of GRE in hospitals [23]. A study by Bonilla et al. [24] determined rectal colonization with GRE in residents of a long-term care facility in Michigan, and found that almost all colonized patients had had some previous contact with the local acute-care hospital.

There have been relatively few studies of genotypic variation and stability of GRE isolates obtained from long-term carriers. Bonten et al. [25] studied the PFGE profiles of 455 GRE isolates from 106 patients colonized over a period of 4–160 days, and found that within individual patients, a strain showed little genetic variation over time, suggesting that individual strain types remain stable. In the 5 long-term carriers examined in our study (all colonized between 11 and 34 weeks) at least 2, and in 1 case, 6 distinct strains were present. When the sequence of strains over time was examined, it appeared that there was a tendency for one strain to predominate for several weeks, before a switch occurred to another predominant strain. Schoonmaker et al. [26] analysed 58 isolates

from 2 patients over 9 weeks – again by PFGE, and demonstrated 12 distinct strains in 1 patient, and 13 in the other. In addition, Montecalvo et al. [27] showed that two-thirds of 36 long-term carriers maintained the same PFGE profile over time, while the other third acquired new strains. The finding that an individual patient can harbour more than one strain of GRE raises the question of how many strains are present at one time. This issue was discussed by Tremlett et al. [28] who isolated 46 GRE from 17 faecal screens from a single patient over a 12 week period. These isolates separated into 6 types by PFGE, and 12 of the 17 screens contained multiple strains of GRE. Thus, if only a single colony from a specimen is taken for analysis, an apparent lack of similarity between strains does not exclude the possibility that spread has occurred from a common source.

Lastly, there was an apparent lack of similarity between the invasive clinical isolate and the colonizing GRE isolate taken by rectal swab at the same time in 4 of 6 patients with GRE infection. These results are not surprising given the evidence that patients may be colonized by more than one strain over time – indeed, the first rectal swab isolate differed from the rectal swab isolate taken at the time of clinical GRE infection in 5 out of the 6 cases. The presence of multiple GRE strains from clinical specimens has also been described [26, 29].

Taken together, these results present a complex picture of GRE epidemiology on a unit where endemicity had been established for at least 2 years. The predominance of some strains when the acquisition rate was high, together with heavy environmental contamination with the same organisms, suggests that cross-infection was an important factor in the spread of GRE when the colonization rate was high. When colonization rates fell, patient and environmental isolates became more heterogeneous. The finding that patients may be colonized by more than one strain of GRE, suggests that multiple colonies should be analysed from a clinical specimen to interpret the results obtained from genotypic analysis adequately.

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