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Molecular epidemiology of glycopeptide-resistant *Enterococcus faecium* on a renal unit

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SUMMARY

The clinical and molecular epidemiology of glycopeptide-resistant *Enterococcus faecium* was investigated during an outbreak on a renal unit. Forty-nine patients were colonized or infected during a 15-month period. Sites of colonization included faeces, urine, intravenous (IV) catheter tips and wound swabs. Ten patients had infections, which included five bacteraemias and three episodes of peritonitis. Pulsed-field gel electrophoresis of 43 patient isolates of glycopeptide-resistant *E. faecium* identified seven strains during the first 7 months of the outbreak. Three of these strains affected five or more patients. One strain accounted for 17/43 isolates. Isolates that were possibly related to another renal unit strain were cultured from patients at two other Manchester hospitals. These isolates were epidemiologically-related, and may represent a single Manchester epidemic strain. Of five patients who had multiple isolates of glycopeptide-resistant *E. faecium*, three had isolates representing a single strain and two were colonized or infected by more than one strain.

INTRODUCTION

Since 1987, when the first outbreak of infections occurred on a London renal unit [1], the epidemiology of glycopeptide-resistant enterococci (GRE) has been intensely studied. A lack of suitable phenotypic typing systems led to the development of molecular-based techniques, including restriction fragment length polymorphisms of total DNA, ribotyping, analysis of plasmid DNA, pulsed-field gel electrophoresis (PFGE) and digestion of amplified fragments of glycopeptide resistance genes [2]. Of these, DNA fingerprinting by PFGE has been widely used to study the epidemiology of GRE as it produces a high degree of discrimination between strains [3, 4]. Criteria for defining strains by PFGE patterns in epidemiological studies have recently been proposed [5].

Use of PFGE to generate DNA macrorestriction profiles has demonstrated that during some clusters of GRE, dissemination of a single strain occurred, usually of *Enterococcus faecium* [6, 7]. However, in other clusters, multiple strains (and species) of GRE have been present [8–10]. These observations have been made for enterococci of both VanA and VanB phenotypes. The spread of genetically-related isolates of glycopeptide-resistant *E. faecium* has been demonstrated both within and between hospitals [11], and the term epidemic vancomycin-resistant *E. faecium* (EVREM) has been proposed for strains isolated from at least two patients in each of two or more hospitals [12].

In this study, the clinical and molecular epidemiology of glycopeptide-resistant *E. faecium* was investigated during an outbreak of colonization and infection of patients on a renal unit.

Hospital setting

The renal unit comprises two 'Nightingale' style wards and a haemodialysis unit. The haemodialysis

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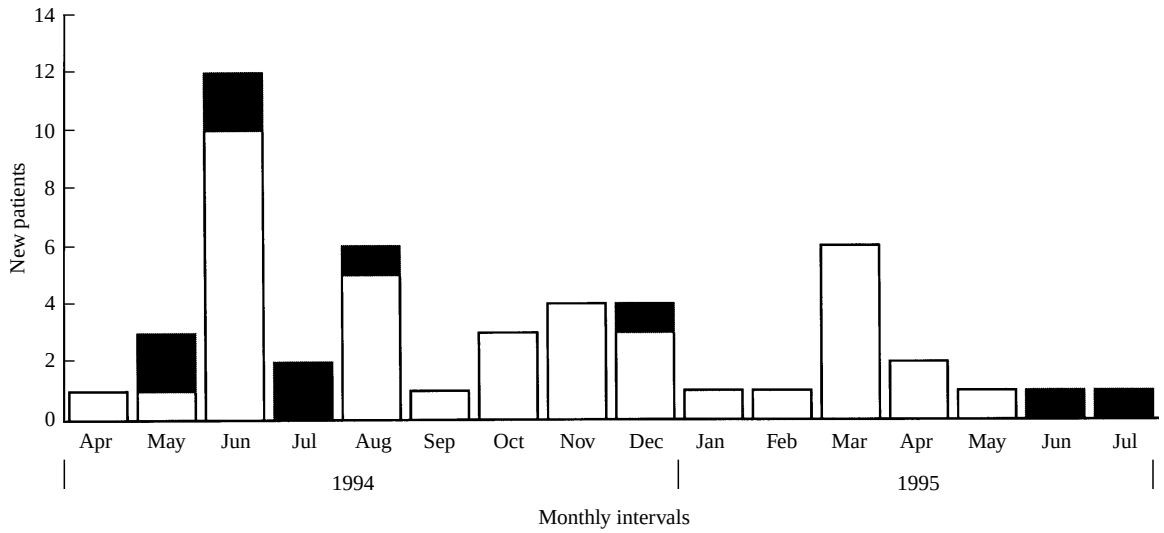

Fig. 1. Epidemic curve for the outbreak: (□) colonized, (■) infected. Note: screening commenced in June 1994.

Table 1. *Details of patients infected by glycopeptide-resistant* E. faecium

Age/sex	Diagnostics	Day of infection*	Site of infection	Specific treatment	Short-term outcome	Comments
61F	Polycystic kidneys CAPD	10	Peritoneum	Tenckhoff catheter removed	Recovered	CAPD peritonitis recent AAC
49F	Renal transplant CAPD	1	Peritoneum	Tenckhoff catheter removed	Recovered	CAPD peritonitis (with coliforms)
41M	Hypertensive CRF haemodialysis	30	Blood (recurrent)	Nil	Recovered	Pancreatitis, GI bleeds multiple laparotomies
44F	Diabetes CAPD	9	Abdominal abscess	Surgical drainage	Died	Mixed organisms
61M	Renal transplant cholecystectomy	52	Blood (recurrent)	Synercid† IV cath. removed	Recovered	Post-op complications multiple organ failure
60M	IgA nephritis, CRF	20	Exit site	Nil	Recovered	Localized infection
32F	CRF, haemodialysis	38	Blood	Nil	Recovered	Recurrent bacteraemia
47F	Hypertensive CRF CAPD	26	Blood (recurrent)	Chloramphenicol	Died	Pseudomembranous colitis, colectomy
54M	Diabetes, CAPD colonic carcinoma	8	Blood	Vancomycin + amp + netilmicin	Died	Colectomy haemodialysed post-op
71F	CAPD, pancytopenia	62	Peritoneum	Tenckhoff catheter removed	Died	CAPD peritonitis

* Days after admission to renal unit.
† Quinupristin/dalfopristin.
CAPD, continuous ambulatory peritoneal dialysis; CRF, chronic renal failure; amp, ampicillin; GI, gastrointestinal; IV, intravenous AAC, antibiotic associated colitis.

unit has 15 beds and maintains approximately 80 patients per week, supporting a further 81 patients on the home haemodialysis programme. Patients are admitted to the unit with new and established renal failure or with 'general medical' problems. The patients with chronic renal failure are maintained on either haemodialysis or peritoneal dialysis and a few have undergone previous renal transplantation: all have a degree of immunosuppression due to their underlying disease or the treatment of their disease. Isolation facilities are poor: the male ward has 24 beds with 3 single rooms and a 2-bed annex. The female ward has 22 beds which include 2 cubicles. Toilet facilities on the wards are shared. Patients in need of intensive care support are transferred to a seven-bed intensive care unit (ICU) which is located in a nearby

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part of the hospital and is an open plan area, admitting medical, surgical and trauma patients. No routine bacteriological surveillance was being carried out at this hospital that would have detected GRE prior to the outbreak.

Outbreak description

GRE were first detected in the urine of an out-patient in April 1994. In May, two continuous ambulatory peritoneal dialysis (CAPD) patients in adjacent beds developed peritonitis within a week of each other. In total, 49 patients were colonized or infected between April 1994 and July 1995 (Fig. 1). There were no clear peaks in incidence of new colonizations (the apparent peak in June 1994 is almost certainly an artefact of screening, which includes previously unrecognized individuals). A new patient colonization rate of 1–6 cases per month continued throughout the period of study, despite infection control interventions. Sites of colonization included faeces, urine, intravenous (IV) catheter tips and wound swabs. In total, 10 patients became infected by GRE, with four deaths (Table 1).

Infection control interventions

Staff and colonized patients were educated about GRE and the importance of handwashing and environmental cleaning were highlighted. Enteric precautions were reinforced for patients with diarrhoea and source isolation was considered on a risk management basis according to the (usually poor) availability of single rooms. There was no change in policy as to where patients were dialysed, but mixing of known colonized and non-colonized individuals was avoided. Management of CAPD catheter exit sites was reviewed and the use of gloves, aprons and alcoholic iodine was instituted. The use of glycopeptide antibiotics was reviewed at a meeting with clinicians, resulting in a decrease in consumption of oral vancomycin and its substitution with oral metronidazole for antibiotic-related diarrhoea. However, the use of IV vancomycin remained high; teicoplanin was not used on this unit.

MATERIALS AND METHODS

Clinical material was obtained according to clinical need and examined by routine laboratory methods. Faeces specimens were additionally cultured on neomycin blood agar, as a selective medium. Environmental swabs were enriched in nutrient broth for 24–48 h, subcultured onto neomycin blood agar and processed in the same way as clinical specimens.

Characterization of isolates

Presumptive enterococci were identified biochemically using standard methods [13, 14] and antimicrobial sensitivity testing was performed following British Society for Antimicrobial Chemotherapy guidelines [15]. Glycopeptide MICs were determined by an agar plate dilutional method; other antibiotics were tested by disk diffusion. In order to define the genetic basis of glycopeptide resistance, the presence of *vanA* or *vanB* resistance genes was determined in six isolates using *vanA* [9] and *vanB* [16] primers in a multiplex PCR assay [10].

DNA fingerprinting by pulsed-field gel electrophoresis (PFGE)

DNA fingerprinting was performed as described previously [7]. DNA was digested with *Sma* I restriction enzyme (New England Biolabs). Switch intervals were either ramped between 5 and 25 s over a 23-h period or a ramped switch interval of 1–10 s over 10 h was followed by a ramped switch interval of 10–25 s over 13 h. DNA fingerprints were compared by visual intragel comparison.

Conjugation studies

An overnight non-quantitative cross-streak method was used, with *E. faecium* strain (GE-1) as the recipient [10]. Transconjugants were selected on brain-heart infusion agar containing vancomycin 10 mg/l, rifampicin 100 mg/l and fusidic acid 25 mg/l.

RESULTS

Isolates

Forty-seven isolates of GRE were recovered from 24 patients. Sites affected included blood, the gastrointestinal tract, intravenous catheter tips, urine, wound swabs, peritoneal fluid and intra-abdominal abscess. Environmental sites from which GRE were recovered included tables, chairs, mattresses, used linen, washbowls, pedal bin lids, commodes, bedpan

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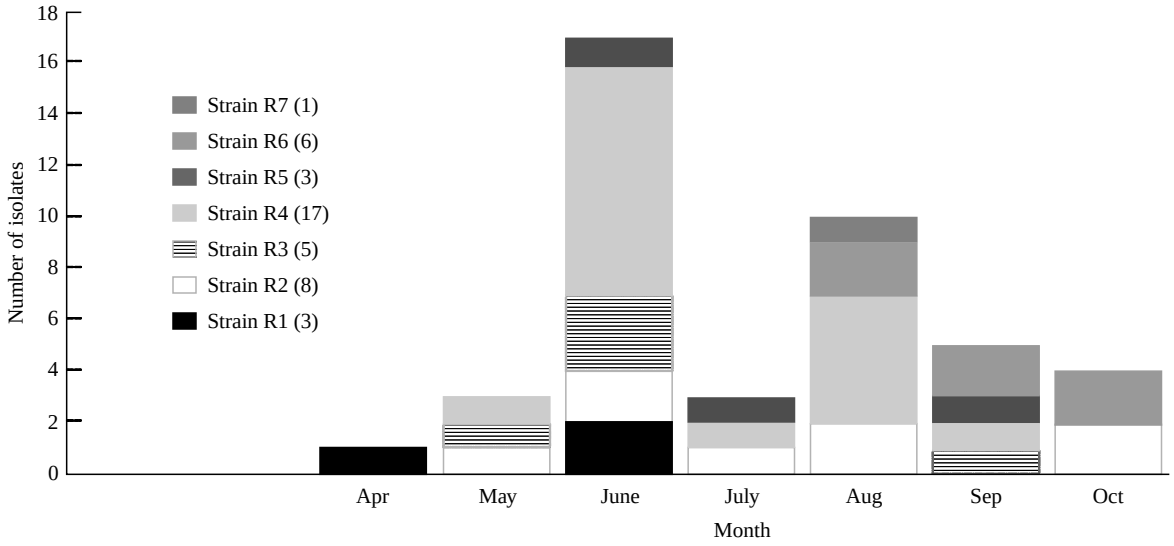

Fig. 2. DNA fingerprinting of 43 glycopeptide-resistant *E. faecium* isolates.

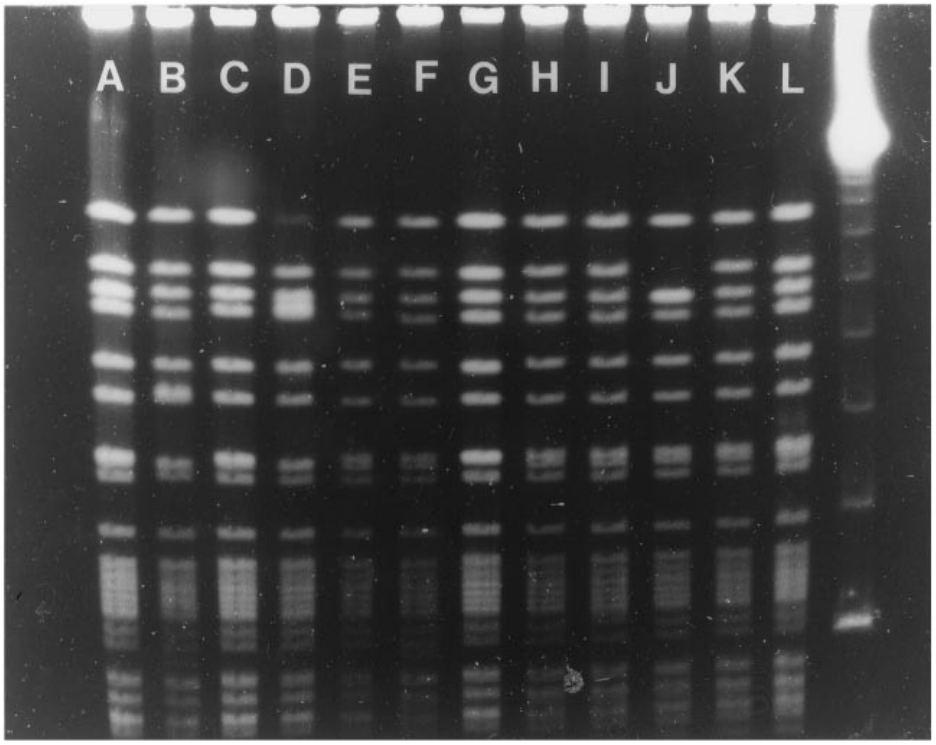

Fig. 3. DNA fingerprints of 12 glycopeptide-resistant *E. faecium* isolates of PFGE type R4. Pulse parameters, 1–10 s for 10 h then 10–25 s for 13 h. Lanes A–L, DNA fingerprints of patient isolates; lane M (not labelled), 48·5 kb lambda ladder. Note that DNA fingerprints are identical or differ by the position of either one or two fragments.

holders, urinary catheter bag holders, taps, toilet seats and toilet flush handles.

Characterization of isolates

Forty-seven patient isolates of presumptive GRE were identified as: *E. faecium* (44), *Enterococcus faecalis* (2) and *Enterococcus raffinosus* (1). Thirteen environmental isolates were identified as: *E. faecium* (9) and *E. faecalis* (4). Forty-six of 47 patient isolates and 11/13 environmental isolates expressed glycopeptide resistance of the VanA phenotype. A single patient isolate of *E. faecalis* and two environmental isolates (1 *E. faecium* and 1 *E. faecalis*) expressed VanB phenotype resistance. All six isolates (which were of the VanA phenotype) that underwent analysis by

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Table 2. *Relationship of strain type to degree of colonization and infection*

Strain	Number of patients colonized/ infected	Clinical infections	Resistance transferable by conjugation (number of isolates)
R1	2	0	+(1/1)
R2	5	1	+(1/1)
R3	5	2	Not tested
R4	11	3	+(1/1)
R5	3	2	+(2/2)
R6	1	1	−(0/1)
R7	1	0	Not tested

PCR, were shown to possess the *vanA* gene. *E. faecium* isolates were resistant to ampicillin and sensitive to chloramphenicol, with variable resistance to gentamicin (high-level) and tetracycline.

DNA fingerprinting

Isolates that generated macrorestriction profiles differing by more than six fragment positions were considered to represent different strains, while isolates producing up to three fragment differences, consistent with a single genetic event, were considered to represent a single strain [5]. Isolates that generated macrorestriction profiles differing by 4–6 fragment differences, consistent with two independent genetic events, were considered to be possibly related [5].

Analysis of 43 isolates of glycopeptide-resistant *E. faecium* from 24 patients affected during the first 7 months of the outbreak identified seven strains, designated R1–R7 (Fig. 2). Three strains (R2–R4) affected five or more patients. Strain R4 (17 isolates) affected 11 patients. Within this strain, 14 isolates (designated subtype R4a) produced identical DNA fingerprints, two isolates (subtype R4b) produced DNA fingerprints that varied from those of R4a isolates by one fragment position and a single isolate (subtype R4c) produced a DNA fingerprint that differed from those of R4a isolates by two fragment positions (Fig. 3). Two strains, R2 and R3, colonized or infected five patients each. Five strains (R2–R6) were identified among the isolates causing clinical infections, with R4 being the most frequent (Table 2). Three subtypes were identified within strain R2. DNA fingerprints generated by subtypes R2a (3 isolates) and R2c (1 isolate) differed from those of subtype R2b (4 isolates) by two fragment positions. DNA fingerprints within strain R2 were similar (6 fragment position differences) to those generated by isolates from a cluster of patients colonized by glycopeptide-resistant *Enterococcus faecium* on the Leukaemia Unit at a nearby hospital (B) [7] and from the renal/haematology unit at a third Manchester hospital (C), suggesting that these isolates were possibly genetically related (Fig. 4).

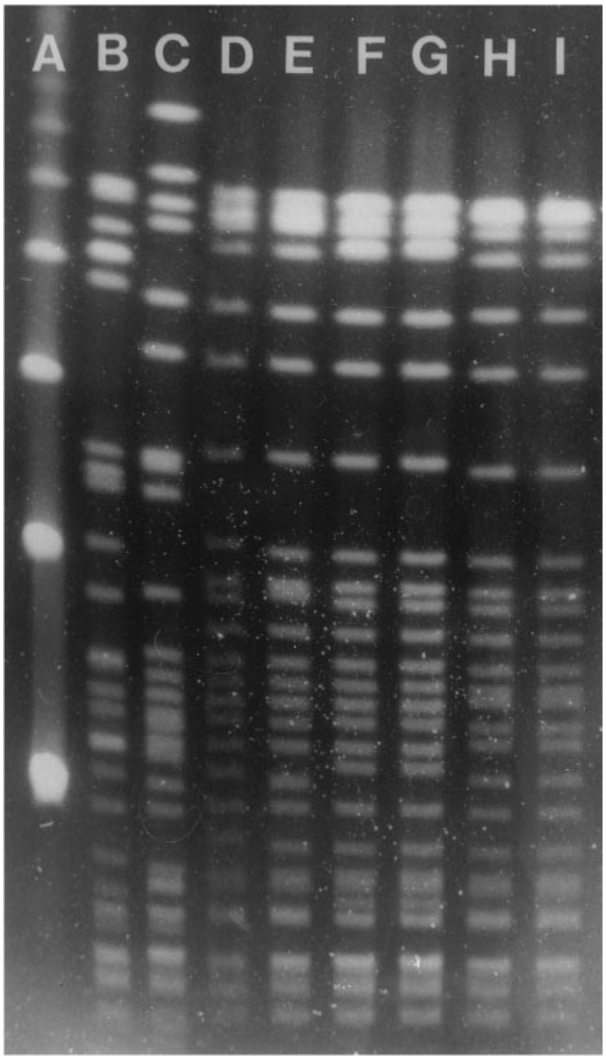

Fig. 4. DNA fingerprints of proposed Manchester epidemic strain isolates. Pulse parameters, 1–10 s for 24 h then 10–25 s for 12 h. Lane A, 48·5 kb lambda ladder; lane B, R1; lane C, R4; lane D, R2b; lane E, R2a; lane F and G, patient isolates from hospital B; lanes H and I, patient isolates from hospital C. Note that DNA fingerprints of isolates in lanes D–I differ by up to six fragment positions.

Five patients had multiple isolates of glycopeptide-resistant *E. faecium* (Fig. 5). Two of these patients (4 and 9) had isolates of two and four strains respectively. Three patients (10, 13 and 19) had recurrent infection and colonization with single strains. One patient (19)

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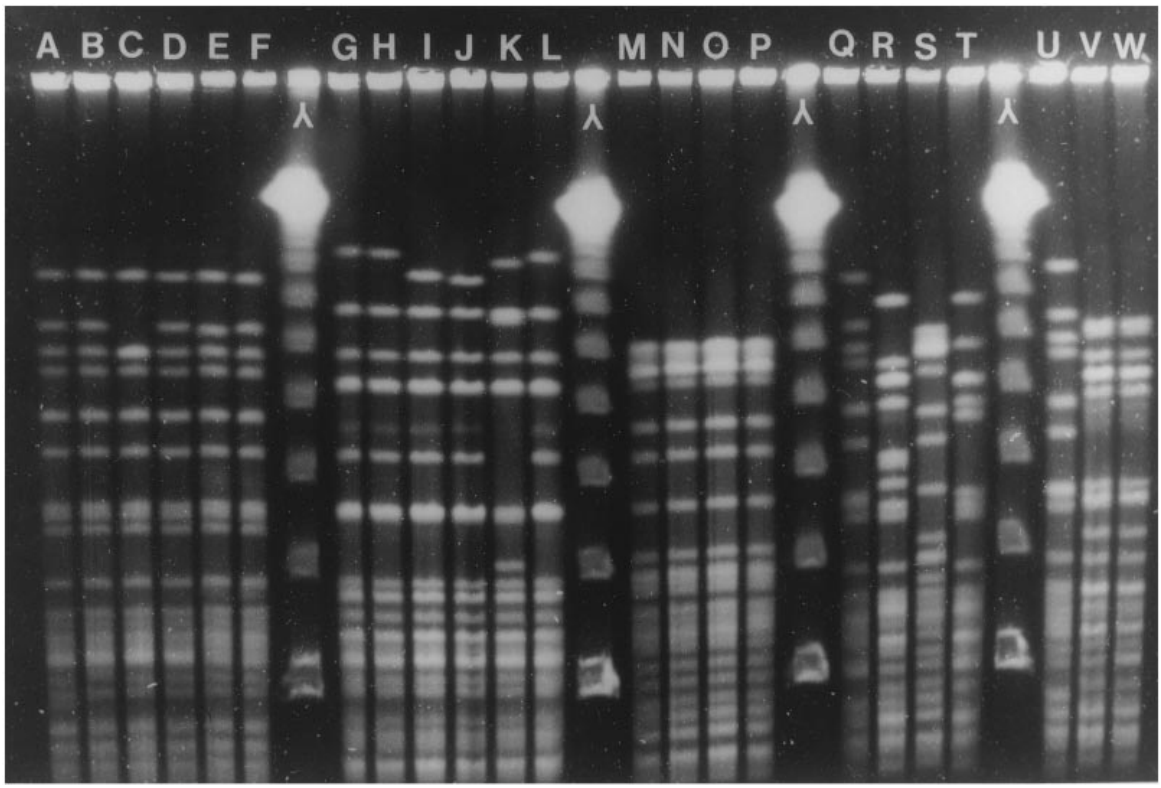

Fig. 5. DNA fingerprints of isolates from patients who had glycopeptide-resistant *E. faecium* cultured from multiple specimens. Pulse parameters, 1–10 s for 10 h then 10–25 s for 13 h. Lane A, blood (R4a); lane B, IV line tip (R4a); lane C, blood (R4c); lane D, blood (R4a); lane E, blood (R4a); lane F, blood (R4a); lane G, faeces (R6a); lane H, blood (R6a); lane I, blood (R6b); lane J, blood (R6b); lane K, blood (R6c); lane L, blood (R6c); lane M, IV line tip (R2b); lane N, urine (R2b); lane O, blood (R2b); lane P, blood (R2b); lane Q, urine (R4a); lane R, IV line tip (R7); lane S, wound (R2a); lane T, blood (R5c); lane U, peritoneal dialysis fluid (R4a); lane V, faeces (R1); lane W, faeces (R1). Note single strains for patients 10 (lanes A–F), 19 (lanes G–L) and 13 (lanes M–P). Patients 9 (lanes Q–T) and 4 (lanes U–W) had isolates of 4 and 2 strains respectively.

had faecal colonization, then recurrent bacteraemia (five blood isolates) with a strain (R6) that was not isolated from any other patient.

Conjugation studies

For five out of six isolates studied, glycopeptide resistance was transferable *in vitro* to the plasmid-free laboratory strain of *E. faecium*. Of five strains tested, R6 was the only one for which transfer of resistance was not demonstrated (Table 2).

DISCUSSION

DNA fingerprinting of GRE from the Renal Unit demonstrated genetic heterogeneity among the isolates over 7 months, with seven strains being identified. However, the demonstration that strain R4 isolates were either identical or genetically closely related, producing the least intragenetic variability of the strains studied (Fig. 4), provides evidence that an outbreak occurred, with dissemination of this strain among patients. In addition, two other strains each affected five patients. The contemporary appearance of GRE isolates (strains R1–R3 and R5–R7) with unrelated macrorestriction profiles to the outbreak R4 strain suggests that *vanA* or its associated resistance genes may have been transferred between strains of *E. faecium* that were present on the unit. Alternatively, several strains of GRE may have been introduced into the renal unit independently over a short period of time, either by transfer from other hospitals or from the community [17]. Although VanA GRE were readily isolated from uncooked meats retailed in the local community [18], none of the food isolates were genetically-related to patient isolates [19]. Supporting evidence for mobility of glycopeptide resistance genes on the renal unit came from the conjugation studies in which the resistance was shown to be transferable for five out of six isolates studied.

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Isolates that were possibly related were cultured from patients at three Manchester hospitals. Since there are epidemiological links between the departments (patients are transferred between the three units for treatment), these isolates may represent a single Manchester strain of epidemic glycopeptide-resistant *E. faecium*. The possibly-related isolates were recovered from hospitals B and C within 3 months of the last known occurrence of the R2 strain on the renal unit. The proposed Manchester epidemic strain is not genetically related to the EVREM strains that have previously been described [12; D. Morrison, personal communication]. The ability of such strains to spread widely between hospitals raises concerns for the control of multiply drug-resistant enterococci. Surveillance of hospital isolates of GRE by molecular characterization and DNA fingerprinting is needed to monitor the dissemination of glycopeptide resistance and to identify clusters of colonization or infection against the background of sporadic colonization [20].

The majority of infections on the renal unit were accounted for by either bacteraemia associated with indwelling IV catheters, or peritonitis associated with CAPD. Thus the organism in most infected sites was likely to have been introduced via an IV or intraperitoneal (Tenckhoff) catheter but could have been either endogenous (prior gastrointestinal colonization) or exogenous (via the hands of health care workers) in origin. During an outbreak of GRE on a liver unit, it was noted that *E. faecium* of the VanA phenotype occurred in clinical specimens in patients who did not have detectable rectal carriage [21]. This finding, together with the clustering of indistinguishable isolates (by ribotyping) led the author to conclude that cross-infection, rather than endogenous acquisition, occurred in these patients. In contrast, during outbreaks on oncology wards, prior colonization of the gut has preceded the development of clinical infections [7, 22, 23]. From our analysis of multiple isolates from single patients, it appears that for different individuals the epidemiology of infection may vary. Some individuals appear to be colonized by strains which are different to the strains causing their infections, suggesting that infection may have been acquired by the exogenous route (via peritoneal and venous catheters), regardless of colonization by GRE at other sites (patients 4 and 9). For other patients with colonization and infection (patients 13 and 19) only a single strain was isolated, suggesting an endogenous source of infection. In practice, it is difficult to determine the relative importance of modes of transmission during GRE outbreaks: environmental contamination is widespread and provides opportunities for both exogenous contamination of wounds and lesions and endogenous acquisition. Infection control efforts must be aimed at reducing both environmental contamination, by physical cleaning [24], and person-to-person transmission, by strict adherence to specific infection control measures [25].

Analysis of chromosomal DNA macrorestriction profiles is becoming increasingly useful in the investigation of outbreaks of nosocomial infection and in monitoring the spread of resistant organisms between health care facilities. This information may help us to target limited infection control resources to where they will be most effective. There is clearly a need for co-ordinated surveillance of such isolates, both at regional and national level.

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REFERENCES

1. Uttley AHC, George RC, Naidoo J, et al. High-level vancomycin-resistant enterococci causing hospital infections. Epidemiol Infect 1989; **103**: 173–81.
2. Woodford N, Johnson AP, Morrison D, Speller DCE. Current perspectives on glycopeptide resistance. Clin Microbiol Rev 1995; **8**: 585–615.
3. Murray BE, Singh KV, Heath DJ, Sharma BR, Weinstock GM. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J Clin Microbiol 1990; **28**: 2059–63.
4. Miranda AG, Singh KV, Murray BE. DNA fingerprinting of *E. faecium* by pulsed-field gel electrophoresis may be a useful epidemiologic tool. J Clin Microbiol 1991; **29**: 2752–7.
5. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
6. Handwerger S, Raucher B, Altarac D, et al. Nosocomial outbreak due to *Enterococius faecium* highly resistant to vancomycin, penicillin and gentamicin. Clin Infect Dis 1993; **16**: 750–5.
7. Chadwick PR, Oppenheim BA, Fox A, Woodford N, Morgenstern GR, Scarffe JH. Epidemiology of an

https://doi.org/10.1017/S0950268897007863 Published online by Cambridge University Press

outbreak of glycopeptide-resistant *Enterococcus faecium* on a leukaemia unit. J Hosp Infect 1996; **34**: 171–82.
8. Bingen EH, Denamur E, Lambert-Zechovsky NY, Elion J. Evidence for unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. J Clin Microbiol 1991; **29**: 1888–92.
9. Woodford N, Morrison D, Johnson AP, Briant V, George RC, Cookson B. Application of DNA probes of rRNA and *vanA* genes to investigation of a nosocomial cluster of vancomycin-resistant enterococci. J Clin Microbiol 1993; **31**: 653–8.
10. Woodford N, Morrison D, Johnson AP, et al. Plasmid-mediated *vanB* glycopeptide resistance in enterococci. Microbial Drug Resistance 1995; **1**: 235–40.
11. Chow JW, Kuritza A, Shlaes DM, Green M, Sahm DF, Zervos MJ. Clonal spread of vancomycin-resistant *Enterococcus faecium* between patients in three hospitals in two states. J Clin Microbiol 1993; **31**: 1609–11.
12. Morrison D, Woodford N, Cookson B. Epidemic vancomycin-resistant *Enterococcus faecium* in the UK. Clin Microbiol Infect 1995; **1**: 146–7.
13. Barrow GI, Feltham RKA, eds. *Cowan and Steel's manual for the identification of medical bacteria*, 3rd edn. Cambridge: Cambridge University Press, 1993.
14. Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J Clin Microbiol 1989; **27**: 731–4.
15. British Society for Antimicrobial Chemotherapy. *A guide to sensitivity testing*. J Antimicrob Chemother 1991; **27** suppl. D.
16. Woodford N, Jones BL, Baccus Z, Ludlam HA, Brown DFJ. Linkage of vancomycin and high-level gentamicin resistance genes on the same plasmid in a clinical isolate of *Enterococcus faecalis*. J Antimicrob Chemother 1995; **35**: 179–84.
17. Jordens JZ, Bates J, Griffiths DT. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J Antimicrob Chemother 1994; **34**: 515–28.
18. Chadwick PR, Woodford N, Kaczmarski EB, Gray S, Barrell RA, Oppenheim BA. Glycopeptide resistant enterococci isolated from uncooked meat. J Antimicrob Chemother 1996; **38**: 908–9.
19. Chadwick PR. Epidemiology of vancomycin-resistant enterococci in South Manchester. MD thesis, The University of Sheffield, 1996.
20. Bodnar UR, Noskin GA, Suriano T, Cooper I, Reisberg BE, Peterson LR. Use of in-house studies of molecular epidemiology and full species identification for controlling spread of vancomycin-resistant *Enterococcus faecalis* isolates. J Clin Microbiol 1996; **34**: 2129–32.
21. Wade JJ. The emergence of *Enterococcus faecium* resistant to glycopeptides and other standard agents – a preliminary report. J Hosp Infect 1995; **30** (suppl.): 483–93.
22. Montecalvo MA, Horowitz H, Gedris C, et al. Outbreak of vancomycin, ampicillin and aminoglycoside-resistant *Enterococcus faecium* in an adult oncology unit. Antimicrob Agents Chemother 1994; **38**: 1363–7.
23. Edmond MB, Ober JF, Weinbaum DL, et al. Vancomycin-resistant *Enterococcus faecium* bacteraemia: risk factors for infection. Clin Infect Dis 1995; **20**: 1126–33.
24. Chadwick C, Oppenheim BA. Cleaning as a cost-effective method of infection control. Lancet 1996; **347**: 1776.
25. Hospital Infection Control Practices Advisory Committee. Recommendations for preventing the spread of vancomycin resistance. Infect Cont Hosp Epidemiol 1995; **16**: 105–13.

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