Journal of Medical Microbiology (2004), **53**, 13–20 DOI 10.1099/jmm.0.05353-0

Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources

Roberta Creti,[1] Monica Imperi,[1] Lucia Bertuccini,[2] Francesca Fabretti,[1] Graziella Orefici,[1] Roberta Di Rosa[3] and Lucilla Baldassarri[2]

[1,2]Laboratorio di Batteriologia[1] and Laboratorio di Ultrastrutture[2], Istituto Superiore di Sanità, Viale Regina Elena, 299-00161 Rome, Italy

[3]Dipartimento di Medicina Clinica, Università 'La Sapienza', P. le Aldo Moro, 5-00185 Rome, Italy

Correspondence
Roberta Creti
roberta.creti@iss.it

Received 19 June 2003
Accepted 20 October 2003

A collection of *Enterococcus faecalis* strains from clinical isolates, healthy individuals and the environment was screened for the presence of virulence factor genes, such as those for collagen-binding protein (*ace*), endocarditis antigen (*efaA*), haemolysin activator (*cylA*), gelatinase (*gelE*), aggregation substances (*asa1* and *asa373*), a surface protein (*esp*) and two novel putative surface antigens (*EF0591* and *EF3314*). Apart from some genes that were present in all strains (*ace*, *efaA* and *EF3314*), the *gelE* gene was the most common factor, although its presence did not correlate with its expression. The genes that encode Esp and CylA were never detected in endocarditis isolates, whereas an association was noted between the *esp* gene and isolates from urinary tract infection (UTI) and bacteraemia. An aggregation substance gene was always present in commensal strains. As for gelatinase, the presence of the *cylA* and *asa* genes did not correlate completely with their phenotypic expression. Generally, isolates from endocarditis, biliary stents and the environment were equipped with fewer virulence factors than isolates from other sources. UTI strains possessed the highest number of factors.

INTRODUCTION

Enterococci are commensals of the intestinal tract of humans and animals and have emerged in recent decades as a major cause of nosocomial infections (Murray & Weinstock, 1999). Enterococcal infections represent the second most common cause of bacteraemia and endocarditis in US hospitals, whereas in Europe, epidemiological evaluations have been less comprehensive (Bonten *et al.*, 2001). In Italian hospitals where active surveillance is operative, enterococci are the third most common cause of infections and cause mainly urinary tract infections (UTIs) and blood infections (Moro *et al.*, 2001).

Mechanisms of acquisition of antibiotic resistance and spread have been well studied (Gilmore, 2002; Shepard & Gilmore, 2002), but enterococcal virulence and pathogenic mechanisms are still largely unknown. Previous studies have analysed the distribution of factors such as haemolysin, gelatinase, aggregation factor or Esp surface protein, although none has been found to be associated significantly with isolates from infection rather than with faecal strains, or with mortality in patients with bacteraemia (Coque *et al.*, 1995; Huycke & Gilmore, 1995; Elsner *et al.*, 2000; Eaton & Gasson, 2001; Archimbaud *et al.*, 2002; Vergis *et al.*, 2002; Waar *et al.*, 2002). Little information is available on the distribution of virulence factor patterns.

Abbreviation: UTI, urinary tract infection.

The purpose of the present report was to perform a molecular epidemiological survey by investigating the presence of known and novel potential virulence factors in *Enterococcus faecalis* isolated from different sources, as well as to study possible correlations between potential virulence factors possessed by strains and their source of isolation.

METHODS

Bacterial strains and growth conditions. Seventy-four *E. faecalis* strains, isolated from invasive (26 strains) and non-invasive (32 strains) hospital infections, as well as from the environment (six strains) and the faeces or throat of healthy individuals (10 strains), were analysed. Invasive infections included endocarditis (nine strains), sepsis (11 strains), endovascular infections (four strains), ascites (one strain) and meningitis (one strain). Isolates from UTIs ($n = 12$), blocked biliary stents ($n = 12$), surgical wounds ($n = 2$), vaginal infections ($n = 2$), eye infections ($n = 1$), pneumonia ($n = 1$) and orthopaedic prostheses ($n = 2$) were considered to be from non-invasive infections. Strains considered in this study had been characterized previously for biofilm formation, antibiotic susceptibility and PFGE profile (Baldassarri *et al.*, 2001b; Dicuonzo *et al.*, 2001).

Bacteria were grown in trypticase soy broth or agar (TSA) or in Todd–Hewitt broth (THB) at 37 °C in 5 % CO_2 without agitation.

PCR. Enterococcal DNA was prepared by suspending a loop of overnight colonies in a tube that contained 500 µl sterile distilled water, boiling for 10 min and then centrifuging at 14 000 ***g*** for 5 min. An aliquot of the supernatant (5 µl) was used as the template in a final volume of 25 µl PCR mixture, which contained: 1× PCR buffer, 2 mM $MgCl_2$, 200 µM each dNTP, 400 nM each primer and 0·25 U *Taq* DNA polymerase (Life Technologies).

Samples were amplified on a DNA thermal cycler (MJ Research) by heating for 5 min at 95 °C, followed by 30 cycles of 95 °C for 60 s, 58 °C for 60 s (52 °C for *gelE* and 63 °C for *esp*, as indicated by Shankar *et al.*, 1999) and 72 °C for 60 s, and a final step of 72 °C for 10 min.

PCR products were analysed by gel electrophoresis in 0·8 % (w/v) agarose gel (Life Technologies).

Primers utilized. Oligonucleotides were synthesized by a custom primer service (Life Technologies) and are described in Table 1.

Phenotypic assays. Production of gelatinase was determined by using TSA supplemented with 1·5 % skimmed milk; a clear halo around colonies after 18 h at 37 °C was considered to be a positive result (Coque *et al.*, 1995).

Haemolysin production was evaluated on Columbia agar base supplemented with 5 % (v/v) fresh human blood. Zones of clearing around colonies after 24 h at 37 °C indicated production of β-haemolysin (Franz *et al.*, 2001).

Expression of aggregation substance was determined for PCR-positive strains by the clumping assay (Dunny *et al.*, 1979) in the presence of sex pheromone, obtained by growing the pheromone-producing *E. faecalis* strain JH2-2 in THB at 37 °C for 18 h. Strains to be tested for aggregation substance expression were grown in THB for 18 h at 37 °C; 20 µl of each culture was used to inoculate 96-well microtitre plates that contained 200 µl pheromone preparation per well, which was diluted serially in THB. Cell clumping was checked at 2, 4, 6 and 18 h. *E. faecalis* OG1XpAD1 and JH2-2 were used as positive and negative controls, respectively.

RESULTS AND DISCUSSION

The presence of genes that encode aggregation substance (*asa1* and *asa373*), cytolysin activator (*cylA*), surface protein (*esp*), gelatinase (*gelE*), collagen-binding protein (*ace*) and endocarditis antigen (*efa*A) was investigated by PCR. The frequency of two novel putative surface antigen genes (*EF0591* and *EF3314*), detected by screening of the *E. faecalis* strain V583 genome (Paulsen *et al.*, 2003), available at the website of The Institute for Genomic Research (http://www.tigr.org/), were also investigated.

Table 1. PCR primers and products for detection of *E. faecalis* virulence determinants

For primers that were not designed in this study, the corresponding reference is given below.

Gene	Sequence (5′⟶3′)	Product size (bp)	GenBank accession no.	Position
16S rRNA*	TGGCATAAGAGTGAAAGGCGC	290	AF070224	179
	GGGGACGTTCAGTTACTAACGT			468
esp†	TTGCTAATGCTAGTCCACGACC	932	AF034779	1217
	GCGTCAACACTTGCATTGCCGA			2149
gelE‡	ACCCCGTATCATTGGTTT	405	M37185	762
	ACGCATTGCTTTTCCATC			1163
cylA	GACTCGGGGATTGATAGGC	688	AD1CLYL	6656
	GCTGCTAAAGCTGCGCTTAC			7344
asa1	CCAGCCAACTATGGCGGAATC	529	SFPASA1	3122
	CCTGTCGCAAGATCGACTGTA			3651
asa373	GGACGCACGTACACAAAGCTAC	619	AJ132039	3094
	CTGGGTGTGATTCCGCTGTTA			3713
ace	GGAATGACCGAGAACGATGGC	616	AF159247	160
	GCTTGATGTTGGCCTGCTTCCG			776
efaA	GCCAATTGGGACAGACCCTC	688	EFU03756	312
	CGCCTTCTGTTCCTTCTTTGGC			1000
EF0591	CGGAAGTATTGCGTTTGGTGGG	844	NC_004668	99
	CGTCTGCTTTAATAGACCCCAG			1003
EF3314	AGAGGGACGATCAGATGAAAAA	566	NC_004668	35
	ATTCCAATTGACGATTCACTTC			601

*Baldassarri *et al.* (2001b).

†Shankar *et al.* (1999).

‡Eaton & Gasson (2001).

In addition, *E. faecalis*-specific 16S rRNA gene primers were included as a control when the biochemical identification for *E. faecalis* was doubtful (Baldassarri *et al.*, 2001b).

Gelatinase, haemolysin and aggregation substance production was tested by phenotypic assays. Individual virulence determinant content of each strain, incidence of virulence factors and their distribution among different classes are reported in Table 2, Table 3 and Table 4, respectively.

Genes that encode Ace and EfaA were found in all strains. For *efaA*, this is in accordance with a previous study in which the gene was always present in medical *E. faecalis* isolates, whereas the majority (89 %) of *E. faecalis* strains from food possessed the *efaA* determinant (Eaton & Gasson, 2001). Both the *efaA* and *ace* genes display allelic sequence variation (with the latter being more variable), suggesting that these variations can influence the ability of surface characteristics among strains of diverse sources. Due to their sequence variability, these genes have recently been used as possible markers for multilocus sequence typing of *E. faecalis* (Nallapareddy *et al.*, 2002).

The *esp* gene was present in 44·6 % of isolates. It was associated more frequently with non-invasive infections (56·2 %, vs 38·4 % of invasive infection isolates) and particularly with UTIs, in accordance with other studies that indicated a possible role of Esp as a colonization factor in UTI (Shankar *et al.*, 2001). Among invasive infections, *esp* was present in most isolates from bacteraemia (72·7 %), but it was never detected in isolates from endocarditis in this study. This is in contrast with the findings of Archimbaud *et al.* (2002), who reported the presence of *esp* in all endocarditis and bacteraemia isolates tested (ten strains in each group).

Even if *esp* is considered to be an infection-associated virulence factor (Shankar *et al.*, 1999), we detected the gene in 20 % of isolates from healthy individuals and in 50 % of environmental strains.

During this study, we also identified *esp*-positive strains among *Enterococcus faecium* isolates (Baldassarri *et al.*, 2001a) that had been identified erroneously as *E. faecalis*. This gene is an *esp* variant (esp_{fm}), the sequence of which is in accordance with the *E. faecium esp* gene sequence determined by others (Willems *et al.*, 2001; Woodford *et al.*, 2001).

The *cylA* gene was present in 23 % of all isolates and was distributed equally among healthy individuals and those with invasive and non-invasive infections. It was never detected in isolates from endocarditis, biliary stents or the environment. Its absence or low prevalence in endocarditis strains has already been reported by other authors (Huycke & Gilmore, 1995; Archimbaud *et al.*, 2002). Haemolytic activity was detected in 64·7 % of *cylA*-positive strains, with a tendency to be present more often among non-invasive (62·5 %) and commensal (100 %) strains than in invasive strains (50 %). The lack of cytolysin phenotypic/genotypic congruence may suggest the occurrence of missing genes in the *cyl* operon among *cylA*-positive/haemolysin-negative strains.

The aggregation substance gene *asa1* was present in 63·5 % of all isolates. It was associated more frequently with non-invasive infections (68·7 %, vs 53·8 % of invasive strains) and present in almost all strains that were derived from healthy individuals. Also, one-third of isolates from the environment were positive for the presence of the *asa1* gene. Previous studies of the incidence of *asa1* in enterococcal isolates are contradictory: some studies indicated a high prevalence of *asa1* in clinical isolates compared to strains from healthy individuals (Coque *et al.*, 1995; Waar *et al.*, 2002), whereas in others, *asa1* did not occur more frequently in invasive than in commensal strains (Huycke & Gilmore, 1995; Archimbaud *et al.*, 2002).

To our knowledge, this is the second study that investigates the occurrence of the aggregation substance gene *asa373* in clinical and commensal isolates. In the previous study (Waar *et al.*, 2002), incidence of this gene was lower (5 and 6 % of isolates from blood cultures and faeces of healthy individuals, respectively) than ours (18·2 and 10 %, respectively). Waar *et al.* (2002) speculated about possible linkage between the *asa1*, *asa373* and *esp* genes, as they were co-present in all isolates tested. Also, in another study of the incidence of the *asa373* gene among enterococci isolated mostly from cheeses, Franz *et al.* (2001) found that the gene always occurred when the *asa1* gene was present. We found no such correlation: nine strains (12·2 %) were positive for *asa373* and the gene was associated with *asa1* and *esp* in six and five strains, respectively. The three genes were co-present in three strains.

Interestingly, we determined the sequence of our *asa373* amplicons and found that they displayed 72 % deduced amino acid similarity with the Asa373 protein (Muscholl-Silberhorn, 1999; De Boever *et al.*, 2000). Further studies on the nature of the plasmid that bears this *asa373* gene variant are ongoing.

By phenotypic assay (Table 3), 66 % of the pAD1 and/or pAM373 PCR-positive strains gave a positive clumping reaction in the presence of sex pheromone produced by *E. faecalis* JH2-2. No correlation could be found between the strength of the clumping reaction and the simultaneous or individual presence of the *asa1* and/or *asa373* genes.

The *gelE* gene was detected in 74·3 % of all isolates and was thus the most common of the factors that we tested. GelE-positive isolates were significantly more frequent among clinical isolates (75–86 %) when compared to commensal strains (40 %), as already reported by Waar *et al.* (2002) and Archimbaud *et al.* (2002). The presence of the *gelE* gene was, however, not strictly correlated with its expression, as gelatinase was produced by only 36·4 % of *gelE*-positive strains.

Generally, a higher proportion of invasive strains (45·5 %) compared to non-invasive strains (33·3 %) produced gelatinase; half of commensal strains expressed it and, more strikingly, even though the majority of envir-

Table 2. Occurrence of the *esp*, *cylA*, *asa1*, *asa373*, *EF0591* and *gelE* genes and production of haemolysin, gelatinase and aggregation substance among *E. faecalis* strains isolated from different sources

The *ace*, *efaA* and *EF3314* genes were always present and have been omitted from the table. Symbols in parentheses indicate expression of the gene. +, Presence/expression of virulence factor; −, absence of virulence factor; ND, not done.

Isolation source and strain	*esp*	*cylA*	*asa1*	*asa373*	Clumping*	EF0591	*gelE*
Endocarditis:							
EFS42	−	−(−)	+	−	−	+	+ (+)
EFS45	−	−(−)	−	−	ND	−	+ (+)
EFS90	−	−(−)	−	−	ND	−	+ (+)
EFS96	−	−(−)	−	−	ND	−	+ (−)
EFS97	−	−(−)	+	−	+	−	+ (−)
EFS100	−	−(−)	+	−	+	−	+ (−)
EFS110	−	−(−)	+	−	+	−	+ (+)
EFS125	−	−(−)	−	−	ND	−	+ (+)
EFS166	−	−(−)	−	−	ND	−	+ (+)
Sepsis:							
EFS83	+	−(−)	+	−	+	−	+ (−)
EFS105	+	−(−)	−	−	ND	−	+ (−)
EFS106	−	−(−)	+	−	−	−	−(−)
EFS108	−	+ (−)	+	−	+	−	+ (+)
EFS109	−	−(−)	−	−	ND	−	+ (−)
EFS113	+	−(−)	−	−	ND	−	+ (−)
EFS121	+	+ (−)	+	−	+	+	+ (+)
EFS127	+	+ (+)	+	+	+	−	−(−)
EFS128	+	+ (+)	+	−	+	−	+ (−)
EFS131	+	−(−)	−	+	−	−	+ (−)
EFS137	+	−(−)	−	−	ND	−	−(−)
Endovascular infection:							
EFS41	−	−(−)	−	−	ND	−	+ (+)
EFS44	−	−(−)	−	+	+	−	−(−)
EFS92	+	−(−)	+	+	+	−	+ (−)
EFS94	−	−(−)	+	−	+	+	+ (−)
Ascites:							
EFS87	+	+ (−)	+	−	+	+	+ (+)
Meningitis:							
EFS122	−	+ (+)	+	+	−	+	+ (+)
Biliary stent:							
EFS12	−	−(−)	+	−	−	+	+ (+)
EFS13	−	−(−)	−	−	ND	−	+ (−)
EFS16	+	−(−)	−	−	ND	−	+ (−)
EFS20	−	−(−)	+	−	−	−	+ (+)
EFS27B	+	−(−)	+	−	−	−	+ (−)
EFS28B	−	−(−)	+	−	+	−	−(−)
EFS29B	−	−(−)	+	−	+	−	−(−)
EFS30D	+	−(−)	+	+	+	−	+ (−)
EFS32	+	−(−)	−	−	ND	−	+ (−)
EFS35D	−	−(−)	−	−	ND	−	+ (+)
EFS38	+	−(−)	−	−	ND	−	−(−)
EFS78	+	−(−)	−	−	ND	−	−(−)
UTI:							
EFS79	+	−(−)	−	−	ND	−	+ (−)
EFS81	+	−(−)	−	−	ND	−	+ (+)

Table 2. cont.

Isolation source and strain	*esp*	*cylA*	*asa1*	*asa373*	Clumping*	EF0591	*gelE*
EFS82	+	−(−)	−	−	ND	−	+ (+)
EFS88	+	−(−)	+	−	+	−	+ (−)
EFS104	+	−(−)	+	−	−	−	+ (−)
EFSU85	+	+ (−)	+	−	+	+	+ (−)
EFSU96	−	−(−)	+	−	−	+	+ (+)
EFSU98	−	−(−)	−	−	ND	−	−(−)
EFSU183	−	+ (−)	+	−	+	−	+ (−)
EFSU184	−	+ (+)	+	+	+	−	+ (−)
EFSU189	+	+ (+)	+	−	+	−	+ (+)
EFSU192	+	+ (+)	+	−	−	−	+ (−)
Vaginal infection:							
EFS49	+	+ (−)	+	−	−	−	−(−)
EFS89	−	−(−)	+	−	+	−	−(−)
Surgical wound:							
EFS80	+	−(−)	+	−	+	−	+ (−)
EFST33	−	+ (+)	+	+	+	−	+ (−)
Pneumonia:							
EFS116	+	−(−)	+	−	+	−	+ (−)
Orthopaedic prosthesis:							
EFS117	−	−(−)	+	−	+	+	+ (+)
EFS118	+	+ (+)	+	−	−	+	−(−)
Eye infection:							
EFS124	−	−(−)	+	−	+	−	+ (−)
Throat:							
EFS62	−	−(−)	+	−	+	−	−(−)
EFS63	−	−(−)	+	−	−	−	−(−)
EFS85	−	+ (+)	+	−	+	−	+ (−)
EFS142	−	−(−)	+	−	−	−	−(−)
EFS143	−	−(−)	+	−	−	−	−(−)
EFS144	−	−(−)	+	−	+	−	−(−)
EFS145	−	−(−)	+	−	+	−	−(−)
Stools:							
EFS148	+	+ (+)	−	+	+	−	+ (−)
EFS149	−	−(−)	+	−	−	−	+ (−)
EFS152	+	+ (+)	+	−	+	−	+ (−)
Wells:							
EFS50	+	−(−)	−	−	ND	−	+ (−)
EFS57	+	−(−)	−	−	ND	−	−(−)
EFS66	+	−(−)	+	−	+	−	+ (−)
EFS67	−	−(−)	−	−	ND	−	+ (−)
EFS68	−	−(−)	+	−	−	−	+ (−)
Sea water:							
EFS140	−	−(−)	−	−	ND	−	+ (−)

*Clumping assay was done only for strains that were positive for the *asa1* and/or *asa373* genes by PCR.

onmental isolates possessed the gene (83·3 %), it was never expressed under our conditions. All endocarditis isolates possessed the *gelE* gene, but only 66·7 % of them expressed it.

Silent *gelE* genes have been observed both in *E. faecalis* isolates from food and in clinical strains, with a higher incidence of silent genes in the latter group (Eaton & Gasson, 2001). Most studies (Huycke & Gilmore, 1995; Elsner *et al.*,

Table 3. Incidence (%) of virulence determinants among *E. faecalis* strains isolated from different sources

Values in parentheses are phenotypic frequencies of gelatinase, haemolytic activity and clumping among gene-targeted PCR-positive strains.

Gene	Total incidence	Infections		Commensals	Environment
		Invasive	Non-invasive		
ace	100	100	100	100	100
efaA	100	100	100	100	100
esp	44·6	38·4	56·2	20	50
cylA	23 (64·7)	23 (50)	25 (62·5)	30 (100)	0
asa1/asa373	63·5/12·2 (66)	53·8/19·2 (75)	68·7/9·3 (63·6)	90/10 (60)	33·3/0 (50)
gelE	74·3 (36·4)	84·6 (45·5)	75 (33·3)	40 (50)	83·3 (0)
EF0591	16·2	46·2	53·8	0	0
EF3314	100	100	100	100	100

Table 4. Incidence (%) of virulence factors among *E. faecalis* strains isolated from different types of infection

Type of infection	*esp*	*cylA*	*asa1*	*asa373*	*gelE*
Invasive:					
Endocarditis	0·0	0·0	44·4	0·0	100·0
Bacteraemia	72·7	36·4	54·5	18·2	72·7
Other	33·3	33·3	66·7	50·0	83·3
Non-invasive:					
Biliary stents	50·0	0·0	50·0	8·3	66·7
UTIs	66·7	41·7	66·7	8·3	90·9
Other	50·0	37·5	100·0	12·5	62·5

2000; Franz *et al.*, 2001; Archimbaud *et al.*, 2002; Vergis *et al.*, 2002; Waar *et al.*, 2002) investigated only gelatinase production, perhaps thereby underestimating the real incidence of the gene that encodes GelE among strains tested.

In our hands, results obtained by phenotypic tests always revealed a lower percentage of strains that produced haemolysin, gelatinase or aggregation substance, compared to genotypic characterization. This may be due to the presence of silent genes that are expressed only under *in vivo* conditions, to the presence of undetected gene mutations or to the fact that detection by PCR of a single gene inside an operon, as is the case of *cylA* for haemolysin production, may overlook the absence of other genes that are necessary for phenotypic expression. Techniques such as RT-PCR may provide useful information on the level of expression of the target DNA.

We also focused our attention on two novel putative *E. faecalis* cell-associated antigens by screening the V583 genome. One gene (*EF0591*) has also recently been found in an *E. faecalis* clinical isolate inside a pathogenicity island associated with the *esp* gene (Shankar *et al.*, 2002). The incidence of *EF0591* in our collection was rather low (16·2 %), but was associated exclusively with clinical isolates. The *EF0591* gene was always associated with the *asa1* gene, but was only associated with the *esp* gene in four cases, indicating that in these strains, a spontaneous deletion of the portion that contains the *esp* gene, already observed by Shankar *et al.* (2002), may have occurred.

EF3314 was chosen because of its significant similarity (31·9 %) with biofilm-associated proteins (Cucarella *et al.*, 2001). The PCR survey indicated that this gene is always present and specific for *E. faecalis*; its role as a possible novel *E. faecalis*-restricted antigen is currently under investigation.

On the whole, omitting from the analysis the ever-present genes *ace*, *efaA* and those for the novel putative antigens EF0591 and EF3314, a characteristic distribution in type and number of virulence factors among strains could be noted.

E. faecalis isolates from endocarditis and the environment possessed only one or two factors. Strains that belonged to other categories had between one and all four factors but the total number of factors differed, depending on the source of isolation (Fig. 1). Most strains isolated from biliary stents possessed one or two factors, while commensal isolates generally had only one factor. Strains from bacteraemia did not show any particular propensity for having a particular number of factors, whereas isolates from UTIs usually possessed two to four factors.

Commensal isolates always had a gene for aggregation substance. Of significance, *cylA* was always associated with aggregation substance genes *asa1* or *asa373*, whereas the reverse was not observed.

The presence of virulence factors or their association with a strain from a particular isolation source did not seem to depend on clonal spread of a few enterococcal genotypes. In a recent paper (Dicuonzo *et al.*, 2001), we analysed the PFGE patterns of our *E. faecalis* collection and observed extreme genetic heterogeneity in our isolates. Only in one case could a small cluster that was derived from the same hospital be grouped clonally, whereas the majority of strains, even when

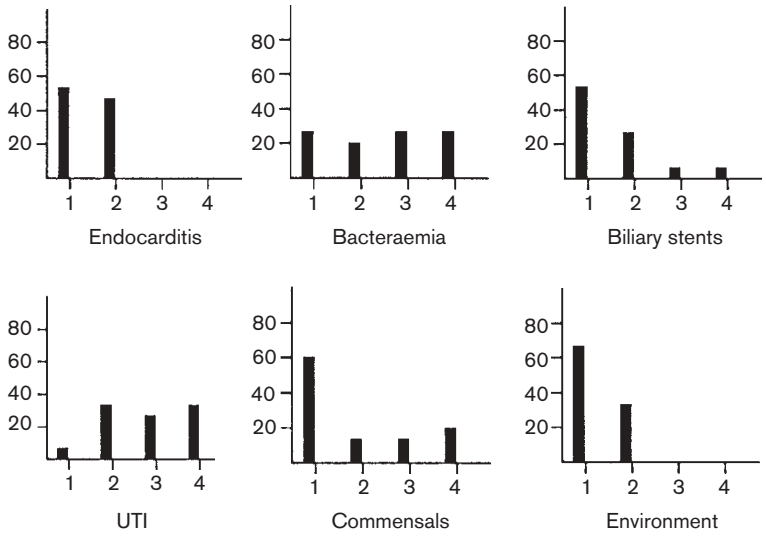

Fig. 1. Incidence of single or multiple virulence determinants possessed by *E. faecalis* isolates. Numbering on the *x* axis represents number of factors; the percentage of strains that possess a certain number of factors is reported on the *y* axis.

isolated from the same hospital unit, clustered in different subtypes and, notably, some environmental strains had the same genotype as clinical isolates.

The ability to form biofilm on inert surfaces was common in the majority of our *E. faecalis* isolates; this characteristic was affected strongly by growth conditions (Baldassarri *et al.*, 2001b). No association between biofilm production and presence of other virulence factors was noted in any of several growth media.

A recent study (Duprè *et al.*, 2003) examined the diffusion of some putative virulence factors in a small collection of enterococcal clinical isolates (15 *E. faecalis* and 32 *E. faecium*) from an Italian region. They found slightly different levels of incidence, particularly of *ace* and *efaA*, which were detected in only 60 and 86·6 % of isolates, respectively.

In conclusion, our data indicate that *E. faecalis* strains isolated from different sources possess distinctive patterns of potential virulence factors, with a larger number of genes that encode potential virulence factors among isolates from UTIs. Further investigations are needed to evaluate the expression of such factors, which may not be revealed by *in vitro* phenotypic tests during the course of infection.

ACKNOWLEDGEMENTS

This work was presented in part at the First International ASM Conference on Enterococci held in Banff, Alberta, Canada, on 27 February–2 March, 2000. We want to thank P. Boccardi for editorial assistance and Federico Giannoni for helpful discussions. This work was supported in part by a grant from CNR (no. CNRC003CE9_004) to G. O. and a grant from the Italian Ministry of Health, Project 1 % no. OAD/F to L. B.

REFERENCES

Archimbaud, C., Shankar, N., Forestier, C., Baghdayan, A., Gilmore, M. S., Charbonné, F. & Joly, B. (2002). In vitro adhesive properties and virulence factors of *Enterococcus faecalis* strains. *Res Microbiol* **153**, 75–80.

Baldassarri, L., Bertuccini, L., Ammendolia, M. G., Gherardi, G. & Creti, R. (2001a). Variant *esp* gene in vancomycin-sensitive *Enterococcus faecium*. *Lancet* **357**, 1802.

Baldassarri, L., Cecchini, R., Bertuccini, L. & 9 other authors (2001b). *Enterococcus* spp. produces slime and survives in rat peritoneal macrophages. *Med Microbiol Immunol* **190**, 113–120.

Bonten, M. J. M., Willems, R. & Weinstein, R. A. (2001). Vancomycin-resistant enterococci: why are they here, and where do they come from? *Lancet Infect Dis* **1**, 314–325.

Coque, T. M., Patterson, J. E., Steckelberg, J. M. & Murray, B. E. (1995). Incidence of hemolysin, gelatinase, and aggregation substance among enterococci isolated from patients with endocarditis and other infections and from feces of hospitalized and community-based persons. *J Infect Dis* **171**, 1223–1229.

Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, Í. & Penadés, J. R. (2001). Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J Bacteriol* **183**, 2888–2896.

De Boever, E. H., Clewell, D. B. & Fraser, C. M. (2000). *Enterococcus faecalis* conjugative plasmid pAM373: complete nucleotide sequence and genetic analyses of sex pheromone response. *Mol Microbiol* **37**, 1327–1341.

Dicuonzo, G., Gherardi, G., Lorino, G. & 7 other authors (2001). Antibiotic resistance and genotypic characterization by PFGE of clinical and environmental isolates of enterococci. *FEMS Microbiol Lett* **201**, 205–211.

Dunny, G. M., Craig, R. A., Carron, R. L. & Clewell, D. B. (1979). Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. *Plasmid* **2**, 454–465.

Duprè, I., Zanetti, S., Schito, A. M., Fadda, G. & Sechi, L. A. (2003). Incidence of virulence determinants in clinical *Enterococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). *J Med Microbiol* **52**, 491–498.

Eaton, T. J. & Gasson, M. J. (2001). Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Appl Environ Microbiol* **67**, 1628–1635.

Elsner, H.-A., Sobottka, I., Mack, D., Laufs, R., Claussen, M. & Wirth, R. (2000). Virulence factors of *Enterococcus faecalis* and *Enterococcus faecium* blood culture isolates. *Eur J Clin Microbiol Infect Dis* **19**, 39–42.

Franz, C. M. A. P., Muscholl-Silberhorn, A. B., Yousif, N. M. K.,

Vancanneyt, M., Swings, J. & Holzapfel, W. H. (2001). Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. *Appl Environ Microbiol* **67**, 4385–4389.

Gilmore, M. S. (editor) (2002). *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance*. Washington, DC: American Society for Microbiology.

Huycke, M. M. & Gilmore, M. S. (1995). Frequency of aggregation substance and cytolysin genes among enterococcal endocarditis isolates. *Plasmid* **34**, 152–156.

Moro, M. L., Gandin, C., Bella, A., Siepi, G. & Petrosillo, N. (2001). A national survey on the surveillance and control of nosocomial infections in public hospitals in Italy (Rapporti ISTISAN 01/4). Rome: Istituto Superiore di Sanità (in Italian).

Murray, B. E. & Weinstock, G. M. (1999). Enterococci: new aspects of an old organism. *Proc Assoc Am Physicians* **111**, 328–334.

Muscholl-Silberhorn, A. (1999). Cloning and functional analysis of Asa373, a novel adhesin unrelated to the other sex pheromone plasmid-encoded aggregation substances of *Enterococcus faecalis*. *Mol Microbiol* **34**, 620–630.

Nallapareddy, S. R., Duh, R.-W., Singh, K. V. & Murray, B. E. (2002). Molecular typing of selected *Enterococcus faecalis* isolates: pilot study using multilocus sequence typing and pulsed-field gel electrophoresis. *J Clin Microbiol* **40**, 868–876.

Paulsen, I. T., Banerjei, L., Myers, G. S. A. & 29 other authors (2003). Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. *Science* **299**, 2071–2074.

Shankar, V., Baghdayan, A. S., Huycke, M. M., Lindahl, G. & Gilmore, M. S. (1999). Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. *Infect Immun* **67**, 193–200.

Shankar, N., Lockatell, C. V., Baghdayan, A. S., Drachenberg, C., Gilmore, M. S. & Johnson, D. E. (2001). Role of *Enterococcus faecalis* surface protein Esp in the pathogenesis of ascending urinary tract infection. *Infect Immun* **69**, 4366–4372.

Shankar, N., Baghdayan, A. S. & Gilmore, M. S. (2002). Modulation of virulence within a pathogenicity island in vancomycin-resistant *Enterococcus faecalis*. *Nature* **417**, 746–750.

Shepard, B. D. & Gilmore, M. S. (2002). Antibiotic-resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. *Microbes Infect* **4**, 215–224.

Vergis, E. N., Shankar, N., Chow, J. W., Hayden, M. K., Snydman, D. R., Zervos, M. J., Linden, P. K., Wagener, M. M. & Muder, R. R. (2002). Association between the presence of enterococcal virulence factors gelatinase, hemolysin, and enterococcal surface protein and mortality among patients with bacteremia due to *Enterococcus faecalis*. *Clin Infect Dis* **35**, 570–575.

Waar, K., Muscholl-Silberhorn, A. B., Willems, R. J. L., Slooff, M. J. H., Harmsen, H. J. M. & Degener, J. E. (2002). Genogrouping and incidence of virulence factors of *Enterococcus faecalis* in liver transplant patients differ from blood culture and fecal isolates. *J Infect Dis* **185**, 1121–1127.

Willems, R. J. L., Homan, W., Top, J. & 9 other authors (2001). Variant *esp* gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. *Lancet* **357**, 853–855.

Woodford, N., Soltani, M. & Hardy, K. J. (2001). Frequency of *esp* in *Enterococcus faecium* isolates. *Lancet* **358**, 584.