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Microarray-Based Detection of 90 Antibiotic Resistance Genes of Gram-Positive Bacteria

Vincent Perreten,[1]* Lorianne Vorlet-Fawer,[1] Peter Slickers,[2] Ralf Ehricht,[2] Peter Kuhnert,[1] and Joachim Frey[1]

Institute of Veterinary Bacteriology, University of Berne, CH-3001 Bern, Switzerland,[1] and Clondiag Chip Technologies GmbH, D-07743 Jena, Germany[2]

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A disposable microarray was developed for detection of up to 90 antibiotic resistance genes in gram-positive bacteria by hybridization. Each antibiotic resistance gene is represented by two specific oligonucleotides chosen from consensus sequences of gene families, except for nine genes for which only one specific oligonucleotide could be developed. A total of 137 oligonucleotides (26 to 33 nucleotides in length with similar physicochemical parameters) were spotted onto the microarray. The microarrays (ArrayTubes) were hybridized with 36 strains carrying specific antibiotic resistance genes that allowed testing of the sensitivity and specificity of 125 oligonucleotides. Among these were well-characterized multidrug-resistant strains of *Enterococcus faecalis*, *Enterococcus faecium*, and *Lactococcus lactis* and an avirulent strain of *Bacillus anthracis* harboring the broad-host-range resistance plasmid pRE25. Analysis of two multidrug-resistant field strains allowed the detection of 12 different antibiotic resistance genes in a *Staphylococcus haemolyticus* strain isolated from mastitis milk and 6 resistance genes in a *Clostridium perfringens* strain isolated from a calf. In both cases, the microarray genotyping corresponded to the phenotype of the strains. The ArrayTube platform presents the advantage of rapidly screening bacteria for the presence of antibiotic resistance genes known in gram-positive bacteria. This technology has a large potential for applications in basic research, food safety, and surveillance programs for antimicrobial resistance.

The intensive use of antibiotics in both public health and animal husbandry has selected for antibiotic-resistant bacteria (39). Under antibiotic selective pressure, bacteria have the ability to develop and exchange resistance genes, making them nonsusceptible to the antimicrobial substances deployed. While antibiotic resistance has emerged in some important animal and human gram-positive pathogens, such as *Staphylococcus* and *Streptococcus* spp. and *Clostridium perfringens*, others, such as *Bacillus anthracis*, are currently still sensitive to antibiotics (15, 24). Nevertheless, *B. anthracis* can acquire resistance genes from other gram-positive bacteria in vitro, as previously described (30, 46) and as demonstrated in this study. It is therefore important to follow the evolution of antibiotic resistance in the bacterial population in order to prevent and repress the emergence of multidrug-resistant strains of those bacteria that can still be treated with antibiotics.

Furthermore, commensal bacteria represent a reservoir of antibiotic resistance genes that have the potential to be transferred to human and animal pathogens. An effort has therefore been made in Europe to reduce the emergence and spread of resistant bacteria. The use of antimicrobial substances for nontherapeutic purposes in animal husbandry has been banned, and surveillance programs for antibiotic-resistant bacteria among both human and animal isolates have been implemented (40). Additionally, it has been proposed that bacteria used as probiotics in food or feed or as starter cultures for the food industry must be free of antibiotic resistance genes (http://europa.eu.int/comm/food/fs/sc/scf/out178_en.pdf). Bacteria used in food preparation are mainly gram positive and include *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Micrococcus*, *Streptococcus*, *Staphylococcus*, and *Propionibacterium* spp. Animal probiotics consist mainly of strains of *Bacillus*, *Enterococcus faecium*, *Pediococcus*, *Lactobacillus*, and *Streptococcus*.

A simple method which allows the rapid detection of antibiotic resistance genes would complement the standard MIC determination for pathogenic and commensal bacteria. In the clinic, this would have the advantage of detecting silent antibiotic resistance genes which might be turned on in vivo or spread to other bacteria and would help in prescribing the appropriate antibiotic. Such a method could also be applied to slow-growing bacteria, for which the MIC determination may cause problems. In the food industry, it would help to determine whether antibiotic-susceptible starter cultures harbor silent antibiotic resistance genes which could directly reach consumers through the food chain. This technology could be used as a tool to survey the antibiotic resistance gene situation in specific bacteria and would enable rapid tracking of newly emerging resistance genes. For these purposes, a convenient and affordable technology should be available.

Today, PCR and hybridization analysis are common methods used to detect antibiotic resistance genes in bacteria. However, the detection of specific resistance genes remains a tremendous amount of work if every possible resistance gene has to be assessed, and therefore microarray technology is most suitable for resistance gene analysis (28). The few microarrays that have been developed to date for identification of antibiotic resistance genes are either restricted to a class of drug or

* Corresponding author. Mailing address: Institute of Veterinary Bacteriology, University of Berne, Längass-Strasse 122, Postfach, CH-3001 Bern, Switzerland. Phone: 41 31 631 2430. Fax: 41 31 631 2634. E-mail: vincent.perreten@vbi.unibe.ch.

TABLE 1. Bacterial strains and plasmids

Strain	Characteristic(s)[a]	Reference or source[b]
Enterococcus faecalis RE25	pRE25 [***erm*(B)**, ***cat*$_{pIP501}$**, ***aph*(3′)-III**, ***sat4***, ***ant*(6)-Ia**]; ***tet*(M)**	48
Enterococcus faecalis JH2-2	Rif^r Fus^r	31
Enterococcus faecalis JHRE25-2	JH2-2 containing pRE25 [*erm*(B), *cat*$_{pIP501}$, *aph*(3′)-*III*, *ant(6)-Ia, sat4*]; Rif^r Fus^r	48
Lactococcus lactis K214	pK214 [***tet*(S)**, ***cat-LM***, ***mdt*(A)**, ***str***]	43, 44
Clostridium perfringens MLP26[c]	***tetA*(P)** *erm*(B) *sat4* ***catP*** *aph*(3′)-*III ant*(6′)-*Ia*	This study
Staphylococcus haemolyticus VPS617[d]	***tet*(K) *mph*(C) *erm*(C) *msr blaZ mecA dfr*(A) *aph*(3′)-III *aph*(2′)-Ia *aac*(6′)-Ie *ant*(6′)-Ia*lnorA*** *sat4*	This study
Bacillus anthracis 4230	pXO2$^+$ [Δ*cap*::***ant*(9)-Ia***, *acpA*]; **pX01$^-$; *bla1 bla2***	23
Bacillus anthracis BR4253	4230 containing pRE25 [*erm*(B), *cat*$_{pIP501}$, *aph*(3′)-*III*, *ant*(6)-*Ia*, *sat4*]; pXO2$^+$ [Δ*cap*::*ant*(9′)-*Ia*, *acpA*]; pXO1$^-$; *bla1 bla2*	This study
Enterococcus faecium SF11770	***aac*(6′)**-*Im aph*(2′)-*Ib aac*(6′)-*Ii ant*(4′)-*Ia ant*(6)-*Ia aph*(3′)-*III erm*(B) *sat4* *tet*(L)-1 *tet*(M) *van*(A) *van*(Z)	11
Enterococcus gallinarum SF9117	***aph*(2′)-Ic *van*(C-1)** *erm*(B)	12
Enterococcus casseliflavus UC73	***aph*(2′)-Id *van*(C)**	53
Bacillus subtilis BR151	pPL708 [***cat-86***, ***ant*(4′)-Ia**]	21
Bacillus subtilis DSM4393	pC194 (***cat-TC***); ***tet*(L)-2 *aadK***	DSMZ
Escherichia coli JIR1905	pWD212 (***catB***)	29
Escherichia coli JIR1597	pJIR235 (***catQ***)	3
Staphylococcus aureus NCTC50582	pC221 (***cat*$_{pC221}$**); *norA*	NCTC
Listeria monocytogenes BM4293	***dfr*(D)**	9; CIP
Bacillus subtilis EC101	pEC101 [***erm*(D)**, *cat-TC*]; *tet*(L)-2 *aadK*	35
Escherichia coli VA831	pVA831 [***erm*(F)**]	35
Escherichia coli/pGERM	pGERM [***erm*(G)**]	50
Staphylococcus warneri VC5	pVC5 [***Inu*(A)**]; *blaZ*	41
Escherichia coli DB10	***Inu*(B)**	7
Streptococcus salivarius Sp6	***mef*(A)** *erm*(B)	51
Streptococcus pyogenes A498	***tet*(T)**	14; CIP
Escherichia coli SC1	pSC1 [***tet*(W)**]	4
Escherichia coli AGHD1	pAGHD1 [***tet*(Z)**]	52
Enterococcus faecium 70/90	***van*(A) *van*(Z) *aac*(6′)-Ii** *tet*(M) erm(B)	33; this study
Enterococcus faecalis DSM12956	***van*(B)** *sat4 ant*(6)-*Ia aph*(3′)-*III erm*(B)	DSMZ
Enterococcus casseliflavus DSM20680	***van*(C)**	DSMZ
Enterococcus gallinarum BM4174	***van*(C-1) *tet*(L)-1 *tet*(U)** *tet*(M) *ant*(6)-*Ia aph*(3′)-*III erm*(B) *sat4*	20
Enterococcus faecium 10/96A	***van*(D4)**	17
Enterococcus faecium N0-0072	***van*(D5)** *sat4 erm*(B) *ant*(6)-*Ia*	6
Enterococcus faecalis BM4405	***van*(E)**	22
Enterococcus faecalis BM4518	***van*(G)** *aac*(6′)-*Ie aph*(2′)-*Ia erm*(B)	18
Staphylococcus aureus BM3093	pIP680 [***vat*(A), *vgb*(A), *vga*(A)**]; *norA*	1; CIP
Staphylococcus aureus BM3318	***vat*(B) *vga*(B) *erm*(A)** *vga*(A)*v aac*(6′)-*Ie ant*(4′)-*Ia ant*(6)-*Ia ant*(9)-*Ia aph*(2′)-*Ia aph*(3′)-*III blaZ mecA sat4 norA*	27; CIP
Staphylococcus cohnii BM10711	pIP1714 [***vat*(C), *vgb*(B)**]; *erm*(C) *mecA tet*(K)	2
Lactobacillus fermentum ROT1	pLME300 [***vat*(E), *erm*(LF)**][e]	26

[a] The genes highlighted in bold are those used as references to validate the microarray. The other genes are those that were additionally detected in the reference strains with the microarray. Rif^r, rifampin resistance; Fus^r, fusidic acid resistance.

[b] NCTC, National Collection of Type Cultures, Centre for Infections, Colindale, London, England; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; CIP, Collection de l'Institut Pasteur, Paris, France.

[c] *C. perfringens* MLP26 was isolated from the intestines of a calf.

[d] *S. haemolyticus* VPS617 was isolated from the milk of a cow with mastitis.

[e] *erm*(LF) is an *erm*(T)-like gene which contains a 260-bp 3′ fragment identical to *erm*(B).

limited to a certain number of genes. Call et al. developed a microarray for detecting 17 tetracycline resistance genes and one β-lactamase gene (8). Recently, a microarray-based system has been optimized for the detection of genes specific to *Staphylococcus aureus*, including 12 resistance genes known to occur occasionally in this species (37).

In this report we describe the first hybridization system using microarray technology for routine microbial investigations that allows rapid and efficient screening of gram-positive bacteria for the presence of up to 90 of the most prevalent and transferable antibiotic resistance genes.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Strains harboring well-characterized resistance genes as well as field strains were used to test the specificity and sensitivity of the microarray-based hybridization system. Hybridization results are shown only for some selected strains (see Fig. 2 and 3). The completely sequenced broad-host-range enterococcal plasmid pRE25 (48), which contains five resistance genes [*cat*$_{pIP501}$, *erm*(B), *sat4*, *aph(3′)-III*, and *ant(6)-Ia*], was used as a gene target to reveal the presence of resistance genes in *Enterococcus* and in an avirulent strain of *B. anthracis*. *Lactococcus lactis* K214, harboring the mosaic resistance plasmid pK214 [*tet*(S), *cat-LM*, *mdt*(A), and *str*] (43), was used as an example of a starter culture. The array was also tested with a vancomycin-resistant *E. faecium* strain harboring a *van*(A) gene and with strains showing a multidrug resistance phenotype but an unknown genotype. For this purpose, one *Staphylococcus haemolyticus* strain isolated from mastitis milk and one *C. perfringens* isolate from cattle were investigated.

All the strains were grown on tryptone soya agar containing 5% defibrinated sheep blood (Oxoid Ltd., Basingstoke, England) at 37°C unless otherwise indicated. *C. perfringens* was incubated under anaerobic conditions. *L. lactis* was grown on M17 agar (Oxoid) at 30°C. *Escherichia coli* and *B. anthracis* strains were grown on Luria-Bertani (LB) agar plates at 37°C. In liquid media, *Enterococcus* and *Staphylococcus* were grown in brain heart infusion broth, *Bacillus* strains in LB broth, and *L. lactis* in GM17 broth. *C. perfringens* was grown in Schädler broth (Oxoid) supplemented with 0.05% (vol/vol) L-cysteine at 37°C under anaerobic conditions. The assays involving *B. anthracis* strains were performed in a biosafety level 3 laboratory using avirulent strains.

TABLE 2. Oligonucleotides used for the detection of resistance genes by PCR analysis

Gene	Primer name	Sequence (5′→3′)	Primer design reference or source
cat_{pIP501}	catF	CCTGCGTGGGCTACTTTA	This study
	catR	CAAAACCACAAGCAACCA	
erm(B)	erm(B)-F	GAAAAGGTACTCAACCAAATA	13
	erm(B)-R	GTAAACAATTTAAGTACCATTACT	
erm(C)	erm(C)-F	AATCGGCTCAGGAAAAGG	This study
	erm(C)-R	ATCGTCAATTCCTGCATG	
mecA	mecA-1	AAAATCGATGGTAAAGGTTGGC	34
	mecA-2	AGTTCTGCAGTACCGGATTTGC	
tet(K)	tet(K)-1	TTAGGTGAAGGGTTAGGTCC	This study
	tet(K)-2	GCAAACTCATTCCAGAAGCA	
tetA(P)	tetA(P)F	CACAGATTGTATGGGGATTAGG	36
	tetA(P)R	CATTTATAGAAAGCACAGTAGC	
tet(L)	tetLF	GTGAATACATCCTATTCA	This study
	tetLR	TTAGAAATCCCTTTGAGA	This study
tet(U)	tetU-F	ATGCAGCTAAGACGTGGC	This study
	tetU-R	TTATTCGGTATCACTTCTCTGTC	
sat4	sat4-F	CGATAAACCCAGCGAACC	This study
	sat4-R	ATAACATAGTATCGACGG	
aph(3′)-*IIIa*	aph3-III-F	CCGCTGCGTAAAAGATAC	This study
	aph3-III-R	GTCATACCACTTGTCCGC	
ant(6)-*Ia*	ant6-I-F	AATTGTGACCCTTGAGGG	This study
	ant6-I-R	GGCATATGTGCTATCCAG	
aac(6′)-*Ie*-*aph*(2′)-*Ia*	aac6-aph2-F	CAGAGCCTTGGGAAGATGAAG	54
	aac6-aph2-R	CCTCGTGTAATTCATGTTCTGGC	
aac(6′)-*Ii*	aac(6)-Ii-F	GAGATACTGATTGGTAGC	This study
	aac(6)-Ii-R	TCTTCACTGACTTCTGCC	
dfr(A)	dfrA-F	CCTTGGCACTTACCAAATG	This study
	dfrA-R	CTGAAGATTCGACTTCCC	
blaZ	blaZ-F	CAGTTCACATGCCAAAGAG	This study
	blaZ-R	TACACTCTTGGCGGTTTC	
mph(C)	mphC-F	CATTGAATGAATCGGGAC	This study
	mphC-R	TTCATACGCCGATTCTCC	
van(E)	vanE-F	AGAATGGTGCTATGCAGG	This study
	vanE-R	TCATGATTTTCCACCGCC	
msr(A)	msrA-F	GCTTAACATGGATGTGG	This study
msr(SA)	msrA-R	GATTGTCCTGTTAATTCCC	
msr(SA′)			
catD	catDPS-F	CCTTGYACATACAGYATGAC	This study
catP	catDPS-R	AACTTGRATKGCSARAGGAAG	
catS			
vgb(B)	vgb(B)-F	GTCTATTCCCGATTCAGG	This study
	vgb(B)-R	TGCAAACCATACGGATCC	

Conjugal transfer. The transfer of plasmid pRE25 (48) from *E. faecalis* RE25 to *B. anthracis* 4230 was performed by filter mating as described previously (42). The transconjugants were selected on LB agar plates containing 19.2 μg of the combination trimethoprim-sulfamethoxazole (1:5) (3.2 μg:16 μg) and 10 μg of erythromycin per milliliter. The transconjugants were identified by colony morphology and by the detection of both the cat_{pIP501} and *erm*(B) resistance genes present on plasmid pRE25 by PCR.

Antimicrobial susceptibility tests. The MICs of erythromycin, clindamycin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, the combination quinupristin-dalfopristin, enrofloxacin, vancomycin, oxacillin, penicillin, the sulfonamide sulfisoxazole, trimethoprim, and the combination amoxicillin-clavulanic acid were determined in Mueller-Hinton broth using custom Sensititre susceptibility plates (Trek Diagnostics Systems, East Grinstead, England; MCS Diagnostics BV, Swalmen, The Netherlands) according to NCCLS guidelines (38).

PCR techniques. The antibiotic resistance genes were amplified by PCR using *Taq* DNA polymerase in accordance with the supplier's directions (Roche Diagnostics, Basel, Switzerland) and using an annealing temperature of 54°C. The oligonucleotides used for PCRs are listed in Table 2.

Genomic DNA isolation. Total DNA was obtained after half a loopful of bacterial cells was lysed in a lysis buffer (0.1 M Tris-HCl, pH 8.5, 0.05% Tween 20, 0.24 mg/ml proteinase K) for 1 h at 60°C, followed by a 15-min denaturation step at 95°C. The lysate was filtered through a 0.2-μm HT Tuffryn membrane (Acrodisc Syringe Filter; Pall Gelman Laboratory, Ann Arbor, MI). Alternatively, DNA was isolated using the guanidium thiocyanate method (45) and was extracted with phenol-chloroform. After addition of ammonium acetate, the cell lysates were purified with 1 volume of phenol:chloroform:isoamyl alcohol (49.5:49.5:1 [vol/vol/vol]). After 5 min of centrifugation at 14,000 rpm (Centrifuge Eppendorf 5415; Eppendorf AG, Hamburg, Germany), the water phase was treated with 1 volume of chloroform:isoamyl alcohol (49.5:1 [vol/vol]). The DNA was precipitated by the addition of 0.6 volume of isopropanol to the aqueous phase and then centrifuged. The DNA pellet was washed once with 80% ethanol and, after a 5-min centrifugation, was dried under a vacuum and resuspended in water.

DNA labeling. The quality of each DNA preparation was assessed by agarose gel electrophoresis using 5 μl of the DNA sample and subsequent ethidium bromide staining. The concentration of DNA was determined spectrophotometrically at 260 nm. Genomic DNA (10 to 100 ng) was labeled by a randomly primed polymerization reaction using Sequenase, version 2.0 (USB Corporation, Cleveland, Ohio) and consisted of three cycles of enzymatic reactions. The labeling reactions were based on the method of Bohlander et al. (5). The protocol, as modified by the DeRisi Laboratory (University of California, San Francisco; www.microarrays.org/pdfs/Round_A_B_C.pdf), was altered as follows. Round A was used unmodified. During Round B, 25 instead of 35 PCR cycles were performed. In Round C, end concentrations of 0.1 mM (each) dATP, dCTP, and dGTP, 0.065 mM dTTP, and 0.035 mM biotin-16-dUTP (Roche Diagnostics) were used instead of the concentrations stated. Furthermore, 35 PCR cycles were run, and a fraction (10 to 20 μl) of the finished reaction product was used for hybridization analysis without further purification steps.

DNA array preparation. The gene sequences and the derived specific oligonucleotides used to prepare the microarray are listed in Table 3. The oligonucleotides were designed from published DNA sequences using the Array Design

TABLE 3. Oligonucleotide sequences of the probes and characteristics and sources of the antibiotic resistance genes represented on the microarray

Spot no.	Identification	Sequence (5′→3′)	Genotype	Resistance phenotype[a]	Mechanism	GenBank accession no.	Gene position[b]	Source
1	be_AAC6-Ie_144	ACATTATACAGAGCCTTGGGAAGATGAAGT	*aac(6′)-Ie*	Tob, Dbk, Ntl, Amk, Ast, 2′Ntl, 5-epi, Siso	Acetyltransferase	M18086	1725–2412	*Staphylococcus aureus*
2	be_AAC6-Ie_475	TTGCCAGAACATGAATTACACGAGGGCAAA						
3	be_AAC6-Ii_71	CTTGGCCGGAAGAATATGGAGACAGCTCGG	*aac(6′)-Ii*	Tob, Dbk, Ntl, Amk, 2′Ntl, 5-epi, Siso	Acetyltransferase	L12710	169–717	*Enterococcus faecium*
4	be_AAC6-Ii_396	AGTGGCTTCCATCCAGAACCTTCGTGAACA						
5	be_AAC6-Im_15	GCGAGTTTCCTTTCGCCCGATGAATGAGGA	*aac(6′)-Im*	Tob, Dbk, Ntl, Amk, 2′Ntl, 5-epi, Siso	Acetyltransferase	AF337947	1215–1751	*Enterococcus faecium*, *E. coli*
6	be_AAC6-Im_286	GCGATGGACCAATTTATCGGTGAGCCGGAA						
7	be_ANT4-Ia_118	CTTGGTCGTCAGACTGATGGGCCCTATTCG	*ant(4′)-Ia*	Tob, Amk, Isp, Dbk	Adenylyltransferase	NC_001565	1390–2151	*Staphylococcus*, *Bacillus*
8	be_ANT4-Ia_197	ATGAATGGACAACCGGTGAGTGGAAGGTGG						
9	be_ANT6-Ia_433	CCAAGCGCAAGGGAGTATGATGATTGCTGC	*ant(6)-Ia*	Sm	Adenylyltransferase	AF516335	14900–15808	*Enterococcus*, *Staphylococcus*
10	be_ANT6-Ia_576	ATCATGGAAGGTCGGCATCGAAACAGGCTT						
11	be_ANT9-Ia_278	GGAGTGAAGTTGTCCCTTGGCAATATCCTCCA	*ant(9)-Ia*	Spc	Adenylyltransferase	X02588	331–1113	*Staphylococcus aureus*
12	be_ANT9-Ia_560	ACCCTAGCTCGAATGTGGCAAACAGTGACT						
13	be_APH2-Ia_149	AAGACAAATGCACGGTTTAGATTATACAGA	*aph(2′)-Ia*	Km, Tob, Nm, Liv, GmC	Phosphotransferase	M18086	2494–3164	*Staphylococcus aureus*
14	be_APH2-Ia_292	TTATGGAAAGACTAAATGCAACAACAGTTT						
15	be_APH2-Ib_317	AGGATGCCCTTGCATATGATGAAGCGACGT	*aph(2′)-Ib*	Km, Tob, Nm, Liv, GmC	Phosphotransferase	AF207840	122–1021	*Enterococcus faecium*, *Escherichia coli*
16	be_APH2-Ib_737	ATCAGCATAAGGCGCCGGAAGTAGCAGAAA						
17	be_APH2-Ic_58	AGCATACAATCCGTCGAGTCGCTTGGTGAG	*aph(2′)-Ic*	Km, Tob, Nm, Liv, GmC	Phosphotransferase	U51479	196–1116	*Enterococcus gallinarum*
18	be_APH2-Ic_346	CTGGCGCTGCAACTTGCTGAGTTCATGAAT						
19	be_APH2-Id_249	GCCATCAGAAACGTACCAAATGTCTTTCGCAGG	*aph(2′)-Id*	Km, Tob, GmC, 2′Ntl, 5-epi, Amk, Dbk	Phosphotransferase	AF016483	131–1036	*Enterococcus casseliflavus*
20	be_APH2-Id_354	GGCAGCTAAGGACCTGGCCCGATTTCTAAG						
21	be_APH3-III_136	ACGGACAGCCGGTATAAAGGGACCACCTAT	*aph(3′)-III*	Km, Nm, Prm, Rsm, Liv, GmB	Phosphotransferase	M36771	293–1084	*Staphylococcus aureus*, *Enterococcus faecalis*
22	be_APH3-III_332	TTATCGAGCTGTATGCGGAGTGCATCAGGC						
23	be_APH3-IVa_20	ATTGGCCGGAGGAACTTCTTGAGCTTCTCG	*aph(3′)-IVa*	Km, Nm, Prm, Rsm, But	Phosphotransferase	X03364	277–1065	*Bacillus circulans*
24	be_APH3-IVa_474	GGAGTACGATTGCACGCCGGAGGAATTGTA						
25	be_NorA_426	AGGACCAGGGATTGGTGGATTTATGGCAGAA	*norA*	Nor,[d] Eno,[d] Ofl,[d] Cip[d]	Quinolones—efflux	D90119	478–1644	*Staphylococcus aureus*
26	be_aadK_61	ATCCGATTGGTCACTTTGGAAGGGTCACGT	*aadK*	Sm	Adenylyltransferase	M26879	90–944	*Bacillus subtilis*
27	be_aadK_175	GATCAGTGGCTCGAAATCTTTGGGAAGCGC						
28	be_bla1_201	AGGTGTATATGCGATTGATACTGGTACAAA	*bla1*	Amp,[c] Amox/clav,[c] Pip[c]	Beta-lactamase	AF367983	626–1555	*Bacillus anthracis*
29	be_bla1_366	AGTGGATTATTCACCTGTTACAGAGAAACA						
30	be_bla2_192	CGGAGAAGCAGTTCCTTCGAACGGTTTA	*bla2*	Amp,[c] Amox/clav,[c] Cfx,[c] Cpd, Cft, Caz, Cax	Beta-lactamase	AF367984	791–1561	*Bacillus anthracis*
31	be_bla2_246	ACTTGTCGATTCTTCTTGGGATGATAAGTT						
32	be_blaZ_718	TTTGTTTATCCTAAGGGCCAATCTGAACCT	*blaZ*	Beta-lactams	Beta-lactamase	M60253	142–987	*Enterococcus faecalis*, *Staphylococcus aureus*
33	be_blaZ_811	AGTGAAACCGCCAAGAGTGTAATGAAGGAA						
34	be_cat-86_367	AGCAGCAACCTATTTCCGAAACCTCATATGCCA	*cat-86*	Cm	Acetyltransferase	K00544	145–807	*Bacillus pumilus*
35	be_cat-86_605	TGAGGTGGCTTATTGAACATTGTGACGAGTGGT						
36	be_cat-DPS_set_114	ATTTGCAGAAAGGATATGATTATTTGATTCCT	*catD*	Cm	Acetyltransferase	X15100	91–729	*Clostridium difficile*
			catP	Cm	Acetyltransferase	U15027	2953–3576	*Clostridium perfringens*
			catS	Cm	Acetyltransferase	X74948	1–492	*Streptococcus pyogenes*
37	be_cat-LM_set_135	AGGATATGAACTGTATCCTGCTTTGA	*cat-LM*	Cm	Acetyltransferase	X68412	1328–1975	*Listeria monocytogenes*
			catpC223	Cm	Acetyltransferase	AY355285	1000–1647	*Staphylococcus aureus*
			catpSCS5	Cm	Acetyltransferase	M58515	213–872	*Staphylococcus haemolyticus*
			catpSCS7	Cm	Acetyltransferase	M58516	90–719	*Staphylococcus aureus*
38	be_cat-TC_set_170	TGACAAGGGTGATAAACTCAAATACAGCT	*cat-TC* and	Cm	Acetyltransferase	U75299	657–1373	*Lactobacillus reuteri*
39	be_cat-TC_set_232	GGTTATTGGGATAAGTTAGAGCCACTTTAT	cat_{pC194}	Cm	Acetyltransferase	NC_002013	1260–1910	*Staphylococcus aureus*
				Cm	Acetyltransferase	NC_002013	1260–1910	*Staphylococcus aureus*
40	be_catB_27	TCATTGGAGTAGAAAGCCATACTTTGAACA	*catB*	Cm	Acetyltransferase	M93113	145–804	*Clostridium butyricum*
41	be_catB_233	TAGGATATTGGGATAGCATGAATCCAAGCT						
42	be_catDP_set_281	TTTCCAGCCTTTGGACTGAGTGTAAGTC	*catP* and *catD*	Cm	Acetyltransferase	U15027	2953–3576	*Clostridium perfringens*
43	be_catDP_set_416	CTATGATACCGTGGTCAACCTTCGATGG		Cm	Acetyltransferase	X15100	91–729	
				Cm	Acetyltransferase	X15100	91–729	
44	be_catQ_66	TGCGGTTAGGTGCACTTACAGTATGACTGCA	*catQ*	Cm	Acetyltransferase	M55620	459–1118	*Clostridium perfringens*
45	be_catQ_186	TAACCGTCACAAGGAGTTCCGCACCTGTTT						
46	be_catS_228	CCTTTGGACACCATACATACCAGATTT	*catS*	Cm	Acetyltransferase	X74948	1–492	*Streptococcus pyogenes*
47	be_catS_383	GCTTTAATCTGAATTTGCAGAAAGGATATGA						
48	be_catpXX_set_196	GTGTTTAGAACAGGAATTAATAGTGAGAATAA	cat_{pSCS1}	Cm	Acetyltransferase	M64281	208–855	*Staphylococcus intermedius*

			cat_{pSCS6}	Cm	Acetyltransferase	X60827	88–735	*Staphylococcus aureus*
			cat_{pIP501}	Cm	Acetyltransferase	X65462	208–855	*Streptococcus agalactiae*
			cat_{pC221}	Cm	Acetyltransferase	X02529	2267–2914	*Staphylococcus aureus*
			cat_{pUB112}	Cm	Acetyltransferase	X02872	208–855	*Staphylococcus aureus*
49	be_cfr_466	GGAATGGGTGAAGCTCTAGCCAACCGTCAA	*cfr*	Cm, Ffc	Unknown	AJ249217	570–1619	*Staphylococcus sciuri*
50	be_cfr_908	GAGAAGCAAACGAAGGGCAGGTAGAAGCCT						
51	be_dfrA_20	TCGCTCACGATAAACAAAGAGTCATTGGGT	*dfr*(A)	Tmp	Dihydrofolate reductase	AF051916	2823–3308:r	*Staphylococcus aureus*
52	be_dfrA_172	AGACGTAACGTCGTACTCACTAACCAAGCT						
53	be_dfrD_140	ACCTTCAATCAATCGGAAGGGCTTTACCTGACA	*dfr*(D)	Tmp	Dihydrofolate reductase	Z50141	94–582	*Staphylococcus haemolyticus*
54	be_ermA_193	TGTCAAGTGACTAAAGAAGCGGTAAACC	*erm*(A)	MLS_B	Methylase	X03216	4551–5282:r	*Staphylococcus aureus*
55	be_ermA_590	AGTGGGTAAACCGTGAATATCGTGTTCT						
56	be_ermB_112	ACAGGTAAAGGGCATTTAACGACGAAACTGGC	*erm*(B)	MLS_B	Methylase	Y00116	262–999	*Enterococcus faecalis*
57	be_ermB_520	AAACTTACCCGCCATACCACAGATGTTCCAGA						
58	be_ermC_149	AGAGGTGTAATTTCGTAACTGCCATTGA	*erm*(C)	MLS_B	Methylase	J01755	2004–2738:r	*Staphylococcus aureus*
59	be_ermC_372	TTTAATCGTGGAATACGGGTTTGCTAAA	*erm*(C)	MLS_B	Methylase			
60	be_ermD_555	AGTGGACTCGGCAATGGTCAGAATAACACGA	*erm*(D)	MLS_B	Methylase	M29832	430–1293	*Bacillus licheniformis*
61	be_ermF_231	TGCCCGAAATGTTCAAGTTGTCGGTTGTGA	*erm*(F)	MLS_B	Methylase	M14730	241–1041	*Bacteroides fragilis*, *Streptococcus*
62	be_ermF_494	GTCCTGAAAGTTTCTTGCCACCGCCAACTG						
63	be_ermG_98	ACATCTTTGAAATAGGTGCAGGGAAAGGTC	*erm*(G)	MLS_B	Methylase	M15332	672–1406	*Bacillus sphaericus*
64	be_ermG_296	TTGGCAGCATACCTTACAACATAAGCACAA						
65	be_ermQ_521	ACTTCCATCCCATGCCTAGTGTAGATTGCGT	*erm*(Q)	MLS_B	Methylase	L22689	262–1035	*Clostridium perfringens*
66	be_ermT_104	TTGAGATTGGTTCAGGGAAAGGTCATTT	*erm*(T)	MLS_B	Methylase	M64090	168–902	*Lactobacillus reuteri*
67	be_ermT_149	AAAGGTGTAATTATGTAACCGCCATTGAAA						
68	be_ermX_231	GGCGGTCGAAGTGGTCCATGATGATTTCCT	*erm*(X)	MLS_B	Methylase	M36726	296–1150	*Corynebacterium diphtheriae*
69	be_ermX_282	TCCCTGCGTCATTGTGGGAAACATTCCCTT						
70	be_ermY_122	AAGGGCATTTCACACTAGAACTGGTTCA	*erm*(Y)	MLS_B	Methylase	AB014481	556–1290	*Staphylococcus aureus*
71	be_ermY_258	ACAGTTTAAGTTCCCAAACAACAAAGCA						
72	be_lnuA_115	AAACAACAAAGAGAACACAGAGATATAGAT	*lnu*(A)	Lm	Transferase	J03947	645–1130	*Staphylococcus aureus*
73	be_lnuA_218	ATTGGATGCCTTCACGTATGGAACTTAA						
74	be_lnuB_169	TCATCCAACTGGTTGTTTGACGTAGCTCCGT	*lnu*(B)	Lm	Transferase	AJ238249	127–930	*Enterococcus faecium*
75	be_mdtA_355	CAGACCGCTCAGATGCCAACAGTCCAATCT	*mdt*(A)	MLS_B, Tet, Min	Efflux	X92946	10534–11790	*Lactococcus lactis*
76	be_mdtA_571	GTCAGGATACCAGAAGTCGCTTCACAGGGC						
77	be_mecA_871	AGCTCCAACATGAAGATGGCTATCGTGTCACA	*mecA*	Met, Oxa	Penicillin-binding protein 2′	AB096217	20340–22346	*Staphylococcus aureus*
78	be_mecA_1042	GCTCAGGTACTGCTATCCACCCTCAAACAGG						
79	be_mef_set_39	AATATGGGCAGGGCAAGCAGTATCATTA	*mef*(A) and *mef*(B)	M	Major facilitator	U70055	314–1531	*Streptococcus pyogenes*
80	be_mef_set_193	GGTGTGCTAGTGGATCGTCATGATAGG		M	Major facilitator	U83667	1–1218	*Streptococcus pneumoniae*
				M	Major facilitator	U83667	1–1218	*Streptococcus pneumoniae*
81	be_mphC_281	CAGGTAAACCCGCAGCCACAATAGATCCAGA	*mph*(C)	M	Phosphorylase	AF167161	5665–6564	*Staphylococcus aureus*
82	be_mphC_555	CGAACTATGGCCTCGACATGCGACCATGAT						
83	be_msr_set_289	ATGCATACAACCGACAGTATGAGTGGTG	*msr*(A) and *msr*(SA)	M, S	ATP-binding transporter	X52085	343–1809	*Staphylococcus epidermidis*
84	be_msr_set_655	GCTAAACGAAATCAAGCGCAACAAATGG	*msr*(SA)	M, S	ATP-binding transporter	AB016613	2005–3471	*Staphylococcus aureus*
			msr(SA′)	M, S	ATP-binding transporter	AB013298	487–1953	*Staphylococcus aureus*
			msr(B)	M, S	ATP-binding transporter	M81802	94–624	*Staphylococcus xylosus*
85	be_sat4_161	AGGATGAAGAGGATGAGGAGGCAGATTGCC	*sat4*	Sth	Acetyltransferase	AF516335	15805–16347	*Enterococcus faecium*
86	be_sat4_338	GCAAGGCATAGGCAGCGCGCTTATCAAT						
87	be_tetK_259	AGTTTGAGCTGTCTTGGTTCATTGATTGC	*tet*(K)	Tet	Efflux	M16217	305–1684	*Staphylococcus aureus*
88	be_tetK_351	TGCTGCATTCCCTTCACTGATTATGGT						
89	be_tetL_1_151	ACAAACTGGGTGAACACAGCCTTTATGT	*tet*(L)	Tet	Efflux	M11036	189–1565	*Bacillus stearothermophilus*
90	be_tetL_1_676	TCTTATCGTTAGCGTGCTGTCATTCCTG						
91	be_tetL_2_269	GCTTAGGGTCGATCATTGGATTTGTTGG	*tet*(L)	Tet	Efflux	X08034	188–1564	*Bacillus subtilis*
92	be_tetL_2_504	GTCGTATTTGCTGCTTATTCCAACTGCA						
93	be_tetM_1033	CTGCTGCAAACGACTGTTGAACCGAGCAAA	*tet*(M)	Tet, Min	Ribosomal protection	X04388	131–2050	*Enterococcus faecalis*
94	be_tetM_1308	TCCACCGAATCCTTTCTGGGCTTCCATTGG						
95	be_tetAP_1193	TATCAGTGGCTCGCTTGAAGCTTGGATTGC	*tetA*(P)	Tet, Min	Ribosomal protection	L20800	207–2120	*Clostridium perfringens*
96	be_tetAP_1266	GGAGCACAAGCAGGGCAGATAGGAGCATTT						
97	be_tetS_18	CGGTATCTTAGCACATGTTGATGCAGGA	*tet*(S)	Tet, Min	Ribosomal protection	L09756	447–2372	*Listeria monocytogenes*
98	be_tetS_776	CAGATGATGGTCAACGGCTTGTCTATGT						

Continued on following page

TABLE 1—*Continued*

Spot no.	Identification	Sequence (5′→3′)	Genotype	Resistance phenotype[a]	Mechanism	GenBank accession no.	Gene position[b]	Source
99	be_tetT_232	CACATGGATTTCATAGCCGAAGTTGAGC	*tet*(T)	Tet, Min	Ribosomal protection	L42544	478–2433	*Streptococcus pyogenes*
100	be_tetT_1326	GGTTCCACCAAATCCTTATTGGGCATCT						
101	be_tetU_133	GCTGAGCCTTCTAATTGGTCGATAATTGCT	*tet*(U)	Tet, Min	Unknown	U01917	413–730	*Enterococcus faecium*
102	be_tetW_66	CCTGCTATATGCCAGCGGAGCCATTTCAGA	*tet*(W)	Tet, Min	Ribosomal protection	AJ222769	192–2111	*Butyrivibrio fibrisolvens*
103	be_tetW_455	TTATCATCAAGCAGACGGTGTCGCTGTCCC						
104	be_tetZ_43	GTGATGCCGATCTTGCCTACCCTTCTCGAC	*tet*(Z)	Tet	Efflux	AF121000	11880–13034:r	*Cornynebacterium glutamicum*
105	be_tetZ_93	CATGATCCCACTGCACGTCGGACTACTGAC						
106	be_vanA_192	CTATTCAGCTGTACTCTCGCCGGATAAA	*van*(A)	Van, Tei	Ligase	M97297	6979–8010	*Enterococcus faecium*
107	be_vanA_884	TACAAGATAACGGCCGCATTGTACTGAA						
108	be_vanB_set_65	AATCCGCAATAGAAATTGCTGCGAACAT	*van*(B) and	Van	Ligase	U00456	62–1090	*Enterococcus faecalis*
109	be_vanB_set_151	CTATGCAAGAAGCCATGTACGGAATGGG	*van*(B2)	Van	Ligase	AF310953	1–1029	*Enterococcus faecium*
				Van	Ligase	AF310953	1–1029	*Enterococcus faecium*
110	be_vanC-1_77	TCCAAGCTATTGACCCGCTGAAATATGA	*van*(C-1)	Van	Ligase	AF162694	1411–2442	*Enterococcus gallinarum*
111	be_vanC-1_497	ACCATGGATTCCCGATCTTTATCAAGCC						
112	be_vanC_set_37	CCGGAATACACCGTTTCTTTAGCTTCAG	*van*(C-2) and	Van	Ligase	L29638	33–1085	*Enterococcus casseliflavus*
113	be_vanC_set_184	CAAGACACGTGGTTGTTGGATACGAAAC	*van*(C-3)	Van	Ligase	AY033764	26–1078	*Enterococcus flavescens*
				Van	Ligase	AY033764	26–1078	*Enterococcus flavescens*
114	be_vanD4-5_183	CTATGCGGGATACCCGGCTGTGATTTCTCC	*van*(D4) and	Van	Ligase	AF277571	1262–2293	*Enterococcus faecium*
115	be_vanD4-5_267	GCCTGTAGACGTGGTGCTTCCGATGATTCA	*van*(D5)	Van	Ligase	AY489045	4010–5041	*Enterococcus faecium*
				Van	Ligase	AY489045	4010–5041	*Enterococcus faecium*
116	be_vanE_298	GGAGGTTATGGTGAGAATGGTGCTATGCAGGG	*van*(E)	Van	Ligase	AF430807	2976–4034	*Enterococcus faecalis*
117	be_vanE_357	TGTAGGTTGTGGTATCGGAGCTGCAGCAAT						
118	be_vanG_362	TGGCAGGAATACCTGTTGTTGGCTGCGATA	*van*(G)	Van	Ligase	AF253562	3715–4764	*Enterococcus faecalis*
119	be_vanG_549	ACCTGTTCGTGCAGGCTCTTCCTTTGGAAT						
120	be_vanZ_328	ACAAATACTGTTGGAGGCTTTCTTGGACTG	*van*(Z)	Tei	Unknown	M97297	10116–10601	*Enterococcus faecium*
121	be_vatA_288	TCATCTATTCAGGATGGGTTGGGAGAAGT	*vat*(A)	S_A	Transferase	L07778	258–917	*Staphylococcus aureus*
122	be_vatA_429	AATCATTGCTGCAGAAGCTGTTGTCAC						
123	be_vatB_9	TGGCCCTGATCCAAATAGCATATATCCACA	*vat*(B)	S_A	Transferase	U19459	67–705	*Staphylococcus aureus*
124	be_vatB_109	ACTTACTATTCCGATGTTAACGGAGCTGAA						
125	be_vatC_474	TTCAGTTGTTGGCGGTAATCCTTCACGATT	*vat*(C)	S_A	Transferase	AF015628	1307–1945	*Staphylococcus aureus*
126	be_vatC_552	AAGGTGGTGGGACCTAGAGATAGAGACGAT						
127	be_vatD_453	GCCATACATGTTAGCTGGAGGAAATCCT	*vat*(D)	S_A	Transferase	L12033	162–791	*Enterococcus faecium*
128	be_vatE_349	TGTAGTCGGAAATGACGTGTGGTTTGGGCA	*vat*(E)	S_A	Transferase	AF139725	63–707	*Enterococcus faecium*
129	be_vatE_409	AGGTGACGGTGCCATTATCGGAGCAAATAGT						
130	be_vgaA_834	CTCGGGTACAATTGAAGGACGGGTATTGTGGA	*vga*(A)	S_B	ATP-binding transporter	M90056	909–2477	*Staphylococcus aureus*
131	be_vgaA_886	CGCGGAGGAGACAAGATGGCAATTATCGGA						
132	be_vgaB_569	TGCTTCTACGAAAGCAACAAGAAGAATACG	*vga*(B)	S_B	ATP-binding transporter	U82085	629–2287	*Staphylococcus aureus*
133	be_vgaB_649	GAGAATAAGGCGCAAGGAATGATTAAGCCC						
134	be_vgbA_142	ACAGAGTACCCACTACCGACACCAGATGCA	*vgb*(A)	S_B	Hydrolase	M20129	641–1540	*Staphylococcus aureus*
135	be_vgbA_281	TGCCTAACCCAGATTCAGCACCCTACGGTA						
136	be_vgbB_273	ATATCCATTGCCACAGCCGGATTCTGGTCC	*vgb*(B)	S_B	Lactonase	AF015628	399–1286	*Staphylococcus cohnii*
137	be_vgbB_539	CAAATGCAGCGGCTCCAGTGGGTATCACTA						
138	1×Spottingpuffer							
139	Marken-Mix							

[a] Aminoglycosides: Tob, tobramycin; Dbk, dibekacin; Ntl, netilmicin; Amk, amikacin; 2′Ntl, 2′-*N*-ethylnetilmicin; 5-epi, 5-episisomicin; Siso, sisomicin; Isp, isepamicin; Sm, streptomycin; Spc, spectinomycin; Ast, Astromicin (fortimicin); Km, kanamycin; Nm, neomycin; Liv, lividomycin; GmB, gentamicin B; GmC, gentamicin C; Prm, paromomycin; Rsm, ribostamycin; But, butirosin; The phenotypes were found in references 49 and 56. Fluoroquinolones: Nor, norfloxacin; Eno, enoxacin; Ofl, ofloxacin; Cip, ciprofloxacin. Beta-Lactams and Cephem: Amp, ampicillin; Amox/clav, amoxicillin-clavulanic acid; met, methicillin; Oxa, oxacillin; Ctx, cefoxitin; Cpd, cefpodoxime; Cft, cefotaxime; Caz, ceftazidime; Cax, ceftriaxone. Phenicols: Cm, chloramphenicol; Ffc, florfenicol. Folate pathway inhibitors: Tmp, trimethoprim. MLS: M, macrolides, L, lincosamides; S_B, streptogramins B; S_A, streptogramin A; Lm, lincomycin. Tetracyclines: Tet, tetracycline, Min, minocycline. Glycopeptides: Van, vancomycin; Tei, teicoplanin. Others: Sth, streptothricin.

[b] :r, the gene is found on the complementary strand.

[c] When expressed in *E. coli* (10).

[d] When overexpressed in *S. aureus* (32).

139 ctrl	129 *vat*(E)	130 *vga*(A)	131 *vga*(A)	132 *vga*(B)	133 *vga*(B)	134 *vgb*(A)	135 *vgb*(A)	136 *vgb*(B)	137 *vgb*(B)	138 buffer	139 ctrl
	118 *van*(G)	119 *van*(G)	120 *van*(Z)	121 *vat*(A)	122 *vat*(A)	123 *vat*(B)	124 *vat*(B)	125 *vat*(C)	126 *vat*(C)	127 *vat*(D)	128 *vat*(E)
139 ctrl	107 *van*(A)	108 *van*(B)	109 *van*(B)	110 *van*(C-1)	111 *van*(C-1)	112 *van*(C)	113 *van*(C)	114 *van*(D4-5)	115 *van*(D4-5)	116 *van*(E)	117 *van*(E)
95 *tetA*(P)	96 *tetA*(P)	97 *tet*(S)	98 *tet*(S)	99 *tet*(T)	100 *tet*(T)	101 *tet*(U)	102 *tet*(W)	103 *tet*(W)	104 *tet*(Z)	105 *tet*(Z)	106 *van*(A)
83 *msr*	84 *msr*	85 *sat4*	86 *sat4*	87 *tet*(K)	88 *tet*(K)	89 *tet*(L)_1	90 *tet*(L)_1	91 *tet*(L)_2	92 *tet*(L)_2	93 *tet*(M)	94 *tet*(M)
71 *erm*(Y)	72 *lnu*(A)	73 *lnu*(A)	74 *lnu*(B)	75 *mdt*(A)	76 *mdt*(A)	77 *mecA*	78 *mecA*	79 *mef*	80 *mef*	81 *mph*(C)	82 *mph*(C)
59 *erm*(C)	60 *erm*(D)	61 *erm*(F)	62 *erm*(F)	63 *erm*(G)	64 *erm*(G)	65 *erm*(Q)	66 *erm*(T)	67 *erm*(T)	68 *erm*(X)	69 *erm*(X)	70 *erm*(Y)
47 *catS*	48 *catpXX*	49 *cfr*	50 *cfr*	51 *dfr*(A)	52 *dfr*(A)	53 *dfr*(D)	54 *erm*(A)	55 *erm*(A)	56 *erm*(B)	57 *erm*(B)	58 *erm*(C)
35 *cat-86*	36 *cat-DPS*	37 *cat-LM*	38 *cat-TC*	39 *cat-TC*	40 *catB*	41 *catB*	42 *cat-DP*	43 *cat-DP*	44 *catQ*	45 *catQ*	46 *catS*
23 *aph(3')-IVa*	24 *aph(3')-IVa*	25 *norA*	26 *aadK*	27 *aadK*	28 *bla1*	29 *bla1*	30 *bla2*	31 *bla2*	32 *blaZ*	33 *blaZ*	34 *cat-86*
11 *ant(9')-Ia*	12 *ant(9')-Ia*	13 *aph(2'')-Ia*	14 *aph(2'')-Ia*	15 *aph(2'')-Ib*	16 *aph(2'')-Ib*	17 *aph(2'')-Ic*	18 *aph(2'')-Ic*	19 *aph(2'')-Id*	20 *aph(2'')-Id*	21 *aph(3')-III*	22 *aph(3')-III*
139 ctrl	1 *aac(6')-Ie*	2 *aac(6')-Ie*	3 *aac(6')-Ii*	4 *aac(6')-Ii*	5 *aac(6')-Im*	6 *aac(6')-Im*	7 *ant(4')-Ia*	8 *ant(4')-Ia*	9 *ant(6')-Ia*	10 *ant(6')-Ia*	139 ctrl

FIG. 1. Distribution layout of the oligonucleotides on the microarray. The detectable genes are italicized, and details are given in Table 3. The following gene abbreviations include a family of genes: *catDPS* detects *catD*, *catP*, and *catS*; *catDP* detects *catD* and *catP*; *catpXX* detects cat_{pC221}, cat_{pUB112}, cat_{pSCS1}, cat_{pSCS6}, and cat_{pIP501}; *cat-LM* detects *cat-LM*, cat_{pSCS5}, and cat_{pSCS7}; *cat-TC* detects *cat-TC* and cat_{pC194}; *mef* detects *mef*(A) and *mef*(B); *msr* detects *msr*(A), *msr*(SA), *msr*(SA′), and *msr*(B); *van*(B) detects *van*(B) and *van*(B2); *van*(C) detects *van*(C-2) and *van*(C-3). The position controls (ctrl) consist of biotin-labeled oligonucleotides.

Software Package (Clondiag Technologies, Jena, Germany). They consist of 26- to 33-mers with similar physicochemical parameters. The probes were spotted onto a 3- by 3-mm glass surface with a Microgrid II spotting machine (BioRobotics Inc./Apogent Discoveries Europe, Cambridge, England) as described previously (37). The glass substrates were incorporated into standard microreaction tubes. The layout of the spotted probes in the microarray is shown in Fig. 1.

DNA hybridization and detection. The microarray tubes were positioned in a Thermomixer comfort (Eppendorf AG, Hamburg, Germany) and washed twice with QMT hybridization buffer (Quantifoil, Jena, Germany) for 5 min at 30°C and 550 rpm. The labeled genomic DNA (10 to 20 μl) was mixed with QMT hybridization buffer to obtain a final volume of 100 μl, denatured for 5 min at 94°C, kept on ice for 3 min, and hybridized for 1 h at 60°C and 550 rpm. The arrays were washed in 500 μl 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) containing 0.2% sodium dodecyl sulfate solution for 5 min at 30°C and 550 rpm, in 500 μl 2× SSC for 5 min at 20°C and 550 rpm, and in 500 μl 0.2× SSC for 5 min at 20°C and 550 rpm. The arrays were blocked with 100 μl 6× SSPE (60 mM sodium phosphate, 1.08 M NaCl, 6 mM EDTA, pH 7.4) solution containing 0.005% Triton X-100 and 2% (wt/vol) milk powder for 15 min at 30°C and 550 rpm; then 100 μl of conjugate buffer (6× SSPE, 0.005% Triton X-100, 100 pg/μl of streptavidin-peroxidase conjugate [Clondiag]) was added, and the array tubes were incubated for 15 additional minutes at 30°C and 550 rpm. The arrays were washed in 2× SSC–0.01% Triton X-100 at 30°C for 5 min and in 2× SSC and then 0.2× SSC for 5 min at 20°C. The arrays were kept at 20°C in the last washing solution until visualization. The hybridized probes were enhanced using 100 μl of tetramethylbenzidine peroxidase substrate (Clondiag). The peroxidase staining procedure and the online detection were performed in an *atr01* array tube reader (Clondiag) for 15 min at 25°C according to the manufacturer's specifications. The hybridization analyses were performed in duplicate.

The data were analyzed using Iconoclust software (Clondiag). Signal intensity and local background were measured for each spot on the array. Extinctions of local backgrounds were subtracted from extinctions of spots. A threshold was determined so that each value above zero was considered a signal. Resulting values below 0.1 were considered negative (−), and those above 0.3 were considered positive (+). Values between 0.1 and 0.3 were regarded as ambiguous (+/−).

RESULTS

Construction of the gene array. A total of 90 resistance genes that had already been characterized in gram-positive bacteria were selected from the GenBank database to be represented on the microarray (Table 3). Only extrinsic potentially transmissible resistance genes were included. Antibiotic resistance due to single-base mutations of the target genes could not be considered, since highly stringent annealing temperatures would be necessary to obtain a specific hybridization with these oligonucleotides. Each antibiotic resistance gene or group of genes was represented on the array by two different oligonucleotides situated apart from each other within the protein coding sequence. The oligonucleotides were chosen according to their high specificity for the related resistance genes. Consensus sequences were used to design the oligonucleotides specific for several subtypes of resistance genes sharing DNA identities higher than 89%. Hence, the chloramphenicol acetyltransferase genes *catD* and *catP* (99.5% DNA identitity) were represented by the catDP oligonucleotides be_catDP_set_281 and be_catDP_set_416, the genes *cat-LM*, cat_{pC223}, cat_{pSCS5}, and cat_{pSCS7} (DNA identity, ≥90.6%) by the oligonucleotide be_cat-LM_set_135, the genes *cat-TC* and cat_{pC194} (99.7%) by the cat-TC oligonucleotides cat-TC_set_170 and cat-TC_set_232, the genes cat_{pC221}, cat_{pUB112}, cat_{pSCS1}, cat_{pSCS6}, and cat_{pIP501} (≥96.9%) by the oligonucleotide be_catpXX_set_196, the macrolide efflux genes *mef*(A) and *mef*(E) (89.9%) by the mef oligonucleotides be_mef_set_39 and be_mef_set_193, the vancomycin resistance genes *van*(B) and *van*(B2) (95.6%) by the vanB oligonucleotides be_vanB_set_65 and be_vanB_set_151, the *van*(C-2) and *van*(C-3) genes (98.7%) by the vanC

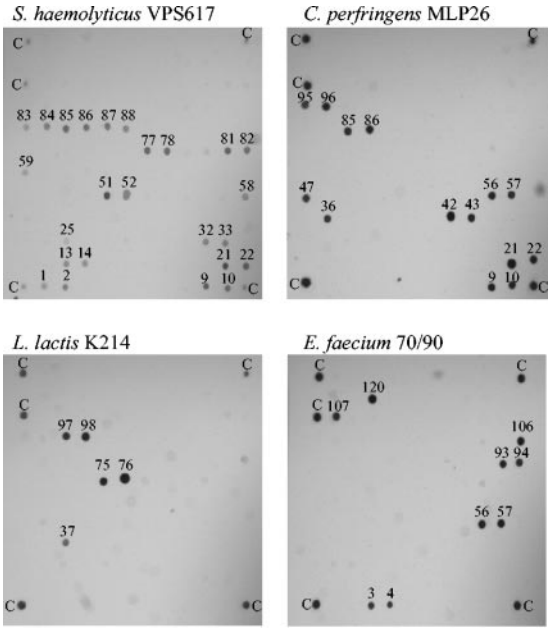

FIG. 2. Microphotographs of microarrays hybridized with genomic DNAs of *S. haemolyticus* VPS617, *C. perfringens* MLP26, *L. lactis* K214, and *E. faecium* 70/90. Spots: 1 and 2, *aac(6′)-Ie*; 3 and 4, *aac(6′)-Ii*; 9 and 10, *ant(6′)-Ia*; 13 and 14, *aph(2″)-Ia*; 21 and 22, *aph(3′)-III*; 25, *norA*; 32 and 33, *blaZ*; 36, *cat-DPS*; 37, *cat-LM*; 42 and 43, *catDP*; 47, *catS*; 51 and 52, *dfr*(A); 56 and 57, *erm*(B); 58 and 59, *erm*(C); 75 and 76, *mdt*(A); 77 and 78, *mecA*; 81 and 82, *mph*(C); 83 and 84, *msr*; 85 and 86, *sat4*; 87 and 88, *tet*(K); 93 and 94, *tet*(M); 95 and 96, *tetA*(P); 97 and 98, *tet*(S); 106 and 107, *van*(A); 120, *van*(Z); C, biotin position marker. The layout of the array and the description of the genes are presented in Fig. 1 and Table 3, respectively.

oligonucleotides be_vanC_set_37 and be_vanC_set_184, the *van*(D4) and *van*(D5) genes (93.6%) by the be_vanD4-5_183 and be_vanD4-5_267 oligonucleotides, and the ATB-binding transporter genes *msr*(A), *msr*(SA), *msr*(SA′), and *msr*(B) (≥98.5%) by the msr oligonucleotides be_msr_set_289 and be_msr_set_655 (Table 3). For a few genes, including *nor*(A), *cat-LM*, *dfr*(D), *erm*(Q), *lnu*(B), *tet*(U), *van*(Z), *vat*(D), and the genes of the catpXX family, only one oligonucleotide could be designed. The bifunctional *aac(6′)-Ie–aph(2″)-Ia* gene has been considered as two individual targets for the microarray design, since these genes have also been shown to confer resistance when expressed separately (47). Additionally, the *aac(4‴)* gene, mediating aminoglycoside resistance in *S. aureus*, was described as a functional *aac(6′)-Ie–aph(2)-Ia* gene lacking the *aph(2)-Ia* site (25). The sequence of each oligonucleotide, with the corresponding genes and the specified phenotypes, is given in Table 3. The microarray possesses five position controls (see Fig. 2 and 3), which consist of biotin-labeled oligonucleotides. Certain antibiotic resistance genes, such as the tetracycline resistance gene *tet*(O) (GenBank accession no. M18896), the streptomycin resistance gene *str* (X06627), the macrolide resistance genes *mre*(A) (U92073) and *msr*(C) (AJ243209 and AF313494), and the vancomycin resistance genes *van*(D1) (AF130997), *van*(D2) (AF153050), and *van*(D3) (AF175293), were omitted and will be included in a second generation of the microarray.

Detection of resistance genes in *Staphylococcus*. *S. haemolyticus* VPS617, isolated from mastitis milk, showed resistance to erythromycin (MIC, >32 μg/ml), tetracycline (MIC, 32 μg/ml), gentamicin (MIC, 32 μg/ml), kanamycin (MIC, >128 μg/ml), streptomycin (MIC, 64 μg/ml), sulfisoxazole (MIC, 1,024 μg/ml), trimethoprim (256 μg/ml), oxacillin (MIC, 32 μg/ml), and penicillin (MIC, 8 μg/ml) and was susceptible to enrofloxacin (MIC, <0.125 μg/ml), cephalotin (MIC, <1 μg/ml), and an amoxicillin-clavulanic acid combination of 2:1 (MICs, <2 and <1 μg/ml, respectively). The MICs were compared with the genes detected by the microarray (Table 4). Hybridization analysis of VPS617 genomic DNA with the microarray revealed 12 acquired antibiotic resistance genes. The erythromycin resistance could be explained by the presence of an *erm*(C) gene conferring resistance to antibiotics including macrolides, lincosamides, and type B streptogramins (MLS_B), an *msr* gene (conferring resistance to macrolides and streptogramins B), and an *mph*(C) gene that inactivates macrolides. *S. haemolyticus* was shown to harbor the tetracycline resistance gene *tet*(K), the aminoglycoside resistance genes *aph(3′)-III*, *aph(2″)-Ia*, *aac(6′)-Ie*, and *ant(6)-Ia*, the streptothricin resistance gene *sat4*, the trimethoprim-resistant dihydrofolate reductase gene *dfr*(A), the beta-lactamase gene *blaZ*, and the methicillin (oxacillin) resistance gene *mecA* (Fig. 2). The staphylococcal housekeeping gene *norA* was also detected. However, this gene is not involved in acquired or transmissible antibiotic resistance. The gene *norA* encodes a membrane-associated protein which causes resistance to hydrophilic quinolones and a variety of other substances such as ethidium bromide, cetrim-

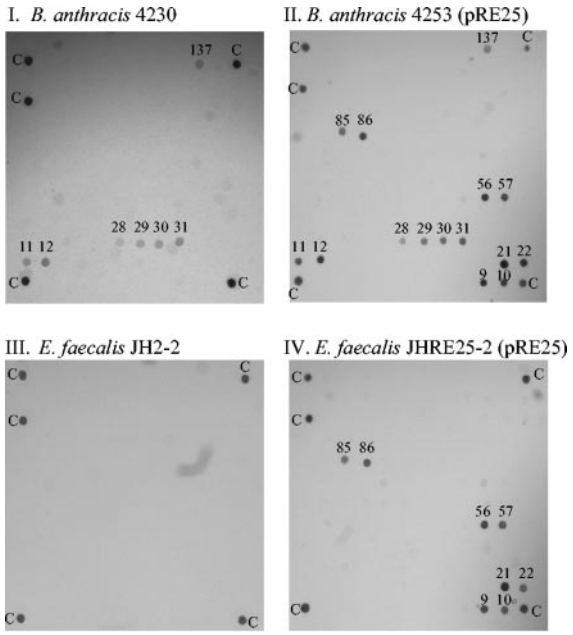

FIG. 3. Microphotographs of microarrays hybridized with DNAs of *E. faecalis* (III and IV) and *B. anthracis* (I and II) before (I and III) and after (II and IV) transformation with plasmid pRE25. Spots: 9 and 10, *ant(6)-Ia*; 11 and 12, *ant(9)-Ia*; 21 and 22, *aph(3′)-III*; 28 and 29, *bla1*; 30 and 31, *bla2*; 56 and 57, *erm*(B); 85 and 86, *sat4*; 137, be_vgbB_539; C, biotin position marker. The layout of the array and the description of the genes are presented in Fig. 1 and Table 3, respectively.

TABLE 4. Relationship between the genes detected in *S. haemolyticus, C. perfringens, L. lactis, E. faecium, E. faecalis* and *B.anthracis* using the microarray and their MICs as determined by broth microdilution

Strain	Genes detected	Antibiotics tested	MIC (μg/ml)[a]	Susceptibility breakpoint[b] (μg/ml)
S. haemolyticus VPS617	emr(C),mph(C), msr	Erythromycin	>32	≤0.5
	tet(K)	Tetracycline	32	≤4
	aac(6′)-Ie-aph(2′)-Ia	Gentamicin	32	≤4
	aph(3′)-III	Kanamycin	>128	≤16
	ant(6)-Ia	Streptomycin	64	≤8[c]
	mecA	Oxacillin	32	≤0.25
	blaZ	Penicillin	8	≤0.12
	dfr(A)	Trimethoprim	256	≤8
	sat4	None	ND	NA
	norA[d]	Norfloxacin	<0.125	≤4
C. perfringens MLP26	*erm*(B)	Erythromycin	>32	NA
		Clindamycin	16	≤2
	tetA(P)	Tetracycline	32	≤4
	catP	Chloramphenicol	64	≤8
	aph(3′)-III	Kanamycin	ND	NA
	ant(6)-Ia	None	ND	NA
	sat4	None	ND	NA
L. lactis K214	*tet*(S)	Tetracycline	>128	≤2[e]
	cat-LM	Chloramphenicol	32	≤4[e]
	mdt(A)	Erythromycin	1	≤0.25[e]
E. faecium 70/90	*tet*(M)	Tetracycline	64	≤4
	erm(B)	Erythromycin	>32	≤0.5
	aac(6′)-Ii	None	ND	NA
	van(A)	Vancomycin	>128	≤4
E. faecalis JHRE25-2	*erm*(B)	Erythromycin	>128	≤0.5
		Clindamycin	>32	≤2[c]
	aph(3′)-III	Kanamycin	>128	64[f]
	ant(6)-Ia	Streptomycin	>128	64[f]
	sat4	None	ND	NA
	Not detected	Chloramphenicol	64	≤8
B. anthracis BR4253	*erm*(B)	Erythromycin	>128	≤0.5
		Clindamycin	>32	≤0.5
	aph(3′)-III	Kanamycin	1	1[f]
	ant(6)-Ia	Streptomycin	1	1[f]
	sat4	None	ND	NA
	Not detected	Chloramphenicol	32	≤8
	bla1 bla2	Penicillin	<0.12	≤0.12

[a] ND, not determined.
[b] Unless otherwise indicated, the breakpoints given are those proposed in the NCCLS guidelines (38). NA, not available.
[c] Breakpoint proposed by the Société Française de Microbiologie (http://sfm.asso.fr).
[d] Confers resistance only when overexpressed (32).
[e] The breakpoints for *Lactococcus* are those defined by the NCCLS for *Streptococcus* spp. other than *Streptococcus pneumoniae.*
[f] MIC for the susceptible strains used as recipients (Table 5).

ide, benzalkonium chloride, tetraphenylphosphonium bromide, and acriflavine only when overexpressed (32).

Detection of resistance genes in *Clostridium*. *C. perfringens* MLP26 was isolated from the intestines of a calf. The strain showed resistance to tetracycline (MIC, 32 μg/ml), erythromycin (MIC, >32 μg/ml), clindamycin (MIC, 16 μg/ml), chloramphenicol (MIC, 64 μg/ml), and kanamycin (MIC, >128 μg/ml), and the MICs were compared to the genotype revealed by the microarray (Table 4). The following genes were detected in *C. perfringens* MLP26: the aminoglycoside resistance genes *aph(3′)-III* and *ant(6)-Ia*, the tetracycline resistance gene *tetA*(P), the streptothricin resistance gene *sat4*, the MLS_B resistance gene *erm*(B), and a chloramphenicol acetyltransferase gene, one of the closely related *catD*, *catP*, and *catS* genes (Fig. 2). Further differentiation of the latter by PCR and sequence analysis revealed the gene *catP* (see below).

Detection of resistance genes in *Lactococcus*. *L. lactis* K214 harbored plasmid pK214, which confers resistance to chloramphenicol (MIC, 32 μg/ml), tetracycline (MIC, >128 μg/ml), and streptomycin (MIC, >128 μg/ml) and decreased susceptibility to erythromycin (MIC, 1 μg/ml) (44). The tetracycline resistance gene *tet*(S), the chloramphenicol acetyltransferase gene *cat-LM*, and the multidrug transporter gene *mdt*(A), involved in erythromycin efflux, could be detected by the corresponding oligonucleotide targets in the microarray (Fig. 2). The streptomycin resistance gene *str*, present on plasmid pK214, was not revealed by the hybridization, since oligonucleotides specific to this target gene were not included on the array. The relationship between the phenotype and the genotype of *L. lactis* K214 is presented in Table 4.

Detection of resistance genes in vancomycin-resistant *E. faecium*. Microarray hybridization of *E. faecium* 70/90 confirmed the presence of the vancomycin and teicoplanin resistance genes *van*(A) and *van*(Z) in this clinical isolate. Additional resistance genes, such as the tetracycline resistance gene *tet*(M), the MLS_B resistance gene *erm*(B), and the aminoglycoside resistance gene *aac(6′)-Ii*, were identified (Fig. 2). The antimicrobial susceptibility test for this strain confirmed the phenotypic expression of the genes detected (Table 4). *E. faecium* 70/90 showed resistance to vancomycin (MIC, >128 μg/ml),

TABLE 5. Susceptibilities of *E. faecalis, B. anthracis*, and transconjugants containing plasmid pRE25 to different antibiotics

Strain	MIC (μg/ml)[a] of:				
	ERY	CLI	CHL	KAN	STR
E. faecalis RE25	>128	>32	64	>128	>128
E. faecalis JH2-2	<0.25	2	<1	64	64
E. faecalis JHRE25-2	>128	>32	64	>128	>128
B. anthracis 4230	1	<0.25	4	1	1
B. anthracis BR4253	>128	>32	32	1	1

[a] ERY, erythromycin; CLI, clindamycin; CHL, chloramphenicol; KAN, kanamycin; STR, streptomycin.

tetracycline (MIC, 64 μg/ml), erythromycin (MIC, >32 μg/ml), and clindamycin (MIC, >32 μg/ml). The MICs of the aminoglycosides that can be affected by *aac(6′)-Ii*, e.g., amikacin and tobramycin (16), were not determined.

Detection of the genes present on the multidrug resistance plasmid pRE25. Plasmid pRE25 was used as a gene target for the detection of antibiotic resistance genes in both *E. faecalis* and *B. anthracis* strains. In *E. faecalis* JHRE25-2, plasmid pRE25 confers resistance to erythromycin, clindamycin, chloramphenicol, and the aminoglycoside antibiotics kanamycin and streptomycin (Table 5). The resistance of strain JHRE25-2 to these antibiotics results from the presence of genes *aph(3′)-III*, *ant(6)-Ia*, *erm*(B), and *sat4* on plasmid pRE25 (48) (Table 4). They could be detected with the microarray (Fig. 3). No signal was obtained with the chloramphenicol acetyltransferase gene target catpXX, although cat_{pIP501} is present in *E. faecalis* JHRE25-2, as confirmed by PCR using genomic DNA.

Detection of resistance genes in *B. anthracis*. The avirulent *B. anthracis* strain 4230, which lacks the virulence plasmid pXO1 and contains the spectinomycin resistance gene *ant(9)-Ia* instead of the capsule genes on pXO2, was used as a model for the detection of resistance genes in *B. anthracis*. Microarray-based analysis of *B. anthracis* 4230 DNA revealed the presence of the β-lactamase genes *bla1* and *bla2* and the spectinomycin resistance gene *ant(9)-Ia* (Fig. 3). It should be noted that both the *bla1* and *bla2* genes are endogenous to *B. anthracis* but are not expressed (10). One hybridization signal was obtained with only one of the two oligonucleotides specific to the *vgb*(B) gene. The *vgb*(B) gene, however, could not be amplified from *B. anthracis* by PCR, confirming that this gene was not present in the strain.

Plasmid pRE25 was then transferred from *E. faecalis* RE25 to *B. anthracis* 4230 by conjugation in order to obtain *B. anthracis* strains carrying acquired resistance genes. The MICs of different antibiotics were determined for the donor strain *E. faecalis* RE25, the recipient strain *B. anthracis* 4230, and the resulting *B. anthracis* transconjugants by a broth microdilution test (Table 5). The MIC for the *B. anthracis* transconjugant BR4253 was then compared to the antibiotic resistance genes detectable by microarray hybridization (Table 4). In the *B. anthracis* transconjugant BR4253, plasmid pRE25 conferred resistance only to erythromycin, clindamycin, and chloramphenicol, not to kanamycin or streptomycin, although the aminoglycoside resistance genes *aph(3′)-III* and *ant(6)-Ia* could be detected by DNA hybridization with the microarray (Fig. 3). The resistance genes *erm*(B) and *sat4* of plasmid pRE25, as well as the *B. anthracis* genes *bla1*, *bla2*, and *ant(9)-Ia*, were also detected. As with *E. faecalis* JHRE25-2, the cat_{pIP501} gene of pRE25 was not detected in *B. anthracis* BR4253 by microarray hybridization (Fig. 3) but could be amplified by PCR.

Specificity testing of the microarray using reference strains. The specificity and sensitivity of the oligonucleotides present on the microarray in detecting antibiotic resistance genes were tested using reference strains that harbor specific antibiotic resistance genes (Table 1). Twenty-nine strains in addition to those presented in Fig. 2 and 3 were hybridized with the microarray. Each of these strains harbors 1, 2, or 3 reference antibiotic resistance genes, for a total of 43 genes. All of these genes could be detected with the specific oligonucleotides present on the microarray, with the exception of the oligonucleotide be_vanC_set_184, which did not hybridize with the *van*(C)-carrying *Enterococcus casseliflavus* strains UC73 and DSM20680. The *van*(C) gene was revealed in these strains with a second oligonucleotide, be_vanC_set_37. The hybridization analyses of the reference strains revealed, besides the reference antibiotic resistance genes, the presence of additional antibiotic resistance genes (Table 1). Overall, a total of 125 oligonucleotides (out of 137) were tested by hybridization of 71 different antibiotic resistance genes.

Confirmation of the resistance genes by PCR. The resistance genes detected in the field strains *S. haemolyticus* VPS617 and *C. perfringens* MLP26 and in the transconjugants *E. faecalis* JHRE25-2 and *B. anthracis* BR4253 by the microarray hybridizations were confirmed by PCR amplification using specific oligonucleotide primers situated apart from the hybridization oligonucleotides. The chloramphenicol acetyltransferase determinant of *C. perfringens* MLP26 was determined by PCR using primers catDPS-F and catDPS-R, which allowed the amplification of either *catD*, *catP*, or *catS*, and by sequence analysis. The *tet*(L) and *tet*(U) genes of *Enterococcus gallinarum* BM4174 and the *aac(6′)-Ii* gene of *E. faecium* 70/90 were first detected with the microarray, then confirmed by PCR and sequence analysis, and used as references. The PCR primers are listed in Table 2.

DISCUSSION

The microarray was designed with oligonucleotides of 26 to 33 bases. This enabled us to find consensus sequences within a family of genes sharing high DNA identities (Table 3). The consensus sequences do not allow for identification of the few different bases which distinguish these genes but indicate to which family they belong. The exact identification of these genes can then be performed using either a more specialized array, PCR, or sequencing if required. The use of oligonucleotides instead of PCR products as used by Call et al. (8) facilitated and accelerated the elaboration of the microarray, since no PCRs and no template DNA of reference strains were necessary. The oligonucleotides show higher hybridization specificity than PCR products and allow a shorter hybridization time. They were found to be highly specific for the target genes by hybridization at a temperature of 60°C in 1 h only.

Two different oligonucleotides were chosen for each resistance gene, with the exception of nine genes where only a single specific oligonucleotide could be found. The use of two different oligonucleotides for the detection of resistance genes has the advantage of increased specificity and sensitivity of the

method. Hence, a hybridization signal was obtained with *B. anthracis* DNA (Fig. 3) that was shown to be free of the *vgb*(B) gene by PCR but that hybridized with the oligonucleotide be_vgbB_539 and not with be_vgb_273. Similarity searches of nucleotide data banks using the BLAST search for short, nearly exact matches (National Center for Biotechnology Information) revealed an exact match of 14 nucleotides for the oligonucleotide be_vgbB_539 with genomic DNA of *B. anthracis* strains. These 14 nucleotides may have hybridized to *B. anthracis* DNA despite the use of a high hybridization temperature of 60°C. Lack of sensitivity was found with two probes only: the probe be_vanC_set_184, which could not detect the *van*(C) gene in either of the *E. casseliflavus* strains UC73 and DSM20680, and the probe be_catpXX_set_196, which could not detect the cat_{pIP501} gene of plasmid pRE25 (Fig. 3). However, the be_catpXX_set_196 target was able to detect a PCR product of the cat_{pIP501} gene labeled with biotin-16-dUTP as well as the cat_{pC221} of plasmid pC221 (Table 1). This demonstrated that the be_catpXX_set_196 oligonucleotide was effectively spotted on the microarray and indicated that the detection of the cat_{pIP501} may depend on the labeling procedure. Additionally, formation of DNA hairpins and/or auto-annealing of the randomly amplified DNA fragment may also affect the hybridization procedures. Further investigations are now necessary to elucidate this technical gap. In an effort to obtain at least two oligonucleotide targets for each antibiotic resistance gene, new sequence alignments are currently under way.

The specificity and sensitivity of the microarray in detecting resistance genes was tested with gram-positive bacteria of eight different genera (*Bacillus*, *Clostridium*, *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Listeria*, *Staphylococcus*, and *Streptococcus*) harboring different antibiotic resistance genes and with resistance genes cloned into *E. coli* vectors. The hybridization analysis using genomic DNAs of these bacteria enabled verification of the sensitivity of 125 of the 137 oligonucleotide targets and identification of 71 resistance genes. All the genes known to be present in the reference strains listed in Table 1, except cat_{pIP501} in *E. faecalis*, could be recovered and identified with the microarray. The microarray also identified additional genes that were present in the reference strains. Additionally, it identified 12 resistance genes involved in the multidrug resistance of *S. haemolyticus* VPS617 and 8 genes in *C. perfringens* MLP26. The antibiotic resistance phenotypes correlated in both strains with the genes detected.

The resistance gene array allowed us to characterize in less than 24 h a collection of resistance genes in two important pathogenic bacterial species of animal origin, namely, *S. haemolyticus* and *C. perfringens*. For example, the erythromycin resistance in *S. haemolyticus* could be explained by the presence of three different genes [*erm*(B), *msr*, and *mph*(C)] known to be involved in resistance to macrolide antibiotics (Fig. 2 and Table 4). This is, to our knowledge, the first report of the detection of *sat4*, *aph(3′)-III*, and *ant(6)-Ia* genes in a *C. perfringens* strain, suggesting the presence of a Tn*5405*-like structure. Transposon Tn*5405* carries an *ant(6′)-Ia–sat4–aph(3′)-III* cluster which is widespread among staphylococci and enterococci (19, 48, 55) and might have been transferred from one of these species to *C. perfringens*. This demonstrated the efficiency of this technology to rapidly characterize antibiotic resistance genes in strains whose resistance genotype was completely unknown. Furthermore, automation of the hybridization procedures is conceivable, since all the hybridization steps are performed in the same tube. The microarray technology will then facilitate and speed the analysis of antibiotic resistance genes.

The microarrays have the particular advantage of detecting the presence of antibiotic resistance genes that are not phenotypically expressed in vitro. Indeed, *B. anthracis* BR4253 does not phenotypically express either of the aminoglycoside resistance genes *aph(3′)-III* and *ant(6)-Ia* present on plasmid pRE25. The expression of these genes might be repressed in *B. anthracis*, as is the case for both β-lactamase genes *bla1* and *bla2*, whose expression is not sufficient to confer penicillin resistance on *B. anthracis* (10).

Antibiotic-resistant bacteria today are present in a large variety of ecological niches such as hospitals, the environment, and food. The microarray presented in this study has been shown to be an efficient prototype that allows for rapid screening of resistance genes in gram-positive bacteria. This technology should rapidly find application in surveillance programs of antibiotic resistance genes, industry, and research in order to limit the emergence and spread of antibiotic resistance genes and extend the therapeutic action of existing drugs.

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REFERENCES

1. **Allignet, J., and N. El Solh.** 1999. Comparative analysis of staphylococcal plasmids carrying three streptogramin-resistance genes: *vat-vgb-vga.* Plasmid **42:**134–138.
2. **Allignet, J., N. Liassine, and N. El Solh.** 1998. Characterization of a staphylococcal plasmid related to pUB110 and carrying two novel genes, *vatC* and *vgbB*, encoding resistance to streptogramins A and B and similar antibiotics. Antimicrob. Agents Chemother. **42:**1794–1798.
3. **Bannam, T. L., and J. I. Rood.** 1991. Relationship between the *Clostridium perfringens catQ* gene product and chloramphenicol acetyltransferases from other bacteria. Antimicrob. Agents Chemother. **35:**471–476.
4. **Barbosa, T. M., K. P. Scott, and H. J. Flint.** 1999. Evidence for recent intergeneric transfer of a new tetracycline resistance gene, *tet*(W), isolated from *Butyrivibrio fibrisolvens*, and the occurrence of *tet*(O) in ruminal bacteria. Environ. Microbiol. **1:**53–64.
5. **Bohlander, S. K., R. Espinosa III, M. M. Le Beau, J. D. Rowley, and M. O. Diaz.** 1992. A method for the rapid sequence-independent amplification of microdissected chromosomal material. Genomics **13:**1322–1324.
6. **Boyd, D. A., P. Kibsey, D. Roscoe, and M. R. Mulvey.** 2004. *Enterococcus faecium* N03-0072 carries a new VanD-type vancomycin resistance determinant: characterization of the VanD5 operon. J. Antimicrob. Chemother. **54:** 680–683.
7. **Bozdogan, B., L. Berrezouga, M. S. Kuo, D. A. Yurek, K. A. Farley, B. J. Stockman, and R. Leclercq.** 1999. A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. Antimicrob. Agents Chemother. **43:**925–929.
8. **Call, D. R., M. K. Bakko, M. J. Krug, and M. C. Roberts.** 2003. Identifying antimicrobial resistance genes with DNA microarrays. Antimicrob. Agents Chemother. **47:**3290–3295.
9. **Charpentier, E., and P. Courvalin.** 1997. Emergence of the trimethoprim resistance gene *dfrD* in *Listeria monocytogenes* BM4293. Antimicrob. Agents Chemother. **41:**1134–1136.
10. **Chen, Y., J. Succi, F. C. Tenover, and T. M. Koehler.** 2003. β-Lactamase genes of the penicillin-susceptible *Bacillus anthracis* Sterne strain. J. Bacteriol. **185:**823–830.

11. **Chow, J. W., V. Kak, I. You, S. J. Kao, J. Petrin, D. B. Clewell, S. A. Lerner, G. H. Miller, and K. J. Shaw.** 2001. Aminoglycoside resistance genes *aph(2″)-Ib* and *aac(6′)-Im* detected together in strains of both *Escherichia coli* and *Enterococcus faecium.* Antimicrob. Agents Chemother. **45:**2691–2694.
12. **Chow, J. W., M. J. Zervos, S. A. Lerner, L. A. Thal, S. M. Donabedian, D. D. Jaworski, S. Tsai, K. J. Shaw, and D. B. Clewell.** 1997. A novel gentamicin resistance gene in *Enterococcus.* Antimicrob. Agents Chemother. **41:**511–514.
13. **Chung, W. O., C. Werckenthin, S. Schwarz, and M. C. Roberts.** 1999. Host range of the *ermF* rRNA methylase gene in bacteria of human and animal origin. J. Antimicrob. Chemother. **43:**5–14.
14. **Clermont, D., O. Chesneau, G. de Cespedes, and T. Horaud.** 1997. New tetracycline resistance determinants coding for ribosomal protection in streptococci and nucleotide sequence of *tet*(T) isolated from *Streptococcus pyogenes* A498. Antimicrob. Agents Chemother. **41:**112–116.
15. **Coker, P. R., K. L. Smith, and M. E. Hugh-Jones.** 2002. Antimicrobial susceptibilities of diverse *Bacillus anthracis* isolates. Antimicrob. Agents Chemother. **46:**3843–3845.
16. **Costa, Y., M. Galimand, R. Leclercq, J. Duval, and P. Courvalin.** 1993. Characterization of the chromosomal *aac(6′)-Ii* gene specific for *Enterococcus faecium.* Antimicrob. Agents Chemother. **37:**1896–1903.
17. **Dalla Costa, L. M., P. E. Reynolds, H. A. Souza, D. C. Souza, M. F. Palepou, and N. Woodford.** 2000. Characterization of a divergent *vanD*-type resistance element from the first glycopeptide-resistant strain of *Enterococcus faecium* isolated in Brazil. Antimicrob. Agents Chemother. **44:**3444–3446.
18. **Depardieu, F., M. G. Bonora, P. E. Reynolds, and P. Courvalin.** 2003. The *vanG* glycopeptide resistance operon from *Enterococcus faecalis* revisited. Mol. Microbiol. **50:**931–948.
19. **Derbise, A., S. Aubert, and N. El Solh.** 1997. Mapping the regions carrying the three contiguous antibiotic resistance genes *aadE*, *sat4*, and *aphA-3* in the genomes of staphylococci. Antimicrob. Agents Chemother. **41:**1024–1032.
20. **Dutka-Malen, S., C. Molinas, M. Arthur, and P. Courvalin.** 1992. Sequence of the *vanC* gene of *Enterococcus gallinarum* BM4174 encoding a D-alanine: D-alanine ligase-related protein necessary for vancomycin resistance. Gene **112:**53–58.
21. **Duvall, E. J., D. M. Williams, P. S. Lovett, C. Rudolph, N. Vasantha, and M. Guyer.** 1983. Chloramphenicol-inducible gene expression in *Bacillus subtilis.* Gene **24:**171–177.
22. **Fines, M., B. Perichon, P. Reynolds, D. F. Sahm, and P. Courvalin.** 1999. VanE, a new type of acquired glycopeptide resistance in *Enterococcus faecalis* BM4405. Antimicrob. Agents Chemother. **43:**2161–2164.
23. **Fouet, A., and M. Mock.** 1996. Differential influence of the two *Bacillus anthracis* plasmids on regulation of virulence gene expression. Infect. Immun. **64:**4928–4932.
24. **Frean, J., K. P. Klugman, L. Arntzen, and S. Bukofzer.** 2003. Susceptibility of *Bacillus anthracis* to eleven antimicrobial agents including novel fluoroquinolones and a ketolide. J. Antimicrob. Chemother. **52:**297–299.
25. **Fujimura, S., Y. Tokue, H. Takahashi, T. Kobayashi, K. Gomi, T. Abe, T. Nukiwa, and A. Watanabe.** 2000. Novel arbekacin- and amikacin-modifying enzyme of methicillin-resistant *Staphylococcus aureus.* FEMS Microbiol. Lett. **190:**299–303.
26. **Gfeller, K. Y., M. Roth, L. Meile, and M. Teuber.** 2003. Sequence and genetic organization of the 19.3-kb erythromycin- and dalfopristin-resistance plasmid pLME300 from *Lactobacillus fermentum* ROT1. Plasmid **50:**190–201.
27. **Haroche, J., J. Allignet, and N. El Solh.** 2002. Tn*5406*, a new staphylococcal transposon conferring resistance to streptogramin A and related compounds including dalfopristin. Antimicrob. Agents Chemother. **46:**2337–2343.
28. **Holzman, D.** 2003. Microarray analyses may speed antibiotic resistance testing. ASM News **69:**538–539.
29. **Huggins, A. S., T. L. Bannam, and J. I. Rood.** 1992. Comparative sequence analysis of the *catB* gene from *Clostridium butyricum.* Antimicrob. Agents Chemother. **36:**2548–2551.
30. **Ivins, B. E., S. L. Welkos, G. B. Knudson, and D. J. LeBlanc.** 1988. Transposon Tn*916* mutagenesis in *Bacillus anthracis.* Infect. Immun. **56:**176–181.
31. **Jacob, A. E., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes.* J. Bacteriol. **117:**360–372.
32. **Kaatz, G. W., S. M. Seo, and C. A. Ruble.** 1993. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus.* Antimicrob. Agents Chemother. **37:**1086–1094.
33. **Klare, I., H. Heier, H. Claus, R. Reissbrodt, and W. Witte.** 1995. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol. Lett. **125:**165–171.
34. **Louie, L., J. Goodfellow, P. Mathieu, A. Glatt, M. Louie, and A. E. Simor.** 2002. Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. J. Clin. Microbiol. **40:**2786–2790.
35. **Luna, V. A., M. Heiken, K. Judge, C. Ulep, N. van Kirk, H. Luis, M. Bernardo, J. Leitao, and M. C. Roberts.** 2002. Distribution of *mef*(A) in gram-positive bacteria from healthy Portuguese children. Antimicrob. Agents Chemother. **46:**2513–2517.
36. **Lyras, D., and J. I. Rood.** 1996. Genetic organization and distribution of tetracycline resistance determinants in *Clostridium perfringens.* Antimicrob. Agents Chemother. **40:**2500–2504.
37. **Monecke, S., I. Leube, and R. Ehricht.** 2003. Simple and robust array-based methods for the parallel detection of resistance genes of *Staphylococcus aureus.* Genome Lett. **2:**116–128.
38. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed., vol. 23, no. 2. Approved standard M7–A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
39. **Neu, H. C.** 1992. The crisis in antibiotic resistance. Science **257:**1064–1073.
40. **Perreten, V.** 2003. Use of antimicrobials in food-producing animals in Switzerland and the European Union (EU). Mitt. Lebensm. Hyg. **94:**155–163.
41. **Perreten, V., N. Giampa, U. Schuler-Schmid, and M. Teuber.** 1998. Antibiotic resistance genes in coagulase-negative staphylococci isolated from food. Syst. Appl. Microbiol. **21:**113–120.
42. **Perreten, V., B. Kollöffel, and M. Teuber.** 1997. Conjugal transfer of the Tn*916*-like transposon Tn*FO1* from *Enterococcus faecalis* isolated from cheese to other gram-positive bacteria. Syst. Appl. Microbiol. **20:**27–38.
43. **Perreten, V., F. Schwarz, L. Cresta, M. Boeglin, G. Dasen, and M. Teuber.** 1997. Antibiotic resistance spread in food. Nature **389:**801–802.
44. **Perreten, V., F. V. Schwarz, M. Teuber, and S. B. Levy.** 2001. Mdt(A), a new efflux protein conferring multiple antibiotic resistance in *Lactococcus lactis* and *Escherichia coli.* Antimicrob. Agents Chemother. **45:**1109–1114.
45. **Pitcher, D. G., N. A. Saunders, and R. J. Owen.** 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. **8:**151–156.
46. **Puzanova, O. B., A. S. Stepanov, S. V. Gavrilov, I. V. Bragin, T. M. Grigor'eva, and R. R. Azizbekian.** 1990. Conjugation transfer of the pAMβ1 plasmid to *Bacillus anthracis.* Mol. Gen. Mikrobiol. Virusol. **4:**19–21.
47. **Rouch, D. A., M. E. Byrne, Y. C. Kong, and R. A. Skurray.** 1987. The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn*4001* from *Staphylococcus aureus*: expression and nucleotide sequence analysis. J. Gen. Microbiol. **133:**3039–3052.
48. **Schwarz, F. V., V. Perreten, and M. Teuber.** 2001. Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from *Enterococcus faecalis* RE25. Plasmid **46:**170–187.
49. **Shaw, K. J., P. N. Rather, R. S. Hare, and G. H. Miller.** 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol. Rev. **57:**138–163.
50. **Shoemaker, N. B., G. R. Wang, and A. A. Salyers.** 2000. Multiple gene products and sequences required for excision of the mobilizable integrated *Bacteroides* element NBU1. J. Bacteriol. **182:**928–936.
51. **Stadler, C., and M. Teuber.** 2002. The macrolide efflux genetic assembly of *Streptococcus pneumoniae* is present in erythromycin-resistant *Streptococcus salivarius.* Antimicrob. Agents Chemother. **46:**3690–3691.
52. **Tauch, A., A. Puhler, J. Kalinowski, and G. Thierbach.** 2000. TetZ, a new tetracycline resistance determinant discovered in gram-positive bacteria, shows high homology to gram-negative regulated efflux systems. Plasmid **44:**285–291.
53. **Tsai, S. F., M. J. Zervos, D. B. Clewell, S. M. Donabedian, D. F. Sahm, and J. W. Chow.** 1998. A new high-level gentamicin resistance gene, *aph(2″)-Id*, in *Enterococcus* spp. Antimicrob. Agents Chemother. **42:**1229–1232.
54. **Vakulenko, S. B., S. M. Donabedian, A. M. Voskresenskiy, M. J. Zervos, S. A. Lerner, and J. W. Chow.** 2003. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. Antimicrob. Agents Chemother. **47:**1423–1426.
55. **Werner, G., B. Hildebrandt, and W. Witte.** 2003. Linkage of *erm*(B) and *aadE–sat4–aphA-3* in multiple-resistant *Enterococcus faecium* isolates of different ecological origins. Microb. Drug Resist. **9:**9–16.
56. **Wright, G. D., and P. R. Thompson.** 1999. Aminoglycoside phosphotransferases: proteins, structure, and mechanism. Front. Biosci. **4:**D9–D21.