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Microarray-based Detection of Antibiotic Resistance Genes in *Salmonella*

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Abstract In the presented study, 143 *Salmonella* isolates belonging to 26 different serovars were screened for the presence of antibiotic resistance genes by microarray analysis. The microarray contained a total of 223 oligonucleotides representing genes encoding for resistance to the following antibiotic classes: aminoglycoside, β-lactam, chloramphenicol, MLS, sulfonamide, tetracycline, trimethoprim, and vancomycin. To a large extent, the microarray data were consistent to the general findings concerning antibiotic resistance in *Salmonella*. Most of the analyzed isolates, harbored three or more resistance genes with the highest numbers found in isolates belonging to the *Salmonella* serovars Typhimurium, Paratyphi B var. Java, Bredeney, Saint Paul and Heidelberg and the only Give isolate investigated.

Keywords Antibiotic Resistance · Microarray · Oligonucleotides · *Salmonella* · Serovars · Typhimurium

Introduction

The intensive use of antimicrobial agents in both animal husbandry and public health has resulted in the drastic increase in antibiotic-resistant bacteria (WHO 1997). Drug resistance is becoming a worldwide problem, which is also demonstrated by the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug resistance, and extended spectrum beta-lactamase (ESBL) producing bacteria (de Neeling et al. 1998; WHO 2005; Stürenburg and Mack 2003). To combat this problem, it will be necessary to increase knowledge on the mechanisms, diversity, and distribution underlying the observed antibiotic resistance (AR) characteristics (Aarts et al. 2006). Molecular tools rather than phenotypic methods can provide this kind of information. Furthermore, molecular research will give a more precise insight on reservoirs of antibiotic resistance (AR) genes and will support the assessment of the dissemination risk of these determinants by horizontal gene transfer. As a result of the ongoing research on resistance, the number of described AR genes and AR-related mutations has increased enormously. Consequently, multi-detection and screening methods will be necessary, rather than single-plex assays, to identify the gene(s) or mutation (s) responsible for the resistance phenotype. A promising tool is the microarray, which allows the detection of a large set of DNA targets simultaneously. This multitarget technique has already proven to be a powerful and rapid method to screen for the presence of multiple antibiotic resistance genes (Aarts et al. 2006 and Holzman 2003). Initially, microarrays were restricted to detect particular classes of AR genes, for example tetracycline or erythromycin resistance determinants (Call et al. 2003 and Volokhov et al. 2003). However, the increase in multidrug-resistant bacteria, the fact that over 500 AR genes have been described, excluding mutation-based resistance, and new resistance determinant are still being identified, require microarrays for broad screening purposes (Frye et al. 2006 and Perreten et al. 2005). Consequently, the previously described thematic AR genes microarray (van Hoek et al. 2005) was expanded with a considerable number of oligonucleotides and now contains 223 oligonucleotides representing over 430 AR genes. These genes confer resistance determinants belonging to the following antibiotic classes: aminoglycoside, β-lactam, chloramphenicol, macro-

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lide lincosamide and streptogramin (MLS), sulfonamide, tetracycline, trimethoprim, and vancomycin. They are found in both gram-positive and gram-negative bacteria. This expanded microarray was used for the screening of a large set of *Salmonella* strains belonging to 26 different serovars including *Salmonella* Typhimurium, represented by various different phage types such as DT104.

Materials and Methods

Salmonella Isolates

In total, 143 *Salmonella* isolates were included in this study. The serovars and number of isolates were *S*. Agona (11), *S*. Anatum (4), *S*. Bareilly (1), *S*. Blockley (4), *S*. Brandenburg (1), *S*. Bredeney (3), *S*. Derby (4), *S*. Dublin (1), *S*. Enteritidis (8), *S*. Give (1), *S*. Hadar (10), *S*. Heidelberg (7), *S*. *enterica* subsp. *houtenae* 48:g.z51:- (1), *S*. Infantis (5), *S*. Kentucky (1), *S*. Livingstone (2), *S*. London (3), *S*. Mandaka (4), *S*. Newport (2), *S*. Paratyphi B var. Java (10), *S*. Rough (1), *S*. Saint Paul (3), *S*. Senftenberg (2), *S*. Tennessee (1), S. Typhimurium (44), and *S*. Virchow (9). Isolates were obtained from various sources in Europe, i.e., BFR, Germany; CIDC-Lelystad, The Netherlands; FRIKI, The Netherlands; INRA, France; Institut Pasteur, France; ISS, Italy; National Institute of Public Health and the Environment (RIVM), The Netherlands. Part of the strains investigated belonged to the RIKILT collection. All *Salmonella* isolates were grown overnight in Brain Heart Infusion broth (Merck, Haarlem, The Netherlands) at 37° C.

DNA Isolation and Labeling

DNA was isolated from pure cultures using the Wizard® genomic DNA purification kit (Promega Benelux b.v., Leiden, The Netherlands) according to the manufacturer's manual. A total of 4 μg of isolated DNA was fluorescently labeled according to van Hoek et al. (2005).

Oligonucleotide Design and Microarray Fabrication

Oligonucleotide probes were designed according to van Hoek et al. (2005) representing genes belonging to the following antibiotic resistance classes: aminoglycosides, β-lactams, chloramphenicol, MLS, sulfonamides, tetracyclines, trimetoprim, and vancomycin. Furthermore, oligonucleotides were included, identifying genes from the *mar* operon, integron-specific integrases, the left border of the first characterized *Salmonella* Genomic Island (SGI1) and *Salmonella* species-specific sequences. Several polymerase chain reaction (PCR) primers were designed to verify the hybridization results obtained by microanalysis.

Fig. 1 Thematic AR genes oligonucleotide microarray: (**a**) the microarray layout, (**b**) examples of hybridization results. On the surface of one slide, two microarrays were spotted. The microarray consists of two grids, each of which contains three subgrids (i.e., the oligonucleotides are spotted in triplicate except the *Salmonella* specific spots which are six times present on a microarray). The gray marked oligonucleotide names in the subgrids are the *Salmonella*-specific spots. These spots are encircled in the hybridization examples (**b**). The black marked names were not included in the analysis of the *Salmonella* strains. They represent oligonucleotides specific for other bacterial species or which do not work properly (nonspecific, high-background signals)

From the GenBank public DNA database, genes of interest and homologous sequences were retrieved. Related sequences were aligned using the ClustalX Software. Potentially gene-specific primers and oligonucleotides were designed based on the regions within the open reading frame with the highest level of homology using the Generunner software package (Hasting Software, Inc. Hastings-on-Hudson, NY). The main criteria designing the oligonucleotides were the absence of secondary structures at the hybridization temperature that was used, very long GC stretches (>10) and repeats. To check for potential interference with other sequences, Blast searches were performed with the selected primers and oligonucleotides against the public DNA databases.

All oligonucleotides were modified with a 5′ C6-amine linker to enhance binding to the microarray glass slides. The oligonucleotides were manufactured by Biolegio (Nijmegen, The Netherlands) and spotted at a concentration of 50 μM in 5×SSC on SCHOTT Nexterion E slides (Isogen Life Science, IJsselstein, The Netherlands) using a MicroGrid II Microarrayer (BioRobotics Ltd., Cambridge, UK). After spotting, the microarrays were washed and blocked according to the manufacturer's instructions (SCHOTT JENA^{er} GLAS GmbH, Jena, Germany). The microarrays were prehybridized in hybridization buffer (5×SSC, 0.2% sodium dodecyl sulfate (SDS), 5×Denhardt's solution, 50% (*v*/*v*) formamide, 0.2 mg/ml denatured herring sperm DNA) in a humid hybridization chamber at 42° C for at least 4 h. Subsequently, the slides were rinsed as described by van Hoek et al. (2005).

Microarray Hybridization and Analysis

Hybridization conditions of the microarray with fluorescently labeled total DNA, scanning, and analysis of the resulting images were performed according to Franssen-van Hal et al. (2002) and van Hoek et al. (2005) All hybridization signals (determined as arbitrary units by the ArrayVision™ Software) were first corrected for spot area and background signal surrounding each individual spot.

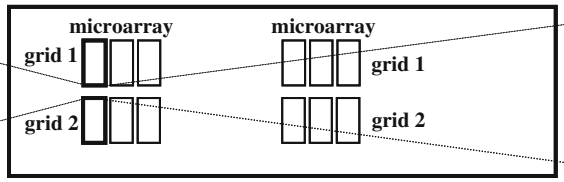
a

sub-grid 1

blaACC 50mer_I	blaFOX 50mer	blaPER 50mer_II
blaACC 50mer_II	blaIMP 50mer	blaPSE 50mer
blaACC 50mer_III	blaKPC 50mer	blaPSE 60mer
blaACT 48mer	blaMIR 47mer	blaROB 50mer_I
blaCARB 50mer_I	blaMOR 50mer_I	blaTEM-60mer
blaCARB 50mer_II	blaOXA 50mer_I	blaVIM 50mer_I
blaCARB 50mer_III	blaOXA 50mer_II	blaVIM 50mer_II
blaCARB 50mer_IV	blaOXA 50mer_III	blaVIM 50mer_III
blaCMY 50mer_I	blaOXA 50mer_IV	sipB/C 60-mer
blaCMY 50mer_II	blaOXA 50mer_V	marA 50mer
blaCTX-M 50mer_I	blaOXA 50mer_VI	marB 50mer
blaCTX-M 50mer_II	blaOXA 50mer_VII	*E. coli*
blaCTX-M 50mer_II	blaOXA 50mer_VIII	marC 50mer
blaCTX-M 50mer_III	blaOXA 50mer_IX	*E. coli*
blaDHA 50mer_I	blaOXA 50mer_X	marR 50mer
blaDHA 50mer_II	blaPER 50mer_I	*E. coli*

dfrA1 60mer	dfrA17 50mer
dfrA2 60mer	dfrA19 60mer
dfrA3 50mer_I	dfrA 50mer_I
dfrA3 50mer_II	dfrB 50mer_I
dfrA5 50mer	dfrB 50mer_II
dfrA6 50mer	dfrD 50mer
dfrA7 60mer	dfrA12 60mer_I
dfrA8 50mer	*E. coli*
dfrA9 50mer	*E. coli*
dfrA10 60mer	*Lactobacillus*
	Bifidobacterium
dfrA 50mer_II	*Lactococcus lactis*
dfrA14 50mer	invA 60mer_II
dfrA14 60mer	Stmm 50mer
dfrA15 50mer	
dfrA16 60mer	

tetA 60mer	tetQ 50mer	Sspp 60mer	
	tetS 50mer	Sspp 50mer	
tetB 60mer	tetT 50mer	*Streptococcus*	
tetC 50mer	tetU 50mer	sul1 60mer_I	
tetD 60mer	tetV 60mer	sul1 50mer	
tetE 60mer	tetW 50mer	sul1 60mer_II	
tetG 60mer	tetX 50mer	sul2 50mer	
tetH 50mer	tetY 50mer		
tetJ 50mer	tetZ 50mer	sulA 50mer	
tetL 50mer	tet30 60mer	*Enterococcus*	
tetK 50mer	tet31 60mer	*Staphylococcus*	
tetM 50mer	tet32 60mer	tet36 60mer	tetK 60mer
tetO 50mer_I	tet33 60mer	tet37 60mer	tetL 60mer
tetO 50mer_II	tet34 60mer	otrA 50mer	tetM 60mer
tetA(P) 50mer	tet34 50mer	otrB 60mer	tetS 60mer
tetB(P) 50mer	tet35 60mer	otrB 50mer	tetW 60mer



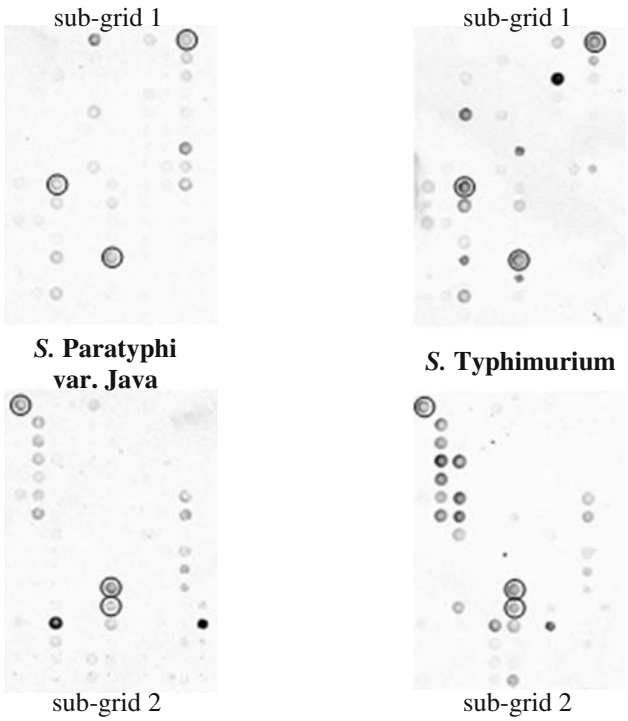
sub-grid 2

sipB/C 60-mer	aacC 50mer_VII	aphA 50mer_IV
aac6-aph2 50mer	aadA1 50mer	aphA 50mer_V
aacA 50mer_I	aadA1 60mer_I	aphA 50mer_VI
aacA 50mer_II	aadA2 60mer_I	strA 50mer
aacA 50mer_III	aadA2 60mer_II	strB 50mer
aacA 50mer_IV	aadA 60mer_I	strA 60mer
aacA 50mer_V	aadA1 60mer_II	strB 60mer
aacA 50mer_VI	aadB 50mer	strA-strB 60mer
aacA 50mer_VII	aadD 50mer	aphA-3 60mer
aacA 50mer_VIII	aadE 50mer	aadE 60merI
aacC 50mer_I	aph(2') 50mer_I	aadE 60merII
aacC 50mer_II	aph(2') 50mer_II	intI1 59mer
aacC 50mer_III	aph(2') 50mer_III	intI2 60mer
aacC 50mer_IV	aphA 50mer_I	intI3 60mer
aacC 50mer_V	aphA 50mer_II	intI4 60mer
aacC 50mer_VI	aphA 50mer_III	SGI1 LB 50mer

catB 50mer_I	vanA 60mer_I
catB 50mer_II	vanB 50mer
catB 50mer_III	vanC1 50mer
catB 50mer_IV	vanC2/C3 50mer
catB 50mer_V	vanD 50mer
cat 50mer_I	vanE 50mer
cat 50mer_II	dfrA12 60mer_II
cat 50mer_III	vanC2/C3 60mer
cat 50mer_IV	vanA 60merII
cat 50mer_V	vanB 60mer
cat 50mer_VI	invA 60mer_I
cat 50mer_VII	Sspp 60mer
cmlA 50-mer	Sspp 50mer
cmlB 50-mer	
floR 50mer	Seis 60mer
floR 60mer	Stmm 60mer

ereA 50mer_I	msr 50mer_II	mefE 60-mer	
ereA 50mer_II	vat 50mer_I	vat 60mer_I	
ereB 50-mer	vat 50mer_II		
ermA 50mer	vat 50mer_III		
	vat 50mer_IV		
erm 50mer_I	vat 50mer_V	*Bifidobacterium*	
erm 50mer_II	vga 50mer_I	*Bifidobacterium*	
erm 50mer_III	vgb 50mer_I	*Lactococcus lactis*	
erm 50mer_IV	vgb 50mer_II	*Lactobacillus*	
erm 50mer_V	ermA 60mer_II	*Bifidobacterium*	
erm 50mer_VI	ermB 60mer_II	*Streptococcus*	
	ermC 60mer_II	*Lactococcus lactis*	aphE 60mer
mefE 50mer	ermA 60mer_I	*Bifidobacterium*	sat 60mer_I
mph 50mer_I	ermB 60mer_I	*Streptococcus*	sat 60mer_II
mph 50mer_II	ermC 60mer_I	*Enterococcus*	sat 60mer_III
msr 50mer_I	mefA 59mer	*Staphylococcus*	

b



Only hybridization signals with a signal-to-noise ratio higher than three (S/N>3) were taken into account for further analysis in Excel. With the Excel software the fluorescent signals were corrected for labeling and hybridization conditions. The average hybridization signals obtained with the *Salmonella* specific oligonucleotides (invA 60mer_I, invA 60mer_II, sipB/C 60mer, and Sspp 60mer) were used for this correction factor. As a rule, corrected hybridization signals with a ratio of 0.5 or more indicated the presence of a particular antibiotic resistance gene.

PCR

The PCR tests to verify the microarray hybridization results were performed in a total volume of 50 μl containing approximately 40 ng of bacterial DNA, 10 pmol of each primer, 1×PCR buffer (Invitrogen BV, Breda, The Netherlands), 3 mM $MgCl_2$, 0.2 mM of each deoxyribonucleoside triphosphate (dNTP), 1 U *Taq* DNA polymerase recombinant (Invitrogen BV, Breda, The Netherlands). The following PCR program was used: 95° C for 3 min; 35 cycles of 95° C for 30 s, 55° C for 30 s, 72° C for 30 s; 72° C for 10 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel.

Results

In a previous study, the successful application of microarray screening for the presence of antibiotic resistance genes in *Salmonella* was described (van Hoek et al. 2005). In the present paper, both the number of oligonucleotides on the microarray and the amount of *Salmonella* strains investigated were increased. The oligonucleotides have been designed in such a way that they are gene-specific and not species-specific. Consequently, the microarray is able to detect and identify AR genes in various species. The microarray now contains 223 oligonucleotide probes identifying over 430 AR genes encoding for resistance against aminoglycosides (#43), β-lactams (#39), chloramphenicol (#16), MLS (#32), sulfonamides (#5), tetracyclines (#42), trimethoprim (#23), and vancomycin (#9). Furthermore, oligonucleotides representing the multiple antibiotic resistance (mar) locus, different integron classes, and the *Salmonella* genomic island 1 (SGI1) were also added to the microarray (Fig. 1). The oligonucleotide names, lengths, sequences, GC %, and represented gene(s) are given in Table 1. Oligonucleotides already described in Mättö et al. (2007) and van Hoek et al. (2005) are indicated. Phenotypic and genotypic well-defined control strains were used to check whether the designed oligonucleotides could detect the corresponding gene(s) (indicated with an asterisk in Table 1). Furthermore, for confirmation purposes part of the microarray data was verified by PCR using gene-specific assays (Table 2).

A total of 143 *Salmonella* isolates encompassing 26 different serovars were screened for the presence of AR genes using the oligonucleotide microarray. For serovar Typhimurium, a total number of 44 isolates were investigated, which belonged to 14 different phage types, including the multiresistant phage type DT104. An overview of the microarray screening results is shown in Table 3. The data are presented per serovar and if applicable (*S.* Typhimurium) by phage type. The amount of strains investigated is indicated and the number of isolates showing a hybridization signal with a certain oligonucleotide. Only results from oligonucleotides that gave a hybridization response with at least one of the *Salmonella* isolates are included in Table 3. As a result of the lack of control strains for all oligonucleotides present on the microarray, some results might be less reliable and this could lead to an overestimation of the number of AR genes present in an isolate. In contrast, the number of detected AR genes could also be underestimated by not knowing whether the oligonucleotides are actually able to detect the corresponding AR gene(s). However, various experiments with DNA from control strains available resulted in hybridization signals with oligonucleotides representing the AR gene(s) present in these bacteria (data not shown), indicating that the chosen criteria for the design of the oligonucleotides were appropriate.

Some antibiotic resistance genes were represented by two or three oligonucleotides that differed in length (see Table 1). In the case of *aadA1*, *tet*(A), and *sul1* the 50-mer oligonucleotide resulted in lower numbers of positive strains in comparison to the corresponding 60-mer. However, for other genes, such as *dfrA14* and *floR*, the 50- and 60-mer oligonucleotides gave similar results. As some of the AR genes were only represented by a 50-mer oligonucleotide, this could have influenced the number of positive strains.

A total of 51.4% of the microarray data was checked by PCR (approximately 2,500 PCR tests were performed). More than 95% of the PCR results were in agreement with the microarray data (not shown). Similar percentages were found by Malorny et al. (2007) with an oligonucleotide multiprobe microarray developed for the molecular characterization of *Salmonella*. In those cases of discordant results, the observed hybridization values were often very low and close to the cutoff values between positive and negative hybridization signals.

In general, multiple AR genes were detected per isolate, especially in those belonging to the serovars Typhimurium, Paratyphi B var. Java, Bredeney, Saint Paul, and Heidelberg, and the only Give isolate investigated. Common AR genes found in the different serovars were *strA* conferring

Table 1 Microarray oligonucleotides

Oligonucleotide name[a]	Sequence (5′–3′)	GC %	Gene(s) represented[d]
Aminoglycosides			
aac6-aph2 50mer[b]	gattgttatttaatggaatatagatatgatgataatgccacaaatgttaa	24.0	*aac(6′)-aph(2″)**, *aacA/aphD*
aacA 50mer_I	ttgtcagaccagattatcaaaataaaggtattggcaagatcctgcttaag	36.0	*aacA1*
aacA 50mer_II	cacgcccgacacttgcYgacgtacaggaacagtacttgccaagcgtttta	52.0	*aacA4*, *aac(6′)-Ib*
aacA 50mer_III	tgctgtagcacccgacggagaagcactagggtttgccgagctttcgatcc	58.0	*aacA5*, *aacA7*
aacA 50mer_IV[b]	ccggccgcacatcgttgagtggtggggtggYgacgaagagcgaccgactc	66.0	*aac(6′)-II*
aacA 50mer_V	atcggtttgcttgacggaactccattcgcgttcgcacagtcgtacgttgc	54.0	*aac(6′)-IIb*
aacA 50mer_VI	agttaaacaaggggttgggtacaaagctcgtacgctcgctcgttgaactc	50.0	*aac(6′)-IIc*
aacA 50mer_VII	gcatcatttattgcgatggcagacggggtggcgattggctttgcggatgc	56.0	*aac(6′)-Iy*
aacA 50mer_VIII	tatgcttgggaatatgtctggatgataaactgattggMtggaccggatta	40.0	*aac(6′)-Ib*, *aac(6′)-Il*, *aac(6′)-Iq*
aacC 50mer_I	caaagttaggtggctcaagtatgggcatcattcgcacatgtaggctcggc	52.0	*aac(3)-Ia, aacC1*
aacC 50mer_II	catcgaaggctaggagtcgcaactgccctgattagccacctgaagcgtgt	56.0	*aac(3)-Ib*
aacC 50mer_III[c]	agctgaaacgctgacggagcctcacgaactcggtcacgccttggggaaag	60.0	*aac(3)-IIa*, *aacC2*
aacC 50mer_IV	ctggtggcaatagaaggatacgtgctgatgcttggcgcgccgctggatac	58.0	*aac(3)-IIIa*, *aacC3*
aacC 50mer_V	gccgttcgcgacacctacaggcacgcaatggcgcatgatcgaggagttcg	62.0	*aac(3)-IIIb*
aacC 50mer_VI	cgtcgtgggagagcggggaaccctgatggtgacctgcggctggaacgacg	72.0	*aac(3)-VII*
aacC 50mer_VII	ggacctcagtgaggcggactacaataatggtcgtctcccagaagcgctgc	64.0	*aacC9*
aadA 60mer_I	agccatacagtgatattgatttgctggttactgtggctgcacggctcgatgagactgtcc		*aadA6, aadA10, aadA11, aadA13*
aadA1 50mer[c]	ggcctgaagccacacagtgatattgatttgctggttacggtgaccgtaag	50.0	*aadA1**
aadA1 60mer_I[c]	ggcctgaagccacacagtgatattgatttgctggttacggtgaccgtaaggcttgatgaa	48.3	
aadA1 60mer_II	cccgtcatacttgaagctagacaggcttatcttggacaagaagaagatcgcttggcctc	49.2	
aadA2 60mer_I[c]	ctttgacccggttcctgaacaggatctattcgaggcgctgagggaaaccttgaagctatg	51.7	*aadA2**
aadA2 60mer_II	ctaagcaagcttatctgggacaaaaagaagatcacttggcctcacgcgcagatcacttgg	48.3	
aadB 50mer	gtccgttgtaacagctgggaggcgatcatctgggattacttttactatgc	53.3	*aad*B*
aadD 50mer	aggcaaatggcgtaatattcgtgtgcaaggaccgacaacatttctaccat	49.2	*aad*D
aadE 50mer[b]	cgtttatatctaatgatgactggcttaatcaatttgggaatataataatg	28.0	*aad*E*
aadE 60mer_I[b]	caagggagtatgatgattgctgcaatgaattttggaatgtaacaccttatgttattaaag	33.3	
aadE 60mer_II[b]	ttaagtgtaggcaagaactataagtttattgaaaggtatatatccgaggatttgtgggag	35.0	
aph(2′) 50mer_I	ggatgcccttgcatatgatgaagcgacgtttttgaaagagttacattcca	42.0	*aph(2′)-Ib*
aph(2′) 50mer_II	gaaggcttaagggcaagggatcaggactggatttctgaagggttagagct	50.0	*aph(2′)-Ic*
aph(2′) 50mer_III	ccggaggtggtttttacaggaatgccatcagaaacgtaccaaatgtcttt	44.0	*aph(2′)-Id*
aphA 50mer_I	tctatcgattgtatgggaagcccaatgcgccagagttgtttctgaaacat	44.0	*aphA1**, *aphA1-IAB*
aphA 50mer_II	caggatctcctgtcatctcaccttgctcctgccgagaaagtatccatcat	50.0	*aph(3′)-IIa*, *aphA2*, *nptII**
aphA 50mer_III[b]	gatctggccgatgtggattgcgaaaactgggaagaagacactccatttaa	46.0	*aphA3**
aphA 50mer_IV	attgcttgctatctataaggaggcactcaatctgttaaattcaattgcta	34.0	*aphA6*
aphA 50mer_V	catgagtgagttaaggggaaacacatagattgctttattgatcatccaa	38.0	*aphA7*
aphA 50mer_VI	ctgatttcttgcgccgcctgcatgagatcccaacgattgaatgcccttc	52.0	*aph(3″)-Id*
aphA 60mer_I[b]	tttgctcggaagagtatgaagatgaacaaagccctgaaaagattatcgagctgtatgcgg	43.3	*aphA3**
aphE 60mer[b]	acacggtcgtctgccacggtgatctctgcctgcccaacatcgtcctccatccggagaccc	63.3	*aphE*
sat 60mer_I	gtgaagtgttcgatgtgcacctatccgaccaaggctttgaactatctaccagaagtgtga	46.7	*sat*2[e]
sat 60mer_II[b]	aagcgatgccgacgttcttcagcttgcgcggtgcgatttctctttcgaggtcacagctga	55.0	*sat*3
sat 60mer_III[b]	cccagcgaaccatttgaggtgataggtaagattataccgaggtatgaaaacgagaattgg	43.3	*sat*4
strA 50mer[c]	acgcgccgttgatgtggtgtcccgcaatgccgtcaatcccgacttcttac	58.0	*strA**
strB 50mer	cggttgctcggtcgtgagaacaatctgatgttgctcgaatatgccgggga	54.0	strB*
β-*lactams*			
blaACC 50mer_I	gcctacagctatttatgccggaagatattaaaaataccacacagctgatg	40.0	$bla_{\text{ACC-1}}$*
blaACC 50mer_II	tgagcaaaccatccttcctctattaggcatgaatcaaacctacctgaagg	44.0	$bla_{\text{ACC-2}}$
blaACC 50mer_III	atatcggttacttcaagtcaggcaaaatcactcaggatctgatgtgggaa	42.0	$bla_{\text{ACC-3}}$
blaACT 48mer	gaagccggactcccttcaggataattcactcaggaaaggccttaccct	52.1	$bla_{\text{ACT-1}}$, $bla_{\text{ACT -2}}$
blaCARB 50mer_I	tctcccgtaatagaaaagcaagtaggacaagcaataacgctcgatgatgc	44.0	$bla_{\text{CARB-4}}$
blaCARB 50mer_II	aaattggtgagcaaatagcgaagacagtattaatggagaatagccgtaac	38.0	$bla_{\text{CARB-5}}$, $bla_{\text{CARB-8}}$
blaCARB 50mer_III	caactcctaaggcaatagccagcacgttaaatcaattattatttggttcc	38.0	$bla_{\text{CARB-6}}$, $bla_{\text{CARB-7}}$, $bla_{\text{CARB-9}}$
blaCARB 50mer_IV	tctagatcgtgtcgagcctgagctcaatgaaggtaaactcggtgatttga	46.0	$bla_{\text{CARB-7}}$, $bla_{\text{CARB-9}}$
blaCMY 50mer_I	atcaagaccagctcggcggatctgctgcgttttgtgaaggccaacatcgg	56.0	$bla_{\text{CMY-1}}$, $bla_{\text{CMY-10}}$, $bla_{\text{CMY-11}}$
blaCMY 50mer_II	ggcgagcagcctgaagcagccatttgcccagttgatggagcagaccctgc	62.0	$bla_{\text{CMY-8}}$, $bla_{\text{CMY-9}}$, $bla_{\text{CMY-19}}$, $bla_{\text{MOX-1}}$
blaCTX-M 50mer_I	ggcttaccgacgtcgtggactgcaggtgataagaccggcagcggcgacta	62.0	$bla_{\text{CTX-M-9}}$ group[f]

Table 1 (continued)

Oligonucleotide name[a]	Sequence (5′–3′)	GC %	Gene(s) represented[d]
blaCTX-M 50mer_II	gctaaatcagcgcgttgaaatcaagaagagcgacctggttaactacaatc	44.0	$bla_{\text{CTX-M-2}}$ group[g]
baCTX-M 50mer_III	gctgatggcgacggcaaccgtcacgctgttgttaggaagtgtgccgctgt	60.0	$bla_{\text{CTX-M-1}}$ group[h]
blaDHA 50mer_I	aggtgccggatgcggtaaaaagccgtgcggatctgctgaatttctatcag	52.0	$bla_{\text{DHA-1}}$, $bla_{\text{DHA-2}}$, bla_{MOR}
blaDHA 50mer_II	atgatcattaacggcgtgaccaacgaggtcgcactgcagccgcacccggt	60.0	
blaFOX 50mer	atagtctgggccagccatttgagMaactgatgagccagaccctgctgccc	57.0	bla_{FOX}
blaIMP 50mer[c]	ctctcatttycatagYgacagcacRggBggaatagagtggcttaattctc	46.3	$bla_{\text{IMP*}}$
blaKPC 50mer	gcgcgccgtgacggaaagcttacaaaaactgacactgggctctgcactgg	58.0	bla_{KPC}
blaMIR 47mer	tgccaaaaccgtcgtcggaggcagtgataacaaggtggcgctggcac	59.6	bla_{MIR}, $bla_{\text{ZEG-1}}$
blaMOR 50mer_I	ggaaggggatcacactgctggatctggctacctacaccgcaggcgggctg	64.0	bla_{MOR}, $bla_{\text{DHA-1}}$, $bla_{\text{DHA-2}}$
blaOXA 50mer_I	taccaatgacttagctcgtgcatcaaaggaatatcttccagcatcaacat	40.0	$bla_{\text{OXA-10}}$ group[i]
blaOXA 50mer_II	tcaacattcaaaattcctaatgctctaataggtcttgaaaccggcgccat	40.0	$bla_{\text{OXA-5}}$
blaOXA 50mer_III	ccattaaaggttacgcctattcaagaggtagagtttgtttcccaattagc	40.0	$bla_{\text{OXA-23}}$, $bla_{\text{OXA-27}}$, $bla_{\text{OXA-49}}$, $bla_{\text{OXA-73}}$
blaOXA 50mer_IV	cgatgaccttgcacataaccgattacctttaaattagaaactcaagaag	36.0	$bla_{\text{OXA-24}}$, $bla_{\text{OXA-25}}$, $bla_{\text{OXA-26}}$, $bla_{\text{OXA-33}}$, $bla_{\text{OXA-40}}$, $bla_{\text{OXA-72}}$
blaOXA 50mer_V	ttcgtgatgagttccagatttttcgatgggacggcgttaacaggggcttt	48.0	$bla_{\text{OXA-02}}$, $bla_{\text{OXA-15}}$, $bla_{\text{OXA-32}}$, $bla_{\text{OXA-34}}$, $bla_{\text{OXA-102}}$
blaOXA 50mer_VI	gagctatttgcaaaagagatcggtgaagacaaggctcgacgctatttgaa	44.0	$bla_{\text{OXA-03}}$, $bla_{\text{OXA-21}}$
blaOXA 50mer_VII	ttgataatccgatttctagcactgcttttctcagctgttgtacttgtctc	40.0	$bla_{\text{OXA-20}}$, $bla_{\text{OXA-37}}$
blaOXA 50mer_VIII	ggaacagcaatcatacaccaaagacgtggatgcaattttctgttgtttgg	42.0	$bla_{\text{OXA-01}}$, $bla_{\text{OXA-04}}$, $bla_{\text{OXA-30}}$*, $bla_{\text{OXA-31}}$, $bla_{\text{OXA-47}}$
blaOXA 50mer_IX	acacaacaattaggtatgactcgatttaagaattatgttgatgcatttca	30.0	$bla_{\text{OXA-29}}$
blaOXA 50mer_X	gcccgcttccaccttcaagctggcgctggccgtcatgggcttcgaccacg	68.0	$bla_{\text{OXA-22}}$
blaPER 50mer_I	ttataaaagctgtagttactgcctcgacgctactgatggtatcttttagt	38.0	$bla_{\text{PER-1}}$, $bla_{\text{PER-3}}$
blaPER 50mer_II	tctgttactgttaatcgtgctgcagtattacaaaatacctggtcgccaat	40.0	$bla_{\text{PER-2}}$
blaPSE 50mer[c]	gtcactggtaatttactacgttcagtattgccggcgggatggaacattgc	48.0	$bla_{\text{PSE-1}}$(=$bla_{\text{CARB-2}}$)*
blaPSE 60mer[c]	cactggtaatttactacgttcagtattgccggcgggatggaacattgcggatcgctcagg	50.0	
blaROB 50mer_I	atgacgattgcccaattatgtgaagcagccgtgcggtttagcgacaacag	68.0	$bla_{\text{ROB-1}}$
blaTEM 60mer[c]	ctcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgc	45.0	bla_{TEM}*
blaVIM 49mer_I	atgttaaaagttattagtagtttattggtctacatgaccgcgtctgtca	34.7	$bla_{\text{VIM-1}}$, $bla_{\text{VIM-4}}$, $bla_{\text{VIM-5}}$
blaVIM 50mer_II	ttttgagtaagttattggtctatttgaccgcgtctatcatggctattgcg	40.0	$bla_{\text{VIM-2}}$, $bla_{\text{VIM-3}}$, $bla_{\text{VIM-6}}$, $bla_{\text{VIM-8}}$, $bla_{\text{VIM-9}}$, $bla_{\text{VIM-10}}$, $bla_{\text{VIM-11}}$, $bla_{\text{VIM-14}}$
blaVIM 50mer_III	aattcgcagctttctggttggtatcagtgcattcgtcatggccgtacttg	48.0	$bla_{\text{VIM-7}}$
Chloramphenicol			
cat 50mer_I	cttttagaactggttacaatagcgacggagagttaggttattgggataag	40.0	*cat(pC194),cat-TC*, cat*
cat 50mer_II	cacctgaatatatcatcattaccctgggtgagttttgacggatttaacct	40.0	*catII*
cat 50mer_III	aacaccagaaaatcatttaaatatttcagcattaccttgggttaattttg	28.0	*catIII, catA3*
cat 50mer_IV	tatttaaacaatgtgaaatgtacttacagtatgactgccaatatagaaat	26.0	*catB*
cat 50mer_V	cagcctttggactgagtgtaagtctgactttaaatcatttttagcagatt	36.0	*catD, catP*
cat 50mer_VI	aattaccttgaggatattagaaactatagcgacgttttgaatttcatgcc	34.0	*catQ*
cat 50mer_VII	gcaagatgtggcgtgttacggtgaaaacctggcctatttccctaaagggt	50.0	*catA1, cat*
catB 50mer_I	gcaaaatcagtcgatgcattccagcgggctggcgacacagttataggaag	52.0	*catB2*
catB 50mer_II	gttgataagttgatcatcggtagtttctgctctatcgggagtggggcttc	48.0	*catB3*
catB 50mer_III	caaaattggagacggtgccgtgataggtagtcgctcgttggtgacaaaag	50.0	*catB8**
catB 50mer_IV	catgcaagaagagccagctttttcaagttcaacggacgcctttcaaaagg	46.0	*catB6*
catB 50mer_V	caagggcatcgcagtgattggataagtacattcccatttttctatcagga	42.0	*catB9*
cmlA 50mer	aatggtcgcagctgctactccccgttaagtgcctgaacttctggttgtac	52.0	*cmlA, cmlA1*, cmlA4, cmlA5, cmlA6, cmlA7*
cmlB 50mer	tcatctacggcttgcttggctctatgcttgctatggttccggcgataggc	54.0	*cmlB*
floR 50mer[c]	gcgttgcttgtttgcggagcggtcctgttggggatcggcgaactttacgg	60.0	*floR**
floR 60mer	gcgtgggatggcgttgcttgtttgcggagcggtcctgttggggatcggcgaactttacgg	61.7	
marRAB locus			
marA 50mer	tccaaatggcacctgcaacggatgtttaaaaaagaRaccggtcattcatt	50.0	*marA Salmonella*, *E. coli*, *Shigella*
marB 50mer	gggtctgttacttatcgcctccggatatggcattgcagaacaaactttgt	50.0	*marB Salmonella*
marC 50mer	attatttttcttgccgtggcggtgatcctgtggggatgcttacgcagttc	50.0	*marC Salmonella*
marR 50mer	ggacggcgcggcaatttgtgagcaatgtcatcaacgaccagggcaagacc	58.0	*marR Salmonella*

Table 1 (continued)

Oligonucleotide name[a]	Sequence (5′–3′)	GC %	Gene(s) represented[d]
MLS			
ereA 50mer_I	catgaaaccgcacgttgatatgttgactcacttgttggcgtccattgatg	46.0	*ere*(A)*
ereA 50mer_II	gttcccatgggacagcatctcgcagagagggaggaggggattaccgtgc	62.0	*ere*(A2)
ereB 50mer	tgatatacccagaaatggaggttcatacttaccaaatatggagatagtgc	38.0	*ere*(B)*
ermA 50mer[b]	gtgactaaagaagcggtaaacccctctgagaatataaaagtgattcaaac	38.0	*erm*(A)*
ermA 60mer_I[b]	gttcttttcactaaaaaccaattccgacaggctttgaagcatgcaaatgtcactaatatt	35.0	
ermA 60mer_II[b]	caacgagctttRggtttRctRttaatggtggaRatggatataaaaatKctYaaaaaagta	30.0	
ermB 60mer_I[b]	attcgtgtcactttaattcaccaagatattctacagtttcaattccctaacaaacagagg	35.0	*erm*(B)*
ermB 60mer_II[b]	ggattctacaagcgtaccttggatattcaccgaacactagggttgctcttgcacactcaa	46.7	
ermC 60mer_I[b]	ggcagaagttgatatttctatattaagtatggttccaagagaatattttcatcctaaacc	31.7	*erm*(C)*
ermC 60mer_II[b]	acgcaaaattgtttttgatagtatagctaatgagatttatttaatcgtggaatacgggtt	30.0	
erm 50mer_I	ccttcaattagaacctacacaaaagttattttcgaggaagctttacaatc	34.0	*erm*(F), *erm*(FS), *erm*(FU)
erm 50mer_II	caaatagtaaatgatgatatactgaaatttacatttcctagccacaatcc	30.0	*erm*(G)
erm 50mer_III	taagttcccaaacaacaaagcatataaaatctacggtaatatacctttatt	28.0	*erm*(GM)
erm 50mer_IV	gctaaaaggttgctaaatacaaatcgttcactagcactatttttaatgac*	32.0	*erm*(GT)*, *erm*(T)
erm 50mer_V[c]	aacattacattgcgtttcttgggcttgccgaatatgcgctaaaggagccg	48.0	*erm*(D), *erm*(J), *erm*(K)
erm 50mer_VI	agattattaagaaattattattagaagagttaaattcaccaactgatatg	22.0	*erm*(Q)
mefA 59mer[b]	tcctttgcatctggaatgttgataggggtctattattagggttatttgggaattacca	39.0	*mef*(A)*
mefE 50mer[c]	aagggagatgaaagaaggagttgtggttctgagacaaaacaaaggattgt	40.0	*mef*(E)*
mefE 60mer[b]	gctagcaggaggcttattattaggaagattaggggcttcgaaaagcatgtattactaat	40.0	
mph 50mer_I	cgcagccacaatagatccagaaatacaaaattatgtatgggaaattgaac	36.0	*mph*(BM), *mph*(C)
mph 50mer_II	gctgactgtcaatgagcttgggctcgactataggatcgtgatcgccaccg	56.0	*mph*(A), *mph*(K)
msr 50mer_I	agtgaactccatatacctatgcatacaaccgacagtatgagtggtggtga	44.0	*msr*(A), *msr*(SA)
msr 50mer_II	taggtgcaaatggtgtaggtaagacaactttacttgaagctatttaccac	38.0	*msr*(A), *msr*(SA), *msr*(B)
vat 50mer_I	tattgaaattggggactacacctattatgatgacccagtaaatcccaccg	42.0	*sat*(G), *vat*(E), vat(E-3)–vat(E-8)
vat 50mer_II	ttttagaaaaattagaaaatgttgaggttggagaatactcatattatgat	24.0	*sat*(A), *vat*(D)
vat 50mer_III	gaatgaattcaataacaacttatccttttaatataatgggaaatggttgg	28.0	*vat*(B)
vat 50mer_IV	attgtcggtggaaatcccttaaaatttataagaaaaaggttttctgatgg	32.0	*vat*(A), *vat*
vat 50mer_V	ggtgggacctagagatagagacgataaatgaaaatattgattgcatcctg	40.0	*vat*(C)
vat 60mer_I	cggtgccaaccacgtaatgaaaggtatttcgacttatccatttaatattttaggtggcga	40.0	*sat*(G), *vat*(E), *vat*(E-3)–*vat*(E-8)
vga 50mer_I	gatgaaccaacaaactttcttgatatggaRgctatagaggcgtttgaatc	30.0	*vga*(A), *vga*(A)$_{LC}$, *vga*
vgb 50mer_I	aaatatgataagttgcatcaatttagatggaaaaattacagagtacccac	46.0	*vgb*
vgb 50mer_II	cagggaaattagaagaatatcctctaccaacaaatgcagcggctccagtg	42.0	*vgb*(B)
Sulfonamide			
sul1 50mer[c]	gccccgcaccggaaacatcgctgcacgtgctgtcgaaccttcaaaagctg	60.0	*sul1**
sul1 60mer_I[c]	tttcttgagccccgcaccggaaacatcgctgcacgtgctgtcgaaccttcaaaagctgaa	53.3	
sul1 60mer_II	gatttttcttgagccccgcaccggaaacatcgctgcacgtgctgtcgaaccttcaaaagc	53.3	
sul2 50mer[c]	gcgctcaaggcagatggcattcccgtctcgctcgacagttatcaMcccgc	61.0	*sul2**
sulA 50mer	caccctagctcgctcatMttYcctcattttggttttggWcaagcttttac	44.0	*sulA*
Tetracycline			
otrA 50mer[b]	tcgtcctggacgatctcaaggtcaacctcatcgacaccccgggccactcc	62.0	*otr*(A)*
otrB 50mer[b]	ggtcaacttcaccatcggcgtcggcatcttcggcacggtcaccaccctgc	64.0	*otr*(B)
otrB 60mer[b]	cgcaagcccatgtacctgatctccatcgtggtgttcatcggcggctcggtgctgtgcggc	63.3	
tet30 60mer	ccctgttcaatggcctcaacctgttcgtcgcgctgtttgttctgccggaaagccgaaagg	56.7	*tet*(30)
tet31 60mer	gctcttatcatggtcattatcctcttccctaaagagcaatcacgcccaaaagaaatcgag	43.3	*tet*(31)
tet32 60mer	gttattttagctgatgatactttgaaactgaacgacattctgggaaatgaaaaactcctg	35.0	*tet*(32)
tet33 60mer	ggtggactgttcggtgccatctcgccacatctgccgtttttgctcgctgctcttctctca	56.7	*tet*(33)
tet34 50mer	atcatgatcaccagcgtgatatgaccgtgctaaaagcgccagaaggtgat	48.0	*tet*(34)
tet34 60mer	cagcttgctgaaaaacagatgccagctgaacagtggaaaggtatttgggcggtgagccgt	51.7	
tet35 60mer	tatcgacgcagctaccttatgcattatcagtggcgtttgtgtcatgtattggctttatca	41.7	*tet*(35)
tet36 60mer	attaacatctagctgtacaatacactacatatcagaaaagaacagagaaacaattttaga	28.3	*tet*(36)
tet37 60mer	tgaagaaacttgcaggtattcacattgattatatctgttcaacacatggtcctgtatggc	38.3	*tet*(37)*
tetA 60mer[c]	ccaggcaggtggatgaggaacgtcaggggcagctgcaaggctcactggcggcgctcacca	66.7	*tet*(A)*
tetA(P) 50mer[b]	ttgtagcacagattgtatggggattagggtctacttttatcagtggctcg	44.0	*tetA*(P)
tetB 60mer[c]	tatgcgttaatgcaggttatctttgctccttggcttggaaaaatgtctgaccgatttggt	41.7	*tet*(B)*

Table 1 (continued)

Oligonucleotide name[a]	Sequence (5′–3′)	GC %	Gene(s) represented[d]
tetB(P) 50mer[b]	agaaatacaagaaaagctttcattatatgcaagagaaggaagtctatatc	30.0	*tetB*(P)
tetC 50mer[b]	tgcggtattcggaatcttgcacgccctcgctcaagccttcgtcactggtc	58.0	*tet*(C)*
tetD 60mer[b]	agcgcaggtatcagctttatcacactgcttaaacctctggcgctgttgctgtttgtctttt	46.7	*tet*(D)*
tetE 60mer[b]	tgggtatggataattggctgcggattatatgtagttgcattgattatactgaggtttttc	36.7	*tet*(E)*
tetG 60mer[c]	cgcttttcgcagttttcttcattattcaactgatcggccaagtgcctgcagccctatggg	58.3	*tet*(G)*
tetH 60mer[b]	gggcgaaaaaaccaccattatgatcagtatgtctattgatatgatgggct	40.0	*tet*(H)
tetJ 60mer[b]	ttttcattactttgtttccaagaaactcaaaccacaaaaatttcgactga	30.0	*tet*(J)
tetK 50mer[b]	atgtttatatttgttatgggcggattatctttttactaaaacagttatatc	26.0	*tet*(K)*
tetK 60mer[b]	gttagtacaaggagtaggatctgctgcattcccttcactgattatggtggttgtagctag	45.0	
tetL 50mer[b]	gtaatggttgtagttgcgcgctatattccaaaggaaaatagggtaaagc	42.0	*tet*(L)*
tetL 60mer[b]	cggctacattggtgggatacttgttgatagaagaggtcctttatacgtgttaaacatcgg	43.3	
tetM 50mer[b]	aaagctggaacagaattgttagagccatatcttagttttaaaRtttatgc	30.0	*tet*(M)*
tetM 60mer[b]	gaagtKattacKaataaattttattcatcaacacatcgaggtcMgtctgaactttgcgga	36.7	
tetO 50mer_I[b]	tcatcaacgcctgaaggtcaatctgaactatgcgggcaggtttttaagat	44.0	*tet*(O)*
tetO 50mer_II[b]	ctttctgggcttctgtcgggttgtccatagagccgctccctattggaagc	56.0	
tetQ 50mer[b]	acattgtggattgaagacccgtctttgtccttttccataaactcatatag	38.0	*tet*(Q)*
tetS 50mer[b]	gacatcataattaagcagactgtgaatctaaatttgaaaccttatgtaat	28.0	*tet*(S)*
tetS 60mer[b]	tatgtagatacagtaactcacgaaattgtgctatctttttttaggtgaggtccaaatggag	36.7	
tetT 50mer[b]	aattgacaaatgtaagggatgataaagtaattcaaagtcaaataatagag	26.0	*tet*(T)*
tetU 50mer[b]	attggtcgataattgctagacatacaaaatataaatatcggaatttgctg	30.0	*tet*(U)
etV 60mer[b]	gcggaccggatcaaccagcgcaccatcatcattgccgtcgaggtggtcaacttcgtcacg	60.0	*tet*(V)
tetW 50mer[b]	ggataagctctccgccgatattatcatcaagcagacggtgtcgctgtccc	54.0	*tet*(W)*
tetW 60mer[b]	cattcaagcggcagtcacttccttccagtggcacagatgtaaagtYaacattgtggatac	45.0	
tetX 50mer[b]	aaaagcgggattgttacaaacttattatgacttagccttaccaatgggtg	54.0	*tet*(X)*
tetY 50mer[b]	gccagctttttgcgttgttttttcttatgcagctgattgggcaggcgcc	50.0	*tet*(Y)
tetZ 50mer[b]	atcgactacctcgtgctcgcactgacggacacgctgtgggtcttttacct	56.0	*tet*(Z)
Trimethoprim			
dfrA 50mer_I	acaactgaccactgggaatacacttgtaatggcacggaaaacttttaatt	38.0	*dfrA, dfrC*
dfrA 50mer_II	aggctcaccgagagcaaagtggtcgttatgggccgcaagacatttgagtc	54.0	*dfrA13*, *dfrA21**, *dfrA22*, *dfrA23*
dfrA1 60mer[c]	gcggtcgtaacacgttcaagttttacatctgacaatgagaacgtaKtgatctttccatca	40.8	*dfrA1**
dfrA2 60mer	atcgcgtRcgcaagaaatctggYgccgcttggcagggtcaagtYgtcggKtggtaYtgca	57.5	*dfrA2*
dfrA3 50mer_I	gaagtggatttgtctgtggaaggagatgcatttttccccgcaatagaccg	48.0	*dfrA3*, chromosomal
dfrA3 50mer_II	cattgacgctcagttgaacggtgataccctttcccggattacctatcgc	50.0	*dfrA3*, plasmid
dfrA5 50mer	cctggacggccgataatgacaacgtaatagtattcccgtcgatcgaagag	50.0	*dfrA5*
dfrA6 50mer	atgaaaatatctcttatggcagctgtttccgagaatggagtaattggctc	40.0	*dfrA6*
dfrA7 60mer	gtgttcttccaaatcgaaaatatgcagtagtgtcgaggaaaggaatttcaagctcaaatg	38.3	*dfrA7*
dfrA8 50mer	cttgcccgtcaaattagaaggtcgcacctgcatcgttatgacgcgccaag	54.0	*dfrA8*
dfrA9 50mer	gtgggaagagtgcgtacgagaacttagctgcctacgtggacaaactctac	52.0	*dfrA9*
dfrA10 60mer[c]	tcattggtggtgttggtttattatctgaagcgatagaacatgctagcactgtttacatga	38.3	*dfrA10**
dfrA12 60mer_I	tcgcagactcactgagggaaaagtcgttgtcatggggcgaaagacctttgagtctatcgg	51.7	*dfrA12**
dfrA12 60mer_II	cgtagttgtttcaacgctgtcgcacgctatcgctttggcatccgaactcggcaatgaact	51.7	
dfrA14 50mer[c]	aatgatgacaatgtagttgtatttcagtcaatcgaagaggccatggacag	40.0	*dfrA14**
dfrA14 60mer[c]	ttggacatcaaatgatgacaatgtagttgtatttcagtcaatcgaagaggccatggacag	40.0	
dfrA15 50mer	gccgttgtaactcgttcaagcttcacttccagtgatgagaatgtattggt	50.0	*dfrA15**
dfrA16 60mer	gagatggagacatagtttttcctgaaatcccagatacattcaagttggtatttgagcaag	38.3	*dfrA16**
dfrA17 50mer	aaaacgtcctagtttttccttcaatagaaaatgctttgaaagagctatca	32.0	*dfrA17*
dfrA19 60mer	atcttcgtgctgtgatcaacctgtatgagaataccgatcaacgcattgctgtcattggtg	45.0	*dfrA19*
dfrB 50mer_I	aacgctcccgttgcagggcagtttgcgcttcccctgagtgccacctttgg	62.0	*dfrB2*
dfrB 50mer_II	caacacaacaatggagtcagtactctagttgctggccagtttgcgctccc	52.0	*dfrB3*
dfrD 50mer	gagtaatcggcaaggataacgacattccatggagaatttctagtgattgg	42.0	*dfrD*
Vancomycin			
vanA 60mer_I[b]	gcggaatgggaaaacgacaattgctattcagctgtactctcgccggataaaaaaatgcac	45.0	*vanA**
vanA 60mer_II[b]	cgtgtSgatatgtttttacaagataacggccgcattgtactgaacgaagtcaatacYctg	41.7	
vanB 50mer[b]	taccctgtctttgtgaagccggcacggtcaggttcgtcctttggcgtaac	56.0	*vanB**
vanB 60mer[b]	cgcactacatcggaattacaaaaaacggYgtatggaagctatgcaagaagccatgtacgg	45.0	
vanC1 50mer[b]	tggttgccatgtcgctgcctccgcattatgtatgaacaaatggctcttgc	50.0	*vanC-1*

Table 1 (continued)

Oligonucleotide name[a]	Sequence (5′–3′)	GC %	Gene(s) represented[d]
vanC2/C3 50mer[b]	tttgactgtcggtgcttgtgacgccatttcattagtagacggctttttcg	46.0	*vanC-2/3*
vanC2/C3 60mer[b]	actctttgactgtcggtgcttgtgacgccatttcattagtagacggctttttcgattttg	43.3	
vanD 50mer	gagattgccgcaaacatagatacaaaaaaatatcagccttattatattgg	32.0	*vanD*
vanE 50mer	aagggacaagacctacaaaaagtcgatgcgtttgcgaaaatacatggatt	40.0	*vanE*
Integron related			
intI1 59mer[c]	cgagcagctgtcgcgtgcacgggcatggtggctgaaggaccaggccgagggccgcagcg	72.9	*intI1**
intI2 60mer[c]	atgaatgcttgcgtttgcgggttaaagattttgattttgataatggctgcatcactgtgc	40.0	*intI2**
intI3 60mer	acccacttgctgcaagcaggcacagacatccgaacggtgcaagagttgttggggcattcg	56.7	*intI3*
intI4 60mer	cgccgccatcatatgaacgaaacagtactacaaaaagcggtgagaagatcggctcaagaa	46.7	*intI4*
SGI1 LB 50mer	ttctgtattgggaagtaaatctcctaaattaaattaaaaacgaagtaaaa	24.0	SGI1 left border*
Salmonella specific			
invA 60mer_I	taagcgaacgtgtttccgtgcgtaatatgaaattaattatggaagcgctcgcattgtggg	40.0	*invA Salmonella**
invA 60mer_II	tgctttctctacttaacagtgctcgtttacgaccYgaattMctgatYctggtactaatgg	40.0	
Seis 60mer[c]	gggagccaatattaatgaccaagcaaatactggaattgacgggcttgcaggttggcgagt	48.3	*sefA S.* Enteritidis*
sipB/C 60mer[c]	agcgctaaagatattctgaatagtattggtattagcagcagtaaagtcagtgacctgggg	41.7	*sipB/C Salmonella**
Sspp 50mer[c]	tgaaggaaattacgctgcatttattatggatcagaatacgccccgttcgg	44.0	putative DNA/RNA endonuclease *Salmonella**
Sspp 60mer[c]	cgtcaaaaagtgaaggaaattacgctgcatttattatggatcagaatacgccccgttcgg	43.3	
Stmm 50mer[c]	actgaggatgtgaaaaatgtacaagttgcaaatgctgatttgacagaggc	40.0	*fliC S.* Typhimurium*
Stmm 60mer[c]	actgaggatgtgaaaaatgtacaagttgcaaatgctgatttgacagaggctaaagccgca	41.7	

[a] The antibiotic class is indicated in bold and italics.
[b] Described by Mättö et al. (2007).
[c] Described by van Hoek et al. (2005).
[d] For genes with an asterisk, control strains were available.
[e] Also called *sat1* (Partridge and Hall 2005).
[f] The $bla_{\text{CTX-M-9}}$ group includes $bla_{\text{CTX-M-9}}$*, $bla_{\text{CTX-M-13}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-16}}$, $bla_{\text{CTX-M-17}}$, $bla_{\text{CTX-M-19}}$, $bla_{\text{CTX-M-21}}$, $bla_{\text{CTX-M-24}}$, $bla_{\text{CTX-M-27}}$*, $bla_{\text{CTX-M-38}}$, $bla_{\text{CTX-M-51}}$, $bla_{\text{TOHO-2}}$, $bla_{\text{UOE-2}}$ (Bonnet 2004).
[g] The $bla_{\text{CTX-M-2}}$ group includes $bla_{\text{CTX-M-2}}$*, $bla_{\text{CTX-M-5}}$, $bla_{\text{CTX-M-20}}$, $bla_{\text{CTX-M-31}}$, $bla_{\text{CTX-M-35}}$, $bla_{\text{CTX-M-43}}$, $bla_{\text{CTX-M-56}}$, $bla_{\text{CTX-M-59}}$, bla_{KLUA}, $bla_{\text{TOHO-1}}$ (Bonnet 2004).
[h] The $bla_{\text{CTX-M-1}}$ group includes $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-10}}$, $bla_{\text{CTX-M-11}}$, $bla_{\text{CTX-M-12}}$, $bla_{\text{CTX-M-15}}$*, $bla_{\text{CTX-M-22}}$, $bla_{\text{CTX-M-28}}$, $bla_{\text{CTX-M-32}}$, $bla_{\text{CTX-M-33}}$, $bla_{\text{CTX-M-34}}$, $bla_{\text{CTX-M-36}}$, $bla_{\text{CTX-M-37}}$, $bla_{\text{CTX-M-38}}$, $bla_{\text{CTX-M-42}}$, $bla_{\text{CTX-M-52}}$, $bla_{\text{CTX-M-53}}$, $bla_{\text{CTX-M-54}}$, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-57}}$, $bla_{\text{CTX-M-58}}$, $bla_{\text{CTX-M-60}}$, $bla_{\text{CTX-M-61}}$, $bla_{\text{CTX-M-64}}$, $bla_{\text{CTX-M-66}}$, $bla_{\text{UOE-1}}$ (Bonnet 2004).
[i] The $bla_{\text{OXA-10}}$ group includes $bla_{\text{OXA-7}}$, $bla_{\text{OXA-10}}$ (=$bla_{\text{PSE-2}}$), $bla_{\text{OXA-13}}$, $bla_{\text{OXA-14}}$, $bla_{\text{OXA-16}}$, $bla_{\text{OXA-17}}$, $bla_{\text{OXA-19}}$, $bla_{\text{OXA-28}}$, $bla_{\text{OXA-35}}$, $bla_{\text{OXA-56}}$, $bla_{\text{OXA-74}}$, $bla_{\text{OXA-101}}$.

resistance to the aminoglycoside antibiotic streptomycin, an extended-spectrum-β-lactamase bla_{TEM} gene, the genes *marA*, *marC*, and *marR* belonging to the multiple antibiotic resistance (mar) locus and the tetracycline resistance gene *tet*(A). Also general, but less frequently present, were the aminoglycoside resistance genes *aadA1* and *aadA2*, the sulfonamide resistance gene *sul1*, and the trimethoprim resistance gene *dfrA1*. MLS and vancomycin resistance genes were nearly absent in the *Salmonella* investigated, with one exception, i.e., *mph*(A) or *mph*(K) was demonstrated in one Heidelberg and three Blockley isolates.

In contrast to the high frequency of *strA*, the related *strB* gene was not detected by microarray analysis, although an increased incidence in streptomycin-resistant *Salmonella* has been reported because of the presence of both *strA* (*aph(3")-Ib*) and *strB* (*aph(6")-Id*) (Sundin and Bender 1996 and Pezzella et al. 2004). The absence of a hybridization signal is probably the result of the bad performance of the designed strB 50-mer oligonucleotide. This was confirmed by the design of three new oligonucleotides, i.e., strA 60mer (5′-gccatggtgatccctgcatgccgaacttcatggtggaccc taaaactcttcaatgcacgg-3′) strA-strB 60mer (5′-tgcgattg gaccctctgacttggggKtgatgttcatgccgcctgtttttcctgctcattg-3′, specific for the 3′ end of *strA* and 5′ end of *strB*) and strB 60mer (5′-gatgagcaatgcctcggaactgcgtgggctacatggcgatctg catcatgaaaacatcat-3′). All three oligonucleotides gave a hybridization signal when tested with a control strain containing both *strA* and *strB*.

As listed in Table 1, the microarray contained a considerable number of oligonucleotides representing the four classes of ESBLs, e.g., class A: $bla_{\text{CTX-M}}$ type genes; class B: bla_{VIM} type genes; class C: bla_{CMY} type genes; class D: bla_{OXA} type genes. Forty-nine out of the 143 examined *Salmonella* harbored a bla_{TEM} gene, whereas $bla_{\text{PSE-01}}$ was

Table 2 PCR primers

Antibiotic class or integrase	Primer name	Sequence (5′–3′)	Primer name	Sequence (5′–3′)	Gene(s)	Length PCR product
Aminoglycoside	aacA4_F	tggggcggagaagaagc	aacA4-R	tgcattcgccagtaact	*aacA4*	184 bp
	aacC2_F	tgggtgcgccgctaaac	aacC2-R	caaagcaatcgagaatg	*aacC2*	195 bp
	aadA1 _F[a]	atgagggaagtggtgatcgc	aadA1 _R[a]	ttccaaaaggtcgttgatcaaa	*aadA1*	216 bp
	aadA2_F[a]	gcagcgcaatgacattcttg	aadA2_R[a]	catccttcggcgcgattttg	*aadA2*	284 bp
	aphA1_F	gatttatatgggtatag	aphA1_R	cggaagaggcataaatg	*aphA1*	223 bp
	sat2_F	aagactctgcttgctatggc	sat2_R	tcctgtgctcccgagaac	*sat2*	346 bp
	strA_F	cgaacgagagctaccgg	strA_R	ttccgagccgcccaagg	*strA*	140 bp
	strB_F	tgctgatgaactgcgcg	strB_R	ggagaagggcagaaggc	*strB*	227 bp
β-lactam	blaACC-1_F	tgaagctgttatccgtg	blaACC-1_R	tgttttgccgctaaccc	bla_{ACC-1}	202 bp
	blaCTX-M-g1_F	gtacagcaaaaacttgccg	blaCTX-M-g1_R	ctttcacttttcttcagc	$bla_{CTX-M-1}$, $bla_{CTX-M-3}$, $bla_{CTX-M-10}$, $bla_{CTX-M-11}$, $bla_{CTX-M-12}$, $bla_{CTX-M-15}$*, $bla_{CTX-M-22}$, $bla_{CTX-M-28}$, $bla_{CTX-M-32}$, $bla_{CTX-M-33}$, $bla_{CTX-M-34}$, $bla_{CTX-M-36}$, $bla_{CTX-M-37}$, $bla_{CTX-M-38}$, $bla_{CTX-M-42}$, $bla_{CTX-M-52}$, $bla_{CTX-M-53}$, $bla_{CTX-M-54}$, $bla_{CTX-M-55}$, $bla_{CTX-M-57}$, $bla_{CTX-M-58}$, $bla_{CTX-M-60}$, $bla_{CTX-M-61}$, $bla_{CTX-M-64}$, $bla_{CTX-M-66}$, bla_{UOE-1}	170 bp
	blaCTX-M-g2_F	cgctgcatgcgcaggcg	blaCTX-M-g2_R	gcaaaacgttcatcggcacg	$bla_{CTX-M-2}$, $bla_{CTX-M-5}$, $bla_{CTX-M-20}$, $bla_{CTX-M-31}$, $bla_{CTX-M-35}$, $bla_{CTX-M-43}$, $bla_{CTX-M-56}$, $bla_{CTX-M-59}$, bla_{KLUA}, bla_{TOHO-1}	136 bp
	blaCTX-M-g5_F	gagcttggcgcagcg	blaCTX-M-g5_R	cgtcactttatcgggc	$bla_{CTX-M-5}$, bla_{KLUA}	87 bp
	blaCTX-M-g9_F	ggcaatacgaccggcgc	blaCTX-M-g9_R	cagcggcgcacgaccctg	$bla_{CTX-M-9}$, $bla_{CTX-M-13}$, $bla_{CTX-M-14}$, $bla_{CTX-M-16}$, $bla_{CTX-M-17}$, $bla_{CTX-M-19}$, $bla_{CTX-M-21}$, $bla_{CTX-M-24}$, $bla_{CTX-M-27}$*, $bla_{CTX-M-38}$, $bla_{CTX-M-51}$, bla_{TOHO-2}, bla_{UOE-2}	135 bp
	blaOXA-g1_F	tatggcatttgatgcgg	blaOXA-g1_R	gttttctatggctgag	bla_{OXA-01}, bla_{OXA-04}, bla_{OXA-30}, bla_{OXA-31}, bla_{OXA-47}	352 bp
	blaPSE_1_F[a]	cgctatctgaaatgaaccag	blaPSE_1_R[a]	tttcgctctgccattgaagc	bla_{PSE-1}	229 bp
	blaTEM_F[a]	tgggtgcacgagtgggttac	blaTEM_R[a]	gttagctccttcggtcctcc	bla_{TEM}	328 bp
Chloramphenicol	catB3_F	gtagtttctgctctatc	catB3_R	cttcttagcgggattgct	*catB3*	302 bp
	cmlA_F	cgctcgtcgacatgtggc	cmlA_R	gccaagctgagacacacc	*cmlA, cmlA1, cmlA4, cmlA5, cmlA6, cmlA7*	272 bp
	floR_F[a]	ccttcttcgtcttcttctcg	floR_R[a]	ggtaggatgaaggtgaggaa	*floR*	255 bp
Sulfonamide	sul1_F[a]	gccttgcgacggagcggggt	sul1_R[a]	aggcatgatctaaccctcgg	*sul1*	363 bp
	sul2_F	caaggcagatggcattccc	sul2_R	gtcgccagcgggtgcctc	*sul2*	211 bp
Tetracycline	tetA_F[a]	gccggcgctgcaagcaattt	tetA_R[a]	ccacgttgttatagaagcc	*tet*(A)	148 bp
	tetB_F[a]	acgtgaatttattgcttcgg	tetB_R[a]	atacagcatccaaagcgcac	*tet*(B)	205 bp
	tetC_F	gcgggatatcgtccattccg	tetC_R	cgtagaggatccacaggacg	*tet*(C)	206 bp
	tetD_F	aaaccggcggtacagacaga	tetD_R	aaaccgaccgccgcgctgtc	*tet*(D)	200 bp
	tetG_F[a]	gactggcttcgttcttctgg	tetG_R[a]	ttgcgaatggtctgcgtagt	*tet*(G)	308 bp
Trimethoprim	dfrA1_F[a]	ccaaaggtgaacagctcctg	dfrA1_R[a]	atatgtagtgtatctacttg	*dfrA1*	271 bp
	dfrA2_F	gttgcWgggcagtttgcgct	dfrA2_R	gcagccacaggataaat	*dfrA2*	185 bp
	dfrA10_F	tttgagagcttcttagaa	dfrA10_R	accggtacatacacatcagc	*dfrA10*	158 bp
	dfrA12_F	cctcgttttgacgcgctc	dfrA12_R	attggcgccgggaagaacg	*dfrA12*	204 bp
	dfrA14_F[a]	tctggtgggtcgcaagacg	dfrA14_R[a]	atgggtaatgtttctcgg	*dfrA14*	204 bp
	dfrA16_F	tacaaaagcttgatttcc	dfrA16_R	aatagttaatgttagact	*dfrA16*	142 bp
Integrase	intI1_F[a]	atcgggccttgatgttac	intI1_R[a]	gcgcgctgaaaggtctgg	*intI1*	256 bp
	intI2_F[a]	gcaggttatggatactcg	intI2_R[a]	gctgtttctgcttttccc	*intI2*	157 bp

[a] Described by van Hoek et al. (2005)

Table 3 Summary of the microarray hybridization data of the *Salmonella* isolates

Serovar	S. *Agona*	S. *Anatum*	S. *Bareilly*	S. *Blockley*	S. Brandenburg	S. Bredeney	S. Derby	S. Dublin	S. Enteritidis	S. Give	S. Hadar	S. Heidelberg	S. *enterica* subsp. *houtenae* 48:g,z51:-	S. Infantis	S. Kentucky	S. Livingstone	S. London	S. Mandaka	S. Newport	S. Paratyphi B var. Java	S. Rough	S. Saint Paul	S. Senftenberg	S. Tennessee	S. Virchow	S. Typhimurium														Total
Phage type																										DT104	Pt401	Unknown	Pt296	Pt350	ARS	DT012	DT041	DT120	DT208	Pt3	Pt353	Pt507	Pt510	
# isolates	11	4	1	4	1	3	4	1	8	1	10	7	1	5	1	2	3	4	2	10	1	3	2	1	9	19	5	5	3	2	2	1	1	1	1	1	1	1	1	143
Aminoglycoside																																								
aacA 50mer_II																2									1															3
aacC 50mer_III					1				1							1									1															4
aadA1 50mer	1						1			1		2								10						2				1										18
aadA1 60mer_I	2	2				1	1			1		2								10					1	3				1										24
aadA1 60mer_II	2	2				1	1			1		2								9					1	3				1										23
aadA2 60mer_I	3	1					1					1							1	4	1	3			1	15		2					1	1						35
aadA2 60mer_II	3	1					1					1							1	4	1	3				15		2					1	1						34
aadB 50mer												1										3																		4
aphA 50mer_I				3		1	1	1			2	3							1		1														1					14
sat 60mer_I																				10																				10
strA 50mer	3	2	1	3		3	3			1	7	4			1		3		1		1		1	1	1	4	1				2		1		1					45
strB 50mer																																								0
ESBL																																								
blaACC 50mer_I			1													1																								2
blaCTX-M 50mer_I																1		4			1																			6
blaCTX-M 50mer_II																									1															1
blaCTX-M 50mer_III															1			4																						5
blaOXA 50mer_VIII															1																									1
blaPSE 50mer	1																									15	1	1					1	1						20
blaPSE 60mer	1																									15	1	1					1	1						20
blaTEM 60mer	2	1		1		2			2	1	7	5		1	1	1	2		1	6	1	3			2	3	2			1	2				1	1				49
Chloramphenicol																																								
catB 50mer_II															1																									1
cmlA 50mer		1																								1														2
cmlB 50mer																										1														1
floR 50mer	1																									12	1	1					1	1						17
floR 60mer	1																									12	1	1					1	1						17
mar operon																																								
marA 50mer	9	2	1	4	1	3	4	1	8	1	10	7	1	5			3	2	1	9	1	3	2	1	9	16	4	5	3	2	2	1	1	1	1	1		1	1	127
marB 50mer																																								0
marC 50mer	11	4	1	4	1	3	4	1	8	1	10	7	1	5		1	3	4	2	9	1	3	2	1	9	19	5	5	3	2	2	1	1	1	1	1		1	1	139
marR 50mer	11	4	1	4	1	3	4	1	8	1	10	7	1	5	1	2	3	4	2	10	1	3	2	1	9	19	5	5	3	2	2	1	1	1	1	1		1	1	142
MLS																																								
mph 50mer_II				3								1																												4
Sulfonamide																																								
sul1 50mer	3	1					2			1		1				1			1	1		3				15	1	2			1		1	1						35
sul1 60mer_I	4	2				1	2		1	1		3				2			1	3	1	3			1	16	1	2		1	1		1	1						48
sul1 60mer_II	4	2				1	2			1		3				1			1	3	1	3			1	16	1	2		1	1		1	1						46
sul2 50mer	2					1	1								1		1		1	7			1	1		2	1		1		2				1					23
Tetracycline																																								
tetA 60mer	3	2		3		1	1		1	1	8	6					3	2	2		1				3	4	4			1								1		47
tetB 60mer	1					1	1						1													1			1		1		1		1		1			10
tetC 50mer									1										1			3								2		1							1	9
tetD 60mer															1																									1
tetG 60mer	1																									12		2					1	1						17
Trimethoprim																																								
dfrA 50mer_II																1		3																						4
dfrA1 60mer	3	1				1	1		1	1		1	1							10					1	2														23
dfrA10 60mer	1																	1												1										3
dfrA12 60mer_I	1										1			1												1														4
dfrA12 60mer_II	1										1			1												1														4
dfrA14 50mer	1					1	1					1											1	1		1			1		1									9
dfrA14 60mer	1					1	1					1											1	1		1			1		1									9
dfrA15 50mer									1																															1
dfrA16 60mer																					1																			1
Integron related																																								
intI1 59mer	2	1					2								1	1			1	2	1	2			1	15	1	2		1			1	1						35
intI2 60mer	1											2	1							10										1										15
SGI1 LB 50mer																										7	1	2					1	1						12

almost exclusively found in *S.* Typhimurium DT104. The plasmid-encoded CTX-M type genes were occasionally detected, the class C β-lactamase gene bla_{ACC-1} was detected twice and a class D ESBL bla_{OXA} was found once.

The microarray also included oligonucleotides representing sequences of class 1 and class 2 integrons (integrase genes) and sequences of SGI1. As summarized in Table 3, most of the DT104 isolates gave hybridization signals with oligonucleotide intI1 59mer representing the integrase of a class 1 integron. Class 2 integrons were almost exclusively detected in Paratyphi B var. Java, but, also two Agona, the investigated *houtenae* strain, and one of the DT104 isolates harbored *intI2*.

A total of 44 Typhimurium strains have been investigated by microarray analysis, including 19 DT104 or Pt506 isolates. The DT nomenclature is according to the English and the Pt based on the Dutch phage-typing system. DT104 corresponds with Pt506 and they are grouped as DT104 in Table 3.

The microarray hybridization data of the Typhimurium isolates revealed a high frequency of the commonly SGI1 localized AR determinants: the *aadA2* gene coding for an adenyltransferase mediating resistance to the aminoglycoside streptomycin and the aminocyclitol spectinomycin, the ESBL bla_{PSE-1} gene, the sulfonamide resistance gene *sul1*, the chloramphenicol/florfenicol resistance gene *floR* and the tetracycline resistance gene *tet*(G). Most of the DT104 strains investigated here also harbored these AR genes; however, some of them only showed hybridization signals with a subset of these determinants, which suggests the presence of variant SGI1. From four of the investigated isolates (S/921495, S/960275, S/954435, and S/960081), it was already known that they contain variable SGI1 (Boyd et al. 2002). These strains had already been analyzed on a smaller oligonucleotide microarray (see van Hoek et al. 2005).

In *S.* Paratyphi B var. Java, the streptomycin resistance gene *aadA1* was found in all strains, although, for one of them one of the 3 *aadA1* oligonucleotides (i.e., aadA1 60mer_II) did not give a hybridization signal. The related resistance genes *aad*A2 and *strA* were also detected in this serovar, but less frequent. In addition, all investigated Paratyphi B var. Java isolates contained *dfrA1* and *sat2*.

Among the 10 investigated Hadar isolates, seven contained bla_{TEM} and *strA*; furthermore, in eight *tet*(A) was identified. Less frequently *aphA1* and *sul1* were demonstrated. The genes *strA*, $bla_{TEM,}$ and *tetA* were found in similar incidences in isolates belonging to the serovars Heidelberg and London. Within *S.* Enteritidis, responsible for the greatest part of the human cases of salmonellosis, most likely related to the consumption of raw shell eggs, the number of AR genes per isolate was relatively low on average.

Discussion

Taking into account the number of antibiotic resistance genes described in the literature so far, it would take quite an effort to investigate bacterial isolates by PCR. Microarray analysis allows the screening of a large number of targets simultaneously and as such circumvents the shortcomings of PCR. The potential of miniaturization in addition to multiplexing offers a considerable advantage of microarray analysis over other molecular methods for clinical or epidemiological applications. As summarized by Garaizar et al. (2006), microarrays have been shown to be helpful for quick detection of antibiotic resistance, determination of virulence and pathogenicity, species determination, genome comparison, and molecular epidemiological typing of strains.

In this paper, the successful application of microarray analysis was demonstrated again using a microarray containing 223 oligonucleotides representing more than 430 AR genes for the screening of a large set of *Salmonella* isolates. To a large extent, the obtained hybridization results confirmed the general findings concerning antibiotic resistance in *Salmonella*. Although many antibiotic resistance genes are represented by the microarray, the used setup is, however, not suited for the detection of mutation-mediated resistance, like for instance mutations within the gyrase gene leading to nalidixic acid resistance.

The level and extent of resistance among *Salmonella* varies in different geographical locations. Nevertheless, there is a correlation between the length of time an antimicrobial agent has been used and its corresponding resistance (McDermott 2006). A general increase is noticed with respect to *Salmonella* strains with a multidrug resistance phenotype including resistance to ampicillin, chloramphenicol, sulfonamides, streptomycin, and tetracycline (Brisabois et al. 1997). The microarray data presented here support those findings because in the majority of the isolates investigated multiple AR genes were detected. The most frequently detected genes in this study belong to the inducible *mar* operon, which controls the intrinsic levels of susceptibility to structurally different antibiotics. The occurrence of these genes within *Salmonella enterica* have been described by, for instance, Sulavik et al. (1997) and Kunonga et al. (2000), who demonstrated the presence of the *marR*, *marA*, and *marB* genes in *S.* Typhimurium and 30 different serovars, respectively, and by Randall and Woodward (2001) describing 44 serovars of *Salmonella* with a conserved *marA*. The results reported here concerning the widespread occurrence of *mar* genes in the genus of *Salmonella* are in agreement with the data mentioned above, although the *mar*-related oligonucleotides were not tested with well-defined control strains, nor were the results confirmed by PCR. Probably as a consequence *marB* was not detected, whereas *marR* and

marA, also part of the transcriptional unit TU2 called *marRAB* and *marC,* a gene belonging to transcriptional unit TU1, were found.

The second most frequently detected gene was the ESBL bla_{TEM}, although *strA*, *sul1*, and *tet*(A) were also common among the *Salmonella* strains investigated. It is a well-known fact that like other Enterobacteriaceae, also ESBL-producing *Salmonella* strains have been emerging worldwide during the last decade (Bonnet 2004 and Hasman et al. 2005). Integrons (in particular class 1) are frequently found in *Salmonella* and are often associated with AR genes cassettes. These elements have the ability to integrate and excise genes and play a role in the dissemination of AR among different bacteria (Miko et al. 2003; Rowe-Magnus and Mazel 2002; Carattoli 2001 and Martinez-Freijo et al. 1998). Within the Typhimurium DT104 isolates, recognized as an emergent and multiresistant pathogen, different combinations of AR genes were found in this study that are most likely associated with the class 1 integron elements located on the SGI1. Strains belonging to phage type DT104 are very commonly characterized by the clustering of five resistance genes, i.e., bla_{PSE-1}, *floR aadA2*, *sul1*, and *tet*(G), on the so-called Salmonella Genomic Island 1, resulting in the penta resistance type ACSSuT (Boyd et al. 2001). However, the phenomenon of variant SGI1 elements has been identified by Boyd et al. (2002) and the described strains were also analyzed here. The microarray results were in full accordance with the published data (see also van Hoek et al. 2005).

Class 2 integron sequences were found in the Paratyphi B var. Java isolates investigated, all harbored *aadA1*, *sat2* and *dfrA1*, which are known to be part of the *Tn*7 transposon a known class 2 integron (Partridge and Hall 2005). The microarray results are in good agreement with Miko et al. (2003). These authors investigated 85 multi-resistant D-tartrate positive *Salmonella enterica* subsp. *enterica* serovar Paratyphi B (also known as *S*. Paratyphi B var. Java) isolates for the presence of integrons and detected in all of them the chromosomally located *Tn*7-like class 2 integron harboring the same *dfrA1-sat2-aadA1* gene cassette.

S. Hadar is often found as a cause of human infections in many European countries, and high frequencies of resistance are reported for ampicillin, streptomycin, and tetracycline (Threlfall et al. 2000). These phenotypic data were supported by the obtained microarray results, as bla_{TEM}, *strA*, and *tet*(A), respectively, were identified in nearly all *S.* Hadar investigated. These multiple AR genes, however, could not be linked to the presence of a class 1 or 2 integron; this phenomenon has been reported before in this serovar (Randall et al. 2004).

According to the Dutch monitoring program of antimicrobial resistance and antibiotic usage in animals and other reports (for review, see McDermott) the highest level of resistance is found in *Salmonella* isolates belonging to serovar Typhimurium (McDermott 2006; Mevius et al. 2004 and Mevius and van Pelt 2005). The most common phenotypes in *S.* Typhimurium display resistance to amoxicillin, tetracycline, sulphamethoxazole, trimethoprim, chloramphenicol, and florfenicol with the highest levels of resistance in strains with a DT104 phage type. This is also reflected by the microarray screening presented here as AR genes related to the above-mentioned phenotypes were found in high frequencies within serovar Typhimurium and phage type DT104. Besides *S.* Typhimurium, also within *S.* Paratyphi var. Java and *S*. Derby, the percentage of resistance to amoxicillin, tetracycline, sulphamethoxazole, trimethoprim, and chloramphenicol/florfenicol is relatively high (Martinez-Freijo et al. 1998; Mevius et al. 2004 and Mevius and van Pelt 2005) compared to the other serovars. As a consequence a high number of AR genes within isolates of these serovars were identified with the microarray screening.

Part of the oligonucleotides presented in this study have already been described in previous studies (van Hoek et al. 2005 and Mättö et al. 2007). The latter publication demonstrated the applicability of the designed oligonucleotide microarray not only for pathogens, but also for the screening of gram-positive *Bifidobacterium* spp. strains that are generally associated with good intestinal health. Broad screening of the presence of AR genes both within the pathogenic and in the environmental or food-associated (harmless) bacterial population will help to identify reservoirs for AR determinants and to critically evaluate the use of antibiotics both in agriculture and for the treatment of infections in humans.

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