Applied and Environmental Microbiology, Oct. 2011, p. 6991–6999 Vol. 77, No. 19
0099-2240/11/$12.00 doi:10.1128/AEM.00567-11

Microarray-Based Analysis of IncA/C Plasmid-Associated Genes from Multidrug-Resistant *Salmonella enterica*▽†

Rebecca L. Lindsey,* Jonathan G. Frye, Paula J. Fedorka-Cray, and Richard J. Meinersmann

U.S. Department of Agriculture, Agricultural Research Service, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Richard B. Russell Agricultural Research Center, 950 College Station Road, Athens, Georgia 30605-2720

Received 11 March 2011/Accepted 28 July 2011

In the family *Enterobacteriaceae*, plasmids have been classified according to 27 incompatibility (Inc) or replicon types that are based on the inability of different plasmids with the same replication mechanism to coexist in the same cell. Certain replicon types such as IncA/C are associated with multidrug resistance (MDR). We developed a microarray that contains 286 unique 70-mer oligonucleotide probes based on sequences from five IncA/C plasmids: pYR1 (*Yersinia ruckeri*), pPIP1202 (*Yersinia pestis*), pP99-018 (*Photobacterium damselae*), pSN254 (*Salmonella enterica* serovar Newport), and pP91278 (*Photobacterium damselae*). DNA from 59 *Salmonella enterica* isolates was hybridized to the microarray and analyzed for the presence or absence of genes. These isolates represented 17 serovars from 14 different animal hosts and from different geographical regions in the United States. Qualitative cluster analysis was performed using CLUSTER 3.0 to group microarray hybridization results. We found that IncA/C plasmids occurred in two lineages distinguished by a major insertion-deletion (indel) region that contains genes encoding mostly hypothetical proteins. The most variable genes were represented by transposon-associated genes as well as four antimicrobial resistance genes (*aphA*, *merP*, *merA*, and *aadA*). Sixteen mercury resistance genes were identified and highly conserved, suggesting that mercury ion-related exposure is a stronger pressure than anticipated. We used these data to construct a core IncA/C genome and an accessory genome. The results of our studies suggest that the transfer of antimicrobial resistance determinants by transfer of IncA/C plasmids is somewhat less common than exchange within the plasmids orchestrated by transposable elements, such as transposons, integrating and conjugative elements (ICEs), and insertion sequence common regions (IS*CR*s), and thus pose less opportunity for exchange of antimicrobial resistance.

The control of bacterial infections is threatened by the apparent increase in the number of bacteria that carry antimicrobial resistance (AR) genes (1). Plasmids are transmissible extrachromosomal genetic material that can carry AR genes (4, 7, 17). Other mobile elements responsible for the transfer of genetic material like AR genes include transposable elements such as transposons, integrating and conjugative elements (ICEs), and insertion sequence common regions (IS*CR*s) (5, 21, 37, 38, 40). In the family *Enterobacteriaceae*, plasmids have been classified into incompatibility (Inc) groups that are based on the inability of plasmids with the same replication mechanism to be maintained in the same cell lineage. In *Enterobacteriaceae*, there are 27 described Inc or replicon types (8). IncA/C plasmids are associated with multidrug resistance (MDR) (16, 39). We have previously found that IncA/C plasmids are prevalent in multidrug-resistant *Salmonella enterica* (23).

Population genetics studies that have included data on the distribution of plasmids are common, but there have been very few studies on the distribution and flow of genes within a set of plasmids. Cataloging the genetic composition of plasmid populations can be used to make inferences about their history, which may be useful in understanding the selective pressures that have led to the prevalence of the plasmids and an appreciation of how anthropogenic effects may continue to influence their evolution. Such a study may not be meaningful with small plasmids, but a study of large plasmids may reveal how the plasmid evolved, accumulating and discarding various genes. IncA/C plasmids tend to be relatively large, generally 130 to 180 kb, with a complement of 160 to 210 genes (6, 16). Available DNA sequences indicate that there is heterogeneity in the complement of genes (6, 16). Welch et al. (39) presented data on the presence or absence of 12 loci based on PCR analysis of IncA/C plasmids from 51 isolates of *S. enterica* and 19 isolates of other members of the family *Enterobacteriaceae*, but they did not analyze the data for linkage disequilibrium. Here we used a previously described microarray with oligonucleotide probes for all the unique genes in five IncA/C plasmids that have been previously sequenced (24) to evaluate the gene content (286 loci) of IncA/C plasmids from a population of 59 different *S. enterica* isolates and to report on the core and accessory genome. We then used linkage disequilibrium analysis to examine the population genetics of these genes in these isolates.

MATERIALS AND METHODS

Bacterial isolates and plasmids. The 59 *Salmonella enterica* isolates in this study were selected from a diverse subset of 98 IncA/C-positive diagnostic isolates with known pulsed-field gel electrophoresis (PFGE) patterns (23). Diverse isolates were selected based on cluster analysis, antibiotic resistance, plasmid profile, source animal, and region. Cluster analysis revealed 48 unique PFGE types among the 59 isolates studied here, suggesting a variable population. These

* Corresponding author. Mailing address: USDA-ARS-BEAR, Richard J. Russell Research Center, P.O. Box 5677, Athens, GA 30604-5677. Phone: (706) 546-3676. Fax: (706) 546-3018. E-mail: rebecca.lindsey@ars.usda.gov.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 12 August 2011.

isolates represented 17 serovars from 14 different sources, including canines (dogs) ($n = 4$), cattle ($n = 17$), chickens ($n = 3$), dairy cattle ($n = 7$), equines (horses) ($n = 6$), felines (domestic cat) ($n = 1$), reptiles (turtle and lizard) ($n = 3$), swine ($n = 12$), turkeys ($n = 3$), mammals (alpaca and another mammal) ($n = 2$), and an unknown source ($n = 1$). Strains were obtained from all five regions in the United States as defined for the NARMS program (http://www.ars.usda.gov/Main/docs.htm?docid=6750).

Salmonella isolates were grown on brilliant green sulfa agar (BGSA) or blood agar plates (BAPs) at 37°C after recovering from frozen stock cultures stored at −70°C by standard methods (http://www.ars.usda.gov/Main/docs.htm?docid=6750&page=1).

DNA extraction and labeling. Total DNA from *S. enterica* isolates was extracted from 5 ml of overnight cultures grown in LB broth (Hardy Diagnostics, Santa Maria, CA) using the GenElute bacterial genomic DNA kit (Sigma, St. Louis, MO) following the manufacturers' instructions for Gram-negative bacteria. DNA was labeled using random priming with N_{15} (Operon, Huntsville, AL), and Cy3- or Cy5-dCTP (Amersham, Piscataway, NJ) was incorporated during extension with Klenow fragment (New England BioLabs, Beverly, MA) as previously described (28). DNA targets were purified following the manufacturer's directions using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and eluted in 1 mM Tris-HCl (pH 8.0); the sample was then dried down to a volume of 20 μl. Positive-control DNA from *Yersinia ruckeri* YR71 (39) was labeled with Cy3, while *Salmonella enterica* serovar Typhi CT18 (26), and negative-control DNA from *Escherichia coli* DH10B (11) was labeled with Cy5 and hybridized to arrays. The 59 study isolates were labeled with Cy3 or Cy5 and hybridized to arrays.

Microarray construction, hybridization, and data analysis. The plasmid/antimicrobial resistance (PAR) microarray was constructed as previously described with 70-mer oligonucleotide probes for 286 unique IncA/C genes, 207 IncH1 plasmid genes, and 775 antimicrobial resistance genes (18, 24, 42) printed in triplicate. This study focused exclusively on the 286 unique IncA/C probes that were designed based on sequence obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) from five IncA/C plasmids: pYR1 (*Yersinia ruckeri* strain YR71) (NCBI accession no. CP000602), pIP1202 (*Yersinia pestis* biovar Orientalis strain IP275) (NCBI accession no. CP000603), pP99-018 (*Photobacterium damselae* subsp. *piscicida*) (NCBI accession no. AB277723), pSN254 (*Salmonella enterica* subsp. *enterica* serovar Newport strain SL254) (NCBI accession no. CP000604), and pP91278 (*Photobacterium damselae* subsp. *piscicida*) (NCBI accession no. AB277724). The array was comprised of probes for all of the genes identified in pYR1 plus all the unique genes from the remaining plasmids.

Protocols for hybridizations in formamide buffer were used for hybridization and the posthybridization wash processes as previously described (18, 19, 24, 28). The hybridized slides were scanned with a GenePix 4100A laser scanner (Axon, Foster City, CA) using a laser with a light wavelength of 532 nm or 635 nm to excite the Cy3 or Cy5 dye, respectively. Fluorescent images were captured as separate single-image tagged image file (TIF) format and analyzed with ScanArray Express software version 1.1 (Packard BioChip Technologies). Hybridization signal intensities were measured, the means of the triplicate spots were recorded, and a cutoff two times the median of the mean hybridization intensity to all 70-mer probes was used. Microsoft Excel (Microsoft Corporation, Redmond, WA) was used to process hybridization results and to make comparisons of microarray hybridizations as previously described (19, 24). A positive hybridization to an oligonucleotide probe on the array was interpreted to indicate that the gene was present.

Relationships between isolates were determined by hierarchical cluster analysis using open source CLUSTER 3.0 with Euclidean distance for gene content (10, 12).

Statistical analysis. Linkage disequilibrium (LD) of probes on the array was calculated as an extension of Fisher's exact probability test on contingency tables (31) as instituted by the program Arlequin (13). Standard settings, such as 10,000 steps in the Markov chain and 1,000 dememorization steps, were used, and calculations of D (LD coefficient), D' (maximum value standardized LD coefficient), and r^2 coefficients were made with a significance level of 0.05. LD was not calculated for probes associated with hypothetical genes or genes that displayed all positive or all negative hybridizations.

PCR detection of bla_{SHV-1}. The bla_{SHV-1} gene was amplified using primers, conditions, and controls by the method of Rasheed et al. (29).

RESULTS

DNA isolated from 59 *S. enterica* isolates was hybridized to the plasmid/antimicrobial resistance (PAR) microarrays (23, 24). These isolates represented 17 serovars from 14 different host sources and were obtained from five regions of the United States (24).

Fifty-nine different hybridization profiles were found. Hybridization results are illustrated in Fig. 1. Isolates are ordered across the top of Fig. 1 based on hierarchical cluster analysis that was performed with results of the microarray hybridizations. Analysis of the 180 oligonucleotide probes on the microarray that correspond specifically to the region 1 section of the array (the pYR1 genes; Fig. 1, region column, black or gray bar) reveals the presence of a region of insertions or deletions (indel) affecting 53 of those probes (pYR1x0087 to pYR1x0142). The first 18 isolates in Fig. 1 showed no or reduced hybridization (except for isolate 552) to the probes in the indel that consist mostly of probes that correspond to hypothetical protein genes (35 of 53 [66%]) and *traCNUW*, a single-stranded DNA-binding protein (*ssb*), assorted domain genes (cyclic diguanylate phosphodiesterase, von Willebrand factor type A, [2Fe-2S]-binding protein), and the *insB1* transposase gene, to name a few. The remaining 41 isolates showed positive hybridizations to the probes in the indel.

A second notable indel in region 1 of the array consists of 13 contiguous probes (pYR1x0147 to pYR1x0160) that correspond to genes for three hypothetical proteins, resolvase *tnpR* for transposon Tn*5393*, transposase *insC* for insertion sequence (IS) IS*903*, transposase *insD* for IS*1133*, transposase *insE1* for IS*10R*, tetracycline resistance *tetA* (class B), *tetCD*, transcriptional regulator (ArsR family), tetracycline repressor *tetR* (class B), and a sodium/glutamate symporter. Analysis of the remaining 127 oligonucleotide probes on the microarray in region 1 showed large regions of hybridizations (Fig. 1, region column, black bar); 114 of these probes bound to DNA from 53 of 59 (90%) or more of the isolates and 106 probes bound to DNA from 95% or more of the isolates.

Region 2 (Fig. 1, region column, blue bar) of the microarray contains 40 oligonucleotide probes that correspond specifically to pIP1202 genes. Analysis of region 2 (pIP1202x0044 to pIP1202x0198) showed most of these genes were present in two contiguous blocks: mercury resistance (8 oligonucleotide probes) and tetracycline resistance-associated regions. We also

FIG. 1. Microarray hybridization results for this study. The leftmost column, the Name[a] column, lists the probe names, and the yellow-highlighted probes (listed top to bottom) correspond to the locations of the Welch et al. (39) primers along the IncA/C backbone. Column 2, the region[b] column, is color coded to indicate regions 1 to 5 on the array; region 1 with pYR1 probes is black or gray, region 2 with pIP1202 probes is blue, region 3 with pP99-018 probes is red, region 4 with pSN254 probes is yellow, and region 5 with pP91278 probes is orange. Columns 3 to 62 show hybridization results; blue indicates that the trait is present, and red indicates that the trait is absent. Isolate numbers are arranged across the top according to CLUSTAL 3.0 analysis. The last column, the Class[c] column, is the probe class, and the gene elements (6, 16) are color coded (*merA* [light blue], *aadA* [light brown], beta-lactamase [light yellow], and *floR* [light pink] from Call et al. [6]).

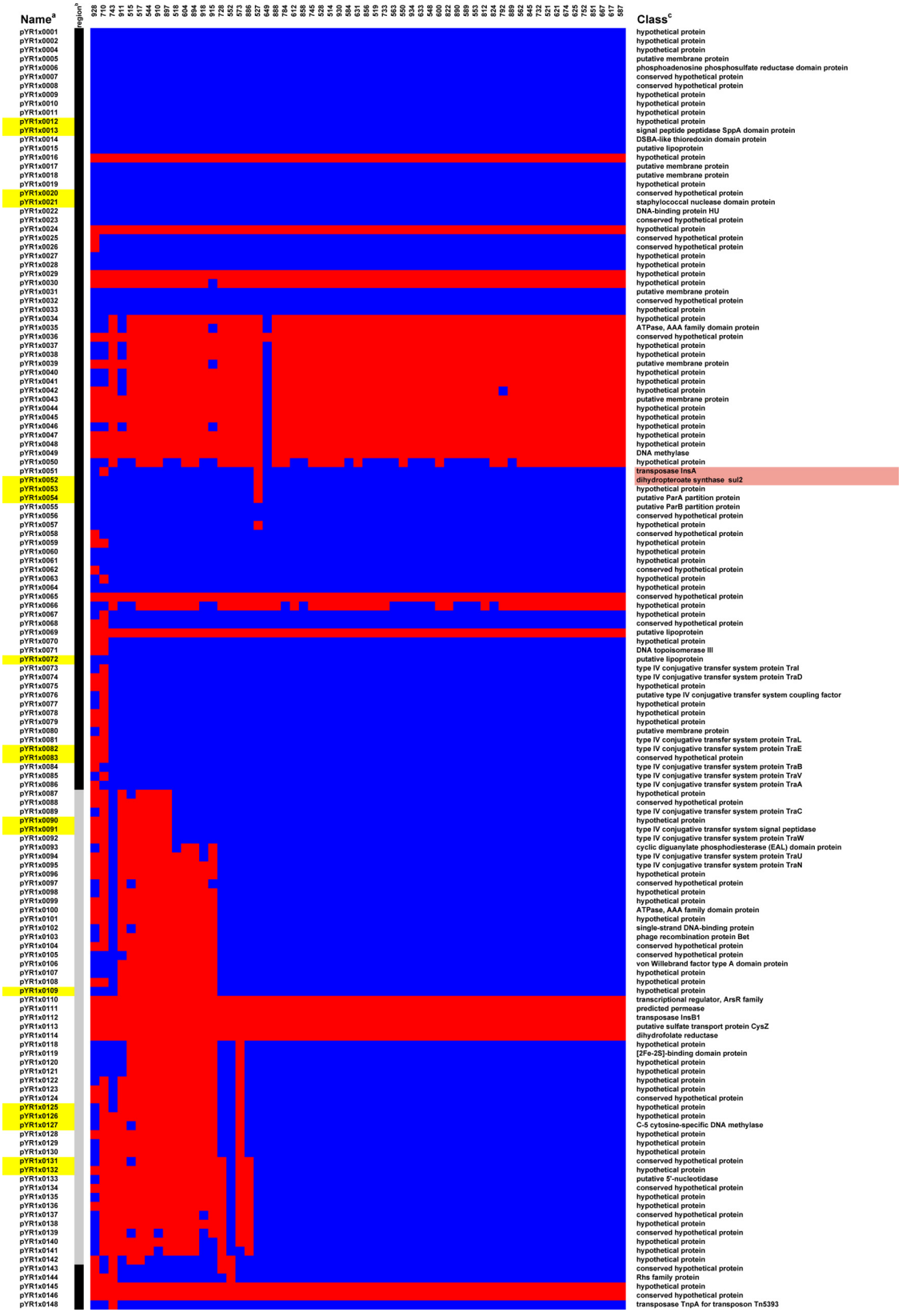
Name[a]
Region[b]
928 710 743 511 515 517 544 910 897 515 604 894 918 915 729 552 573 886 527 649 888 784 612 858 745 508 514 930 584 631 856 519 733 563 560 934 633 548 600 822 880 899 553 812 824 792 899 562 845 732 521 627 674 625 752 851 657 617 587
Class[c]
pYR1x0001 hypothetical protein
pYR1x0002 hypothetical protein
pYR1x0004 hypothetical protein
pYR1x0005 putative membrane protein
pYR1x0006 phosphoadenosine phosphosulfate reductase domain protein
pYR1x0007 conserved hypothetical protein
pYR1x0008 conserved hypothetical protein
pYR1x0009 hypothetical protein
pYR1x0010 hypothetical protein
pYR1x0011 hypothetical protein
pYR1x0012 hypothetical protein
pYR1x0013 signal peptide peptidase SppA domain protein
pYR1x0014 DSBA-like thioredoxin domain protein
pYR1x0015 putative lipoprotein
pYR1x0016 hypothetical protein
pYR1x0017 putative membrane protein
pYR1x0018 putative membrane protein
pYR1x0019 hypothetical protein
pYR1x0020 conserved hypothetical protein
pYR1x0021 staphylococcal nuclease domain protein
pYR1x0022 DNA-binding protein HU
pYR1x0023 conserved hypothetical protein
pYR1x0024 hypothetical protein
pYR1x0025 conserved hypothetical protein
pYR1x0026 conserved hypothetical protein
pYR1x0027 hypothetical protein
pYR1x0028 hypothetical protein
pYR1x0029 hypothetical protein
pYR1x0030 hypothetical protein
pYR1x0031 putative membrane protein
pYR1x0032 conserved hypothetical protein
pYR1x0033 hypothetical protein
pYR1x0034 hypothetical protein
pYR1x0035 ATPase, AAA family domain protein
pYR1x0036 conserved hypothetical protein
pYR1x0037 hypothetical protein
pYR1x0038 hypothetical protein
pYR1x0039 putative membrane protein
pYR1x0040 hypothetical protein
pYR1x0041 hypothetical protein
pYR1x0042 hypothetical protein
pYR1x0043 putative membrane protein
pYR1x0044 hypothetical protein
pYR1x0045 hypothetical protein
pYR1x0046 hypothetical protein
pYR1x0047 hypothetical protein
pYR1x0048 hypothetical protein
pYR1x0049 DNA methylase
pYR1x0050 hypothetical protein
pYR1x0051 transposase InsA
pYR1x0052 dihydropteroate synthase sul2
pYR1x0053 hypothetical protein
pYR1x0054 putative ParA partition protein
pYR1x0055 putative ParB partition protein
pYR1x0056 conserved hypothetical protein
pYR1x0057 hypothetical protein
pYR1x0058 conserved hypothetical protein
pYR1x0059 hypothetical protein
pYR1x0060 hypothetical protein
pYR1x0061 hypothetical protein
pYR1x0062 conserved hypothetical protein
pYR1x0063 hypothetical protein
pYR1x0064 hypothetical protein
pYR1x0065 conserved hypothetical protein
pYR1x0066 hypothetical protein
pYR1x0067 hypothetical protein
pYR1x0068 conserved hypothetical protein
pYR1x0069 putative lipoprotein
pYR1x0070 hypothetical protein
pYR1x0071 DNA topoisomerase III
pYR1x0072 putative lipoprotein
pYR1x0073 type IV conjugative transfer system protein TraI
pYR1x0074 type IV conjugative transfer system protein TraD
pYR1x0075 hypothetical protein
pYR1x0076 putative type IV conjugative transfer system coupling factor
pYR1x0077 hypothetical protein
pYR1x0078 hypothetical protein
pYR1x0079 hypothetical protein
pYR1x0080 putative membrane protein
pYR1x0081 type IV conjugative transfer system protein TraL
pYR1x0082 type IV conjugative transfer system protein TraE
pYR1x0083 conserved hypothetical protein
pYR1x0084 type IV conjugative transfer system protein TraB
pYR1x0085 type IV conjugative transfer system protein TraV
pYR1x0086 type IV conjugative transfer system protein TraA
pYR1x0087 hypothetical protein
pYR1x0088 conserved hypothetical protein
pYR1x0089 type IV conjugative transfer system protein TraC
pYR1x0090 hypothetical protein
pYR1x0091 type IV conjugative transfer system signal peptidase
pYR1x0092 type IV conjugative transfer system protein TraW
pYR1x0093 cyclic diguanylate phosphodiesterase (EAL) domain protein
pYR1x0094 type IV conjugative transfer system protein TraU
pYR1x0095 type IV conjugative transfer system protein TraN
pYR1x0096 hypothetical protein
pYR1x0097 conserved hypothetical protein
pYR1x0098 hypothetical protein
pYR1x0099 hypothetical protein
pYR1x0100 ATPase, AAA family domain protein
pYR1x0101 hypothetical protein
pYR1x0102 single-strand DNA-binding protein
pYR1x0103 phage recombination protein Bet
pYR1x0104 conserved hypothetical protein
pYR1x0105 conserved hypothetical protein
pYR1x0106 von Willebrand factor type A domain protein
pYR1x0107 hypothetical protein
pYR1x0108 hypothetical protein
pYR1x0109 hypothetical protein
pYR1x0110 transcriptional regulator, ArsR family
pYR1x0111 predicted permease
pYR1x0112 transposase InsB1
pYR1x0113 putative sulfate transport protein CysZ
pYR1x0114 dihydrofolate reductase
pYR1x0118 hypothetical protein
pYR1x0119 [2Fe-2S]-binding domain protein
pYR1x0120 hypothetical protein
pYR1x0121 hypothetical protein
pYR1x0122 hypothetical protein
pYR1x0123 hypothetical protein
pYR1x0124 conserved hypothetical protein
pYR1x0125 hypothetical protein
pYR1x0126 hypothetical protein
pYR1x0127 C-5 cytosine-specific DNA methylase
pYR1x0128 hypothetical protein
pYR1x0129 hypothetical protein
pYR1x0130 hypothetical protein
pYR1x0131 conserved hypothetical protein
pYR1x0132 hypothetical protein
pYR1x0133 putative 5′-nucleotidase
pYR1x0134 conserved hypothetical protein
pYR1x0135 hypothetical protein
pYR1x0136 hypothetical protein
pYR1x0137 conserved hypothetical protein
pYR1x0138 hypothetical protein
pYR1x0139 conserved hypothetical protein
pYR1x0140 hypothetical protein
pYR1x0141 hypothetical protein
pYR1x0142 hypothetical protein
pYR1x0143 conserved hypothetical protein
pYR1x0144 Rhs family protein
pYR1x0145 hypothetical protein
pYR1x0146 conserved hypothetical protein
pYR1x0148 transposase TnpA for transposon Tn5393

Fig. 1–*Continued.*

TABLE 1. IncA/C core genome based on positive hybridization of microarray probes

Set or subset of isolates	Total no. of genes detected	Probe description (no. of positive probes representing genes or genetic elements with the same description)
All 59 isolates (core genome)	42	Hypothetical protein (17), conserved hypothetical protein (7), putative membrane protein (4), putative lipoprotein (2), mercuric resistance *merE* (1), outer membrane lipoprotein *blc1* (1), phosphoadenosine phosphosulfate reductase domain protein (1), putative *parB* partition (1), quaternary ammonium compound resistance *sugE1* (1), signal peptide peptidase *sppA* domain (1), staphylococcal nuclease domain protein (1), streptomycin resistance *strA* (1), transposase *insB2* for insertion sequence (IS) IS*26* (1), transposase *insC3* for IS*Ec9* (1)
58 of 59 isolates	39	Hypothetical protein (14), conserved hypothetical protein (7), type IV conjugative transfer system *traABGIV* (5), transposase *tnpA* for transposon Tn*21* and Tn*5393* (2), beta-lactamase bla_{CMY-2} (1), dihydropteroate synthase *sul2* (1), mercuric transport *merT* (1), Ner-like DNA-binding protein (1), putative membrane protein (1), putative *parA* partition (1), putative type IV conjugative transfer system coupling factor (1), streptomycin resistance *strB* (1), tetracycline repressor, *tetAR* class A (2), transposase *insD1* for IS*4321R* (1)
57 of 59 isolates	25	Hypothetical protein (9), conserved hypothetical protein (5), type IV conjugative transfer system *traDEHL* (4), DNA topoisomerase III (1), phage integrase (1), putative exonuclease (1), putative membrane protein (2), resolvase (1), transposase *insA* (1)
31 to 56 isolates of 59 isolates	89	Hypothetical protein (32), conserved hypothetical protein (15), [2Fe-2S]-binding domain protein (1), alcohol dehydrogenase class III (1), alkylmercury lyase *merB* (1), aminoglycoside 3-*N*-acetyltransferase *aac* (1), aminoglycoside 3′-phosphotransferase (1), aminoglycoside adenylyltransferase *aad* (1), ATPase of the AAA family (1), C-5 cytosine-specific DNA methylase (1), cyclic diguanylate phosphodiesterase (EAL) domain (1), dihydropteroate synthase 1 *sul* (1), DNA replication terminus binding site (1), EAL domain *urf2* (2), florfenicol/chloramphenicol resistance *floR*, Hg(II)-responsive transcriptional regulator *merR* (2), integrase *intI1* for transposon Tn*21* (1), mercuric reductase *merA* (1), mercuric resistance *merE* (3), mercuric resistance transcriptional repressor *merD* (2), mercuric transport *merT* (1), mercuric transport periplasmic component *merP* (2), modulator *tnpM* for transposon Tn*21* (1), phage recombination *bet* (1), putative 5′-nucleotidase (1), quaternary ammonium compound resistance protein (1), protein family HMM PF00893 (1), Rhs family protein (1), single-stranded DNA-binding protein (1), transcriptional regulator (1), LysR family protein (1), transglycosylase SLT domain protein (1), transposase *insC* for IS*903* (1), transposase *insE*, transposase *tnpA* for transposon Tn*21* (1)

found positive hybridizations to *aphA* (aminoglycoside 3′-phosphotransferase).

Region 3 (Fig. 1, region column, red bar) of the microarray contains 13 oligonucleotide probes that correspond specifically to pP99-018 genes. Analysis of region 3 showed variability in an alcohol dehydrogenase and one of nine hypothetical proteins that were present. The oligonucleotide probe that corresponds to a transposase (P99018ORFx148) was not present in any of the isolates.

Region 4 (Fig. 1, region column, yellow bar) of the microarray contains 47 oligonucleotide probes that correspond specifically to pSN254 genes. Analysis of region 4 showed one contiguous block from pSN254x0143 to pSN254x0157, the *aadA* region. Region 4 contains oligonucleotides for genes associated with *aadA* and showed evidence of multiple, apparently unlinked insertions/deletions. Oligonucleotide probes associated with tetracycline repressor *tetAR*, bla_{CMY-2}, and *tnpA* for transposase Tn*21* showed positive hybridizations in 98% of the isolates. Oligonucleotide probes associated with *floR* showed positive hybridizations in 90% of the isolates. Oligonucleotide probes associated with mercury resistance (*merA*), aminoglycoside (*aadA*), transposases *insEF* (IS*CR2* and IS*CR16*) and *insAG*, and transposition *tniB* showed the most variability in our population.

Region 5 (Fig. 1, region column, orange bar) of the microarray contains 5 oligonucleotide probes that correspond specifically to pP91278 genes. Analysis of region 5 showed that three of four hypothetical proteins were present in 95% of the isolates. The oligonucleotide probe that corresponds to dihydrofolate reductase (NCBI accession no. YP_908609) was present only in isolate 858.

A summary of positive probes is listed in Table 1. The first row lists the 42 positive probes for the IncA/C core genome of our isolates (i.e., found in all analyzed isolates). These include probes for genes encoding seven membrane proteins, including *blc1*, putative membrane proteins, a putative lipoprotein, and an outer membrane lipoprotein, two antimicrobial resistance genes (*sugE1* and *strA*), heavy metal resistance (*merE*), a signal peptide peptidase (*sppA*), two domain-containing proteins, partition (*parB*), and a few transposases. Twenty-four probes present in all isolates were of hypothetical function. The second row lists positive probes for 39 accessory genes (i.e., genes present in only 58 of 59 analyzed plasmids), including conjugation genes (*traABGIV*), antimicrobial resistance genes (bla_{CMY-2}, *tetAR*, and *sulII* were not present in isolate 527) (*strB*), 3 transposon-related genes, and 21 genes with hypothetical function (Table 1). Some of the 114 additional accessory genes (i.e., genes not present in 31 to 57 analyzed isolates) include conjugation genes (*traDEHL*), antimicrobial resistance genes (*aac*, *aad*, *floR*, *qac*, *strB*, and *sulI*), heavy metal resistance (*merABDERT*), six transposon-related genes, and 61 genes with hypothetical function (Table 1).

IncA/C plasmid core regions were described by Welch et al. and numbered 1 to 12 in numerical order around the plus strand of the IncA/C plasmid (39). We used our microarray hybridization results to detect the presence or absence of these core regions (Table 2). The majority of our plasmids (42 of 59 hybridizations) were positive for all of the probes.

TABLE 2. Summary of positive hybridizations to IncA/C plasmid core regions as detected by microarray

Isolate	IncA/C core regions detected[a]												n[b]
710	1	2	3	4	–	–	7	–	–	10	–	12	1
544[c]	1	2	3	4	5	–	–	–	–	10	11	12	6
604[d]	1	2	3	4	5	6	–	–	–	10	11	12	6
928	1	2	3	4	–	–	7	8	–	10	–	–	1
743	1	2	3	4	5	6	7	–	–	–	–	–	1
728[e]	1	2	3	4	5	6	7	8	–	10	11	12	2
527	1	2	–	4	5	6	7	8	9	10	11	12	1
552[f]	1	2	3	4	5	6	7	8	9	10	11	12	41
Total on array													59

[a] IncA/C plasmid core regions are based upon the assay by Welch et al. (39), in which core regions are numbered in order 1 to 12 around the plus strand of the IncA/C plasmid sequence. Core regions are scored as present if the probes (highlighted in yellow in Fig. 1) were detected by microarray analysis of the isolate. The core region number is shown if the probe was detected by microarray analysis of the isolate. –, absent by microarray analysis.

[b] n is the number of isolates with the same pattern.

[c] Isolates 544, 517, 910, 897, 515, and 911 show the same pattern.

[d] Isolates 604, 894, 518, 918, 915, and 573 show the same pattern.

[e] Isolates 728 and 886 show the same pattern.

[f] Isolates 552, 528, 674, 617, 587, 562, 845, 745, 514, 631, 930, 584, 519, 856, 633, 851, 752, 625, 550, 521, 621, 667, 600, 824, 934, 822, 563, 889, 812, 732, 733, 553, 548, 890, 888, 858, 589, 792, 784, 612, and 649 are positive for all 12 core regions. These regions are identified with the full hybridization data in Fig. 1.

Pairwise linkage disequilibrium (LD) was calculated among all the probes on the array that were not hypothetical or invariantly positive or negative (see Fig. S1 in the supplemental material). The PAR3 array contained 1,395 (22.4%) probe pairs positive for linkage at P values of <0.05 out of the total of 6,216 pairs that were analyzed. The PAR3 array contained nine pairs of antimicrobial gene probes that were contiguous, eight (88.9%) of which showed linkage disequilibrium; 162 pairs were not contiguous, and 26 (16%) of these pairs were linked. The PAR3 array contained 35 pairs of conjugation gene probes that were contiguous, 23 (66%) of which were linked; 85 pairs were not contiguous, and 42 (49%) of these pairs were linked. The PAR3 array contained 25 pairs of transposon gene probes that were contiguous, 11 (44%) of which were linked; 132 pairs were not contiguous, and 23 (17%) of these pairs were linked. The PAR3 array contained 3 pairs of DNA-binding protein gene probes that were contiguous, all 3 (100%) of which were linked; 63 pairs were not contiguous, and 6 (9.5%) of these pairs were linked.

DISCUSSION

The objective of this study was to learn more about the population genetics of IncA/C plasmids by characterizing a diverse population of 59 *S. enterica* isolates from different animal sources. Isolates were hybridized to a plasmid/antimicrobial resistance (PAR) hybridization array with 286 unique oligonucleotide plasmid probes from IncA/C plasmids, and the results were analyzed to determine the core genome and accessory genome in our isolates (Fig. 1 and Table 1). The universally present core gene set appeared to be very limited in expected plasmid maintenance-related genes; *parB* was the only core gene recognized as needed for replication, but there were 30 genes of unknown function in the core set, some of which are likely to be used in plasmid maintenance. The accuracy of the PAR hybridization array was previously analyzed (24) with control strains *S.* Typhi CT18 (26), *Y. ruckeri* strain YR71 (39), and *S.* Typhimurium LT2 (25). All the probes on the array originated from IncA/C sequences; however, it is possible for chromosomal DNA from the tested strains to give a positive hybridization result to some of the IncA/C-associated probes.

Cluster analysis of the oligonucleotide probes on the microarray showed two lineages of IncA/C plasmids that differed mainly in the presence of an insertion, deletion, or combination of the two (indel) of 53 (pYR1x0087 to pYR1x0142) of the 180 oligonucleotide probes that correspond to region 1 (Fig. 1, region column, black bar). The lineage that consists of isolates 928 to 915 (Fig. 1, top row) was 30% (18 of 59) of the total isolates and did not hybridize to approximately 53 oligonucleotide probes from the pYR1 backbone section of the array. Interestingly, this indel occurred at the same location as an integrating and conjugative element (ICE) hot spot 2 (HS2) (40). The indel in the lineage did not hybridize to the probe for the single-strand DNA-binding protein gene (*ssb*); in previous findings, the sequence for *ssb* was present in pYR1, pSN254, pIP1202, p99-018, and p91278 but missing in pRA1 (16). Because the indel was not identical in all the plasmids, it is likely that it was shaped by more than one genetic event. Welch et al. used 12 loci along the pYR1 IncA/C backbone for a PCR-based screening assay (Fig. 1, loci highlighted in yellow) to investigate the core backbone of IncA/C plasmids (39). In this study, we found an indel dividing our IncA/C plasmids into two lineages in which loci 6 to 9 (pYR1x0087 to pYR1x0142) had no or reduced hybridization in one lineage of 17 isolates, and loci 6 to 9 were present in the second lineage of 42 isolates (Fig. 1 and Table 2) (39). In the lineage of 17 isolates with no or reduced hybridization, six isolates were negative for the core regions encompassed by Welch primers 6 to 9 (Fig. 1, loci highlighted in yellow), another six isolates were negative for core regions 7 to 9, and the five remaining isolates were negative for at least one locus in core regions 7 to 9 (Table 2). Core regions 6 through 9 are within a previously defined insertion/deletion (indel) for IncA/C plasmids and are in the same location as an ICE HS2. Forty-one isolates had genes detected in all 12 core regions located around the genome of IncA/C plasmids (Fig. 1 and Table 2). Welch et al. examined 51 IncA/C-positive *Salmonella* isolates recovered from retail meats from 2000 to 2005, and 16% of these isolates did not

contain loci 6 through 9. Here the lineage without Welch loci 6 to 9 by microarray hybridizations comprises 30% of our population. The differences in the two studies could be due to the different isolate sources (retail meats versus veterinary clinical isolates from sick animals) or time frame (2000 to 2005 versus 2005).

In region 1 (Fig. 1), there were 16 contiguous probes (pYR1x0148 to pYR1x0163) associated with a combined Tn*10* and Tn*5393* transposon cassette previously found in IncA/C plasmids (15, 39). In this study, three genes associated with the Tn*5393* transposon were highly conserved, 98% (58 of 59) of our isolates showed positive hybridization to *tnpA* for Tn*5393* (pYR1x0148) and to *strA* and *strB* (pYR1x0162 and pYR1x0163). These genes are part of the Tn*5393* transposon cassette that confers aminoglycoside resistance and that has widespread dissemination (34). The Tn*5393* cassette has been found in the United States associated with the apple and pear tree pathogen *Erwinia amylovora* (9), in Italy from multidrug-resistant *S. enterica* of mostly poultry origin (27), in Norway in the fish pathogen *Aeromonas salmonicida* (20), and in *Pseudomonas syringae* and *Xanthomonas campestris* (35). The remaining 127 oligonucleotide probes in region 1 of the microarray showed large regions of similarity among the 59 isolates hybridized.

In addition to plasmids, there are other mechanisms for the transfer of genes responsible for antibiotic or heavy metal resistance, including transposable elements, such as transposons and their corresponding integrons, ICEs, and insertion sequence common regions (IS*CR*s) (5, 21, 36–38, 40). Probes on the array associated with transposable elements like transposons, ICEs, and IS*CR*s showed the most variability. Analysis showed that the four antimicrobial or heavy metal resistance probes on the array with the greatest variability were aminoglycoside 3′-phosphotransferase (*aphA*, region 2, pIP1202x0052), mercuric transport protein periplasmic component (*merP*, region 2, pIP1202x0159), mercuric reductase (*merA*, region 2, pIP1202x0161), and aminoglycoside adenyltransferase (*aadA*, region 4, pSN254x0144). All four probes had significant linkage to transposon-related probes (see Fig. S1 in the supplemental material). Aminoglycoside 3′-phosphotransferase (*aphA*) provides resistance to kanamycin-type aminoglycoside antibiotics (30). Hybridizations to this array showed 54% (32 of 59) of the isolates with positive hybridization to the *aphA* (pIP1202x0156) probe from *Y. pestis*. Aminoglycoside adenyltransferase (*aadA*, pSN254x0144), which confers resistance to streptomycin, is within an integron designated In*2* (32) and is part of the Tn*21* transposon family (21). Linkage disequilibrium (LD) analysis of our microarray results found that *aadA* was significantly linked to the five Tn*21*-related probes on the array. In pSN254, the *aadA* region includes the *aadA*, *aacC*, *qac*Δ, *sul1*, *groS*, and *groL* genes (6, 16, 39) and had a high degree of variability in our isolates.

This array contains two distinct variants of *mer* operons that contain *merP*, in region 2 from pIP1202 (*Y. pestis* biovar Orientalis strain IP275) and another from region 4 from pSN254 (*S. enterica* subsp. *enterica* serovar Newport strain SL254). Both *mer* operons contain the basic set of Gram-negative *mer* genes, *merRTPADE*, and are associated with transposons. The *Y. pestis* probe set also contains the mercuric transporter *merC*, which is redundant to *merT*. Here we found *merC* less frequently than *merT*, which corresponds with the results from an earlier study (22). The *S. enterica* probe set contains the organomercurial lyase MerB and the corresponding organomercurial responsive version of MerR. It is interesting to note that the mercury resistance genes on the IncA/C backbone were highly conserved in both lineages. The periplasmic MerP protein (*merP*, pIP1202x0159) and the cytosolic mercuric reductase (*merA*, pIP1202x0161) have frequently been found in IncA/C backbones (6). We found significant linkage disequilibrium between *merA* or *merP* probes and tetracycline resistance- and transposon-related probes (see Fig. S1 in the supplemental material). In a phylogenetic study of 213 bacterial *merA* sequences, Barkay et al. found that bacterial and plasmid MerA loci were often associated with insertion elements and integrase or transposase genes (3). Barkay et al. examined MerA evolution and concluded that MerA originated in a thermophilic bacterial lineage after the divergence of the *Archaea* and *Bacteria* domains from the most-recent common ancestor approximately 2.4 billion years ago (3). Mercury resistance could have evolved in the microflora of animals living in or near natural mercury deposits. However, resistance to other heavy metals may be the driving force for selection of these genes. The selective pressure for mercury resistance in humans and animals like the ones in the present study could be attributed to the use of mercury as a therapy for parasites and syphilis from the 17th century to the early 20th century and the current exposure of humans to mercuric mercury from "silver" amalgam dental fillings which is continuously leached into the saliva and gut contents of humans with these fillings (33). Currently, ~35 metric tons of mercury is used each year in the United States in dental fillings.

It is interesting to note the positive hybridization of probes *insE* (IS*CR* element 2 [IS*CR2*]) and *insF* (IS*CR16*) in this study that were present in 34 and 4 isolates, respectively (Fig. 1, region 4). IS*CR*s include elements capable of antibiotic resistance gene capture and movement and can construct clusters of antibiotic resistance genes on chromosomes and in plasmids (36–38). Toleman et al. noted that IS*CR16* had been found in only three IncA/C plasmids at that time (38); here we found it present in four additional IncA/C plasmids. LD analysis of IS*CR2* and IS*CR16* revealed significant linkage to transposon-related mercury resistance and antibacterial resistance probes (see Fig. S1 in the supplemental material).

All of the *S. enterica* isolates in this study except for isolate 527 showed a positive hybridization to the beta-lactamase $bla_{\text{CMY-2}}$ probe. More than one copy of bla_{CMY} is frequently found in *S. enterica* isolates (6, 39); however, this microarray does not distinguish how many copies of each gene is present, it can only distinguish the presence or absence of hybridization to an oligonucleotide that is representative of a gene. Isolate 888 displayed a positive hybridization to the beta-lactamase $bla_{\text{SHV-1}}$ located in region 2 of the array (NCBI accession number YP_001102238). This isolate was assayed by PCR, because bla_{CMY} and bla_{TEM} have been associated with beta-lactam-resistant isolates from *S. enterica* (16, 39, 41), while bla_{SHV} is found in *Klebsiella pneumoniae* (2, 14). The assay failed to detect bla_{SHV}, indicating a false-positive hybridization possibly due to the bla_{SHV} probe cross hybridizing with other detected genes.

Hybridization analysis of the 47 oligonucleotide probes in region 4 of the array displayed the most variability of all re-

gions of the array (Fig. 1). This is interesting because DNA hybridized to the array was extracted from *S. enterica* isolates, which is presumably more closely related than the sequences from the other species. Region 4 of the microarray contains oligonucleotides of genes associated with *merA*, *aadA*, *floR*, and transposases. As expected, oligonucleotide probes associated with tetracycline repressor proteins TetA and TetR and with bla_{CMY-2} and TnpA for transposase Tn*21* were highly conserved with positive hybridizations in 98% of the isolates in this study.

LD analysis revealed that linkage for all groups (antibacterial, conjugation, transposon, and DNA binding) was enriched if the genes were contiguous. Conjugation genes that were not contiguous also showed enrichment for linkage (Fig. 1; see Fig. S1 in the supplemental material). This may reflect selective pressures from the functional properties of the gene products.

In summary, we found that the IncA/C core genome of our isolates (i.e., positive hybridizations in all analyzed isolates) consists of 42 genes of which 24 were for genes encoding hypothetical proteins. IncA/C plasmids occur in two lineages separated by a major insertion-deletion (indel) that contains mostly genes encoding hypothetical proteins. The most variable genes were represented by transposable element-like transposons, IS*CR*s, and ICEs as well as four antimicrobial or mercury resistance genes. Mercury resistance genes are highly conserved in these IncA/C plasmids. Our previous study showed that the associations of *Salmonella* species, IncA/C plasmids, and multidrug resistance (MDR) genes are very old (23). Mercury resistance is also very old (3) and has been associated with plasmids and transposable elements for a long time, so it is not surprising that they have been found together on IncA/C plasmids. On the basis of differences in linkage disequilibrium, our studies suggest that the transfer of antimicrobial resistance determinants by transfer of IncA/C plasmids is somewhat less common than exchange within the plasmids orchestrated by transposable elements. Thus, the IncA/C plasmids have been remodeled by transposons more often than the plasmid was exchanged with other bacterial lineages. The donors of the transposons remain unknown, but they may be other plasmids or conjugative units that are more transient in the bacterial population.

ACKNOWLEDGMENTS

We gratefully acknowledge Anne O. Summers for critically reading the manuscript and for her expertise and input on the mercury resistance operon. We thank Linda Genzlinger, LaShanda Glenn, Tiffanie Woodley, and Tyler Wilcher for technical assistance.

The mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

1. **Alanis, A. J.** 2005. Resistance to antibiotics: are we in the post-antibiotic era? Arch. Med. Res. **36:**697–705.
2. **Babini, G. S., and D. M. Livermore.** 2000. Are SHV beta-lactamases universal in *Klebsiella pneumoniae*? Antimicrob. Agents Chemother. **44:** 2230.
3. **Barkay, T., K. Kritee, E. Boyd, and G. Geesey.** 2010. A thermophilic bacterial origin and subsequent constraints by redox, light and salinity on the evolution of the microbial mercuric reductase. Environ. Microbiol. **12:**2904–2917.
4. **Bennett, P. M.** 2008. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br. J. Pharmacol. **153**(Suppl. 1)**:**S347–S357.
5. **Burrus, V., G. Pavlovic, B. Decaris, and G. Guedon.** 2002. Conjugative transposons: the tip of the iceberg. Mol. Microbiol. **46:**601–610.
6. **Call, D. R., et al.** 2010. bla_{CMY-2}-positive IncA/C plasmids from *Escherichia coli* and *Salmonella enterica* are a distinct component of a larger lineage of plasmids. Antimicrob. Agents Chemother. **54:**590–596.
7. **Carattoli, A.** 2001. Importance of integrons in the diffusion of resistance. Vet. Res. **32:**243–259.
8. **Carattoli, A.** 2009. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob. Agents Chemother. **53:**2227–2238.
9. **Chiou, C. S., and A. L. Jones.** 1993. Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance genes in *Erwinia amylovora* and other Gram-negative bacteria. J. Bacteriol. **175:**732–740.
10. **de Hoon, M. J., S. Imoto, J. Nolan, and S. Miyano.** 2004. Open source clustering software. Bioinformatics **20:**1453–1454.
11. **Durfee, T., et al.** 2008. The complete genome sequence of *Escherichia coli* DH10B: insights into the biology of a laboratory workhorse. J. Bacteriol. **190:**2597–2606.
12. **Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein.** 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. U. S. A. **95:**14863–14868.
13. **Excoffier, L., G. Laval, and S. Schneider.** 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. Online **1:**47–50.
14. **Ford, P. J., and M. B. Avison.** 2004. Evolutionary mapping of the SHV beta-lactamase and evidence for two separate IS26-dependent blaSHV mobilization events from the Klebsiella pneumoniae chromosome. J. Antimicrob. Chemother. **54:**69–75.
15. **Fricke, W. F., et al.** 2009. Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic *Escherichia coli* strains in *Salmonella enterica* serovar Kentucky isolates from poultry. Appl. Environ. Microbiol. **75:**5963–5971.
16. **Fricke, W. F., et al.** 2009. Comparative genomics of the IncA/C multidrug resistance plasmid family. J. Bacteriol. **191:**4750–4757.
17. **Frost, L. S., R. Leplae, A. O. Summers, and A. Toussaint.** 2005. Mobile genetic elements: the agents of open source evolution. Nat. Rev. Microbiol. **3:**722–732.
18. **Frye, J. G., et al.** 2006. DNA microarray detection of antimicrobial resistance genes in diverse bacteria. Int. J. Antimicrob. Agents **27:**138–151.
19. **Frye, J. G., et al.** 2010. Development of a DNA microarray to detect antimicrobial resistance genes identified in the National Center for Biotechnology Information database. Microb. Drug Resist. **16:**9–19.
20. **L'Abee-Lund, T. M., and H. Sorum.** 2000. Functional Tn*5393*-like transposon in the R plasmid pRAS2 from the fish pathogen *Aeromonas salmonicida* subspecies *salmonicida* isolated in Norway. Appl. Environ. Microbiol. **66:**5533–5535.
21. **Liebert, C. A., R. M. Hall, and A. O. Summers.** 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol. Mol. Biol. Rev. **63:**507–522.
22. **Liebert, C. A., A. L. Watson, and A. O. Summers.** 2000. The quality of merC, a module of the mer mosaic. J. Mol. Evol. **51:**607–622.
23. **Lindsey, R. L., P. J. Fedorka-Cray, J. G. Frye, and R. J. Meinersmann.** 2009. IncA/C plasmids are prevalent in multidrug-resistant *Salmonella enterica* isolates. Appl. Environ. Microbiol. **75:**1908–1915.
24. **Lindsey, R. L., J. G. Frye, P. J. Fedorka-Cray, T. J. Welch, and R. J. Meinersmann.** 2010. An oligonucleotide microarray to characterize multidrug resistant plasmids. J. Microbiol. Methods **81:**96–100.
25. **McClelland, M., et al.** 2001. Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature **413:**852–856.
26. **Parkhill, J., et al.** 2001. Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18. Nature **413:**848–852.
27. **Pezzella, C., A. Ricci, E. DiGiannatale, I. Luzzi, and A. Carattoli.** 2004. Tetracycline and streptomycin resistance genes, transposons, and plasmids in *Salmonella enterica* isolates from animals in Italy. Antimicrob. Agents Chemother. **48:**903–908.
28. **Porwollik, S., J. Frye, L. D. Florea, F. Blackmer, and M. McClelland.** 2003. A non-redundant microarray of genes for two related bacteria. Nucleic Acids Res. **31:**1869–1876.
29. **Rasheed, J. K., et al.** 1997. Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob. Agents Chemother. **41:**647–653.
30. **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.
31. **Slatkin, M.** 1994. Linkage disequilibrium in growing and stable populations. Genetics **137:**331–336.
32. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3:**1669–1683.
33. **Summers, A. O., et al.** 1993. Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. Antimicrob. Agents Chemother. **37:**825–834.
34. **Sundin, G. W.** 2002. Distinct recent lineages of the strA-strB streptomycin-

resistance genes in clinical and environmental bacteria. Curr. Microbiol. **45:**63–69.
35. **Sundin, G. W., and C. L. Bender.** 1995. Expression of the *strA-strB* streptomycin resistance genes in *Pseudomonas syringae* and *Xanthomonas campestris* and characterization of IS*6100* in *X. campestris*. Appl. Environ. Microbiol. **61:**2891–2897.
36. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. ISCR elements: novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70:** 296–316.
37. **Toleman, M. A., and T. R. Walsh.** 2008. Evolution of the ISCR3 group of ISCR elements. Antimicrob. Agents Chemother. **52:**3789–3791.
38. **Toleman, M. A., and T. R. Walsh.** 2010. ISCR elements are key players in IncA/C plasmid evolution. Antimicrob. Agents Chemother. **54:**3534.
39. **Welch, T. J., et al.** 2007. Multiple antimicrobial resistance in plague: an emerging public health risk. PLoS One **2:**e309.
40. **Wozniak, R. A., et al.** 2009. Comparative ICE genomics: insights into the evolution of the SXT/R391 family of ICEs. PLoS Genet. **5:**e1000786.
41. **Zhao, S., et al.** 2009. β-Lactam resistance in *Salmonella* strains isolated from retail meats in the United States by the National Antimicrobial Resistance Monitoring System between 2002 and 2006. Appl. Environ. Microbiol. **75:**7624–7630.
42. **Zou, W., et al.** 2009. Microarray analysis of antimicrobial resistance genes in Salmonella enterica from preharvest poultry environment. J. Appl. Microbiol. **107:**906–914.