Molecular Epidemiology of Ampicillin Resistance in Salmonella spp. and Escherichia coli from Wastewater and Clinical Specimens

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Abstract

Molecular epidemiology at local scale in Sicily (Italy) of ampicillin resistance in *Salmonella* spp. isolates from municipal wastewater ($n = 64$) and clinical specimens ($n = 274$) is described in comparison with previously examined *Escherichia coli* isolates ($n = 273$) from wastewater. High prevalence of antibiotic resistance (28.9%) with highest resistance rates against ampicillin (22.7%) was observed in *E. coli* isolates. Different resistance rates were observed in *Salmonella* according to the serovars, with prevalences of the same order in both wastewater and clinical isolates belonging to the same serovar (e.g., 91.7% ampicillin resistance in wastewater isolates vs. 70.8% in clinical isolates of the *Salmonella* serovar Typhimurium and 0% ampicillin resistance in both wastewater and clinical isolates of the *Salmonella* serovar Enteritidis). The β-lactam resistance gene *bla*$_{TEM}$ was present in both wastewater and clinical *Salmonella* spp. isolates, with the exception of *Salmonella enterica* serovar Typhimurium isolates with a typical six-drug resistance pattern AmpChlSulTeStrSp that had the *bla*$_{PSE-1}$ gene. The *bla*$_{TEM}$ gene was present in all the *E. coli* isolates but one had the *bla*$_{SHV}$ gene. Several *E. coli* and some *Salmonella* isolates were positive for class 1 integrons with variable regions of 1.0 or 1.5 kb containing *aadA1*, *dfrA17-aadA5*, or *dfrA1-aadA1* gene cassettes, whereas *Salmonella* serovar Typhimurium isolates with the six-drug resistance pattern were positive for both 1.0 and 1.2 kb integrons. Polymerase chain reaction replicon typing demonstrated the presence of multireplicon resistance plasmids in several isolates of *E. coli*, containing two to four of the replicons IncF, IncI1, IncFIA, and IncFIB, whereas other isolates showed resistance plasmids with only IncF, IncP, or IncK replicons. Replicon IncI1 was detected in one *Salmonella* isolate, whereas other

isolates belonging to different serovars had IncN replicons. Analysis of isolates from wastewater can be a useful epidemiologic tool to monitor the prevalence of antibiotic resistance and genetic elements related to antibiotic resistance in *Salmonella* clones circulating in the human population.

Introduction

Different serovars of *Salmonella* are among the most common bacterial pathogens causing foodborne diseases in developing as well as in developed countries. Misuse and overuse of antimicrobial agents in human and veterinary medicine have caused a dramatic spread of resistances in several of the most frequent serovars. Diverse mobile elements such as plasmids and integrons have played a major role in the horizontal transmission of drug resistance not only among *Salmonella* serovars but also among different bacterial species by intraspecies and interspecies transfer (Hawkey and Jones, 2009). More generally, it is believed that bacteria participate in common gene pools because genetic exchange occurs among bacterial populations coming from different habitats. Municipal wastewater treatment plants, in particular, can constitute a widely accessible drug resistance gene pool allowing genetic exchange owing to the high bacterial densities in their biofilms and flocks (Moura *et al.*, 2007; Schluter *et al.*, 2007).

Commensal enteric bacteria are known to constitute reservoirs of genes determining antibiotic resistance (Shehabi *et al.*, 2006; Su *et al.*, 2006). In particular, commensal *Escherichia coli* can transfer their resistances in the gastrointestinal environment, especially when antimicrobial exposure occurs, determining high prevalence of antibiotic resistance; when this occurs, mobile elements contribute significantly to the resistance spread (Smith *et al.*, 2007). Resistance plasmids (R plasmids) and integrons have been implicated in this spread among the Gram-negative bacteria, especially enterobacteria, including various *Salmonella* serovars. The ability of bacteria to acquire and disseminate exogenous genes via mobile genetic elements such

as plasmids and transposons has been the major factor in the development of multiple drug resistance over the last 50 years (Hawkey and Jones, 2009).

Molecular methods applied to the study of R plasmids and integrons are of capital importance to understand the epidemiology of antibiotic resistance among bacterial and human populations. However, understanding the epidemiological role of different genetic elements implicated in antibiotic resistance is complex because of the diversity and promiscuity of these elements (Lévesque *et al.*, 1995). In some instance, molecular typing has been proved to be able to describe the dissemination and to follow the evolution of particular resistance plasmids at large scale (Carattoli *et al.*, 2002; Hopkins *et al.*, 2006). At local scale, the study of enterobacteria isolates from urban wastewater could be a useful tool to easily determine the range of antibiotic resistance in the bacterial populations colonizing the intestine of residents.

In a previous paper (Pignato *et al.*, 2009) we have evaluated the prevalence of antibiotic resistance among *E. coli* isolates from raw and treated municipal wastewater used in agriculture, showing high rates of multiple resistance, including resistance against ampicillin. Several ampicillin-resistant isolates were able to transfer *en bloc* their resistance patterns by conjugative R plasmids and some were proved to carry class 1 integrons with gene cassettes of size 1.0 or 1.5 kb. On the basis of these results we concluded that antibiotic-resistant *E. coli* surviving treatment processes can act as a reservoir of antibiotic resistance genes, particularly ampicillin resistance, which can be spread to the pathogenic and commensal enteric bacteria in human and animal consumers of crop irrigated by wastewater.

As resistance against ampicillin was the most frequent resistance in the E. coli isolates previously examined, the objectives of this study were (i) to determine the prevalence of ampicillin resistance among Salmonella spp. isolates from municipal wastewater and clinical specimens obtained in the same geographic location during 1 year of monitoring; (ii) to detect

and characterize bla genes, class 1 integrons, and replicon types in ampicillin-resistant Salmonella spp. and in E. coli isolated from the same wastewater effluents; and (iii) to investigate eventual relatedness among plasmids and integrons implicated in ampicillin resistance of the Salmonella spp. and E. coli isolates.

These objectives were aimed at investigating whether the study of isolates from wastewater can be a useful and easy to perform epidemiologic tool to monitor the prevalence of antibiotic resistance and genetic elements related to drug resistance in Salmonella clones circulating in the human population.

Materials and Methods

Isolation and identification of bacteria

Wastewater samples (108 samples in all, 36 from crude, 36 from treated, and 36 from refined wastewater) from two towns (Caltagirone and San Michele di Ganzaria) located in the district of Catania, in eastern Sicily (Italy), were collected from the respective municipal treatment plants and processed every 2 weeks (Plant A) or every month (Plant B) over a 1-year period, from January to December 2004, as previously described (Pignato *et al.*, 2009). No wastes from farms or slaughterhouses inflowed to the urban wastewater that were treated in the two plants. *E. coli* isolates were those analyzed in a previous study (Pignato *et al.*, 2009). They were isolated by standard membrane filtration technique (APHA AWWA WEF, 1995) using 47-mm acetate membrane filters (Sartorius AG, Goettingen, Germany) with a nominal pore size of 0.45 μm, which were placed on the surface of plates of CHROMagar *E. coli* (CHROMagar, Paris, France), a chromogenous selective medium. After 24 h of incubation at 37°C, plates were inspected for growth and two or three colonies per plate showing the typical morphological characteristics of *E. coli* were selected for biochemical identification by the API 20E system (bioMérieux, Marcy

L'Etoile, France). *Salmonella* spp. was detected and quantified from wastewater by a most probable number procedure using Salmosyst broth base and Salmosyst selective supplement tablets (Merck, Darmstadt, Germany) as a liquid medium for preenrichment (6 h at 37°C in Salmosyst broth base) and selective enrichment (18 h at 37°C in the same tube of Salmosyst broth base after adding a tablet of selective supplement) and Rambach agar (Merck) for plating according to a procedure for rapid detection and isolation of these bacteria (Pignato *et al.*, 1995). Red colonies that developed after 24 h of incubation at 37°C (five colonies from each plate) were submitted to biochemical identification by API 20E system and serotyping by polyvalent and monovalent anti-O and anti-H sera (either Bio-Rad, Marnes la Coquette, France, or Biogenetics, Ponte San Nicolò, Padova, Italy). One isolate of each serovar obtained from each positive sample was retained for the resistance study. During the same 1-year period, *Salmonella* spp. was also isolated from clinical specimens (feces) of patients hospitalized for gastroenteritis at the district hospital (Gravina Hospital) of one of the two towns (Caltagirone, CT) conferring its wastewater to the monitored plant A. Feces were processed by standard microbiological methods and plated on Rambach agar. Red colonies that developed on Rambach agar from wastewater or feces after 24 h of incubation at 37°C were submitted to biochemical identification as described earlier.

Isolates from wastewater

A total of 273 previously examined *E. coli* isolates and 64 *Salmonella* isolates from wastewater were studied. The following serovars were examined: *S. enterica* subsp. *enterica* serovars Typhimurium ($n = 36$), Enteritidis ($n = 6$), Blockley ($n = 4$), Napoli ($n = 4$), Newport ($n = 3$), Bovismorbificans ($n = 2$), Braenderup ($n = 2$), Corvallis ($n = 1$), Infantis ($n = 1$), Kenya ($n = 1$), Montevideo ($n = 1$), and Muenster ($n = 1$), *S. enterica* subsp. *salamae* serovar 6,7:b:z_{39} ($n = 1$), and *S. enterica* subsp. *diarizonae* serovar 61:r:z ($n = 1$).

Isolates from clinical specimens

A total of 274 *Salmonella* isolates from feces of patients suffering from gastroenteritis were studied. The following serovars were identified: *S. enterica* subsp. *enterica* serovars Typhimurium ($n = 144$), Enteritidis ($n = 62$), Blockley ($n = 18$), Napoli ($n = 13$), Muenchen ($n = 9$), Newport ($n = 7$), Goldcoast ($n = 3$), Heidelberg ($n = 3$), Bovismorbificans ($n = 2$), Braenderup ($n = 2$), Infantis ($n = 2$), Bredeney ($n = 1$), Derby ($n = 1$), Hadar ($n = 1$), Isangi ($n = 1$), Kenya ($n = 1$), Muenster ($n = 1$), and Panama ($n = 1$) and *S. enterica* subsp. *salamae* serovars $41:z_{10}:1,2$ ($n = 1$) and $42:b:e,n,x,z_{15}$ ($n = 1$).

Antibiotic susceptibility testing

Isolates were screened for resistance to eight antibiotics (Sigma-Aldrich, Steinheim, Germany) by streaking a loopfull of 18-h broth cultures on Mueller–Hinton agar (Oxoid, Hampshire, England) plates containing ampicillin (Amp) 32 µg/mL, chloramphenicol (Chl) 32 µg/mL, sulphamethoxazole (Sul) 512 µg/mL, tetracycline (Te) 16 µg/mL, trimethoprim 10 µg/mL, streptomycin (Str) 64 µg/mL, kanamycin 64 µg/mL, and nalidixic acid (Nal) 64 µg/mL. Plates were incubated for 24 h at 37°C and isolates that yielded bacterial growth were recorded as resistant to the corresponding antibiotic. The antibiotic concentrations were some above the recommended cutoff points. These concentrations were adopted to avoid ambiguous results. Therefore, the prevalence of resistant isolates might have been underestimated. A selected number of ampicillin-resistant isolates (36 *Salmonella* and 30 *E. coli* isolates) exhibiting representative antibiotic resistance patterns were tested by the disk diffusion method with 32 antimicrobial drugs (Bio-Rad). Isolates were categorized as susceptible, intermediate, or resistant, according to the Antibiogram Committee of the French Society for Microbiology cutoff values (www.sfm.asso.fr/publi/general.php?pa=1).

Resistance transfer determination

In total, 66 ampicillin-resistant isolates (30 *E. coli*, 8 *Salmonella* from wastewater, and 28 *Salmonella* from feces), representative of different antibiotic resistance patterns, were investigated for the transferability of their resistance traits to *E. coli* C1a (*nalA*) and J5 (*rif*). The donor and recipient strains were grown in TSB (Difco, Detroit, MI) to logarithmic phase and then mixed in equal volumes (1 mL + 1 mL) and incubated at 37°C for 18 h. Transconjugant clones were selected on Mueller–Hinton agar containing ampicillin (100 μg/mL) and nalidixic acid (64 μg/mL) or rifampicin (250 μg/mL). Transfer of the resistances was confirmed by testing the transconjugants against the antibiotics corresponding to the donor resistances.

PCR amplification and DNA sequencing

Total DNA was extracted using the InstaGene matrix kit (Bio-Rad) according to the manufacturer's recommendations. The ampicillin resistance genes, bla_{TEM}, bla_{SHV}, bla_{PSE-1}, and bla_{OXA-1} group, and class 1 integron gene cassettes were amplified by polymerase chain reaction (PCR) as described previously (Lévesque *et al.*, 1995; Weill *et al.*, 2006).

Sequencing was performed at the "Plateforme de Génotypage des Pathogènes et Santé Publique, PF8" (Institut Pasteur, Paris, France). The nucleotide sequences and the deduced protein sequences were analyzed with EditSeq and Megalign software (Dnastar, Madison, WI). The BLASTN program of National Center for Biotechnology Information was used for database searches (www.ncbi.nlm.nih.gov/BLAST/).

Replicon typing

PCR-based replicon typing was performed according to Carattoli et al. (2005) to type the R plasmids carried by 17 E. coli C1a transconjugants obtained from isolates of Salmonella and E. coli. Eighteen primer pairs were used to perform simplex PCRs, which recognized HI1, HI2, I1-IY, X, L/M, N, FIA, FIB, W, Y, P, FIC, A/C, T, FIIA, F, K, and B/O replicons.

Results

The numbers and percentages of Salmonella and E. coli isolates resistant to one or more antibiotics and also numbers and percentages of isolates resistant to ampicillin are reported in Table 1. Isolates of Salmonella showed different degrees of antibiotic resistance according to the serovars, with prevalences, including ampicillin resistance prevalence, of the same order in isolates from wastewater and in isolates from clinical specimens. Also, resistance profiles were similar in isolates from wastewater and clinical specimens according to the serovar. In particular, the six-drug resistance pattern AmpChlSulTeStrSp was observed with a similar order of frequency in Salmonella serovar Typhimurium isolates from wastewater and clinical specimens, as further specified below.

Antibiotic resistance of isolates from clinical specimens

Out of 144 S. enterica serovar Typhimurium isolates, 24 were sensitive to all tested antibiotics (16.7%), whereas resistance to ampicillin, alone or associated with other resistances, was shown by 102 (70.8%). The resistance pattern AmpChlSulTeStrSp was detected in 32 (22.2%) isolates. Ampicillin resistance alone or associated with other resistances was also detected in Salmonella serovars Blockley, Heidelberg, Goldcoast, and Napoli. Ampicillin resistance was observed neither among the 62 isolates of Salmonella serovar Enteritidis nor among isolates of other less frequent serovars.

Antibiotic resistance of isolates from wastewater

Out of 36 *S. enterica* serovar Typhimurium isolates, only one (2.8%) was sensitive to all tested antibiotics, 35 (97.2%) were resistant to one or more antibiotics, including 33 (91.7%) resistant to ampicillin, and 18 (50%) were resistant to six antibiotics (resistance type

AmpChlSulTeStrSp). Ampicillin resistance alone or associated with other resistances was also detected in *S. enterica* subsp. *enterica* serovars Blockley and Napoli, and in *S. enterica* subsp. *salamae* serovar 6,7:b:z$_{39}$. No resistant isolates were found among isolates of other serovars. Antibiotic resistance of 273 *E. coli* isolates has been reported in a preceding study (Pignato *et al.*, 2009).

Beta-lactam resistance genes

The blaTEM gene was present in the Salmonella spp. isolates from wastewater and clinical specimens, with the exception of one isolate that did not have any of the bla genes tested and the Salmonella serovar Typhimurium isolates exhibiting resistance patterns AmpChlSulTeStrSp or AmpChlSulTeStrSpNal had the blaPSE-1 gene. The blaTEM gene was also present in all but one ampicillin-resistant E. coli isolates that had the blaSHV gene (Table 2).

Resistance transfer

Salmonella transconjugants were recovered from two isolates of serovar Blockley and one each of serovars Goldcoast and Heidelberg (Table 2). Among 30 E. coli isolates from wastewater, 13 transferred their resistances as previously reported (Pignato et al., 2009).

Integron detection and characterization

The presence of class 1 integrons, the size of their variables regions, and their gene cassettes are shown in Table 2. Class 1 integrons of 1.0 and 1.2 kb together were detected in all the *Salmonella* serovar Typhimurium isolates with resistance pattern AmpChlSulTeStrSp from wastewater and clinical specimens. Of the *Salmonella* spp. isolates from wastewater, only *S. enterica* subsp. *salamae* serovar 6,7:b:z$_{39}$ was positive for a class 1 integron with *dfrA1-aadA1*

gene cassettes. The same gene cassettes, *dfrA1-aadA1*, were detected in the *Salmonella* serovar Goldcoast isolate from clinical specimen. Of the 30 *E. coli* isolates, 9 (30%) were positive for class 1 integrons with a variable region of 1.0 kb (2 isolates) or 1.5 kb (7 isolates). In all, three distinct integron profiles were identified. The two 1.0-kb integrons contained only one gene cassette, *aadA1*, whereas the integrons of 1.5 kb contained two gene cassettes: *dfrA17-aadA5* (two isolates) or *dfrA1-aadA1* (five isolates).

Replicon typing

All the transconjugants were successfully typed by PCR replicon typing, and some isolates proved to carry multireplicon plasmids (Table 2). The majority of the E. coli plasmids (11 out of 13), independently from the carried resistance genes, were positive for the IncF replicon alone or associated with IncFIB (four replicons), IncI1 (three replicons), or IncFIA (two replicons), whereas other plasmids were positive for IncP or IncK replicons. The IncI1 replicon was also detected in Salmonella serovar Goldcoast R plasmid, whereas IncN replicons were detected in the R plasmids of two Salmonella serovar Blockley and one Salmonella serovar Heidelberg isolates sharing similar resistance patterns.

Discussion

Our study was primarily focused on the prevalence of ampicillin resistance in *Salmonella* isolates from wastewater compared with those from clinical specimens. Quite different rates of ampicillin resistance were observed in the *Salmonella* isolates according to the serovar. In particular, isolates of the more prevalent serovars, Typhimurium and Enteritidis, differed distinctly for their susceptibility to antibiotics. All the wastewater and clinical *Salmonella* serovar Enteritidis isolates were susceptible to ampicillin, with low percentages of resistance to other

antibiotics, whereas high percentage of ampicillin resistance was found in *Salmonella* serovar Typhimurium isolates of both origin.

An increase of ampicillin-resistant S. enterica serovar Typhimurium isolates has been observed during the 1990s because of the emergence of an epidemic multidrug-resistant (MDR) strain of definitive phage type (DT) 104. This MDR DT104 clone first emerged in the United Kingdom at the end of the 1980s and has become a major cause of illness in humans and animals in Europe, including Italy, and in the United States (Casin et al., 1999; Glynn et al., 1999; Threlfall, 2000; Carattoli et al., 2002; Mammina et al., 2002; Weill et al., 2006). The multiple antibiotic resistance was due to chromosomal integration of a 43-kb structure called Salmonella genomic island 1 (SGI1), which comprises the blaPSE-1 gene coding for resistance to ampicillin (Boyd et al., 2001). The multidrug resistance region is located at the 3′ end of the SGI1 on a 13-kb region corresponding to a large class 1 integron (Boyd et al., 2001; Levings et al., 2005).

In Italy, MDR *Salmonella* serovar Typhimurium DT104 is of particular concern, as it represents 20% of all human *Salmonella* isolates (Cawthorne *et al.*, 2006). Indeed, the *Salmonella* serovar Typhimurium is the most prevalent serovar accounting for about 40% of all human *Salmonella* isolates each year and 50% of its isolates belong to the DT104 clone (Cawthorne *et al.*, 2006). In this study, isolates that likely belong to the DT104 clone (similar resistance patterns, presence of the bla_{PSE-1} gene, and class 1 integrons with two variable regions of 1.0 and 1.2 kb), although phage typing was not performed, represented about 50% (18/36) of the *Salmonella* serovar Typhimurium isolates from wastewater. These DT104-like isolates were recovered during the 1 year of monitoring from both the treatment plants, indicating the persistent endemic circulation of this clone in the population of the cities conferring their wastewater to the monitored plants. Further, DT104-like isolates were also isolated from clinical specimens although at lower frequency (32/144, 22.2%). Beside this clone, at least another ampicillin-

resistant clone characterized by more restricted resistance patterns, presence of the bla_{TEM} gene, and absence of class 1 integrons was endemic in the same population.

Molecular characterization of selected ampicillin-resistant *E. coli* isolates from wastewater showed that they constituted a rich reservoir of transposable genetic elements. According to the results of other studies (Thomas and Nielsen, 2005; Schluter *et al.*, 2007), it appeared that genetic exchanges could have been occurred among these isolates during their circulation in the intestinal and/or in the wastewater environment, because they shared quite common genetic traits, such as β-lactamase gene type bla_{TEM} and integrons of 1.0 or 1.5 kb with resistance cassettes carrying *dfrA1* or *dfrA17* genes encoding resistance to trimethoprim and *aadA1* or *aadA5* genes encoding resistance to streptomycin. The results of PCR replicon typing showed a variety of conjugative R plasmids that shared some more prevalent replicons of the same Inc type. PCR replicon typing has been proved as a sensitive and specific method for identifying phylogenetically related plasmids, opening the possibility of successfully detecting and tracing the diffusion of plasmids and resistance genes (Carattoli *et al.*, 2006). In our experiments, some replicon types were shared by different *E. coli* and *Salmonella* isolates showing other common genetic traits. This suggests the possibility that intraspecies and interspecies genetic exchanges have occurred in our environmental and epidemiologic conditions within enteric pathogen and commensal (not only *E. coli*) microorganisms. Further, considering the variability of the resistance patterns exhibited by the different isolates, gain and/or loss of additional resistance determinants and gene-associated functions have probably occurred across different bacterial clones in humans and/or in the wastewater.

In conclusion, our study indicates that analysis of isolates from wastewater can be a useful and easy epidemiologic tool to monitor the prevalence of antibiotic resistance and genetic elements related to antibiotic resistance in *Salmonella* clones circulating in the human population.

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Table 1. Antibiotic resistance of *Salmonella* spp. isolates from wastewater (WW) and clinical specimens (faeces = F) and *Escherichia coli*

Species and serovars	Origin	No. of isolates	No. of resistant isolates^	No. of isolates resistant to ampicillin
Salmonella enterica subsp. *enterica* serovar Typhimurium				
	WW	36	35 (97.2%)	33 (91.7%)
	F	144	120 (83.3%)	102 (70.8%)
serovar Enteritidis				
	WW	6	0	0
	F	62	11 (17.7%)	0
serovar Blockley				
	WW	4	4 (100%)	4 (100%)
	F	18	18 (100%)	18 (100%)
serovar Napoli				
	WW	4	4 (100%)	2 (50%)
	F	13	10 (76.9%)	6 (66.7%)
serovar Muenchen				
	F	9	0	0
serovar Newport				
	WW	3	0	0
	F	7	0	0
serovar Goldcoast				
	F	3	3	3
serovar Heidelberg				
	F	3	3	3
Salmonella enterica subsp. *salamae* serovar 6,7:b:z_{39}				
	WW	1	1	1
Other serovars*				
	WW	10	0	0
Other serovars**				
	F	15	0	0
*Escherichia coli****				
	WW	273	79 (28.9%)	62 (22.7%)

^ Isolates resistant to one or more antibiotics
* *S. enterica* subsp. *enterica* serovars Bovismorbificans, Braenderup, Corvallis, Infantis, Kenya, Montevideo, Muenster and *S. enterica* subsp. *diarizonae* serovar 61:r:z
** *S. enterica* subsp. *enterica* serovars Bovismorbificans, Braenderup, Bredeney, Derby, Hadar, Infantis, Isangi, Kenya, Muenster, Panama and *S. enterica* subsp. *salamae* serovars 41:z_{10}:1,2 and 42:b:e,n,x,z_{15}
*** Resistances of *E. coli* isolates have been reported in a previous paper (Pignato *et al.*, 2009)

Table 2. Antibiotic resistance patterns, *bla* gene and class 1 integron characterization, resistance traits transfer to *Escherichia coli* C1a and J5, and replicon typing of a selected number of ampicillin resistant *E. coli* and *Salmonella* spp. isolates from wastewater (WW) and clinical specimens (faeces = F)

Species and strains	Origin	Resistance pattern	*bla* gene	Class 1 Integron (size in kb) [gene cassette]	Transferred resistance traits	Replicons typed by PCR
Plant A						
Escherichia coli						
DE43	WW	Amp	TEM	ND	-	
BR41	WW	AmpTe	TEM	ND	AmpTe	F, FIA, FIB, I1
RA3	WW	AmpStrTe	TEM	ND	-	
BR35	WW	AmpTeKan	TEM	ND	AmpTeKan	F, FIA, I1
RA10	WW	AmpSulTe	TEM	ND	-	
BR76	WW	AmpSulTe	TEM	ND	-	
BR77	WW	AmpSulTe	TEM	ND	-	
BR52	WW	AmpSulStr	TEM	ND	-	
DE18	WW	AmpChlSulStr	TEM	1.0 [*aadA1*]	AmpChlSulStr	F
BR75	WW	AmpChlSulStr	TEM	1.0 [*aadA1*]	-	
RA8	WW	AmpTeStrNal	TEM	ND	-	
BR5	WW	AmpChlSulTeStrKan	TEM	ND	-	
BR51	WW	AmpChlSulStrTmp	TEM	ND	AmpSulStrTmp	F
BR21	WW	AmpSulTeStrTmp	TEM	1.5[*dhfrA1-aadA1*]	AmpSulTeStrTmp	F, FIB
DE3	WW	AmpSulTeStrTmp	TEM	1.5[*dhfrA1-aadA1*]	AmpSulTeStrTmp	F, FIB
BR23	WW	AmpSulTeStrTmp	TEM	1.5[*dhfrA1-aadA1*]	AmpSulTeStrTmp	F, FIB
DE45	WW	AmpChlSulTeStrTmp	TEM	1.5[*dhfrA1-aadA1*]	AmpChlSulTeStrTmp	P
RA82	WW	AmpSulTeStrKanTmp	TEM	ND	AmpSulTeStrKanTmp	F, I1
DE17	WW	AmpSulTeStrTmpNal	TEM	1.5[*dhfrA17-aadA5*]	-	
RA17	WW	AmpSulTeStrTmpNal	TEM	1.5[*dhfrA17-aadA5*]	-	
Salmonella enterica subsp. *enterica* serovar Typhimurium						
1316	F	Amp	TEM	ND	-	
1305	F	AmpSulStr	TEM	ND	-	
1278	WW	AmpTeStr	TEM	ND	-	
1355	F	AmpTeStr	TEM	ND	-	
1390	F	AmpTeStr	TEM	ND	-	
1352	F	AmpChlTeStr	NI	ND	-	
1285	F	AmpSulTeStr	TEM	ND	-	
1287	F	AmpSulTeStr	TEM	ND	-	
1292	F	AmpSulTeStr	TEM	ND	-	
1298	F	AmpSulTeStr	TEM	ND	-	
1303	F	AmpSulTeStr	TEM	ND	-	
1306	F	AmpSulTeStr	TEM	ND	-	
1307	F	AmpSulTeStr	TEM	ND	-	
1251	WW	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1309	WW	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1414	WW	AmpChlSulTeStrSpNal	PSE-1	1.0, 1.2	-	
1286	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1296	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1297	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1324	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1347	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1349	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1351	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1354	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1392	F	AmpChlSulTeStrSpNal	PSE-1	1.0, 1.2	-	
1403	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
1410	F	AmpChlSulTeStrSpNal	PSE-1	1.0, 1.2	-	
1411	F	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
Salmonella enterica subsp. *salamae* serovar 6,7:b:z$_{39}$						
1	WW	AmpSulStrKanTmp	TEM	1.5[*dhfrA1-aadA1*]	-	
Salmonella enterica subsp. *enterica* serovar Goldcoast						
1284	F	AmpSulTeStrKanTmp	TEM	1.5[*dhfrA1-aadA1*]	AmpSulStrTmp	I1

Salmonella enterica subsp. *enterica* serovar Napoli

24	WW	Amp	TEM	ND	-	

Salmonella enterica subsp. *enterica* serovar Blockley

1315	F	AmpSulTeTmp	TEM	ND	AmpSulTeTmp	N
1317	F	AmpSulTeTmp	TEM	ND	AmpSulTeTmp	N

Salmonella enterica subsp. *enterica* serovar Heidelberg

1348	F	AmpSulTeStrTmp	TEM	ND	AmpSulTeStrTmp	N

Plant B

Escherichia coli

BR80	WW	AmpTe	TEM	ND	AmpTe	F
BR81	WW	AmpTe	TEM	ND	AmpTe	F
DE86	WW	AmpTe	TEM	ND	-	
BR79	WW	AmpChlSulStr	SHV	ND	-	
RA56	WW	AmpChlSulTeStr	TEM	ND	-	
RA59	WW	AmpSulStrTmp	TEM	1.5[*dhfrA1-aadA1*]	AmpSulStrTmp	K
BR66	WW	AmpTeStrNal	TEM	ND	-	
RA56	WW	AmpSulTeStrNal	TEM	ND	-	
BR65	WW	AmpSulTeStrNal	TEM	ND	-	
RA70	WW	AmpSulTeStrTmp	TEM	ND	AmpStr	F

Salmonella enterica subsp. *enterica* serovar Typhimurium

2	WW	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	
9	WW	AmpChlSulTeStrSp	PSE-1	1.0, 1.2	-	

Amp = ampicillin; Chl = chloramphenicol; Sul = sulphamethoxazole; Te = tetracycline; Str = streptomycin; Sp = spectinomycin; Kan = kanamycin; Tmp = trimethoprim; Nal = nalidixic acid

ND = None detected

NI = None of the *bla* genes tested was identified

- = No resistance transfer