RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Analysis of in-plant Factors and Genotyping of Antimicrobial Resistance among Four Organisms Isolated from Swine Processing Plants **- NPB-06-162** revised

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Industry Summary

We investigated the role of factors within processing plants including environmental and body site sources for carriage of for food safety significant pathogens and dissemination of antimicrobial resistance factors among them. The objectives of the study were to determine the role of plant size and other risk factors on prevalence of resistant foodborne and commensal organisms, identify the clonality of major foodborne pathogens particularly *Salmonella* and *Campylobacter* recovered from small and large size processing plants in two predominant pig producing states (NC and IA) and to determine the similarities of antimicrobial resistance genes to better understand the likelihood of dissemination among the four important pathogens. In all four pathogens the proportion of positive samples from lairage was higher (30 to 52%) compared to other locations. It was also found that small plants had a higher percentage of pansusceptible isolates (27.4%) compared to the large processing plants (10.6%). All organisms were highly resistant to tetracycline, with the lowest prevalence in *Salmonella* at 69.4%. Multidrug resistance was common among isolates from both small and large processing plants, however, relatively higher in later ones. Further investigation showed that about half (47.3%) of resistance genes tested were found in at least two different bacterial organisms. *Escherichia* and *Salmonella* from the same animal were most commonly found to be carrying the same resistance genes.

Scientific Abstract

The importance of *Salmonella*, *Campylobacter*, *Escherichia,* and *Enterococcus* as carriers of antimicrobial resistance is well known, but limited work has been done to examine the relationship between this phenotypic characteristic and genotypic attributes among strains isolated in similar settings and time points. The specific objectives of the study were to determine the role of plant size and origin of samples on prevalence of resistant foodborne and commensal organisms, identify the clonality of major foodborne pathogens particularly Salmonella and Campylobacter recovered from small and large size processing plants in two predominant pig producing geographic locations (NC and IA) and to determine the distribution and similarity of specific antimicrobial resistance genes and class-1 integrons among the four organisms from swine production. Isolates were collected from processing plants in North Carolina and Iowa. Phenotypic characterization of antimicrobial resistance patterns was completed on 1,284 samples. DNA microarray was used for gene detection in 19 selected samples. PCR of resistance genes and class-1 integrons was performed on 128 isolates. Select resistance genes were sequenced based on PCR results. In all four pathogens the proportion of positive samples from lairage was higher (30 to 52%) compared to other locations. It was also found that small plants had a higher percentage of pansusceptible isolates (27.4%) compared to the large processing plants (10.6%). Phenotypically, all organisms were highly resistant to tetracycline, with the lowest prevalence in *Salmonella* at 69.4%. Multidrug resistance was common among isolates from both small and large processing plants, however, relatively higher in later ones. Microarray results showed that 47.3% of resistance genes tested were found in at least two different bacterial organisms. Within the samples that tested PCR positive for *tetA(B), strA, strB,* and *apha1-Iab*, there were multiple groups of organisms that were from the same animal. After sequencing, *strB and aphA1-Iab* genes with 98.6% or greater homology were found in different organisms (*Salmonella* and *E. coli*) from the same animal host. Class-1 integrons were found in 18.2% of the samples. Large (4kb) integrons were found in *Salmonella* serovar Havana.Large integrons among MDR *Salmonella* is of high significance since integrons facilitate the carriage and dissemination of multi-drug resistance. Overlapping of resistance genes found within the four organisms may be the result of horizontal resistance gene transfer within the host. Further characterization via conjugation is planned.

Introduction

The importance of *Salmonella*, *Campylobacter*, *E.coli*, and *Enterococcus* as carriers of antimicrobial resistance is well known and characterized. These pathogenic and commensal organisms are commonly isolated from the intestinal tract of swine. The interactions that occur between these organisms within the host intestines could potentially involve the transmission of

antimicrobial resistance genes via plasmids, transposons, and phages. Examples of such interactions have been reported previously. Such examples include identifying plasmids carrying CMY-2 genes within *E.coli* and *Salmonella,* which were found to be significantly similar through Southern Blot hybridization studies (Winokur, Vonstein, et al. 2001). Also, it has been suggested that the bacteriophages located in the gut may be involved in the horizontal transfer of resistance genes, particularily within Salmonella species. Two bacteriaphages, ES18 and PDT17, have been shown to transduce the antibiotic resistance genes in S. Typhimurium DT104. This observation is consistent with the fact that the core resistance genes in DT104 strains are chromosomally located in a tight cluster as part of Salmonella genomic island I (Cloeckaert, Boumedine, et al. 2000) (Schmieger, Schicklmaier, et al.1999) (Braddan, Hite, et al 2005) . Although there has been some work done, more information is needed to understand the interactions between a broader spectrum of enteric organisms, including both gram negative and gram positive.

STATED OBJECTIVES FROM ORIGINAL PROPOSAL

1) To determine the role of plant size and associated transportation, holding and in-plant practices and intervention measures on prevalence of resistant foodborne and commensal organisms.
2) To determine the clonality of strains (particularly *Salmonella* and *Campylobacter*) identified from different size processing plants in two predominant pig producing geographic locations (NC and IA).
3) To determine the distribution and similarity of specific antimicrobial resistance genes and class-1 integrons among four important organisms (*Salmonella, Campylobacter*, *E. coli* and enterococci) in various processing settings.

Materials and Methods

This project is a collaborative effort among three institutions including the USDA-ARS and also the Research Triangle Institute International (RTI). All the bacterial isolates of the four organisms were initially isolated by the USDA-ARS laboratory in Athens, Georgia as part of a different research project prior to the current one (Table 1. Isolates were collected from small (n=782) and large (n=502) processing plants in North Carolina and Iowa. Samples were taken from the head meat, carcass, and lairage swabs and superficial inguinal and mesenteric lymph nodes. All bacterial isolation was conducted in USDA-ARS laboratory and preliminary results was as shown in Table 1

Sample type	*E.coli* n (%)	*Salmonella* n (%)	*Enterococcus* n (%)	*Campylobacter* n (%)
Head Meat Swab (n=330)	290 (87.9)	74 (22.4)	245 (74.2)	32 (9.7)
Carcass Swab (n=330)*	203 (61.5)	50 (15.2)	205 (62.1)	14 (4.4)
Superficial Inguinal Lymph Node (n=344)	37 (10.8)	9 (2.6)	76 (22.1)	2 (0.6)
Mesenteric Lymph Node (n=330)	48 (14.6)	139 (42.1)	97 (29.4)	56 (17.0)
Lairage Swab (n=330)	308 (93.3)	289 (87.6)	286 (86.7)	46 (13.9)
Total no. of isolates	886	561	909	150

Table 1: Origin of isolates from various tissues and lairage swabs in small and large pork processing plants from North Carolina and Iowa.

Antimicrobial susceptibility testing was done on 1284 samples by the USDA-ARS using microbroth dilution (Sensititre). The panel of antimicrobials tested differed among the four pathogens. For *Campylobacter* a total of nine antimicobirals were tested, including azithromycin (Ar), ciprofloxacin (Cip), clindamycin (Ca), erythromycin (Er), florfenicol (Ff), gentamicin (Gm), nalidixic acid (Ni), telithromycin (Tt), and tetracycyline (Te) were tested. A total of 15 antimicrobials were tested against the *E.coli* isolates, including amikacin (An), amoxicillin/clavulanic acid (Ax), ampicillin (Am), cefoxitin (Fx), ceftiofur (Cf), ceftrixone (Ce), chloramphenicol (Cl), ciprofloxacin (Cip), gentamicin (Gm), kanamycin (Km), naldixic acid (Ni), streptomycin (St), sulfa (Su), tetracycline (Te), and triple sulfa (Ts). *Enterococcus* isolates were tested against a total of 17 antimicrobials, including bactracin zinc (Bc), chloramphenicol (Cl), ciprofloxin (Cip), daptomycin (Dp), erythromycin (Er), flavomycin (Fv), gentamicin (Gm), kanamycin (Km), lincocin (Ln), linezolid (Lz), penicillin (Pn), nitrofurantoin (Nf), streptomycin (St), synercid (Sy), tetracycline (Te), tylosin (Ty), vancomycin (Vc). *Salmonella* isolates were tested against a total of 15 antimicrobials, including amikacin (An), amoxicillin/clavulanic acid (Ax), ampicillin (Am), cefoxitin (Fx), ceftiofur (Cf), ceftriaxone (Ce), chloramphenicol (Cl), ciprofloxin (Cip), gentamicin (Gm), kanamycin (Km), naldixic acid Ni), streptomycin (St), sulfa (Su), tetracycline (Te), and triple sulfa Ts).

The isolates or DNA were retrieved from the USDA-ARS lab. to conduct further molecular characterization (*Salmonella*: n=481; *Campylobacter*: n= 41; *Enterococcus*: n=125; *E.coli*: n=125). The isolates were grown and the cells were lysed in the USDA-ARS laboratory and brought back to our laboratory. DNA was extracted from the isolates.

Specific resistance genes were selected to be identified by PCR and confirm their presence. Resistance to five antimicrobials were selected for further PCR characterization based on the

availability of phenotypic information for that specific drug in at least three of the organisms. These antimicrobials included tetracycline, streptomycin, kanamycin, gentamicin, and chloramphenicol. The selection of specific genes tested was based on previous reports of common genes found within the different organisms. A general overview was obtained by searching Pubmed for genes which have been reported within each organism. In addition, class-1 integrons were detected and sizes were noted in all four organisms.

PCR gene detection was completed on 128 samples. Gene selection was based on phenotypic expression of resistance against antimicrobials which were tested in at least 3 of the 4 species. Resistance selection included gentamicin (*grm* and *aadB*), tetracycline (*tetA*, *tetB*, *tetM*,and *tetO*), streptomycin (*aadA2*, *aadE*, *strA*, and *strB*), kanamycin (*aphA1-Iab* and *kn*), and chloramphenicol (*cat1* and *cmlA*). Specific genes selected were based on commonality of the genes found previously within the various organisms. Integron detection using PCR was completed on 291 isolates. When more than one organism from the same animal was found to carry the same gene, these samples were further characterized by DNA sequencing. These primers were designed to amplify the majority of the genes.

Genes selected for sequencing were amplified and products were purified with a QIAquick gel extraction kit (QIAgen Inc, Mississauga, Ontario, Canada). Sequences were confirmed using Beckman Coulter CEQ 8000 and the Dye Terminator Cycle Sequencing Quick Start Kit.

Microarray was completed by the Bacterial Epidemiology and Antimicrobial Resistance Research Unit at the USDA-ARS. The oligonucleotide microarray was used to detect 130 antimicrobial resistance genes on 19 selected isolates.

Results and Discussion

1). Objective 1: *To determine the role of plant size and associated transportation, holding and in-plant practices and intervention measures on prevalence of resistant foodborne and commensal organisms.*

Although all the four organisms were isolated from each sample location, there were trends found in the location distribution. The majority of *Campylobacter* were found in the mesenteric lymph node (40.7%), Enterococcus and *E.coli* were both most commonly found in the head meat (33.8% and 32.7%, respectively) and lairage swab (33.3% and 33.6%, respectively), and *Salmonella* was predominately found in the lairage swabs (52.7%), Table 1.

Figure 1 show that the four organisms were commonly recovered from the five sample origins (carcass, head meat, lairage, mesenteric and superficial inguinal lymph node). In all four pathogens the proportion of positive samples from lairage was higher (30 to 52%) compared to other locations.

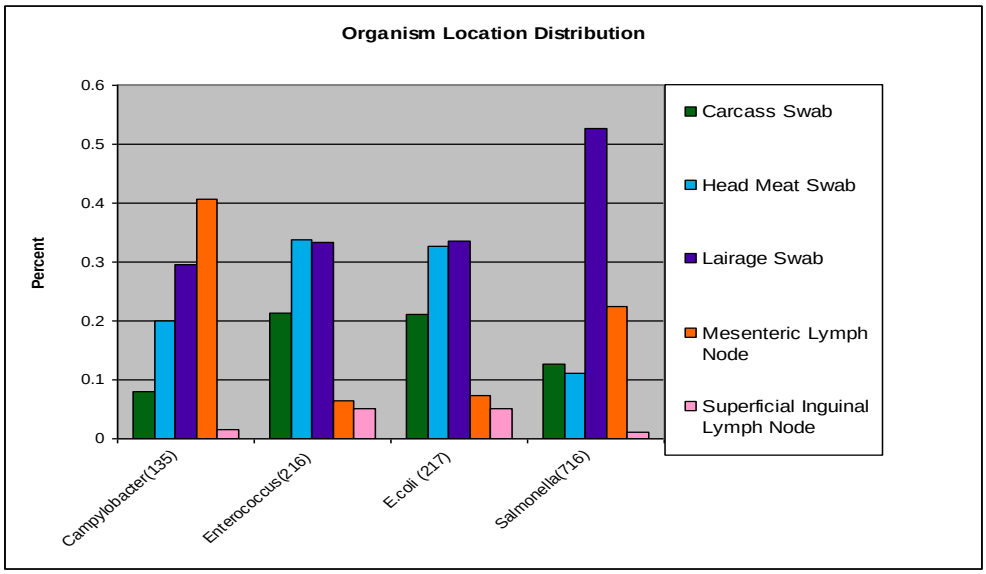


Figure 1: Proportion of the four pathogens by location (carcass, head meat, lymphnodes and lairage swabs)

The proportion of antimicrobial resistant foodborne (*Salmonella* and *Campylobacter*) and commensal (*Enterococcus* and *E.coli*) organisms with respect to plant size and origin of samples (carcass, head meat, lairage and lymph nodes) is presented in detail in Table 2. Of the total isolates tested for antimicrobial susceptibility from small and large plant sizes, 60.7% vs 91.5%, 98.9% vs 100%, 100% vs 87.7% and 88.2% vs 87.7%) of *Salmonella, Campylobacter, Enterococcus* and *E.coli*, respectively were resistant to one or more of the antimicrobial tested. Overall, small plants had a higher percentage of pansusceptible isolates, 27.4% (157/573) compared to the large plants, 10.6% (21/199).

The level of multidrug resistance (resistance to three or more antimicrobials) was higher in foodborne pathogens recovered from large plant sizes (Salmonella: 25.1% vs 61.1% and Campylobacter: 59.8% vs 79.2%) compared to commensal organisms (Enterococcus: 92.5% vs 33.3% and E.coli: 29.4% vs 33.3%).

Multidrug resistance was common among isolates from both small and large processing plants, however, relatively higher in larger ones including higher percentage of isolates carrying StSuTe at 9.55% (19/199), AmKmStTe at 6.03% (12/199), and AmClStSuTe at 7.04% (14/199), Figure 2. Resistance to Nalidixic acid (Ni) was more commonly found in large plants at 5.03%

(10/199), although in both small and large plants resistance to this drug was only found among Salmonella serovar Montevideo. Small plants had a higher percentage of isolates carrying SuTe at 4.4% (25/573) and StTe at 4.4% (25/573). The resistance pattern AmGmKmStSuTeTs was also only found in small plants and furthermore, was only found in 15 Salmonella serovar Havana isolates.

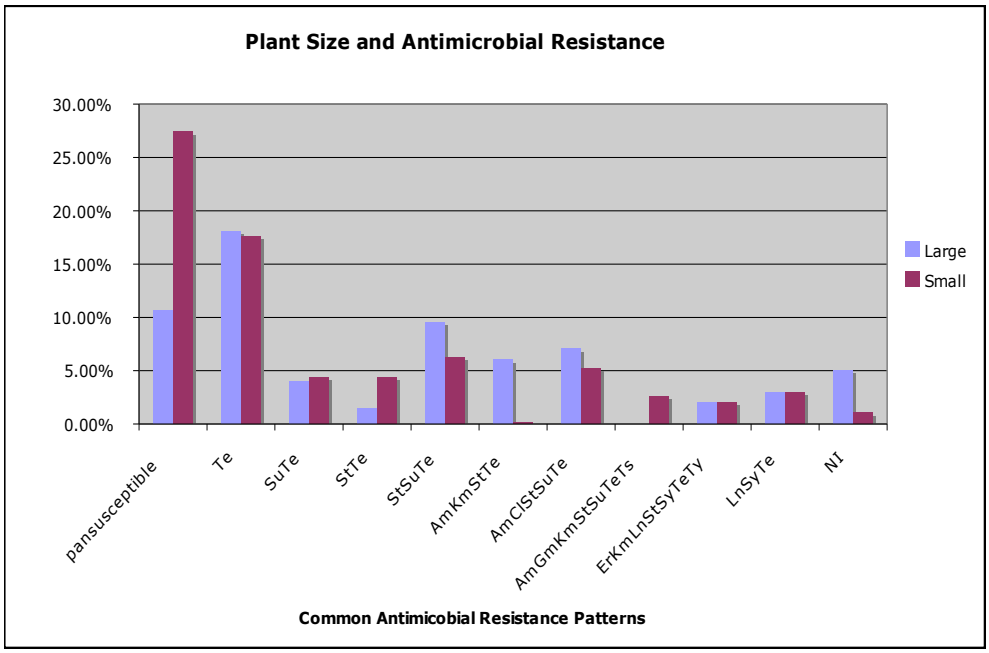


Figure 2: Antimicrobial resistance patterns of the four pathogens by plant size

Plant size								
	Small				**Large**			
Origin of sample	*Salmonella* (n=422)	*Campylobacter* (n=87)	*Enterococcus* (n=134)	*E.coli* (n=136)	*Salmonella* (n=293)	*Campylobacter* (n=48)	*Enterococcus* (n=79)	*E.coli* (n=81)
Carcass	a)* 65.8% (50/76)	a) 100% (11/11)	a) 100% (39/39)	a) 95.1% (39/41)	a) 100% (14/14)	a) 0% (0/0)	a) 5/5	a) 2/5
	b) 30.2% (23/76)	b) 54.5% (6/15)	b) 99.4% (38/39)	b) 43..9% (18/41)	b) 0% (0/14)	b) 0% (0/0)	b) 3/5	b) 1/5
Head Meat	a) 54.9% (28/51)	a) 93.7% (15/16)	a) 100% (43/43)	a) 85.4% (35/41)	a) 90% (27/30)	a) 100% (11/11)	a) 100% (29/29)	a) 76.7% (23/30)
	b) 13.7% (7/51)	b) 56.3% (9/16)	b) 88.4% (38/43)	b) 34.5% (14/41)	b) 76.7% (23/30)	b) 73.6% (7/11)	b) 89.7% (26/29)	b) 36.7% (11/30)
Lairage	a) 54.6% (83/152)	a) 100%% (15/15)	a) 100% (28/28)	a) 89.3% (25/28)	a) 92.3% (205/222)	a) 100% (25/25)	a) 100% (44/44)	a) 100% (45/45)
	b) 14.5% (22/152)	b) 33.3% (5/15)	b) 92.9% (26/28)	b) 14.3% (4/28)	b) 60.8% (135/222)	b) 84% (21/25)	b) 93.2% (41/44)	b) 24.4% (11/45)
MLN[c]	a) 35.4% (89/136)	a) 100% (40/40)	a) 100% (13/13)	a) 80% (12/15)	a) 80% (20/25)	a) 100% (11/11)	a) 1/1	a) 1/1
	b) 36.8% (50/136)	b) 77.5% (31/40)	b) 84.6% (11/13)	b) 6.7.% (1/15)	b) 28% (7/25)	b) 90.9% (10/11)	b) 1/1	b) 0
SILN[d]	a) 6/7	a) 1/1	a) 100% (11/11)	a) 81.8% (9/11)	a) 2/2	a) 1/1	a) 0	a) 0
	b) 5/7	b) 1/1	b) 100% (11/11)	b) 27.3% (3/11)	b) 2/2	b) 0	b) 0	b) 0
Total	**a) 60.7% (256/422)**	**a) 98.9% (86/87)**	**a) 100% (134/134**	**a) 88.2% (120/136)**	**a) 91.5% (268/293)**	**a) 100% (48/48)**	**a) 87.7% (71/81)**	**a) 87.7% (71/81)**
	b) 25.1% (106/422)	**b) 59.8% (52/87)**	**b) 92.5% (124/134)**	**b) 29.4% (40/136)**	**b) 61.1% (179/293)**	**b) 79.2% (38/48)**	**b) 33.3% (27/81)**	**b) 33.3% (27/81)**

*a). Resistance to one or more of the antimicrobials tested; b). Multidrug resistance; c. mesenteric lymph node; d. superficial inguinal lymph node

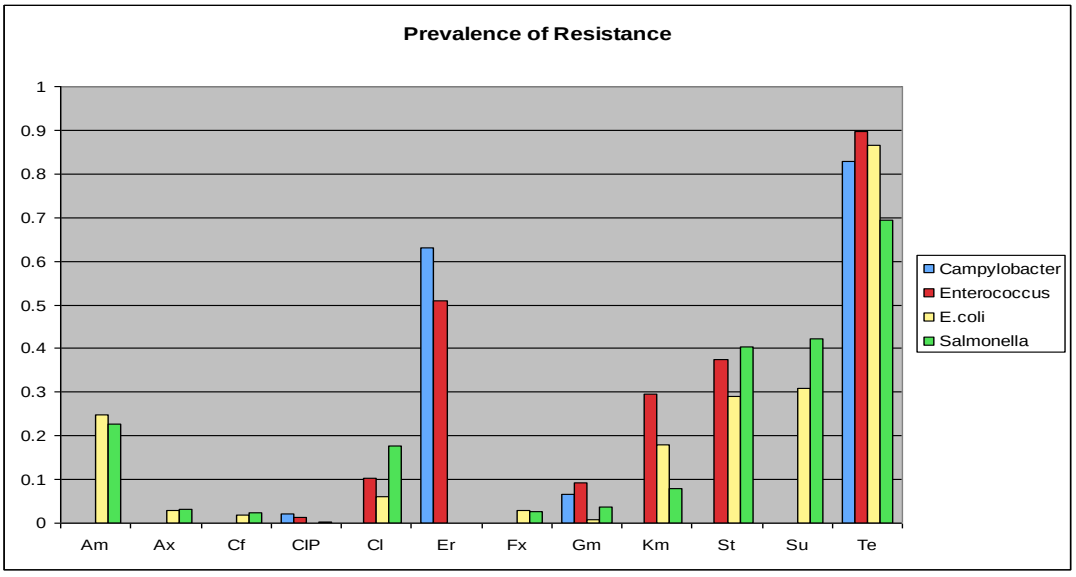
Table 2: Frequency of antimicrobial resistance of foodborne (*Salmonella* and *Campylobacter*) and commensal (*Enterococcus* and *E.coli*) organisms by plant size and sources of samples (carcass, head meat, lairage and lymp nodes)

Even though isolates were obtained from two predominant pig producing geographic locations (North Carolina and Iowa), no isolate was obtained from small size processing plants from Iowa. In large size plants, the proportion of antimicrobial resistant foodborne and commensal organisms is presented in Table 3.

Plant size								
	Small				**Large**			
State	*Salmonella* (n=422)	*Campylob-acter* (n=87)	*Enteroco-ccus* (n=134)	*E.coli* (n=136)	*Salmonella* (n=293)	*Campylob-acter* (n=48)	*Enteroco-ccus* (n=79)	*E. coli* (n=81)
North Carolina	72.5% (306/422)	98.8% (86/87)	100% (134/134)	95.2% (120/136)	96% (143/149)	95% (19/20)	100% (37/37)	84.6% (33/39)
Iowa	-	-	-	-	86.1% (124/144)	100% (28/28)	100% (42/42)	90.5% (38/42)

Table 3: Percentage of antimicrobial resistance of pathogenic (Salmonella and Campylobacter) and commensal (*Enterococcus* and *E. coli*) organisms by state of origin.

Figure 3 below displays the prevalence of resistance in the four different organisms. Resistance to tetracycline was very high (70-90%) in all four pathogens.



There was a great diversity based on resistance patterns found among the organisms with some overlapping patterns between *Salmonella* and *E.*coli (Figure 3). Multi-drug resistance patterns were highly variable, but commonly detected among all four organisms regardless of origin.

In addition to tetracycline resistance to macrolides, some aminogylosides (kanamycin and streptomycin), sulfamethoxazole, and β-lactams was also common. Detailed results of the antimicrobial resistance profiles of the four pathogens to each antimicrobial with respect to plant size is shown in Table 4. Overall, the proportion of antimicrobial resistance of Salmonella and Campylobacter was higher in large processing plants compared to small ones (Table 4). As shown in Table 4, in indicator microorganisms (*Enterococcus* and *E. coli*) the difference in antimicrobial resistance from large and small processing plants was very small compared to Salmonella and Campylobacter.

Plant size								
	Small				Large			
Antimicobial	*Salmonella* (n=422)	*Campylobacter* (n=87)	*Enterococcus* (n=134)	*E.coli* (n=136)	*Salmonella* (n=293)	*Campylobacter* (n=48)	*Enterococcus* (n=79)	*E.coli* (n=81)
Am*	16.4%	ND	ND	25.7%	31.7%	ND	ND	23.5%
An	0	ND	ND	0	0	ND	ND	0
Ax	2.8%	ND	ND	1.5%	3.4%	ND	ND	4.9%
Ar	ND	6%	ND	ND	ND	68.8	ND	ND
BC	ND	ND	8.9%	ND	ND	ND	11.4%	ND
Ca	ND	37.9%	ND	ND	ND	41.7%	ND	ND
Ce	0	ND	ND	0	0.3%	ND	ND	0
Cf	2.7%	ND	ND	0.7%	2.4%	ND	ND	3.7%
Cip	0	0	2.2%	0	0.3%	6.3%	0	0
Cl	11.6%	ND	11.2%	4.2%	26.6%	ND	8.9%	8.6%
Dp	ND	ND	48.5%	ND	ND	ND	55.7%	ND
Er	ND	6%	52.9%	ND	ND	68.8%	49.4%	ND
Ff	ND	0	ND	ND	ND	0	ND	ND
Fv	ND	ND	18.7%	ND	ND	ND	15.2%	ND
Fx	2.8%	ND	ND	1.5%	2.4%	ND	ND	4.9%
Gm	5.2%	6.9%	6.7%	0	1.3%	6.3%	13.9%	2.5%
Km	5.5%	ND	28.4%	15.4%	11.3%	ND	32.9%	22.2%
Ln	ND	ND	99.3%	ND	ND	ND	97.5%	ND
Lz	ND	ND	0	ND	ND	ND	0	ND
Nf	ND	ND	0.7%	ND	ND	ND	2.5%	ND
Ni	1.4%	2.3%	ND	0	3.8%	4.2%	ND	0
Pn	ND	ND	0.7%	ND	ND	ND	0	ND
St	23.2%	ND	46.3%	31.6%	57%	ND	24.1%	24.7%
Su	26.5%	ND	ND	23.5%	56.1%	ND	ND	43.2%
Sy	ND	ND	100%	ND	ND	ND	78.5%	ND
Ts	4.7%	ND	ND	2.2%	2%	ND	ND	6.2%
Te	44.6%	7.9%	94.8%	87.5%	83.3%	89.5%	84.8%	85.2%
Tt	ND	3.7%	ND	ND	ND	39.6%	ND	ND
Ty	ND	ND	55.2%	ND	ND	ND	53.2%	ND
Vc	ND	ND	0	ND	ND	ND	0	ND

Table 4: Antimicrobial resistance profiles of the four pathogens by processing plant size and antimicrobial type

*ampicillin (Am), amikacin (An), amoxicillin/clavulanic acid (Ax), azithromycin (Ar), bactracin zinc (Bc), clindamycin (Ca), ceftrixone (Ce), ceftiofur (Cf), ciprofloxacin (Cip), chloramphenicol (Cl), daptomycin (Dp), erythromycin (Er), florfenicol (Ff), flavomycin (Fv), cefoxitin (Fx), gentamicin (Gm), kanamycin (Km), lincocin (Ln), linezolid (Lz), nitrofurantoin (Nf),nalidixic acid (Ni), penicillin (Pn), streptomycin (St), sulfa (Su), synercid (Sy), triple sulfa (Ts). telithromycin (Tt), and tetracycyline (Te) tylosin (Ty), vancomycin (Vc).

2**). Objective 2**: *To determine the clonality of strains (particularly Salmonella and Campylobacter) identified from different size processing plants in two predominant pig producing geographic locations (NC and IA).*

Clonality of strains was determined by using phenotypic and genotypic characteristics. For *Salmonella*, serotype and antimicrobial resistance patterns were used to compare the clonality of the isolates. Within the large processing plants, ten groups of isolates were identified and classified as highly similar. All ten of these groups contain isolates from at least three different samples. The majority of the groups (8/10) were only isolated from two sources of isolation and no group contains isolates from more than 3 different sample sites. Isolates in these groups were commonly isolated (7/10) from the head meat samples (RH). These isolates were not commonly (2/10) isolated from the carcass samples (RC) or the lymph node samples, including mesenteric lymph nodes (2/10) and superficial lymph nodes (1/10). Overall, 37% (73/199) of isolates analyzed from the large processing plants were found to fall within one of these ten groups. Within the small processing plants, 28 groups containing at least 3 isolates that were characterized as highly similar were identified. Overall, 57% (326/573) of isolates analyzed from the small processing plants fall into one of these groups. The majority of these groups, 61% (17/28), contain isolates from more than 2 sources of isolation, with 46% (13/28) of groups containing isolates from 4 or more isolation sources and 14% (4/28) of groups containing isolates from all 5 sampling sites. The majority, 79% (22/28), of these groups contain isolates from

the mesenteric lymph node (RM). Groups also commonly contain isolates from the lairage at 64% (RL- 18/28), from the head meat at 61% (RH -17/28), and from the carcass at 57% (RC- 16/28) (Table 5).

Large Processing Plants				
Salmonella Serotype	**Resistance Pattern**	**State**	**Source of Isolates**	**# of isolates**
S. Agona	AxAmFxCfClKmStSuTeTs	IA	RH	3
S. Agona	Te	NC	RC, RM	11
S. Derby	StSuTe	NC	RH, RL, RS	18
S. Montevideo	NI	NC	RC	10
S. Copenhagen	AmCLStSuTe	IA	RH, RL	11
S. Copenhagen	AmKmStTe	NC	RH	8
S. Copenhagen	Pansusceptible	IA	RH	3
S. Typhimurium	AmKmStTe	NC	RH, RM	3
S. Worthington	Te	NC	RH, RL	3
S. 4,5,12:I-	Pansusceptible	IA	RM	3
Small Processing Plants				
Salmonella Serotype	**Resistance Pattern**	**State**	**Source of Isolate**	**#of isolates**
S. Adel	Te	NC	RC, RL	3
S. Adel	Pansusceptible	NC	RC, RH, RL, RM	10
S. Agona	StSuTe	NC	RC, RM	3
S. Anatum	Pansusceptible	NC	RL, RM	4
S. Anatum	Te	NC	RC, RH, RL, RM	32
S. Branderup	Pansusceptible	NC	RL, RM	5
S. Derby	Pansusceptible	NC	RC, RH, RM	9
S. Derby	StSuTe	NC	RC, RH, RL, RM, RS	21
S. Hadar	StTe	NC	RC, RH, RL, RM, RS	16
S. Hadar	Te	NC	RC, RH, RL	5
S. Havana	AmGmKmStSuTeTs	NC	RC, RL, RM, RS	15
S. Infantis	Pansusceptible	NC	RH, RL, RM	24
S. Johannesburg	Pansusceptible	NC	RH, RL, RM	4
S. London	Pansusceptible	NC	RH, RL	8
S. London	Te	NC	RC, RH, RL, RM	16
S. Mbandaka	SuTeTs	NC	RM	4
S. Montevideo	NI	NC	RC	6
S. Muechen	Pansusceptible	NC	RC, RH, RL, RM	14
S. Muechen	StSuTe	NC	RM	6
S. Muechen	SuTe	NC	RH, RL, RM	21
S. Ohio	Pansusceptible	NC	RC	11
S. Read	Pansusceptible	NC	RH, RL	3
S. Senftenberg	Pansusceptible	NC	RH, RL, RM	3
S. Stpa	Pansusceptible	NC	RC, RH, RM	4
S. Copehhagen	AmClStSuTe	NC	RC, RH, RL, RM, RS	28
S. Copehhagen	AmClSuTe	NC	RC, RH, RM	6
S. Uganda	Pansusceptible	NC	RC, RH, RL, RM, RS	41

S. Worthington	Pansusceptible	NC	RL, RM	4

Table 5: Phenotypic characterization of *Salmonella* serovars from different sources

Campylobacter clonality was assessed using antimicrobial resistance patterns. A total of 41 isolates were assessed and all isolates were identified as *Campylobacter coli*. Overall, the isolates were highly diverse. Within the large processing plants, one group was identified containing 3 isolates with identical antimicrobial resistance patterns. The isolates from this group were isolated from the head meat (RH), lairage (RL), and superficial inguinal lymph nodes (RS). Overall, a total of 27% (3/11) of the isolates from the large processing plants were characterized as similar. Within the small processing plants, two groups were identified. The first group was a group of 4 isolates all isolated from the mesenteric lymph nodes (RM). The second group was a group of 6 isolates. These isolates were found within carcass samples (RC), head meat samples (RH), and mesenteric lymph node samples (RM). Overall, 33% (10/30) of the isolates from small processing plants fell into one of these groups (Table 6).

Large Processing Plants			
Resistance Pattern	**State**	**Source of isolates**	**# of isolates**
ErTe	IA	RH, RL, RS	3
Small Processing Plants			
Resistance Pattern	**State**	**Source of isolates**	**# of isolates**
ArCaErTe	NC	RM	4
ArErTe	NC	RC, RH, RM	6

Table 6: Phenotypic characterization of C. coli isolates from different sources

The antimicrobial resistance patterns detected varied in the four pathogens. The most common resistance patterns identified in the four pathogens is shown in Table 7.

Campylobacter	**Number (%)**	***Enterococcus***	**Number (%)**
ArCaErTtTe	11 (8.15%)	ClErGmKmLnSyTeTy	8 (3.70%)
ArCaErTe	11 (8.15%)	ErGmKmLnStSyTeTy	6 (2.78%)
ArErTtTe	11(8.15%)	ErKmLnStSyTeTy	25 (11.6%)
ArErTe	16 (11.9%)	ErLnStSyTeTy	25 (11.6%)
AtTe	9(6.67%)	ErLnSyTeTy	13 (6.02%)

		FvLnTe	10 (4.63%)
		LnSyTe	41 (19.0%)
		LnSy	10 (4.63%)
E.coli	**Number (%)**	***Salmonella***	**Number (%)**
AmKmStSuTe	5 (2.30%)	AmGmKmStSuTeTs	17 (2.37%)
AmStTe	8 (3.69%)	AmClStSuTe	88 (12.3%)
KmSuTe	7 (3.23%)	AmClSuTe	14 (2.96%)
AmTe	20 (9.22%)		

Table 7. Common resistance patterns among the four organisms

Among the four organisms phenotypically shared resistance patterns were found only among *Salmonella* and *E.coli* isolates (Table 8)

Shared Patterns	***Salmonella***	***E.coli***
AxAmFxCfClStSuTe	6 (0.838%)	1 (0.461%)
KmStSuTe	1 (0.140%)	8 (2.69%)
AmKmStTe	16 (2.235)	1 (0.461%)
KmStTe	8 (1.12%)	1 (0.461%)
StSuTe	110 (15.36%)	7 (3.23%)
StTe	22 (3.07%)	18 (8.29%)
SuTe	40 (5.59%)	14 (6.45%)

Table 8. Shared resistance patterns between organisms (Salmonella and E. coli).

Salmonella and *Campylobacter* were identified from large size processing plants only in two predominant pig producing geographic locations (NC and IA). No Salmonella and Campylobacter were available from small size plants from Iowa and therefore we compared isolates from two states from large processing plants only. Phenotypically, all Salmonella isolates from large plants from NC (n=149) and IA (n=144) were resistant to one or more of the antimicrobials tested. However, Salmonella isolates recovered from Iowa were showed resistance to almost all the 15 antimicrobials tested compared to those recovered from North Carolina (Table 9A) which showed susceptibility to cefoxitin, ceftiofur, ceftriaxone, ciprofloxacin and triple sulfa.

Table 9: Comparison of phenotypes of antimicrobial resistant *Salmonella* and *Campylobacter* isolates from large processing plants in North Carolina and Iowa

(A). *Salmonella*

Antimicrobial	**North Carolina** (n=149)	**Iowa** (n=144)
Am	21 (14.1%)	70 (48.6%)
An	0	0
Ax	1 (0.7%)	8 (5.6%)
Ce	0	1 (0.7%)
Cf	0	7 (4.9%)
Cip	0	1 (0.7%)
Cl	9 (6%)	68 (47.2%)
Fx	0	7 (4.9%)
Gm	2 (1.3%)	2 (1.3%)
Km	14 (9.4%)	18 (12.5%)
Ni	0	1 (0.7%)
St	75 (50.3%)	91 (63.2%)
Su	77 (51.7%)	87 (60.4%)
Te	121 (81.2%)	123 (85.4%)
Ts	0	6 (4.2%)

(B). *Campylobacter*

Antimicrobial	**North Carolina** (n=20)	**Iowa** (n=28)
Ar	18 (90%)	12 (42.8%)
Cip	0	2 (7.1%)
Ca	10 (50%)	10 (35.7%)
Er	10 (50%)	20(71.4%)
Ff	0	0
Gm	1 (5%)	2 (7.1%)
Ni	1 (5%)	1 (3.6%)
Te	10 (50%)	9 (32.1%)
Tt	19 (95%)	24 (85.7%)

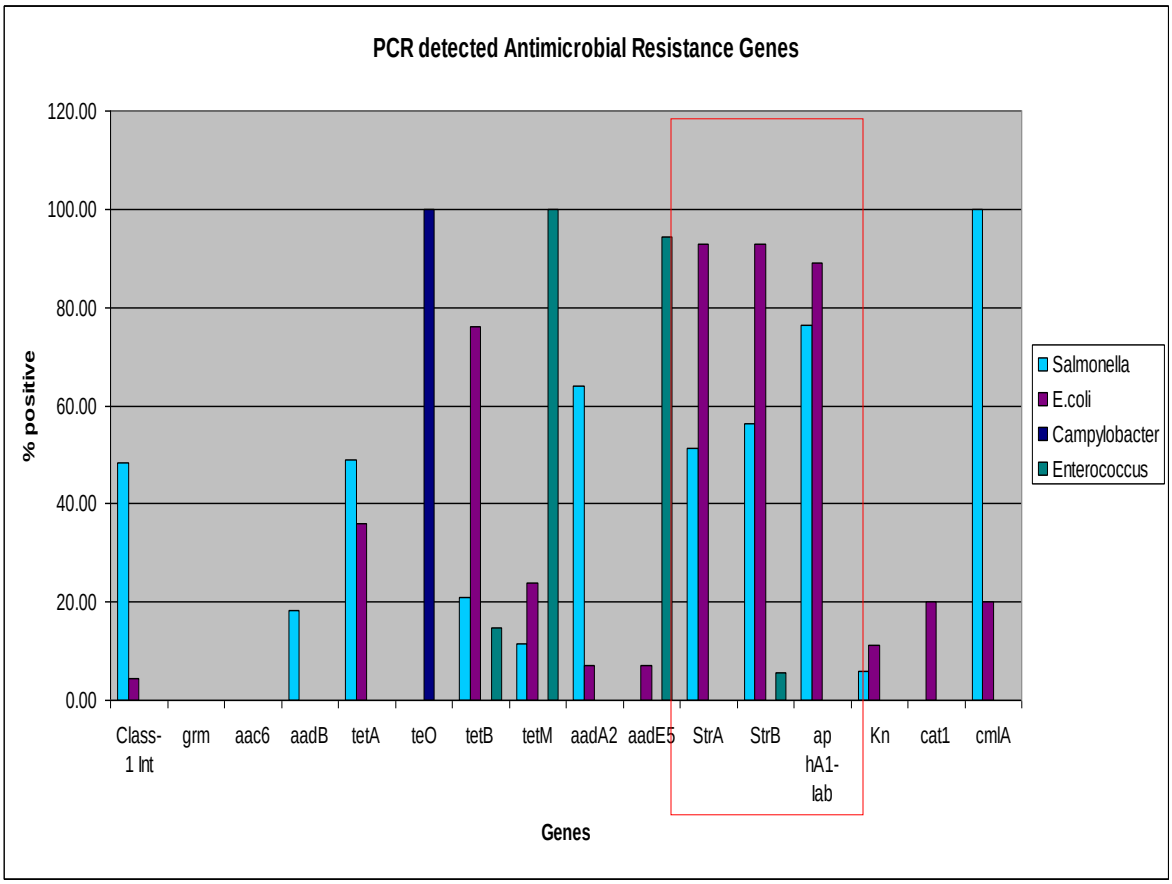
Campylobacter isolates from large processing plants from both states showed comparable and similar resistance profiles to a panel of nine different antimicrobials tested in this study (Table .9B).

3). Objective 3: *To determine the distribution and similarity of specific antimicrobial resistance genes and class-1 integrons among four important*

organisms (Salmonella, Campylobacter, E. coli and enterococci) in various processing settings.

The most common genes found were those encoding for tetracycline and aminoglycoside resistance. Figure 4 shows a summary of the genes detected.

Figure 4: Antimicrobial resistance genes detected by PCR and sequencing in the four pathogens. The highlighted genes are those in which different species from the same animal or same farm were found to carry the same gene



Salmonella species were most commonly found to carry *aadB*, *tetA(A)*, *aadA2*, *strA*, *strB*, *apha1-Iab*, and *cmlA*. *Escherichia* samples most commonly carried *tetB*, *strA*, *strB*, and *apha1-Iab*. *Enterococcus* species most commonly carried *tetM* and *aadE*. *TetO* was the only gene found in *Campylobacter* and all

samples tested positive for this gene. *strA*, *strB*, and *apha1-Iab* genes appear to be linked. 32/33 (96.97%) of the samples which were positive for *strA* were also positive for *strB*, 31/36 (86.11%) of samples positive for *strB* also carried *strA*, and 18/21 (85.71%) of samples positive for *apha1-Iab* were also positive for *strB*. *Escherichia* and *Salmonella* from the same animal were most commonly found to be carrying the same genes. Within the samples that tested positive for *tetA(B), strA*, *strB*, and *AphA1-Iab* there were multiple organisms that were from the same animal. After sequencing these genes, *strA* genes were found to cluster within the species while *strB* and *aphA1-Iab* genes with 97.2% or greater homology were found in different organisms (*Salmonella* and *E. coli*) from the same animal host. The dendrograms below (Figure 5) demonstrate this finding.

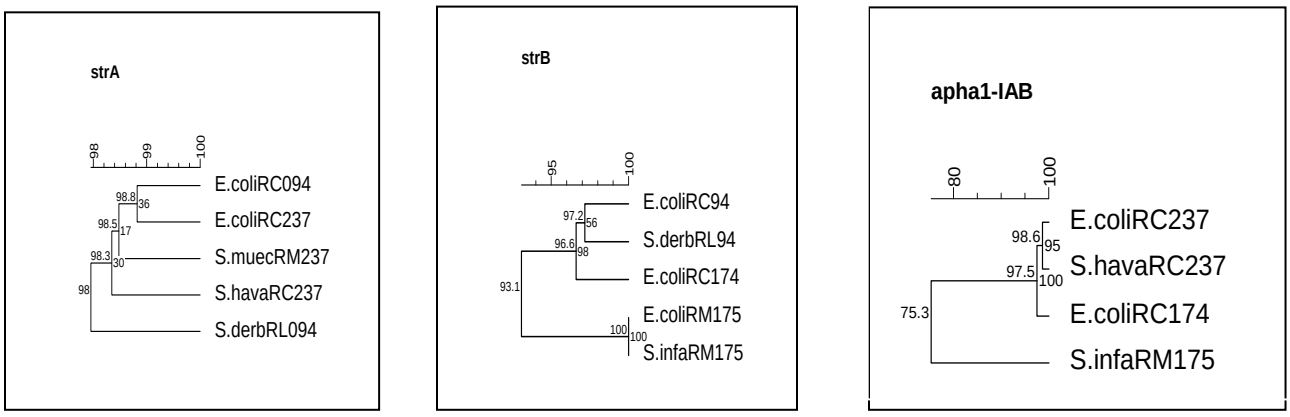


Figure 5: Dendrograms of sequenced resistance genes

Salmonella serovar Infantis *apha1-Iab* gene is noticeably unrelated to the other *apha1-Iab* genes. This gene amplified with the *apha1-Iab* primer but actually matches more to the ***aph3'-I*** gene according to NCBI BLAST.Integrons were found in 17.5% of the samples, with the majority being from *Salmonella*. Integrons were most commonly, 38/51 (74.5%), isolated from small processing plants. Three *Escherichia* samples were found to carry integrons sizing 1.0kb, 2.1kb and 2.8kb. 58.3% (28/48) of the Salmonella carried two integrons sizes

1.0kb and 1.2kb. The majority (25/28) of these integrons were found in serovar Typhimurium, including phage types DT104 and U302. The three others were found in serovars *4,12:I-*, *Hadar*, and *Infanta*. The most common resistance pattern among this group was AmClStSuTe, found in 19/28 (67.86%)

Seven of the nine *Salmonella* serovar Havana samples were found to carry 4kb integrons. Eight of the nine *Havana* samples were collected on the same date from a small processing plant in North Carolina and all nine have the same multidrug resistance pattern, AmGmKmStSuTeTs. Seven of the Havana samples were tested by PCR for specific resistance genes and found 7/7 carrying *aadA2*, 5/7 carrying *tetA(B),* 4/7 carrying *strA*, 6/7 carrying *strB*, and 6/7 carrying *apha1-Iab*. One of the 4kb integrons was sequenced and the following gene cassettes were found (Figure 6):

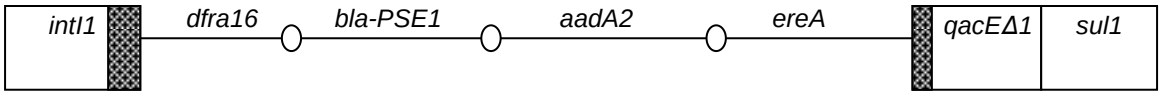
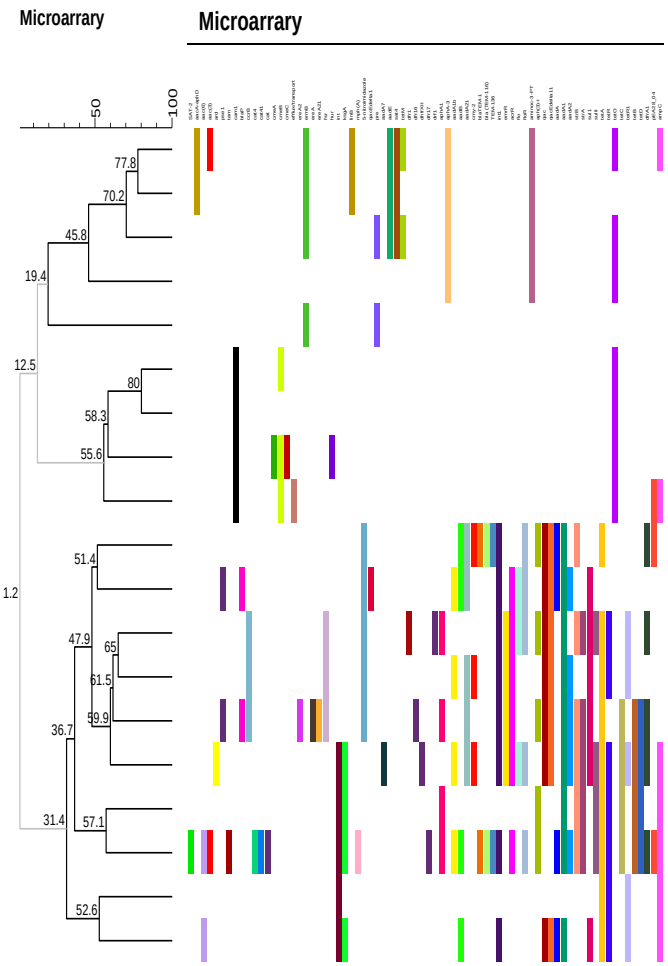


Figure 6: Schematics of 4kb integron sequenced from *Salmonella* serovar Havana

Five others were characterized by using the sequencing primers for PCR. All were found to be identical.

Within those genes selected that were tested using microarray and PCR, there was 87.1% similarity within the results. The differences that were found could be explained. They could be due to the fact that the microarray uses a 19 base fragment probe while PCR amplified a larger portion of the gene, possibly leading to false positives in the microarray picking up non-functional parts of genes found within the organisms. Also, there could be mutations within the primer site selected for PCR, leading to false negatives in the PCR testing. Finally, PCR was not performed on samples that were not phenotypically showing resistance and some organisms were shown to be positive for such genes on the Microarray.

Microarray results gave an over view of where overlaps of resistance may be occurring. Nineteen samples, including 5 *Salmonella*, 5 *Campylobacter*, 5 *Escherichia*, and 4 *Enterococcus* were selected based on their multidrug resistance patterns. The dendrogram below (Figure 7) shows the similarities between the resistance genes carried by each of the samples. *Salmonella* and *Escherichia* isolates were found to carry more of the genes tested and have the most similarities. Of the 74 antimicrobial resistant specific genes on the microarray, 35 (47.3%) were found in more than one species. Of those found within more than one species, 30/35 (85.7%) were shared between *Escherichia* and *Salmonella* and 3/35 (8.57%) were shared between *Enterococcus* and *Campylobacter*. Two resistance genes were found to be carried by three different organisms 1 was shared between *Campylobacter*, *Escherichia*, and *Salmonella*, and 1 was shared between *Campylobacter*, *Escherichia*, and *Enterococcus*. Within the overlapping genes, 11/35 (31.4%) were found to be aminoglycosides, 7/35 (20%) were tetracyclines, and 6/35 (17.1%) were beta-lactamases. Both genes that were carried by three organisms were beta-lactamase genes, AmpC and pEA28_04.



Key	serovar/species	date	plant size	State	R. pattern
EntRH152	faec	100705	large	IA	ErGmKmLnStSyTeTy
EntRH243	faec	101305	small	NC	ClPErFvKmLnStTeTy
EntRS010	faec	92105	small	NC	ClErGmKmLnStSyTeTy
CamRC023	coli	92205	small	NC	ArCaErTtTe
EntRM175	hira	101105	small	NC	BcErFvLnSyTeTy
CamRM221	coli	101505	small	NC	CaErGmTt
CamRM046	coli	92305	small	NC	ArErGmTtTe
CamRH175	coli	101105	small	NC	ArErTtTe
CamRM243	coli	101405	small	NC	ArCaErGmTe
SalRH065	ugan	92705	small	NC	AxAmFxCfClGmKmStSuTe
SalRM298	tcop	101905	large	NC	AmClGmStSuTe
SalRH129	agon	100605	large	IA	AxAmFxCfClKmStSuTeTs
SalRH171	derb	101105	small	NC	StSuTe
SalRL206	hava	101305	small	NC	AmGmKmStSuTeTs
EscRH152	coli	100705	large	IA	AxAmFxCfClStSuTeTs
EscRH059	coli	92705	smal	NC	AxAmFxKmStSuTe
EscRH317	coli	102005	large	NC	AmClKmStSuTeTs
EscRC054	coli	92305	smal	NC	AxAmFxCfTe
EscRL115	coli	100505	large	IA	AxAmFxGmStSuTe

Figure 7: Similarities between the four organisms based on microarray analysis of antimicrobial resistance genes. The colors represent different antimicrobial resistance genes listed across the top of the graph. The presence of color indicates the presence of the gene, while white indicates the absence of that gene.