Title: Characterization of Multiple-Antimicrobial Resistance among Swine Salmonella **NPB #98-218**

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I. Abstract:

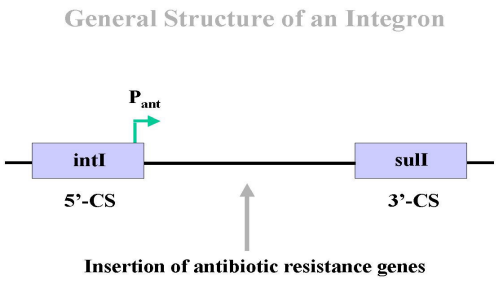
Forty-two Salmonella isolates obtained from diseased swine were genetically characterized for the presence of specific antimicrobial resistance mechanisms. Twenty of these isolates were *S. typhimurium* DT104 and the other 20 were non-DT104 salmonella strains. PFGE was initially employed to determine the genetic relatedness among the *Salmonella* isolates and revealed twenty distinct genetic patterns among the 42 isolates. However, all DT104 isolates fell within two distinct genetic clusters. Other Salmonella isolates genetically grouped together within their specific species.

All DT104 isolates displayed the pentamer resistance phenotype to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT). Resistance to sulfamethoxazole, tetracycline, streptomycin, kanamycin, and ampicillin was most common among the non-DT104 *Salmonella* isolates. The presence of integrons among these Salmonella isolates was also investigated. All DT104 strains contained two chromosomal integrons of 1000 and 1200 base pairs. DNA sequencing revealed that the two integrons contained genes encoding resistance to streptomycin and ampicillin, respectively. None of the non-DT104 strains showed this same pattern although several strains possessed integrons of 1000 bp or larger. Several of the non-DT104 strains did not possess any integrons. This research suggest that integrons contribute to antimicrobial resistance among swine *Salmonella* but are not responsible for all resistance phenotypes observed.

II. Introduction:

The ease with which bacteria become resistant to antimicrobial agents continues to concern clinicians, public health officials, and researchers. Antimicrobial resistance is a problem of both national and international importance, with resistance mechanisms described for all known antibiotics currently available (3). Although the spread of resistant microorganisms is disturbing, the association of resistance determinants with mobile DNA elements, such as plasmids, transposons and integrons, is of much greater concern. These mobile DNA elements assist in the rapid dispersion of resistance genes within bacterial species and between different species (4,6). Many resistance genes in *Escherichia coli* and *Salmonella* species are on large, transferable extrachromosomal DNA

elements, called plasmids. These plasmids may contain other mobile elements, termed transposons. In turn, plasmids and transposons, coding multiple drug resistance, often possess another genetic element, the integron (**Figure 1**).



Integrons contain one or more resistance genes, present as mobile gene cassettes, inserted in various arrangements between two conserved DNA regions, creating arrays of different antimicrobial resistance genes (**Figure 1**) (6). Over 40 gene cassettes and four distinct classes of integrons have been identified to date (4,6). Cassette-associated genes conferring resistance to beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, streptomycin, and quaternary ammonium compounds used as antiseptics and disinfectants, have been found (4). Also, class I integrons include a sulfonamide resistance gene (**sul1**) in the backbone structure (4). Since little is known about the potential contribution of integrons to maintaining multiple antimicrobial resistance among clinical swine *Salmonella* species, integrons were the focus of our study.

We were especially interested in integrons as they occured in *S. enterica* serotype Typhimurium, definitive type 104 (**DT104**), an emerging foodborne pathogen detected in several countries including: the United States (US), the United Kingdom, Canada, France, Germany, and Denmark (2). DT104 isolates often exhibit a multiple antimicrobial resistance pattern (R-Type) with resistance to ampicillin (**A**), chloramphenicol (**C**), streptomycin (**S**), sulfonamides (**Su**), and tetracycline (**T**); (**ACSSuT**) (2,10,11). Molecular studies have demonstrated that in DT104 strains, resistance genes are chromosomally encoded (10,11). Unlike plasmid-mediated resistance, which may disappear in the absence of continued selective pressure, chromosomally-mediated resistance is usually maintained; thus, it is necessary to eliminate the resistant strain to prevent transfer between animals and humans and between humans.

To date, there is no information regarding emergence and characterization of integron-mediated, multiple antimicrobial resistance among swine *Salmonella* isolates from the US. The majority of work published on resistance genes in *Salmonella* has been obtained from European isolates (10,11). Despite much research into the characterization of resistance mechanisms among these isolates, the prevalence and relative contribution of integron-mediated, multiple antimicrobial resistance among veterinary *Salmonella* isolates is far from clear.

III. Objectives:

At present it is unclear if antimicrobial cross resistances observed between animal and human *Salmonella* isolates are due to the transfer of the resistant organisms between hosts or to the transfer of **mobile DNA elements** encoding antimicrobial resistance genes**.**

The aims of our study were to:

1. Identify and characterize mobile DNA elements, namely integrons, possessing antibiotic resistance genes among DT104 and non-DT104 *S. typhimurium* strains isolated from US swine.
2. Map these mobile DNA elements to the chromosome and/or plasmids using the

following techniques, as appropriate: PCR, restriction endonuclease digestion analysis, Southern hybridization, and DNA sequencing.

3. Characterize the salmonella isolates to see if there was clonal dissemination of resistant isolates between animals utilizing Pulsed-field gel electrophoresis (PFGE) methodology.

Our underlying goal was to advance the knowledge concerning development and dissemination of antimicrobial resistance among bacterial pathogens important in swine husbandry and emerging as foodborne pathogens contaminating pork.

IV. Procedures:

We proposed to genetically characterize the antimicrobial resistance mechanisms present among 42 isolates of *Salmonella* isolated from diseased and healthy swine. Twenty of these isolates were *S. typhimurium* DT104 and the other 20 were non-DT104 salmonella strains, all isolated from swine. We obtained these isolates from Kathleen Ferris, Bacterial Identification Group, NVSL, Ames, IA, and they are representative of the *Salmonella* spp. being isolated from swine throughout the United States.

Antimicrobial susceptibility determination. Antimicrobial minimum inhibitory concentrations (MIC) of salmonella isolates were determined via the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, OH) and interpreted according to the National Committee Clinical Laboratory Standards (NCCLS) guidelines for micro-broth dilution methods (8,9). Sensititre susceptibility testing was performed according to the manufacturers instructions. Briefly, each microtiter plate, after inoculation with bacterial strains, is resealed with an adhesive seal, and incubated at 35 C for 18-24 hours. The Sensititre system records the minimum inhibitory concentration (MIC) as the lowest concentration of antimicrobial that inhibits visible growth. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212 were used as quality control microorganisms. A comprehensive antibiogram was determined for the salmonella isolates, using a customized antimicrobial panel of 17 antibiotics with a range of concentrations employed in the National Antimicrobial Resistance Monitoring System (**NARMS**) established by CDC, USDA and FDA (13).

Table 1. Antibiotics used in the National Antibiotic Resistance Monitoring Program.

1. Amikacin	10.Florfenicol
2. Amoxicillin/clavulanic acid	11.Gentamicin
3. Ampicillin	12.Kanamycin
4. Apramycin	13.Nalidixic acid
5. Ceftiofur	14.Streptomycin
6. Ceftriaxone	15.Sulfamethoxazole
7. Cephalothin	16.Tetracycline
8. Chloramphenicol	17.Trimethoprim-sulfamethoxazole
9. Ciprofloxacin	

Preparation and amplification of chromosomal DNA. To determine the extent of integron-mediated multiple antimicrobial resistance among our swine *Salmonella* isolates, PCR primers homologous to conserved DNA sequences in previously described integrons were used (6). Primers were also used to amplify and sequence any integrons detected among our isolates. DNA from *Salmonella* isolates was prepared and purified by routine procedures (10,11). PCR amplification conditions have been previously described (6,10,11).

DNA sequencing of PCR amplicons. PCR amplicons were separated by horizontal gel electrophoresis. Appropriate amplicons were identified by size, excised from the agarose, and purified using the Wizard PCR Clean Up System (Promega, Madison, WI). Sequencing of PCR amplicons was performed according to manufacturer's protocol for cycle sequencing using the
cycle sequencer (Model 377, Perkin Elmer/Applied Biosystems, Foster City, CA). DNA sequencing was performed at the Center for Agricultural Biotechnology of the University of Maryland. DNA sequence data obtained from our studies were analyzed using GCG programs (Genetics Computer Group, Madison, WI), and were compared with published GenBank DNA sequences to determine the presence of specific gene cassettes among the identified integrons.

Pulsed-Field Gel Electrophoresis (PFGE) of Salmonella isolates. PFGE was performed according to a protocol developed by the Centers for Disease Control and Prevention (CDC). Briefly, bacteria were grown on TSA blood agar (Becton Dickinson Microbiology System, Cockeysville, MD) at 37°C for 18 h. Bacterial colonies were suspended in cell suspension buffer (100mMTris HCl, 100mM EDTA, pH8.0) and adjusted to 0.48-0.52 OD using Dade MicroScan Turbidity Meter (Dade Behring Inc., West Sacramento, CA). The cell suspension (200 µL) was mixed with 10 µL of proteinase K (10mg/mL) and equal volume of melted 1% SeaKem Gold agarose (FMC, BioProducts, Rockville, Maine) containing 1% SDS. The mixture was carefully dispensed into a sample mold (Bio-Rad Laboratories, Hercules, CA). After solidification, the plugs were transferred to a tube containing 5 ml of lysis buffer (50mMTris HCl, 50mMEDTA, pH8.0, plus 1% Sarcosyl) and 0.1mg/ml of proteinase K. Cells were lysed overnight in a water bath at 54°C with vigorous agitation. After lysis, the plugs were washed twice with distilled water and four times with TE buffer (10mM Tris HCl, 1mMEDTA, pH8.0) for 15 min per wash at 50°C with vigorous agitation. Agarose-embedded DNA was digested with 50 U of X*ba*I (Boehringer Mannheim corp. Indianapolis, IN) overnight in a water bath at 37°C. The plugs were then placed in an 1% SeaKem Gold agarose (FMC) gel and restriction fragments were separated by electrophoresis in 0.5x TBE buffer at 14°C for 18 h using a Chef Mapper (Bio-Rad) with pulse times of 2.16 -63.8 s. The gel was stained with ethidium bromide, and DNA bands were visualized with UV transillumination. The PFGE images were analyzed using Molecular Analyst Fingerprinting Plus Software (Bio-Rad).

V. Results:

Over 40 *Salmonella* isolates of swine have been assembled and assayed. These organisms include 20 *S. typhimurium* DT104, 2 *S. typhimurium* non-DT104, and 2 *S. typhimurium* Copenhagen nonDT104 strains. The remaining isolates include representatives of the *anatum*, *cholerasuis-kunzendorf*, *derby*, *heidelburg*, *infantis*, and *mbandaka* serotypes. Using Sensititre technology, minimum inhibitory concentrations of 17 different antimicrobials for each isolate have been determined (Table 1). All DT104

isolates displayed the pentamer resistance phenotype to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT). Three of four non-DT104 *S. typhimurium* strains displayed resistance to ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline. Ninety-eight percent (41/42) of *Salmonella* isolates were considered multiply resistant (resistant to at least two different antimicrobial classes). One *S. infantis* (CVM814) isolate was resistant to amoxicillin, apramycin, cephalothin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline (Table 1). Only 1 strain (*Salmonella mbandaka*) was resistant to sulfamethoxazole/trimethoprim. Overall, resistance to sulfamethoxazole, tetracycline, streptomycin, kanamycin, and ampicillin were most common among the non-DT104 *Salmonella* isolates.

The presence of integrons among these Salmonella isolates was also determined. **All** 20 DT104 strains examined by PCR amplification for the presence of integrons have been found to contain two amplicons, one of 1000 bp and the other of 1200 bp (Figure 1). DNA sequencing revealed that the 1000 bp integron contained a gene encoding resistance to streptomycin (*aadA2* aminoglycoside 3" adenyltransferase) where as the 1200 bp integron contained a gene encoding resistance to ampicillin (bla_{PSE-1} β-lactamase). None of the non-DT104 strains showed this same pattern although two strains (CVM805, *S. typhimurium* var copenhagen and CVM817, *S. derby*) produced a 1000 bp integron upon PCR amplification (Figure 1). DNA sequencing revealed that the 1000 bp integron recovered from CVM805-*S. typhimurium* var copenhagen contained the *aadA* gene encoding resistance to streptomycin and spectinomycin, whereas the 1000 bp integron amplified by CVM817-*S. derby* contained the *aadA2* gene as described previously, also encoding resistance to streptomycin and spectinomycin. It is interesting to note that many of the salmonella isolates were resistant to streptomycin but did not possess integrons suggesting other resistant mechanisms at work. The majority of non-DT104 *Salmonella* strains produced no integron amplicons following PCR.

In an added effort to determine the antimicrobial-resistance mechanisms present in these strains, all isolates have been examined for phenotypic characteristics associated with mutation of the *mar* locus (1). Mutations in this chromosomal locus in *Escherichia coli* are associated with acquisition of low-level resistance to certain antimicrobials and tolerance to certain chemicals including cyclohexane. Cyclohexane tolerance was found in one DT104 strain and one *S. typhimurium* Copenhagen non-DT104 isolate (CVM798 and 805, respectively). Interestingly, these two strains differed from the other strains tested in that they were more resistant to ceftiofur and ciprofloxacin (Table 1). This increase in resistance to ciprofloxacin is especially provocative since some researchers have noted that DT104 strains may acquire resistance to this drug (2,5). Resistance to ciprofloxacin by *Salmonella* is especially troubling since it is often the drug of choice in human *Salmonellosis* therapy (2).

RNA from the two isolates (CVM798 and 805) displaying increased organic solvent tolerance were probed with an *E. coli*-derived *mar*A gene probe. *mar*A encodes a transcriptional activator of the *marRAB* operon (7). *marA* was shown not to be overexpressed in these two isolates. Failure to detect *marA* expression in organisms exhibiting a *mar*-like phenotype suggests that these two salmonellae may possess a homolog of the *E. coli mar* locus. Further work to clone and sequence this homolog is needed.

Lastly, genetic relatedness among the swine salmonella isolates was assayed using Pulsed-field gel electorphoresis (PFGE). PFGE revealed twenty distinct genetic patterns among the 42 isolates. However, all *S. typhimurium* DT104 isolates fell within two distinct genetic clusters that were very similar to one another (Figure 2). No correlation was observed between the state of origin of the DT104 isolate and which genetic cluster it fell

into. The *S. typhimurium* non-DT104 and *S. typhimurium* – copenhagen strains did not fall into the DT104 clusters but were the most similar of all the species assayed to the DT104 isolates. Other Salmonella serotypes grouped together within their specific species. Specific genetic clusters were observed for *S. anatum* (N=3), *S. cholerasuis* (N=4), *S. derby* (N=3), *S. heidelburg* (N=3), and *S. infantis* (N=3) (Figure 2).

This research and that of others suggest that antimicrobial resistance among swine *Salmonella* is likely due to multiple mechanisms involving a *mar*-like homolog (shown here), integrons (shown here), and conjugative R plasmids (8,9,12). Additionally, *S. typhimurium* DT104 isolates from diseased swine in the United States are clonal in origin and suggest that this particular species is capable of rapid spread and is widely distributed in the US.

VI. List of References:

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Table 1. Antimicrobial susceptibility patterns of swine *Salmonella* spp.[a] (:g/ml)

Isolate	ST[b]	Amk[c]	Amo	Amp	Apr	Cef	Cet	Cep	Cml	Cip	Ffc	Gen	Kan	Nal	Str	Sul	Tet	Tri
S. typhimurium DT104																		
CVM790	CO	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**128**	**>512**	**>32**	0.25
CVM800	IA	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	8	**128**	**>512**	**>32**	0.50
CVM801	IA	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.25
CVM785	IN	≤4	16	**>32**	4	≤0.50	≤0.25	2	**>32**	≤0.01	>16	0.50	≤16	≤4	**256**	**>512**	**>32**	0.25
CVM793	IN	≤4	16	**>32**	4	≤0.50	≤0.25	8	**>32**	≤0.01	>16	0.50	≤16	≤4	**128**	**>512**	**>32**	0.25
CVM798	IL	≤4	16	**>32**	4	2	≤0.25	8	**>32**	0.06	>16	0.50	≤16	8	**64**	**>512**	**>32**	0.25
CVM799	IL	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**128**	**>512**	**>32**	0.25
CVM789	MN	≤4	16	**>32**	≤2	≤0.50	≤0.25	8	**>32**	≤0.01	>16	0.50	≤16	≤4	**256**	**>512**	**>32**	0.25
CVM787	NC	≤4	16	**>32**	4	≤0.50	≤0.25	2	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.25
CVM803	NC	≤4	16	**>32**	4	≤0.50	≤0.25	2	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.25
CVM786	OH	≤4	16	**>32**	≤2	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.50
CVM795	OH	≤4	16	**>32**	4	≤0.50	≤0.25	2	**>32**	≤0.01	>16	0.50	≤16	≤4	**128**	**>512**	**>32**	0.25
CVM804	OH	≤4	16	**>32**	≤2	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.25
CVM792	OK	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**128**	**>512**	**>32**	≤0.12
CVM797	OK	≤4	16	**>32**	4	≤0.50	≤0.25	8	**>32**	≤0.01	>16	1	≤16	≤4	**64**	**>512**	**>32**	0.25
CVM802	OK	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	≤0.12
CVM791	PA	≤4	16	**>32**	4	≤0.50	≤0.25	2	**>32**	0.03	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.25
CVM796	SC	≤4	16	**>32**	4	≤0.50	≤0.25	2	**>32**	≤0.01	>16	0.50	≤16	≤4	**64**	**>512**	**>32**	0.25

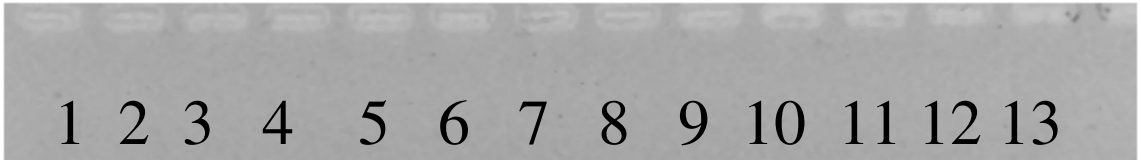
CVM788	VA	≤4	16	**>32**	4	≤0.50	≤0.25	4	**>32**	≤0.01	>16	0.50	≤16	≤4	**128**	**>512**
>32	0.25															
CVM794		8	16	**>32**	8	≤0.50	≤0.25	4	**>32**	≤0.01	>16	2	≤16	≤4	**256**	**>512**
>32	0.25															
***S. typhimurium* non-DT104**																
CVM806	MN	≤4	8	**>32**	4	≤0.50	≤0.25	16	16	≤0.01	4	0.50	**>64**	≤4	**256**	**>512**
>32	0.25															
CVM808	NC	≤4	1	16	8	≤0.50	≤0.25	4	16	≤0.01	4	0.50	≤16	≤4	64	**>512**
8	≤0.12															
***S. typhimurium* – copenhagen**																
CVM807	NC	≤4	8	**>32**	≤2	≤0.50	≤0.25	4	8	≤0.01	4	0.50	**>64**	≤4	**256**	**>512**
>32	0.25															
CVM805	WI	≤4	16	**>32**	4	2	≤0.25	16	16	0.06	16	4	**>64**	16	**256**	**>512**
>32	0.50															
S. anatum																
CVM811	IL	≤4	4	**>32**	≤2	≤0.50	≤0.25	8	8	0.03	4	0.50	≤16	8	≤32	**>512**
>32	≤0.12															
CVM810	NC	≤4	1	≤2	8	1	≤0.25	2	8	0.03	4	≤0.25	≤16	8	≤32	**>512**
32	≤0.12															
CVM813	TX	≤4	1	≤2	≤2	≤0.50	≤0.25	4	8	0.50	4	≤0.25	≤16	8	≤32	**>512**
>32	≤0.12															

Isolate	ST[b]	Amk[c]	Amo	Amp	Apr	Cef	Cet	Cep	Cml	Cip	Ffc	Gen	Kan	Nal	Str	Sul	Tet	Tri
S. cholerasuis – kunzendorf																		
CVM824	IL	≤4	8	**>32**	8	1	≤0.25	8	8	0.03	4	0.50	≤16	8	**>256**	**>512**	**>32**	0.50
CVM823	KS	≤4	1	≤2	4	≤0.50	≤0.25	2	8	0.03	4	0.50	≤16	16	**>256**	**>512**	≤4	0.50
CVM816	MO	≤4	16	**>32**	≤2	1	≤0.25	16	≤4	0.03	4	1	≤16	≤4	**>256**	**>512**	**>32**	≤0.12
CVM821	NE	≤4	1	≤2	4	≤0.50	≤0.25	2	≤4	0.03	4	0.50	**>64**	8	**>256**	**>512**	**>32**	0.25
S. derby																		
CVM817	OH	≤4	1	≤2	4	≤0.50	≤0.25	8	8	≤0.01	4	0.50	32	≤4	**256**	**>512**	**>32**	0.25
CVM819	MN	≤4	1	≤2	≤2	1	≤0.25	2	16	0.03	8	0.50	≤16	≤4	**128**	**>512**	**>32**	0.50
CVM820	MO	≤4	1	≤2	4	1	≤0.25	2	8	0.03	8	0.50	≤16	≤4	≤32	≤128		

≤4	≤0.12														
S. heidelburg															
CVM822	IL	≤4	1	≤2	≤2	≤0.50	≤0.25	4	8	0.03	4	0.50	**>64**	≤4	**128**	**>512**
>32	≤0.12															
CVM815	NC	≤4	8	≤2	≤2	≤0.50	≤0.25	2	16	0.03	8	0.50	**>64**	≤4	**64**	**>512**
>32	≤0.12															
CVM825	OK	≤4	1	≤2	≤2	≤0.50	≤0.25	2	8	≤0.01	4	≤0.25	**>64**	≤4	**128**	≤128
>32	≤0.12															
S. infantis																
CVM812	ID	≤4	1	8	≤2	1	≤0.25	4	8	0.03	8	≤0.25	≤16	≤4	≤32	**>512**
≤4	0.25															
CVM818	IL	≤4	1	≤2	8	1	≤0.25	4	8	≤0.01	8	≤0.25	≤16	≤4	≤32	**>512**
≤4	0.25															
CVM814	ND	≤4	**>32**	16	**>32**	1	≤0.25	**>32**	**>32**	≤0.01	16	**>16**	**>64**	≤4	128	**>512**
>32	0.25															
S. mbandaka																
CVM809	IN	≤4	1	≤2	4	1	≤0.25	4	16	0.03	8	1	≤16	≤4	≤32	**>512**
>32	≤0.12															
CVM826	NC	≤4	1	≤2	4	≤0.50	≤0.25	2	8	≤0.01	8	1	**>64**	8	**128**	**>512**
>32	**>4**															

[a] MIC's determined via microdilution methods according to NCCLS guidelines. Resistance is indicated in **bold**.
[b] State of origin
[c] Amk, Amikacin; Amo, amoxicillin/Clavulanic acid; Amp, Ampicillin; Apr, Apramycin; Cef, Ceftiofur; Cet, Ceftriaxone; Cep, Cephalothin; Cml, chloramphenicol; Cip, ciprofloxacin; Ffc, Florfenicol; Gen, Gentamicin; Kan, Kanamycin; Nal, nalidixic acid; Str, Streptomycin; Sul, Sulfamethoxazole; Tet, Tetracycline; Tri, Trimethoprim/Sulfamethoxazole.
[d] *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Psuedomonas aeruginosa* ATCC 27853 were used as controls in MIC determinations.

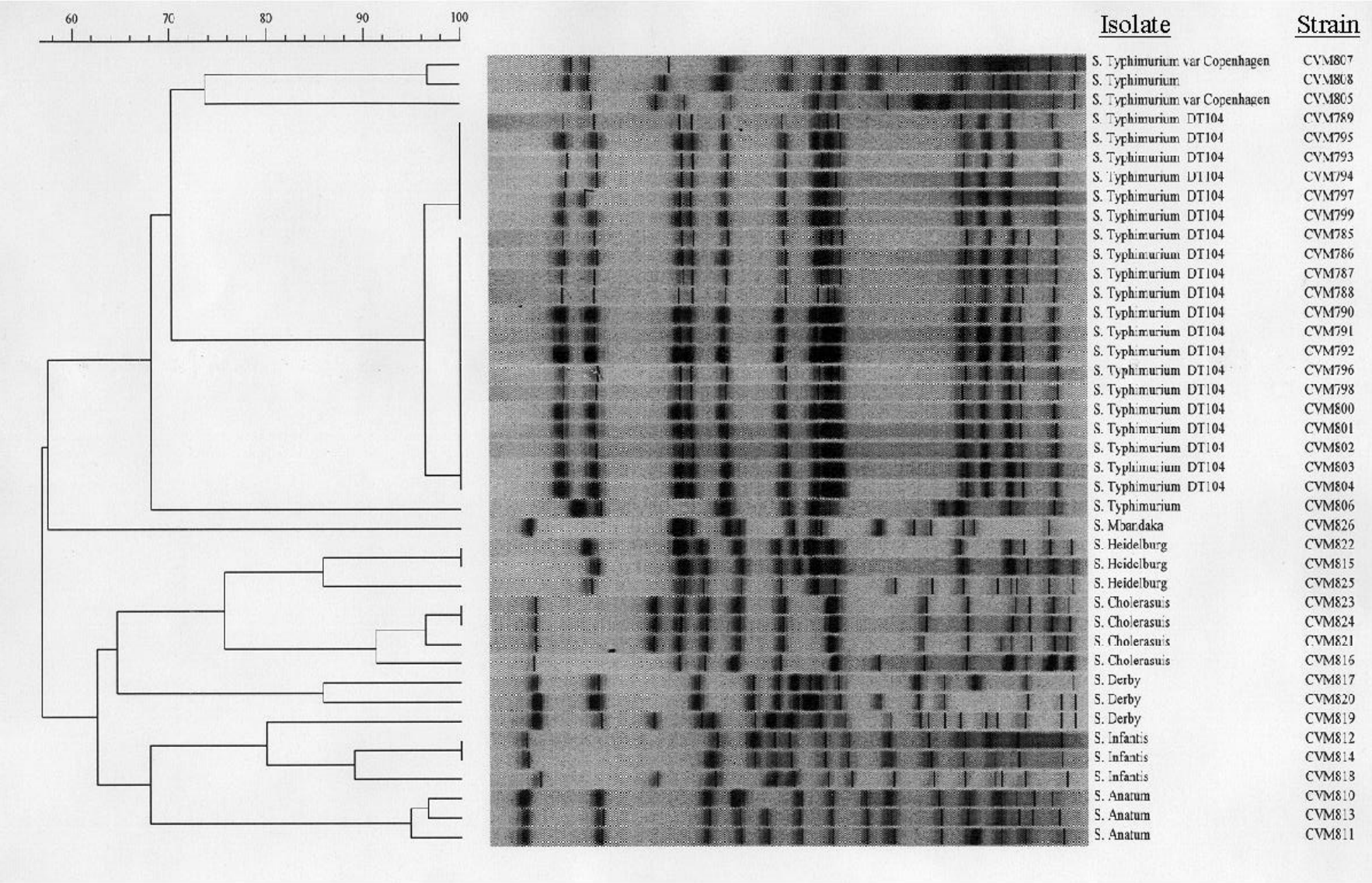
Figure 1. PCR amplification of integrons among swine salmonella isolates



1200 bps
1000 bps

1% agarose gel; PCR using integron specific primers, top band = 1200 base pairs, bottom band = 1000 base pairs
Lanes 1,13,14,26 = 1 KB DNA ladder;
Lanes 2-12, 15-23 = *S. typhimurium* DT104 isolates (CVM785-804, respectively);
Lanes24-25 = *S. typhimuirum* var Copenhagen (CVM805) and *S. derby* (CVM817)

Figure 2. Genetic relatedness among swine *Salmonella* isolates



PFGE patterns of Salmonella species were cleaved with restriction enzyme *Xba1*