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Trends in antimicrobial resistance, phage types and integrons among *Salmonella* serotypes from pigs, 1997–2000

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***Objectives*: The objectives of this study were to determine antimicrobial resistance and to identify phage types and class 1 integrons among non-typhoidal *Salmonella* isolates from 24 pig farms in North Carolina collected between 1997 and 2000.**

***Methods*: A total of 1314 isolates of 30 serotypes from pig faecal samples were collected and analysed over a 3 year period. The isolates were characterized using antimicrobial susceptibility testing, phage typing, PCR and DNA sequencing for class 1 integrons.**

***Results*: A high frequency of resistance to antimicrobial agents including tetracycline (85%), ampicillin (47%), co-amoxiclav (23%) and chloramphenicol (21%) was detected. Two multidrug resistance patterns were common in Typhimurium (including variant Copenhagen): isolates with co-amoxiclav, ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (R-type AxACSSuT) [36%] and isolates with ampicillin, kanamycin, streptomycin, sulfamethoxazole and tetracycline (R-type AKSSuT) [45%] resistance patterns. Definitive Type 104 (DT104) was the most common (34%) among eight phage types identified. AKSSuT was found among non-DT104 phage types, particularly DT21 and DT193. Class 1 integrons were detected among various serotypes including Typhimurium, Derby, Muenchen, Worthington, Bere and Muenster. *aadA* was the most common resistance gene insert, and the *oxa30* β-lactamase resistance gene was also identified among serovar Muenchen.**

***Conclusions*: In this study, two most important multidrug resistance patterns (AxACSSuT and AKSSuT) and phage types of public health significance (DT104 and DT193) constituted two-thirds of the serotype Typhimurium isolates. The findings imply that pigs raised in the commercial production system may pose a risk in serving as reservoirs of resistant *Salmonella*.**

Keywords: swine, food safety, antibiotic resistance, salmonellae

Introduction

Human salmonellosis outbreaks have repeatedly been traced to food products of animal origin, including pork, in many parts of the world.[1–3] The increasing prevalence of multidrug resistance among *Salmonella* and resistance to clinically important antimicrobial agents such as fluoroquinolones and third generation cephalosporins has also been an emerging problem in recent years.[4–6]

The frequency of multidrug-resistant serotypes such as Typhimurium and Newport is reportedly increasing. One major concern to public health has been the emergence of Definitive Type 104 (DT104), which was first recognized in the UK in 1984[4] and later identified in other parts of the world.[2,7–9] This phage type commonly exhibits resistance to five antimicrobial agents: ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (R-type ACSSuT). Recent studies have also shown that this phage type can acquire additional resistance to other, relatively new and potent antimicrobial agents such as fluoroquinolones[4] and higher generation cephalosporins.[5,6]

Studies in Denmark implicated pork products as important sources for human DT104 outbreaks in recent years.[2,10] Other phage

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types of this serotype, such as DT193, have also been implicated as sources of human infections in the UK and Italy.[1,3,11] Outbreaks involving both of these phage types have also been reported to originate from improperly processed contaminated pork.

Even though pork accounts for 25% of the total meat consumption in the United States, reports of human outbreaks of salmonellosis linked to pork consumption are rare. Though North Carolina is the second largest pork producing state in the United States, the relatively few reports on antimicrobial resistance of *Salmonella* in swine were mostly reported from areas with low levels of pig production.[12–15] Information on the potential role of commercial swine production in dissemination of multidrug-resistant salmonellae in the United States is very limited. In addition, the role that food-producing animals play as a primary source of multidrug-resistant *Salmonella* has often been questionable.[11] Prompted by these observations, we conducted a longitudinal study of antimicrobial use and resistance among *Salmonella* isolates collected from pigs. In this study, we investigated antimicrobial resistance and occurrence of multidrug-resistant serotypes, phage types and class 1 integrons among *Salmonella* from pigs in commercial swine operations between 1997 and 2000.

Materials and methods

Bacterial isolates

Samples were obtained from 24 commercial swine production farms in North Carolina that were managed under two major swine production systems (referred to as 'System' in this manuscript). Six nursery and 18 finishing farms were included. Sampling from the same farms was repeated three times between 1997 and 2000. Briefly, 96 faecal specimens were collected from each of 49 groups of pigs (27 from System I and 22 from System II). Details of the sampling scheme were as described earlier.[16,17]

Salmonella isolation and identification

Salmonella were isolated using conventional methods.[18,19] Briefly, 10 g of faecal samples were pre-enriched using buffered peptone water (Difco, Sparks, MD, USA), and then incubated at 37°C for 24 h. A 100-μL suspension from each sample was transferred to Rappaport Vassillidis medium (Difco) at 1:100 dilutions and incubated at 42°C for 24 h. Samples were then plated on Bacto XLT-4 agar base (Difco) and incubated at 37°C for 24 h. Single colonies were then tested for the appropriate biochemical reactions on triple sugar iron (Difco) and urea agar (Difco). Confirmed positive isolates were submitted to the National Veterinary Services Laboratories (Ames, IA, USA) for serotyping and phage typing.

Antimicrobial susceptibility test

Susceptibility testing was carried out initially using the Vitek Jr. semi-automated system (bioMérieux, Hazelwood, MO, USA) using breakpoint panels. Each isolate was first tested against a panel of 10 antimicrobial agents. The antimicrobials and respective resistance MIC break points were amikacin [Ak] (64 mg/L), co-amoxiclav [Ax] (32/16 mg/L), ampicillin [A] (32 mg/L), cefotaxime [Cf] (64 mg/L), cefalothin [Ce] (32 mg/L), chloramphenicol [C] (32 mg/L), ciprofloxacin [Cip] (4 mg/L), gentamicin [G] (16 mg/L), tetracycline [T] (16 mg/L) and trimethoprim/sulfamethoxazole [T/S] (4/76 mg/L).[20,21] In this study, isolates with intermediate MIC breakpoints were grouped with susceptible organisms in order to not to overestimate occurrence of resistance.

Additional susceptibilities to sulfamethoxazole [Su] (0.25 μg), streptomycin [S] (10 μg), kanamycin [K] (30 μg) and ceftriaxone [Cro] (30 μg) were also determined on *S.* Typhimurium, its variant Copenhagen isolates and other serovars (Muenchen, Derby and Worthington), to which further molecular analysis was conducted, by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar plates using conventional techniques.[20,21] Results were interpreted according to the NCCLS criteria. *Escherichia coli* strains ATCC 25922 and 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms in antimicrobial susceptibility testing according to NCCLS recommendations.

Identification of Class 1 integrons

Variable region of class 1 integrons was amplified using the following PCR primers: 5′-CS (5′-GGCATCCAAGCACAAGC-3′) and 3′-CS (5′-AAGCAGACTTGACCTGAT-3′). The smallest expected size of the variable region if no resistance gene cassette is inserted in the integron was 153 bp. Amplification reactions were carried out with 1 μL of purified DNA (Qiagen DNAeasy tissue kit, Valencia, CA, USA), 300 μM deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 50 pmol of primers, and 0.5 U of Gold *Taq* polymerase (Perkin-Elmer, Foster City, CA, USA). Distilled water was added to bring the final volume to 20 μL. The PCR cycle included initial denaturation at 95°C for 5 min and 30 cycles of denaturation for 1 min at 95°C, primer annealing for 1 min at 54°C, and extension for 1 min at 72°C.

DNA sequence analysis

PCR amplicons for class 1 integrons of three serotypes showing multidrug resistance patterns including Derby, Muenchen and Worthington were sequenced. DNA from amplified product was run on agarose gel using 1% agarose and QIAquick Gel extraction kit (Qiagen) was used to purify DNA. Purified samples were submitted to the University of North Carolina Sequencing Facility for sequencing.

Statistical analysis

Comparison of proportions and analysis of association between antimicrobial use and resistance was conducted by χ^2 univariate analysis at type-I error (α) level of 0.05 using commercial software (Minitab 12, Cytel Software, Boston, MA, USA). Odds ratios and 95% confidence intervals were calculated to determine the strength of association.

Results

Antimicrobial use and resistance

Among groups in System I (total of 27 groups), the antimicrobials and proportion of pig groups that were administered antimicrobials in feed, water or as injectables included tetracycline (100%), penicillin (100%), ceftiofur (50%), aminoglycosides (82%) and sulfa drugs (100%). In System II, the antimicrobials and percentage of groups administered were tetracycline (95%), penicillin (60%), ceftiofur (75%), aminoglycosides (10%) and sulfa drugs (22%). Ceftiofur was used only at therapeutic levels whereas the remaining antimicrobials were used at subtherapeutic (feed grade) level as well. More groups in System II (17 of 22) were exposed to ceftiofur than System I (13 of 27). According to the farm records, antimicrobials were used throughout the 3-year study period with no noticeable changes in types, doses or routes of administration.

All the 1314 *Salmonella* isolates were susceptible to ciprofloxacin, cefotaxime and amikacin. Of all the isolates tested, only 188

Table 1. Summary of individual antimicrobial resistance frequency among 30 serotypes in the 3 year study period

Serotype/sample period	n^b	Number of isolates and (percentage) resistance to each antimicrobial[a]						
		Ax	A	Ce	C	G	T	T/S
Copenhagen								
year-I	160	111 (69)	144 (90)	6 (4)	106 (66)	2 (1)	150 (94)	0 (0.0)
year-II	99	67 (68)	94 (95)	5 (5)	68 (69)	0 (0.0)	93 (94)	0 (0.0)
year-III	119	47 (39)	100 (84)	0 (0.0)	47 (39)	0 (0.0)	116 (97)	1 (1)
sub-total	378	225 (59.5)	338 (89.4)	11 (2.9)	221 (58.5)	2 (0.5)	359 (95)	1 (0.26)
Typhimurium								
year-I	73	24 (33)	71 (97)	5 (7)	9 (12)	0 (0.0)	72 (99)	0 (0.0)
year-II	69	4 (6)	65 (94)	1 (1)	3 (4)	0 (0.0)	64 (93)	0 (0.0)
year-III	38	6 (16)	33 (87)	2 (5)	2 (5)	0 (0.0)	36 (95)	0 (0.0)
sub-total	180	34 (18.9)	169 (93.9)	8 (4.4)	14 (7.8)	0 (0.0)	172 (95.6)	0 (0.0)
Derby								
year-I	50	4 (8)	14 (28)	0 (0.0)	13 (26)	14 (28)	49 (98)	0 (0.0)
year-II	32	0 (0.0)	5 (16)	0 (0.0)	5 (16)	5 (16)	26 (81)	0 (0.0)
year-III	26	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	23 (88)	0 (0.0)
sub-total	108	4 (3.7)	19 (17.6)	0 (0.0)	18 (16.7)	19 (17.6)	98 (90.7)	0 (0.0)
All others (27 serotypes)								
year-I	128	6 (4.7)	8 (6.25)	1 (0.78)	5 (3.9)	5 (3.9)	118 (92.2)	1 (0.78)
year-II	242	9 (3.7)	30 (12.4)	8 (3.3)	1 (0.4)	1 (0.4)	166 (68.6)	0 (0.0)
year-III	278	29 (10.4)	48 (17.3)	3 (1.1)	23 (8.3)	0 (0.0)	206 (74.1)	5 (1.8)
sub-total	648	44 (6.8)	86 (13.3)	12 (1.85)	29 (4.5)	6 (0.93)	490 (75.6)	6 (0.93)
Total	1314	307 (23.4)	612 (46.6)	31 (2.4)	282 (21.5)	27 (2.05)	1119 (85.2)	7 (0.53)

Year-I, 1997–1998; Year-II, 1998–1999; Year-III, 1999–2000.
[a]Abbreviations of the antimicrobials shown on the table are: Ax, co-amoxiclav; A, ampicillin; Ce, cefalothin; C, chloramphenicol; G, gentamicin; T, tetracycline and T/S, trimethoprim–sulfamethoxazole.
[b]*n*, number of isolates tested in each respective sampling period.

(14%) were susceptible to all antimicrobials tested. The remaining 1126 isolates (86%) showed resistance to at least one antimicrobial agent. The most common resistance observed was to tetracycline (85%) with a high frequency of resistance noted during the entire 3 year study period (ranging between 74% and 99%). The β-lactams (ampicillin and co-amoxiclav) were the class to which *Salmonella* serotypes were next most frequently resistant. Isolates were most often resistant to ampicillin (47%) and co-amoxiclav (23%). Resistance to both β-lactam agents was more common in the first year of the study than in the second or third year. Frequency of resistance to β-lactams declined significantly, from 35% to 18% for ampicillin, and from 48% to 23% for co-amoxiclav ($P < 0.05$). Fifty isolates derived from 21 groups that were resistant to co-amoxiclav were further tested for resistance to ceftriaxone but none were found to be resistant.

More than 21% of the isolates in the study were resistant to chloramphenicol. Typhimurium variant Copenhagen comprised 78% of all isolates resistant to this antimicrobial agent, whereas serotype Typhimurium comprised only 5% of chloramphenicol-resistant isolates. A decline in the frequency of chloramphenicol resistance was noticed in the 3 year study period in parallel with the decline in resistance to the β-lactam agents. Resistance to cefalothin (2%), gentamicin (2%) and trimethoprim/sulfamethoxazole (<1%) were the least common of the tested antimicrobial agents (Table 1).

Multidrug resistance (MDR) profiles

Among 1126 isolates that showed resistance, 56% were found to be resistant to more than one antimicrobial. Resistance to multiple antimicrobial agents was predominantly seen among the more prevalent serotypes, mainly Typhimurium (94%) and its variant Copenhagen (90%). The next most common serotype, Derby, however, was often found to be resistant to tetracycline alone. Nineteen percent of Derby isolates were multiresistant with the ACGSSuT resistance pattern. Among the relatively rare serotypes, a higher frequency of MDR was found mainly among serotypes Havana (83%) and Muenchen (75%).

Two predominant MDR patterns were found among *S.* Typhimurium and variant Copenhagen isolates: AxACSSuT (36%) and AKSSuT (45%). Among serotype Typhimurium variant Copenhagen (n = 328), both of these resistance patterns were common: AxACSSuT (52%) and AKSSuT (31%). Serotype Typhimurium (n = 156) exhibited predominantly the latter resistance pattern (72%) but rarely the former (3%). The AxACSSuT resistance pattern was more common in the first year of the study (61%) among Copenhagen isolates and its frequency declined by the end of the study period (39%). On the other hand, isolates with the AKSSuT resistance pattern were relatively less common at the beginning of the study (20%) but were more common by the end (46%) indicating a multidrug resistance pattern shift ($P < 0.05$).

Identification of phage types

Phage typing of 369 isolates of serotype Typhimurium (including variant Copenhagen) resulted in identification of eight phage types: DT104, DT21, DT193, DT208, DT12, U302, DT120 and DT169 (Table 2). DT104 was the most common phage type detected (34%). This phage type was commonly found among Typhimurium variant Copenhagen isolates (121 of 125). Isolates of this phage type also

Table 2. Phage types of serotype Typhimurium, variant Copenhagen and respective resistance patterns exhibited

Phage type (369)	Serotype (*n*)	Resistance pattern (number of isolates)
DT104 (125)	Copenhagen (121)	**AxACSSuT(102)**, SSu (10), AxACeCSSuT (2), Other (7)
	Typhimurium (4)	**AxACSSuT (4)**
DT21 (96)	Copenhagen (5)	AxACKSSuT (1), AxACeKSSuT (1), ACeCKSSuT (1), ACeKSSuT (1), AKSSuT (1)
	Typhimurium (91)	**AKSSuT (66),** AxAKSSuT (12), None (3), Other (10)
DT193 (91)	Copenhagen (67)	**AKSSuT (50),** AxAKSSuT (3), AxACeCKSSuT (3), Other (11)
	Typhimurium (24)	**AxASSuT (8),** ASSuT (6), AKSSuT (4), Other (6)
DT208 (18)	Copenhagen (5)	**Te (4),** AKSSuT (1)
	Typhimurium (13)	**AKSSuT (4), AxACKSSuT (4),** Other (5)
DT12 (16)	Copenhagen (16)	**AKSSuT (13)**, Other (3)
U302 (14)	Copenhagen (12)	**AxACSSuT (11),** AxASSuT (1)
	Typhimurium (2)	**AKSSuT (2)**
DT120 (1)	Copenhagen (1)	**AxACSSuT (1)**
DT169 (1)	Copenhagen (1)	**AxACSSuT (1)**
Untypeable (5)	Copenhagen (5)	**AKSSuT (3),** AxACSSuT (2)
RDNC[a] (2)	Copenhagen (1)	**AKSSuT (1)**
	Typhimurium (1)	**AKSSuT (1)**

Predominant resistance patterns associated with each phage type are shown in bold.
[a]RDNC stands for reactive to phages, did not conform with any known type.

often exhibited an expanded MDR with the AxACSSuT (106 of 125) followed by SSu (10 of 125) resistance pattern. Few isolates of this phage type exhibited an expanded spectrum of antimicrobial resistance with cefalothin (two of 125) or gentamicin (one of 125) resistance in addition to the former pattern. The other phage type that commonly showed the AxACSSuT resistance pattern, similar to DT104, was U302. This phage type was found in 14 of the 369 isolates tested, and 11 of the isolates showed this resistance pattern.

Four other phage types predominantly exhibited pentaresistance with the AKSSuT resistance pattern. The two most common phage types exhibiting this resistance pattern were DT21 (26%) and DT193 (25%). DT21 was found predominantly among Typhimurium isolates. Unlike DT104 and DT21, DT193 was not associated with a specific variant but was common in both Typhimurium and variant Copenhagen isolates.

Presence of Class 1 integrons and sequence analysis

The serotypes and respective resistance patterns tested were Typhimurium phage type DT104 (AxACSSuT), phage type DT193 (AKSSuT), Derby (ACGSSuT), Worthington (AxACeSSuT), Muenchen (AxACKSSuT), Muenster (AxACeGT) and Bere (AT). All the six serotypes tested were found to have at least one Class 1 integron. As shown in Figure 1, up to three different sizes of integrons per isolate were detected, ranging between 0.2 and 2.0 kb. Three different size integrons were detected within a single isolate of serotype Worthington ranging between 0.2 and 1.6 kb, whereas hexaresistant Typhimurium DT104 isolate showed two integrons of 1.0 and 1.2 kb, and pentaresistant Typhimurium DT193 exhibited a single 1.0 kb amplicon. The pentaresistant Muenster isolates carried the largest integron of about 2.0 kb. Further sequence analysis of amplicons was carried out on three of the serovars: Derby, Muenchen and Worthington, each of which showed multidrug resistance. As shown in Table 3, the aminoglycoside resistance gene *aadA* was present in all three serovars. The 2 kb amplicon from serovar Muenchen also carried an additional β-lactamase gene, *oxa30*. All the six Worthington isolates tested also carried a 0.2 kb amplicon that, as expected due to the small integron size, did not carry any resistance gene cassette.

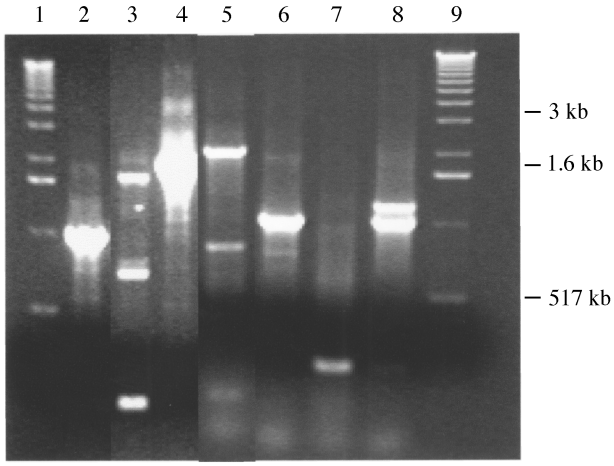

Figure 1. PCR amplification of class 1 integrons among different serotypes of Salmonella. 1 and 9, molecular weight marker; 2, Derby (ACGSSuT); 3, Worthington (AxACeSSuT); 4, Muenchen (ACKSSuT); 5, Muenster (AxACeGT); 6, Typhimurium DT193 (AKSSuT); 7, Bere (AT); 8, Typhimurium DT104 (ACSSuT).

Discussion

In this study, we demonstrated the widespread occurrence of antimicrobial resistance to tetracycline and β-lactams in 24 farms of two modern commercial swine production systems. This finding may not

Table 3. Identification of class 1 integrons and resistance gene cassettes carried on integrons using PCR and sequencing

Serovar	Resistance pattern	Tested by PCR	Integron size (kb)	Isolates carrying integron	Gene(s) detected on sequencing	NCBI accession no.
Derby	ACGSSuT	13	1.0	11	*aadA*	AY171244
Muenchen	ACKSSuT	4	2.0	4	*oxa30* *aadA*	U90945 STY496285
Worthington	AxACeSSuT	6	0.2 0.75 1.6	6 4 4	Class 1 integron CS[a] *aadA* no homology	AF151984 U90945

[a]CS, conserved segment of the Class 1 integron with no resistance gene cassette identified.

be surprising as these antimicrobials have widely been used in pig production, including the groups tested in this study. What was more surprising was the high frequency of chloramphenicol resistance despite no reported usage of any phenicols, including the fluorinated analogue florfenicol, in the groups of pigs studied. This class of antimicrobial agent has not been used in swine production for more than a decade. The common occurrence of chloramphenicol resistance in this study can largely be explained by the emergence and spread of multidrug-resistant Typhimurium that harbour physically linked pentaresistance alleles, the most notable being members of the DT104 phage type.[22–24] We did not find resistance to third generation cephalosporins such as cefotaxime and ceftriaxone, the aminoglycoside amikacin, or the fluoroquinolone ciprofloxacin, among the isolates. Though resistance to these classes of antimicrobials is rare in the United States, a relatively high frequency of resistance has been reported in other countries with fluoroquinolone-resistant Typhimurium DT104 reported in 14% of isolates collected in England.[24,25] Resistance to two β-lactam agents: ampicillin and co-amoxiclav were more common in the first year of study than in the second or the third. In this study, the reduction in the frequency of resistance was concordant with the reduction in the overall frequency of serotype Typhimurium and the Copenhagen variant. Consistent with previous reports, resistant phenotypes appear to be associated with particular serotypes.[15,26,27] The decline in chloramphenicol resistance was concordant with the shift of the predominant multidrug resistance pattern within serotype Typhimurium, particularly variant Copenhagen, from AxACSSuT to AKSSuT over the study period.

Among the most common serotypes Typhimurium and variant Copenhagen, two MDR phenotypes were prevalent throughout the study period: AxACSSuT (36%) and AKSSuT (45%). Pentaresistance patterns (ACSSuT) are commonly reported in numerous outbreaks, mainly of phage type DT104.[4,7,28,29] We found DT104, currently among the most significant public health threat, to be the most common phage type among the pig isolates commonly exhibiting hexaresistance pattern, AxACSSuT. Unlike previous reports, the findings in this study show that DT104 isolates exhibited additional resistance to co-amoxiclav, a more potent β-lactam with β-lactamase inhibitor, clavulanic acid. However, 50 isolates with co-amoxiclav resistance, further tested for ceftriaxone resistance were found to be susceptible implying the lack of AmpC phenotype. PCR on representative isolates from this group revealed that they do not carry bla_{CMY-2} gene (data not shown). We believe the co-amoxiclav resistance is encoded by the previously explained gene, bla_{PSE1}.[22,23] In addition, this hexaresistance pattern was also detected among other less frequent phage types of Typhimurium, mainly U302.[23,30] Phage type DT104 has previously been shown to be prevalent among a number of host species including food animals, pets and wild animals, with several food-borne outbreaks in humans linked to this phage type.[2,9,28,29] However, such a high frequency among apparently healthy pigs has, to our knowledge, not been reported previously.

The other pentaresistance pattern, AKSSuT, is increasingly being reported in numerous geographical locations. According to the National Antimicrobial Resistance Monitoring System's report, more than 9% of Typhimurium isolates from humans exhibited this pentaresistance pattern and increasing numbers of animal isolates also exhibited this pattern.[32,33] A common phage type of interest in this study, which predominantly exhibited the AKSSuT resistance pattern was DT193. Since it was first characterized in 1978,[34] this phage type expanded its MDR spectrum and an increasing number of human infections emerged in the 1980s causing major food-borne outbreaks in the late 1980s and 1990s.[1,3,11] In the United States, no large-scale food-borne outbreak has so far been attributed to this phage type. The high frequency of occurrence among isolates originating from healthy pigs in this study could be of concern since previous reports of human outbreaks due to this phage type were associated with pork products.[1,3] Molecular characterization of strains exhibiting this pentaresistance pattern was done by our group previously, and we have shown that all the resistance genes are carried extrachromosomally on conjugative plasmids and that they were different from those of DT104. Detailed results were published previously.[23]

This study revealed a significant shift over time in the phage type and associated resistance pattern of the most commonly isolated serotype. Early in the study period, most isolates of *S.* Typhimurium variant Copenhagen were of phage type DT104, with its AxACSSuT hexaresistance pattern. However, during the period of study, this phage type was displaced by others, particularly DT21 and DT193, with AKSSuT pattern. There was no indication of a change in the antimicrobial use pattern, and so there was no overt alteration in selective pressure that might have fostered such a change. Nonetheless, it is interesting to note that both DT193/DT21, which finally predominated, and DT104, which was displaced, are resistant to four

antimicrobials representing classes commonly used in pigs (and used in the animals in this study).

Analysis of class 1 integrons among different and some relatively rare serotypes with multidrug resistance phenotypes revealed that these mobile DNA elements are common. Each of the six serotypes tested carried at least one integron sequence in their genome. The lowest expected size of the variable region if no resistance gene cassette is inserted in the integron was 153 bp (GenBank accession number M73819). Finding a variable region of the integron as low as 0.2 kb implies the integrons have no inserted resistance gene cassettes and resistance genes in these isolates are not associated with the integron identified. For isolates of the Worthington serotype, this has been confirmed by DNA sequence analysis as shown in Table 3. This implies these isolates carry an integron, a potential hotspot for the development of multidrug resistance. Amplicons as large as 2.0 kb have also been identified implying multiple resistance genes inserted within the integron. Though the frequency of occurrence of some of the serotypes tested is currently low, their MDR pattern and presence of class 1 integrons have not been described before and may signify the potential for expansion of their MDR spectrum and emergence as important public health hazards. Further sequence analysis of PCR products of class 1 integrons revealed that despite the size variation of these integrons, all the three serovars tested carried aminoglycoside adenyl transferase gene, *aadA*. The 2.0 kb amplicon from serovar Muenchen carried two genes, *aadA* and the β-lactamase, *oxa30*. We have not detected *oxa30* from other serovars, including the most commonly multidrug-resistant serovar Typhimurium.

In summary, in this study, we found the two most important multidrug resistance patterns (AxACSSuT and AKSSuT) and phage types of public health significance (DT104 and DT193) to constitute about two-thirds of the serotype Typhimurium isolates in groups of swine. Recent preliminary data on *Salmonella* serotypes and antimicrobial resistance revealed that hexaresistant isolates with AxACSSuT resistance pattern were very common with 84% frequency among serotype Typhimurium isolates collected in 2003 (Gebreyes and Thakur, unpublished data). As the study was conducted in 24 farms and in repeated samplings within two commercial production systems, it may not be representative of overall contemporary production environments. However, the findings show that pigs may pose a potential risk in serving as reservoirs and disseminating multidrug-resistant *Salmonella.*

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