Changes in Antimicrobial Resistance among *Salmonella enterica* Serovar Typhimurium Isolates from Humans and Cattle in the Northwestern United States, 1982–1997

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We compared antimicrobial resistance patterns of *Salmonella enterica* serovar Typhimurium (ST) of isolates from humans (n = 715) and cattle (n = 378) in the Pacific Northwest from 1982 through 1997. The major changes in antimicrobial resistance can be attributed to the widespread clonal dissemination of multidrug-resistant definitive phage type 104 ST.

Enteritidis is the most frequent *Salmonella* serovar in all regions of the United States, except the Pacific Northwest where Typhimurium is the most frequent. In 1996, 31.1% of all *Salmonella* isolates from human sources in Washington, Oregon, and Idaho were serovar Typhimurium, while 14.6% were serovar Enteritidis (1). Typhimurium is also one of the most common *Salmonella* serovars from animal sources in the United States (2).

Use of antimicrobial drugs in food animals may lead to resistant strains of pathogens, which may be transmitted to humans through food (3,4). Although there is evidence that this transmission occurs, the contribution of antimicrobial use in food animals to resistance in bacteria infecting humans is the subject of debate (5-8).

We compared antimicrobial resistance patterns of *Salmonella enterica* serovar Typhimurium (ST) from human and cattle sources over a 15-year period and examined how these patterns relate to antimicrobial use in livestock and humans.

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The Study

We used all ST (including *S.* Typhimurium var Copenhagen) isolates from clinical bovine samples submitted to the Washington Animal Disease Diagnostic Laboratory from 1986 through 1997 and from cattle herds tested by the Field Disease Investigation Unit during salmonellosis outbreaks over the same period (n = 378). For herds sampled repeatedly over time, all but the first ST isolate (per year) were excluded. Antimicrobial resistance data were also available for ST isolates from cattle for 1982 through 1986 from clinical submissions to the Washington Animal Disease Diagnostic Laboratory. Isolates from human clinical specimens (n = 715) were obtained from the Washington State Department of Health Public Health Laboratory for 1989, 1994, 1996, and 1997, and from the Idaho Division of Health, Bureau of Laboratories for 1997.

Isolates from other laboratories were subcultured onto solid brain heart infusion agar. All isolates were maintained in a -70º C bank freezer in brain heart infusion broth containing 25% to 30% buffered glycerol. Isolates were streaked for isolation on sheep blood agar prior to susceptibility testing. Susceptibility testing for the antimicrobial drugs listed in Table 1 was done by a disk diffusion method (9) on

Table 1. Resistance to individual antibiotics among *Salmonella* Typhimurium isolates from cattle and humans

A. From cattle

	Number (%) resistant by years			
Antimicrobial drug	1982-1985[a] n = 49	1986-1990 n = 116	1991-1994 n = 90	1995-1997 n = 123
Ampicillin	39 (79.6)	99 (85.3)	72 (80.0)	113 (91.9)
Chloramphenicol[a]	2 (4.1)	2 (1.7)	56 (62.2)	90 (73.2)
Gentamicin	1 (2.0)	6 (5.2)	14 (15.6)	5 (4.1)
Kanamycin[a]	NA	103 (90.4)	49 (54.4)	50 (40.7)
Streptomycin	43 (87.8)	109 (94.0)	78 (86.7)	116 (94.3)
Tetracycline	43 (87.8)	101 (87.1)	77 (85.6)	115 (93.5)
Trimethoprim	NA	1 (0.9)	11 (12.2)	6 (4.9)
Trimethoprim-sulfamethoxazole	7 (14.3)	1 (0.9)	11 (12.2)	9 (7.3)
Triple sulfa	39 (79.6)	108 (93.1)	75 (83.3)	117 (95.1)

B. From humans

	Number (%) resistant by year			
Antimicrobial drug	1989 n = 90	1994 n = 189	1996 n = 187	1997 n = 249
Ampicillin[a]	22 (24.4)	107 (56.6)	109 (58.3)	164 (65.9)
Chloramphenicol[a]	3 (3.3)	84 (44.4)	92 (49.2)	116 (46.6)
Gentamicin	2 (2.2)	5 (2.7)	5 (2.7)	2 (0.8)
Kanamycin	25 (27.8)	44 (23.3)	34 (18.2)	47 (18.9)
Streptomycin[a]	42 (46.7)	112 (59.3)	109 (58.3)	174 (69.9)
Tetracycline[a]	36 (40.0)	101 (53.4)	109 (58.3)	158 (63.5)
Trimethoprim	0	7 (5.1)	6 (3.1)	5 (2.0)
Trimethoprim-sulfamethoxazole	0	6 (3.7)	6 (3.2)	5 (2.0)
Triple sulfa[a]	36 (40.0)	123 (65.1)	143 (76.5)	231 (92.8)

[a]Chi-square test for trend, p value <0.001.
NA, data not available.

Mueller-Hinton agar prepared according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (10,11). Ciprofloxacin susceptibility was tested on a subset of isolates systematically selected to include five per species per year.

Data for the analysis were divided into periods 1982-1986, 1987-1990, 1991-1994, and 1995-1997. These periods were chosen to compare isolates from cattle with isolates from humans for the years for which isolates from humans were available. Each isolate was classified as resistant or susceptible to each antimicrobial drug tested by the threshold zone size for resistance, as recommended by NCCLS (10,11). The proportions of isolates resistant to individual drugs and having each antimicrobial resistance pattern were computed by species and period. Significance testing of differences in proportions was done with Epi Info (12) using the chi-square test and the chi-square test for trend.

Marked changes in resistance to chloramphenicol were observed for isolates from both cattle and humans (Tables 1, 2). Before 1991, fewer than 5% of isolates from cattle and only 3% of the 1989 isolates from humans were resistant to chloramphenicol; by the mid-1990s, more than 70% (90 of 123) of isolates from cattle ($p < 0.01$) and almost 50% (92 of 187) of isolates from humans ($p < 0.01$) were resistant to chloramphenicol. Most (79%) isolates from cattle were resistant to ampicillin, streptomycin, tetracycline, and sulfonamides throughout the study period. Among isolates from humans, the proportion resistant to these drugs was significantly lower in 1989 than in 1997 ($p < 0.01$). The proportion of isolates from cattle resistant to kanamycin was significantly less ($p < 0.01$) in 1990 to 1994 than in 1986 to 1990. All isolates tested were susceptible to ciprofloxacin, and average zone sizes showed no evidence of decline during the period (data not shown).

Among isolates from both cattle and humans, the ACSSuT resistance pattern was the most

Table 2. Antimicrobial resistance patterns for *Salmonella* Typhimurium isolates from cattle and humans

A. Cattle

	Number (%)			
	1982-1985[a] n = 49	1986-1990 n = 114	1991-1994 n = 90	1995-1997 n = 123
ACSSuT	1 (2.0)	1 (0.9)	18 (20.0)	55 (44.7)
ACKSSuT		0	25 (27.8)	24 (19.5)
ASSuT	24 (49.0)	0	5 (5.6)	6 (4.9)
AKSSuT		83 (72.8)	4 (4.4)	14 (11.4)
Su	1 (2.0)	1 (0.9)	0	0
All Others	22 (45.0)	24 (21.1)	29 (32.2)	20 (16.2)
Suscept[b]	1 (2.0)	5 (4.4)	9 (10.0)	4 (3.3)

B. Humans

	Number (%)		
	1989 n = 90	1994 n = 189	1996-1997 n = 436
ACSSuT	2 (2.2)	44 (23.3)	156 (35.8)
ACKSSuT	0	26 (13.8)	35 (8.0)
ASSuT	0	14 (7.4)	24 (5.5)
AKSSuT	19 (21.1)	2 (1.1)	33 (7.6)
Su	1 (1.1)	9 (4.8)	80 (18.4)
All Others	25 (27.8)	33 (17.5)	47 (10.8)
Suscept[b]	43 (47.8)	61 (32.3)	61 (14.0)

[a]Data on kanamycin susceptibility not available for 1982-1985.
[b]Susceptible to all antimicrobial drugs tested.
A, ampicillin; C, chloramphenicol; T, tetracycline; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulfonamide; Tmp, trimethoprim.

frequent and increased in frequency over the study period (Table 2). Before 1991, ACSSuT accounted for fewer than 4 (2%) of 255 of isolates from both species, and by the mid-1990s it accounted for more than 55 (40%) of 123 of isolates from cattle and more than 156 (35%) of 436 of isolates from humans. Isolates with the ACSSuT resistance pattern together with ACKSSuT-resistant isolates accounted for 79 (64%) of 123 isolates from cattle and 191 (44%) of 436 isolates from humans by the mid-1990s. As previously reported, 57 (95%) of 60 of these isolates were phage typed and found to be DT104 with a single pulsed-field gel electrophoresis pattern (13).

Isolates susceptible to all drugs tested were more common from human than cattle sources (166 [23.2%] of 715 vs. 19 [5.0%] of 378, $p < 0.01$). The proportion of isolates from humans susceptible to all drugs tested decreased substantially from 1989 to 1997 (chi-square test for trend, $p < 0.01$), while that of isolates from cattle was 10% or less for all periods studied (Table 2).

Conclusions

Antimicrobial resistance has been commonly observed in human and bovine ST isolates since the earliest days of antimicrobial use. This study provides a longitudinal perspective on resistance in ST from cattle and humans in a region and allows insight as to the mechanism of changes in antimicrobial resistance. The greatest changes were in chloramphenicol and kanamycin resistance in isolates from cattle and ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline resistance in isolates from humans. Changes in resistance in isolates from both species were primarily due to the sharply increased occurrence of isolates displaying the ACSSuT resistance pattern, a reliable marker for multidrug-resistant definitive type 104 ST (MR-DT104) (14). This has been shown to be so in the Pacific Northwest through a subset of isolates from this study (13).

MR-DT104 was first detected almost simultaneously in several geographic areas, including the United Kingdom (15), the United States (13), and Canada (16). Molecular genetic studies indicate that the same gene cassette accounts for multiple resistance in isolates from these and other diverse geographic areas (17,18). This study not only provides supporting evidence that MR-DT104 from different regions are clonal in origin, but refutes the notion that the multiple antimicrobial resistance of this clone was due to acquisition of new resistance genes by indigenous ST in each region.

It is possible that local antimicrobial selection pressure played an important role in dissemination of MR-DT104 through cattle populations into the human population. However, several observations argue against this hypothesis. First, the rise in the percentage of resistance to chloramphenicol in isolates from cattle occurred after the withdrawal of the drug for use in food animals in the mid-1980s (19) and before the 1996 approval of florfenicol (a chloramphenicol analog that shares resistance loci with chloramphenicol in MR-DT104 [20]) for therapeutic use in cattle. Second, before the dissemination of MR-DT104, most isolates from cattle were resistant to ampicillin, streptomycin, sulfonamides, and tetracycline. It is not evident how, in the absence of chloramphenicol use, antimicrobial selection pressure would favor R-type ACSSuT over ASSuT, although it is possible

that an unmeasured resistance factor favored the dissemination of MR-DT104 over ASSuT strains. Third, early in its global dissemination, MR-DT104 was isolated from several species of wildlife, which are not exposed to substantial amounts of antimicrobial drugs (13). Finally, reports of broad dissemination of *Salmonella* clones susceptible to antimicrobial drugs commonly used in livestock provide evidence that agent factors other than antimicrobial resistance are necessary for broad dissemination (21,22).

Nevertheless, some selection pressure that likely involved antimicrobial use must explain the high prevalence of antimicrobial resistance among ST. There is strong evidence that livestock are the main reservoir for human salmonellosis in industrialized countries (23); however, it would be an error to assume that the emergence of a globally disseminated clone can be attributed to antimicrobial use in livestock. A human reservoir exists for nontyphoidal *Salmonella*, including serovar Typhimurium, in developing countries (24,25), and there is strong evidence that antimicrobial use in humans has not only driven the emergence of multidrug-resistant clones in these regions but has resulted in an increasingly high prevalence of multiple resistance (26-29). Dissemination of multidrug-resistant *Salmonella* from developing countries, through human traffic, is well documented (30,31) and seems a more likely mode of international transport than the far more limited international livestock traffic.

Multidrug-resistant clones capable of global dissemination can emerge as a result of antimicrobial selection pressure in either livestock or humans; simply restricting antimicrobial use in livestock populations cannot prevent broad dissemination. The problem of globally distributed multidrug-resistant bacterial clones can be compared to the nosocomial scenario: prudent antimicrobial use is a sensible step, but the main effort must go toward preventing dissemination if the program is to be effective.

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