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Antimicrobial Resistance in *Salmonella* in the United States from 1948 to 1995

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We conducted a retrospective study of 2,149 clinical *Salmonella* strains to help document the historical emergence of antimicrobial resistance. There were significant increases in resistance to older drugs, including ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, which were most common in *Salmonella enterica* serotype Typhimurium. An increase in multidrug resistance was observed for each decade since the 1950s. These data help show how *Salmonella* evolved over the past 6 decades, after the introduction of new antimicrobial agents.

Approximately 1.2 million people in the United States contract salmonellosis annually, resulting in 23,128 hospitalizations and >452 deaths (1). Infections caused by antimicrobial-resistant *Salmonella* have been associated with increased hospitalization rates, morbidity, mortality, and economic costs (2–4). Fluoroquinolones or expanded-spectrum cephalosporins are frontline treatments in cases of life-threatening salmonellosis (5). In the United States, systematic monitoring of resistance in foodborne pathogens began in 1996 with implementation of the National Antimicrobial Resistance Monitoring System (NARMS) (6). There are very few data on resistance trends among foodborne pathogens before then. To help document the evolution of resistance in salmonellae prior to NARMS, we tested banked historical isolates, collected over the past 6 decades, for susceptibility to the antimicrobial agents used in NARMS and compared these data with recent surveillance data.

A total of 2,149 *Salmonella* isolates, representing >145 serotypes, obtained from human clinical cases between 1948 and 1995 were included in this study (Table 1). These isolates were mainly from culture collections at the Centers for Disease Control and Prevention. We focused on five serotypes commonly associated with human foodborne infections, namely, *Salmonella enterica* serotypes Enteritidis, Typhimurium, Newport, Heidelberg, and Saintpaul. Most of the isolates were recovered from stool, and a few were recovered from blood and urine. Historical isolates were maintained on Trypticase soy agar stabs sealed with paraffin and stored at room temperature. NARMS isolates were frozen in Trypticase soy broth containing 30% glycerol at −70°C for prolonged storage.

Antimicrobial MICs were determined with the Sensititre system (Thermo Fisher Scientific, Trek Diagnostics, Cleveland, OH). Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines, where CLSI breakpoints are available (7). The tested antimicrobial agents are listed in Table 2. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. The Mann-Kendall test, a nonparametric test, was used to detect the resistance trend over time. The magnitude of the change was estimated by using Sen's nonparametric method and calculated with the Excel template MAKESENS (8). A P value of <0.05 was considered significant.

Overall, 429 of 2,149 (20%) isolates were resistant to at least one antimicrobial, and 165 (7.7%) isolates were MDR, with 62 (2.9%) isolates showing resistance to five or more classes of drugs. Resistance was higher for older drugs, such as streptomycin (12.4%), sulfamethoxazole (10%), tetracycline (9.9%), and ampicillin (7.2%) (Table 2). Resistance to newer agents, such as ceftiofur, ceftriaxone, and ciprofloxacin, was not detected in any isolate. The most common serotype was *S.* Enteritidis (13.2%), followed by *S.* Typhimurium (12.2%), *S.* Newport (8.3%), *S.* Heidelberg (6.9%), and *S.* Saintpaul (4.9%). The frequency of resistance varied by serotype; *S.* Typhimurium often exhibited resistance to more agents than other serotypes, and *S.* Enteritidis exhibited resistance to the fewest agents.

Resistance increased steadily with each decade for streptomycin, sulfamethoxazole, tetracycline, and ampicillin (Table 2), which was consistent with historical trends in *Escherichia coli* (9). Resistance to chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline has long been recognized in *Salmonella* spp. (10), including those causing infections in animals (11). Comparison of these historical trends with recent NARMS data showed that resistance to the older compounds increased steadily until the late

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TABLE 1 Distribution of *Salmonella* isolates, overall and five common serotypes, by decade

S. enterica serotype	No. of isolates in:						
	1940s ($n = 63$)	1950s ($n = 614$)	1960s ($n = 177$)	1970s ($n = 458$)	1980s ($n = 380$)	1990–1995 ($n = 457$)	Total ($n = 2,149$)
Enteritidis	2	23	12	169	46	32	284
Typhimurium[a]	3	2	57	35	112	54	263
Newport	6	1	43	75	20	33	178
Heidelberg	0	12	12	45	49	31	149
Saintpaul	0	6	9	54	20	16	105
Other serotypes	51	549	42	75	66	286	1,069
Not serotyped	1	21	2	5	67	5	101

[a] Includes *S.* Typhimurium variant Copenhagen.

1990s, often linked together in MDR strains, and then began a steady decline (12).

For sulfamethoxazole, resistance increased from 1.6% in the 1940s to 19.7% in the 1980s, followed by a slight drop to 13.8% in the 1990s. A similar decreasing trend in sulfonamide resistance was reported among human nontyphoidal *Salmonella* isolates between 1996 and 2009 (12). Sulfonamides were introduced into human medicine in the 1930s and have been in continuous use for >70 years, usually as a single agent before the 1970s and in combination with trimethoprim since the 1970s. Sulfonamides are still used in the veterinary and agricultural fields (13).

Ampicillin, along with trimethoprim-sulfamethoxazole, was commonly used to treat salmonellosis in the 1980s (14). While we detected ampicillin resistance in isolates from 1949, resistance became a problem in the early 1980s (15), leading to a shift in clinical practice toward the use of quinolones and extended-spectrum cephalosporins. We documented a rise in ampicillin resistance in the 1980s that was followed by a drop in the 1990s and an uptick in the 2000s.

Current statistics on drug use show that tetracycline is the most commonly used antibiotic in food animal production (16), where it has been used historically for production and therapeutic purposes. Out data show that coresistance to tetracycline was frequent. Concurrent resistance to tetracycline-streptomycin was the most common coresistance phenotype (7.3%), followed by resistance to tetracycline-sulfamethoxazole (5.3%), ampicillin-streptomycin (4.9%), tetracycline-ampicillin (4.5%), tetracycline-sulfamethoxazole-streptomycin (4.2%), and tetracycline-ampicillin-streptomycin-sulfamethoxazole (2.8%) (Fig. 1). A total of 52 of 62 (83.9%) chloramphenicol-resistant *Salmonella* isolates were coresistant to tetracycline. Similarly, the use of sulfamethoxazole (and other agents) might help drive coselection of resistance determinants for other antibiotics. In our data, 63.7% and 53% of sulfamethoxazole-resistant *Salmonella* were coresis-

TABLE 2 Antimicrobial resistance phenotypes of *Salmonella*, overall and top five serotypes in strain set

Antimicrobial class	Antimicrobial agent	Resistance breakpoint (μg/ml)	% resistance in:								Timeline for clinical use of antimicrobials
			Overall	Enteritidis	Typhimurium	Newport	Heidelberg	Saintpaul	Other serotypes	Not serotyped	
β-Lactamase inhibitor combinations	Amoxicillin-clavulanic acid	≥32/≥16	0.6	0	2.3	0	1.3	1.9	0.2	1	1984
Aminopenicillins	Ampicillin	≥32	7.2	5.3	22.9	11.2	8.1	12.4	2.4	13.9	1961
Cephems	Cefoxitin	≥32	0.7	0.4	0.8	0.6	0	1	1	0	1977
	Ceftiofur	≥8	0	0	0	0	0	0	0	0	1988
	Ceftriaxone	≥4	0	0	0	0	0	0	0	0	1984
	Cephalothin	≥32	3.6	2.1	8.4	7.9	3.4	7.6	2.2	1	1964
Phenicols	Chloramphenicol	≥32	2.9	1.1	11.8	2.2	2	1.9	0.8	9.9	1947
Aminoglycosides	Gentamicin	≥16	1.1	0	2.3	0	8.1	1	0.5	0	1963
	Kanamycin	≥64	3.1	1.1	10.6	2.3	9.4	6.7	0.3	5.9	1957
	Streptomycin	≥64	12.4	4.2	24.7	13.5	34.2	18.1	7.6	14.9	1943
Quinolones	Nalidixic acid	≥32	0.5	0	1.9	0.6	0	0	0.5	0	1962
	Ciprofloxacin	≥4	0	0	0	0	0	0	0	0	1987
Tetracyclines	Tetracycline	≥16	9.9	2.5	23.6	12.9	20.1	13.3	5.5	17.8	1948
Folate pathway inhibitors	Sulfamethoxazole	≥512	10	2.5	24	15.2	24.2	16.2	4.7	14.9	1936
	Trimethoprim-sulfamethoxazole	≥4/≥76	0.6	0	1.9	0.6	0	1	0.1	5	1968

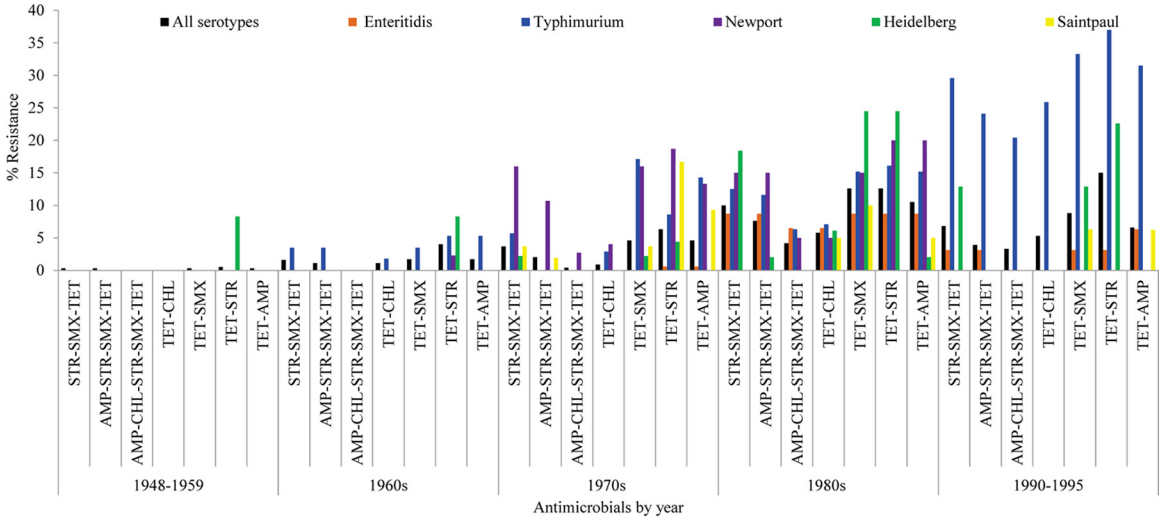

FIG 1 Changes in MDR phenotypes among the top 5 *Salmonella* serotypes in our strain set. STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; AMP, ampicillin; CHL, chloramphenicol.

tant to streptomycin and tetracycline, respectively. The presence of the *sul1* gene as a constituent of class 1 integrons can mediate the spread of sulfonamide resistance in the absence of selective pressure (17).

The frequency of resistance varied by serotype. *S.* Typhimurium often exhibited resistance to more agents than other serotypes, and *S.* Enteritidis exhibited resistance to the fewest agents (Table 2). *S.* Typhimurium has long been known to be more resistant than other common serotypes to antimicrobials (18, 19). Resistance of *S.* Typhimurium to tetracycline, chloramphenicol, and ampicillin increased with each decade. Tetracyclines were approved for use in the 1950s and have been used extensively in the prophylaxis and therapy of human and animal infections. Tetracyclines are by far the most common antibacterial agents currently used in food animal production (16). While chloramphenicol has been available since the 1940s, it is rarely used in the United States today due to toxicity issues. The related compound, florfenicol, is approved for use in swine, cattle, salmonids, and catfish. Chloramphenicol resistance persists in *Salmonella* and other enteric species (9, 12).

Resistance changes were most notable for *S.* Typhimurium. Comparing data from pre-1960 with those from post-1989, resistance rose from 0% to 33.3% for ampicillin, 0% to 25.9% for chloramphenicol, 0% to 42.6% for streptomycin, 20% to 42.6% for tetracycline, and 0% to 37% for sulfamethoxazole (Fig. 2). Comparing data from pre-1960 with those from post-1989, the proportion of pan-susceptible isolates declined from 87.3% to 62.7% for the five serotypes examined here. Simultaneously, MDR increased from 0% (0/55) to 16.9% (28/166). MDR was most common in *S.* Typhimurium, where 61 of 263 (23.2%) isolates were resistant to three or more antimicrobial classes, and 30 (11.4%) isolates were resistant to five or more classes. The other common serotypes with MDR phenotypes were *S.* Newport (22/178), *S.* Heidelberg (22/149), *S.* Saintpaul (12/105), and *S.* Enteritidis (9/284). MDR increased in *S.* Typhimurium (from 0.0% to 37.0%) and *S.* Heidelberg (from 0.0% to 16.1%) between pre-

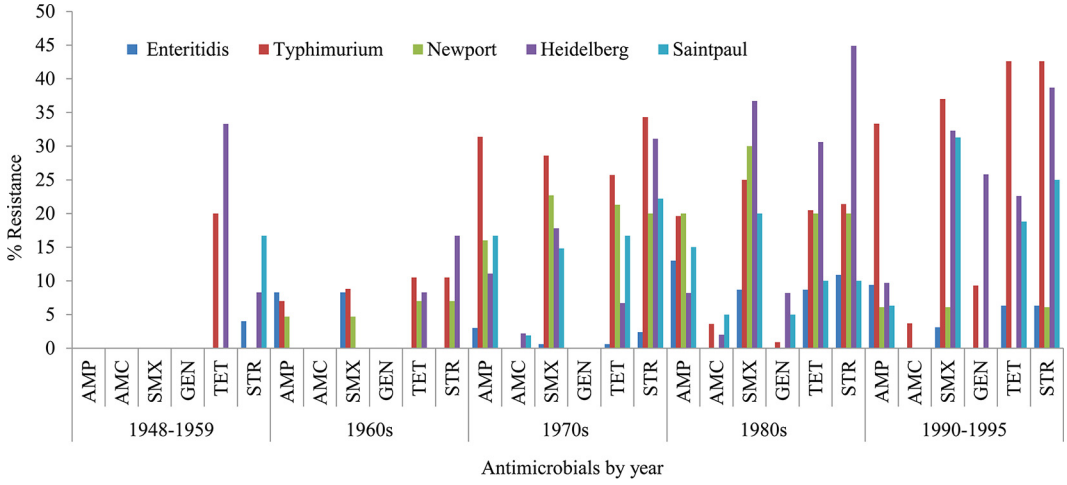

FIG 2 Changes in select antimicrobial resistance among the top 5 *Salmonella* serotypes in our strain set. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; SMX, sulfamethoxazole; GEN, gentamicin; TET, tetracycline; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole.

TABLE 3 Number of isolates with multidrug resistance, by serotype[a]

S. enterica serotype	Total	Pan-susceptible (%)	MDR (%)	MDR, ≥5 drugs (%)	STR-SMX-TET (%)	AMP-STR-SMX-TET (%)	AMP-CHL-STR-SMX-TET (%)
All *Salmonella*	2,149	1,720 (80.0)	165 (7.7)	62 (2.9)	91 (4.2)	60 (2.8)	33 (1.5)
Enteritidis	284	263 (92.6)	9 (3.2)	4 (1.4)	5 (1.8)	5 (1.8)	3 (1.1)
Typhimurium	263	164 (62.4)	61 (23.2)	30 (11.4)	34 (12.9)	28 (10.6)	18 (6.8)
Newport	178	136 (76.4)	22 (12.4)	10 (5.6)	15 (8.4)	11 (6.2)	3 (1.7)
Heidelberg	149	84 (56.4)	22 (14.8)	2 (1.3)	14 (9.4)	1 (0.7)	0 (0)
Saintpaul	105	76 (72.4)	12 (11.4)	4 (3.8)	2 (1.9)	1 (1)	0 (0)
Other serotypes	1,069	920 (86.1)	23 (2.2)	6 (0.6)	13 (1.2)	6 (0.6)	4 (0.4)
Not serotyped	101	77 (76.2)	16 (15.8)	6 (5.9)	8 (7.9)	8 (7.9)	5 (5)

[a] MDR, multidrug resistant; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; AMP, ampicillin; CHL, chloramphenicol.

1960 and post-1989 (Table 3). MDR nontyphoidal *Salmonella* with the ampicillin-chloramphenicol-streptomycin-sulfamethoxazole-tetracycline pattern is of particular concern in the United States (20–22). *S.* Typhimurium DT104 emerged in the United States in the 1990s and has been one of the leading causes of animal and human salmonellosis (23–25). The typical *S.* Typhimurium DT104 exhibits the ampicillin-chloramphenicol-streptomycin-sulfamethoxazole-tetracycline resistance pattern. Medalla et al. (26) reported a significant decline in MDR among NARMS human *Salmonella* isolates between 1996 and 2009, driven mainly by decreased MDR among *S.* Typhimurium. Our study is limited by its reliance on preexisting culture collections, which resulted in a nonrandom sample and an uneven temporal distribution of isolates. In addition, we had no information regarding prior treatment, travel history, or the rationale for preserving the strains. In *Salmonella*, the spread of resistance is mainly via horizontal gene transfer (27). Because most of the historical strains were maintained at room temperature, it is possible that loss of plasmids (28) and any associated resistance traits may have affected our results. Regardless of these unavoidable limitations, the use of the same testing platform with historical strains, coupled with secular surveillance data, provides a broad picture of resistance development over time and helps characterize the evolution of drug resistance in *Salmonella* spp. since the beginning of the antibiotic age.

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