APPLIED MICROBIOLOGY, Feb. 1971, p. 358-362

Vol. 21, No 2
Printed in U.S.A.

Survey of Infectious Multiple Drug Resistance Among *Salmonella* Isolated from Animals in the United States

DOROTHY W. POCURULL, STUART A. GAINES, AND H. DWIGHT MERCER
Division of Veterinary Research, Food and Drug Administration, Beltsville, Maryland 20705

Received for publication 9 November 1970

Salmonella cultures were obtained from outbreaks of animal disease from 37 states and 1 territory. They were screened for resistance to 11 antimicrobial drugs. Of the 1,251 strains studied, 935 were resistant to one or more of these agents. The three most common resistance patterns were ampicillin, dihydrostreptomycin, sulfamethoxypyridazine, tetracycline; ampicillin, dihydrostreptomycin, sulfamethoxypyridazine; dihydrostreptomycin, sulfamethoxypyridazine, tetracycline. Resistance transfer was demonstrated on 267 multiply resistant cultures, of which 181 were able to transfer all or part of their resistance pattern to a drug-sensitive recipient.

The occurrence of transferable drug resistance of the kind mediated by plasmids, called R factors, has been extensively documented since it was first demonstrated in Japan in 1959. These reports from abroad (2, 3, 5, 6, 11, 12) and the United States (7–10, 15–17, 21) are primarily studies of the phenomenon in human beings and show that it is widespread. Less information is available about the incidence of this type of resistance in animal populations. Smith and Halls (20) studied *Escherichia coli* strains isolated from healthy humans, calves, pigs, and fowls; although the survey was limited, their results indicated that infective resistance is probably the most common form of drug resistance among these organisms in Great Britain. Walton's investigation (22) of *E. coli* strains also collected from healthy animals in England led him to conclude that infectious drug resistance has wide distribution in this species in the gut of healthy pigs and calves. He correlated isolation of infectious, multiply resistant strains of intestinal coliforms with types of drugs supplied to the animals.

Smith (18) expanded his investigations to diseased animals (and humans) with an examination of pathogenic *E. coli* isolated from cases of human neonatal diarrhea, from calves and lambs suffering from neonatal diarrhea or bacteremia, from pigs with neonatal or postweaning diarrhea or bowel edema, and from fowls with "colisepticemia." He found a high incidence of drug resistance with complex patterns and mostly of the infective type.

Aden and co-workers (1) noted that their 1969 study was the first on the incidence of transferable drug resistance in domestic animals in this country. They examined *Enterobacteriaceae* obtained from cases of neonatal diarrhea in calves and piglets and showed that this type of drug resistance was common among their cultures. In our survey of *Salmonella* serotypes, we found a high incidence of antimicrobial drug resistance and corresponding resistance-transfer factor.

MATERIALS AND METHODS

Between April 1968 and March 1970, 1,251 serologically identified cultures of *Salmonella* were received. They had all been isolated from cases of animal disease in 37 states and 1 territory a few weeks prior to our laboratory accession. The transmitting laboratories selected the cultures to include as wide a variety of *Salmonella* and host species as possible.

Antimicrobial sensitivity patterns were determined by a standardized single-disc method (4) at the following levels: ampicillin, 10 μg; dihydrostreptomycin, 10 μg; cephalothin, 30 μg; sulfamethoxypyridazine, 0.25 mg; cclistin, 10 μg; chloramphenicol, 30 μg; furazolidone, 100 μg; neomycin, 30 μg; polymyxin B, 300 units; tetracycline, 30 μg; and nalidixic acid, 30 μg.

As a matter of practicality, only cultures which showed resistance to three or more drugs were examined for transferable resistance by the technique of Schroeder et al. (15) which utilized as recipient an *E. coli* (083:k untypable: H14) mutated to high nalidixic acid resistance. A modification by these workers in later studies uses dicloxacillin also in the selective media for conjugation experiments.

RESULTS

Table 1 lists geographical distribution of *Salmonella* species with their animal sources and numbers of drugs in resistance patterns. Screening

TABLE 1. *Distribution of multiple drug resistance in U.S. animals by states, animal, number of Salmonella serotypes, and ability to transfer R factors*

Salmonella serotypes (61)	No. of states represented	Animal source	No. of serotypes resistant to no. of drugs							Resistance transfer		
			0	1	2	3	4	5	>5	Complete	Partial	None
S. alachua (2)	2	Chicken 1, pig 1	2									
S. albany (7)	3	Chicken 1, horse 1, turkey 5	1	4	2							
S. amager (1)	1	Pig 1						1		1		
S. anatum (40)	11	Chicken 4, cow 3, pig 18, turkey 15	4	15	11	9	1			2	6	2
S. bareilly (2)	2	Pig 2	2									
S. berta (1)	1	Pig 1	1									
S. binza (1)	1	Pig 1		1								
S. blockley (15)	5	Chicken 9, pig 4, turkey 2	10	5								
S. braenderup (1)	1	Pig 1		1								
S. bredeney (17)	6	Chicken 6, pig 5, turkey 6	8	7	2							
S. california (5)	6	Chicken 4, turkey 1	4	1								
S. cerro (2)	1	Chicken 1, pig 1	1	1								
S. chester (13)	4	Chicken 2, turkey 11	7	2	3							
S. choleraesuis (6)	1	Pig 6		6								
S. choleraesuis var. *kunzendorf* (340)	25	Chicken 1, pig 339	6	117	179	33	5			7	18	13
S. cubana (6)	5	Chicken 2, pig 2, turkey 2	5		1							
S. derby (53)	18	Chicken 3, cow 4, pig 38, quail 1, turkey 6, sheep 1	22	16	10	4	1				1	4
S. drypool (3)	3	Cow 1, pig 2	1	2								
S. eimsbuettel (15)	3	Chicken 6, cow 2, pig 5, turkey 2	5	10								
S. enteritidis (34)	7	Chicken 2, cow 1, duck 1, horse 1, pig 11, turkey 18	32	1	1							
S. give (6)	5	Chicken 1, pig 5	4	2								
S. heidelberg (89)	17	Chicken 18, cow 1, horse 5, pig 19, turkey 46	21	24	25	9	9	1		3	7	9
S. indiana (1)	1	Turkey 1		1								
S. infantis (40)	13	Anteater 1, chicken 17, cow 3, horse 2, pig 14, turkey 3	26	11	1	1	1				2	
S. java (2)	2	Pig 2	2									
S. javiana (2)	2	Pig 2	1				1					1
S. johannesburg (2)	1	Chicken 1, turkey 1	2									
S. kentucky (2)	2	Pig 1, turkey 1	1		1							
S. kottbus (8)	3	Chicken 6, pig 2	5		2	1					1	
S. lexington (1)	1	Turkey 1				1				1		
S. litchfield (1)	1	Pig 1		1								
S. livingstone (1)	1	Turkey 1	1									
S. lomita (1)	1	Pig 1		1								
S. london (1)	1	Turkey 1		1								
S. manhattan (3)	2	Turkey 3			2	1				1		
S. miami (3)	2	Horse 1, pig 2	3									
S. minnesota (4)	2	Chicken 1, pig 3	1	3								2
S. montevideo (10)	6	Chicken 3, pig 4, turkey 3	5	2	1	1	1					2
S. muenchen (13)	4	Chicken 1, goose 1, pig 8, turkey 3	10	1		2						2
S. muenster (1)	1	Horse 1		1								
S. newington (4)	4	Chicken 3, pig 1		2	1	1				1		
S. newport (24)	12	Cat 1, chicken 1, cow 7, pig 14, turkey 1	13	1	5		2	3		1	3	1

[a] Total for states: Alabama 1, Arizona 3, Arkansas 38, California 1, Colorado 2, Delaware 4, District of Columbia 1, Florida 22, Georgia 4, Illinois 156, Indiana 283, Iowa 63, Kansas 1, Kentucky 18, Lousiana 19, Maryland 38, Massachusetts 3, Michigan 15, Minnesota 203, Mississippi 19, Missouri 31, Nebraska 20, New Hampshire 2, New Jersey 2, North Carolina 46, North Dakota 3, Ohio 50, Oklahoma 14, Pennsylvania 2, Puerto Rico 6, Rhode Island 1, South Carolina 38, South Dakota 8, Tennessee 15, Texas 106, Virginia 1, Washington 1, Wisconsin 11. Animals: Anteater 1, cat 1, chicken 150, cow 90, dog 1, duck 2, goose 4, horse 20, pig 678, pigeon 3, quail 2, turkey 292, sheep 7.

TABLE 1—*Continued*

Salmonella serotypes (61)	No. of states represented	Animal source	No. of serotypes resistant to no. of drugs							Resistance transfer		
			0	1	2	3	4	5	>5	Complete	Partial	None
S. orion (3)	5	Chicken 1, pig 1, turkey 1	2	1								
S. oranienburg (2)		Chicken 1, pig 1	1	1								
S. panama (1)	1	Pig 1					1				1	
S. phoenix (1)	1	Pig 1		1								
S. poona (1)	1	Pig 1	1									
S. reading (10)	7	Chicken 1, pig 1, pigeon 1, turkey 7	4	4	1	1				1		
S. saint paul (96)	18	Chicken 10, cow 4, horse 1, pig 8, turkey 73	27	32	23	6	5	2	1	8	4	2
S. san diego (21)	6	Goose 1, turkey 20	4	8	1	7	1			4	1	3
S. schwarzengrund (6)	6	Chicken 1, pig 2, turkey 3		4	2							
S. senftenberg (31)	4	Chicken 1, pig 5, turkey 25	10	4	7	7	3			1	1	8
S. siegburg (2)	2	Chicken 2	1	1								
S. taksony (1)	1	Turkey 1	1									
S. tennessee (7)	5	Chicken 3, pig 2, turkey 2	2	5								
S. thomasville (6)	1	Chicken 2, cow 2, pig 1, sheep 1	1	5								
S. thompson (18)	9	Chicken 9, pig 7, turkey 2	10	8								
S. typhimurium (223)	25	Chicken 14, cow 59, dog 1, goose 2, horse 7, pig 115, turkey 21, sheep 4	32	25	29	28	87	19	3	46	52	39
S. typhimurium var. *copenhagen* (29)	15	Chicken 9, cow 2, duck 1, horse 1, pig 12, pigeon 1, turkey 2, sheep 1	9	12	2	3	1	2		2	4	
S. typhisuis (1)	1	Pig 1		1								
S. worthington (9)	5	Chicken 2, cow 1, pig 2, pigeon 1, quail 1, turkey 2	5	2	1	1					1	
Total[a] (1,251)			316	355	313	116	119	28	4	79	102	86

for antimicrobial sensitivity showed that 25% of the cultures were completely sensitive to the spectrum, 75% were resistant to one or more drugs, 46% were resistant to two or more drugs, and 21% were resistant to three or more drugs. The last group consisted of 267 cultures divided among 22 *Salmonella* species. Among the multiply resistant cultures, 137 were *S. typhimurium* and 38 were *S. choleraesuis* var. *kunzendorf*. In the group tested for resistance transfer, resistance was most common for dihydrostreptomycin (DS), 96%, followed by sulfamethoxypyridazine (KY), 83.8%; tetracycline (TE), 77.9%; and ampicillin (AM), 69.6%. No resistance was found to chloramphenicol, furazolidone, polymyxin B, or nalidixic acid. The other drugs fell into 24 different patterns with varying transferability (Table 2). Resistance patterns encountered most often were AM, DS, KY, TE appearing 82 times with 68.2% complete or partial transfer; AM, DS, KY (39) with 84.6% transfer; and DS, KY, TE (32) with 71.8% transfer. *S. typhimurium* transferred completely or partially 71% of the time and *S. choleraesuis* var. *kunzendorf* 65% of the time, compared to 67% transfer for all multiply resistant cultures.

DISCUSSION

Within our specified criteria, the survey shows a higher incidence of multiply resistant cultures of *S. typhimurium* (10.9%) than of other *Salmonella* serotypes, whereas the proportion of these cultures transferring resistance was only slightly more than that of the other serotypes. Earlier studies on animal and human isolates of *S. typhimurium* have shown a higher incidence of drug resistance for this serotype within experimental limits (13–17). *S. choleraesuis* var. *kunzendorf* was less often resistant to three or more drugs (3%) but transferred to a similar degree. The incidence of this serotype resistant to

TABLE 2. *Drug resistance patterns and transferability*[a]

Resistance pattern	No. of cultures	Pattern transferred	No. of cultures	Per cent transfer
AM, DS, CF, KY, N, TE	4	AM, DS, CF, KY, N, TE	1	75
		AM, DS, KY, N, TE	1	
		AM, KY, N	1	
AM, DS, CF, KY, TE	7	AM, DS, CF, KY, TE	2	100
		AM, DS, KY, TE	3	
		AM, KY, TE	2	
AM, DS, CF, KY	7	AM, DS, CF, KY	4	85.7
		DS	1	
		KY	1	
AM, DS, KY, N, TE	20	AM, DS, KY, N, TE	4	70
		AM, DS, KY, TE	7	
		AM, DS, N, TE	1	
		AM, DS, TE	1	
		AM, KY, TE	1	
AM, DS, KY, N	1	AM, DS, KY, N	1	100
AM, DS, KY, TE	82	AM, DS, KY, TE	34	63.4
		AM, DS, TE	3	
		AM, KY, TE	15	
AM, DS, KY	39	AM, DS, KY	15	84.6
		AM, DS	9	
		AM, KY	2	
		AM	6	
		KY	1	
AM, DS, N, TE	9	AM, DS, N, TE	5	66
		DS, N, TE	1	
AM, DS, N	4	AM, DS, N	1	
		AM, DS	1	
		AM, N	1	
AM, DS, TE	3	AM, DS, TE	1	33
AM, CF, KY, TE	1			0
AM, CF, TE	1			0
AM, KY, N, TE	1	AM, N, TE	1	100
AM, KY, TE	6	AM, KY, TE	2	50
		KY, TE	1	
AM, KY, N	1	AM, KY	1	100
DS, CF, KY	2			0
DS, CF, CL	1			0
DS, KY, CL, N, TE	1	DS, KY, N, TE	1	100
DS, KY, CL	1			0
DS, KY, N, TE	18	DS, KY, N, TE	5	66
		DS, KY, TE	1	
		DS, N, TE	4	
		DS, TE	1	
		KY, TE	1	
DS, KY, N	1			0
DS, KY, TE	32	DS, KY, TE	9	71.8
		DS, N, TE	4	
		DS, TE	6	
		DS	3	
		KY, TE	1	
DS, CL, N	2			0
DS, N, TE	23	DS, N, TE	8	60
		DS, TE	6	

[a] AM = ampicillin, DS = dihydrostreptomycin, CF = cephalothin, KY = sulfamethoxypyridazine, CL = colistin, N = neomycin, TE = tetracycline.

two or more drugs is higher than that for *S. typhimurium*.

The strains of *Salmonella* studied were apparently invasive pathogens responsible for disease conditions and mortality. The mortality rate as obtained from the partial histories of these disease outbreaks indicates that 7,482 head of livestock, principally pigs and cattle, and 59,241 birds (including turkeys, chickens, geese, and ducks) died. Many history sheets were not complete in that the numbers of animals dead were given at the time of sample collection, and final mortality rate could not be obtained. The figures represent only a portion of actual deaths. There was no provision for recording treatment of illness; thus, we are unable to correlate incidence of resistance with use of antimicrobial drugs.

A recent study (19) of in vivo transfer of antibiotic resistance among organisms isolated from chickens, calves, and pigs shows that many factors are involved in resistance transmission, particularly the ability of organisms to colonize the alimentary tract. The presence of a clinical syndrome in the animals from which the *Salmonella* strains of this survey were isolated would indicate that this requisite condition was met.

This survey indicates that pathogenic salmonellae with infectious multiple resistance have been present in numerous outbreaks of disease in the animal populations of the United States.

ACKNOWLEDGMENTS

We thank Erika Alvarez for her capable technical help; William Blackburn, National Animal Disease Laboratory, Ames, Iowa, and Saul Wilson, Department of Agriculture, Hyattsville, Md., for kindly supplying the *Salmonella* strains; John Bennett, National Communicable Disease Center, Atlanta, Ga., for advice and assistance in many ways; the Winthrop Laboratories, New York, N.Y., for generously providing the nalidixic acid; and Bristol Laboratories, Syracuse, N.Y., for their sample of dicloxacillin.

LITERATURE CITED

1. Aden, D. P., N. D. Reed, N. R. Underdahl, and C. A. Mebus. 1969. Transferable drug resistance among *Enterobacteriaceae* isolated from cases of neonatal diarrhea in calves and piglets. Appl. Microbiol. **18**:961–964.
2. Anderson, E. S., and N. Datta. 1965. Resistance to penicillin and its transfer in Enterobacteriaceae. Lancet **1**:407–409.
3. Anderson, E. S., and M. J. Lewis. 1965. Drug resistance and its transfer in *Salmonella typhimurium*. Nature (London) **208**:579–583.
4. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. **36**:493–496.
5. Datta, N. 1962. Transmissible drug resistance in an epidemic strain of *Salmonella typhimurium*. J. Hyg. (London) **60**:301–310.
6. Datta, N. 1969. Drug resistance and R factors in the bowel bacteria of London patients before and after admission to hospital. Brit. Med. J. **2**:407—411.
7. Gardner, P., and D. H. Smith. 1969. Studies on the epidemiology of resistance (R) factors. Ann. Intern. Med. **71**:1–9.
8. Gill, F. A., and E. W. Hook. 1966. *Salmonella* strains with transferable antimicrobial resistance. J. Amer. Med. Ass. **198**:1267–1269.
9. Gunter, A. C., and T. W. Feary. 1968. Infectious drug resistance among clinically isolated *Escherichia coli*. J. Bacteriol. **96**:1556–1561.
10. Kabins, S. A., and S. Cohen. 1966. Resistance-transfer factor in Enterobacteriaceae. N. Engl. J. Med. **275**:248–252.
11. Lewis, M. J. 1968. Transferable drug resistance and other transferable agents in strains of *Escherichia coli* from two human populations. Lancet **1**:1389–1393.
12. Lebek, G. 1963. The spontaneous loss of episomal transmissible multiple resistance to antibiotics in populations of gram-negative intestinal microorganisms in cultures with and without antibiotics. Z. Hyg. Infektionskr. Med. Mikrobiol. Immunol. Virol. **149**:255–266.
13. Manten, A., P. A. M. Guinee, and E. H. Kampelmacher. 1966. Incidence of resistance to tetracycline and chlor amphenicol among *Salmonella* bacteria found in the Netherlands in 1963 and in 1964. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. Org. **200**:13–20.
14. McWhorter, A., M. C. Murrell, and P. R. Edwards. 1963. Resistance of Salmonellae isolated in 1962 to chlortetracycline. Appl. Microbiol. **11**:368–370.
15. Schroeder, S. A., P. M. Terry, and J. V. Bennett. 1968. Antibiotic resistance and transfer factor in *Salmonella*, United States 1967. J. Amer. Med. Ass. **205**:903.
16. Smith, D. H. 1966. *Salmonella* with transferable drug resistance. N. Engl. J. Med. **275**:625–630.
17. Smith, D. H., and S. E. Armour. 1966. Transferable R factors in enteric bacteria causing infection of the genito-urinary tract. Lancet **2**:15–18.
18. Smith, H. W. 1967. The incidence of infective drug resistance in strains of *Escherichia coli* isolated from diseased human beings and domestic animals. Vet. Rec. **80**:464–469.
19. Smith, H. W. 1970. The transfer of antibiotic resistance between strains of Enterobacteria in chicken, calves, and pigs. J. Med. Microbiol. **3**:165–180.
20. Smith, H. W., and S. Halls. 1966. Observations on infective drug resistance in Britain. Vet. Rec. **78**:415–420.
21. Smith, J. P., and J. H. Marymount. 1967. Transmissible drug resistance. J. Kans. Med. Soc. **68**:425–427.
22. Walton, J. R. 1966. Infectious drug resistance in *Escherichia coli* isolated from healthy farm animals. Lancet **2**:1300–1302.