

Chapter 5

ANTIMICROBIAL SENSITIVITY IN *SALMONELLA*

Salmonellas received for serological identification at VLA Weybridge and Lasswade are tested for their *in vitro* sensitivity to 16 antimicrobials. All these isolates come from animals and their environment in England and Wales. The choice of antimicrobials, which is reviewed periodically, is designed to comprise a core set which has been used in veterinary practice for many years, some of the more recently licensed antimicrobials and some of limited usage in Great Britain which are used in other European countries. In 2001, the 30 µg cefuroxime disc that had been used in previous years, was replaced with a 30 µg ceftazidime disc.

All tests are performed using a disc diffusion technique on Oxoid "Isosensitest" agar using antibiotic discs as follows:

	Antimicrobial	Concentration (µg per ml)	Code
1	Nalidixic acid	30	NA
2	Tetracycline	10	T
3	Neomycin	10	N
4	Ampicillin	10	AM
5	Furazolidone	15	FR
6	Ceftazidime (from 1/1/01)	30	CAZ
6	Cefuroxime (used until 31/12/00)	30	CX
7	Sulphamethoxazole/trimethoprim	25	TM
8	Chloramphenicol	10	C
9	Amikacin	30	AK
10	Amoxycillin/clavulanic acid	30	AMC
11	Gentamicin	10	CN
12	Streptomycin	25	S
13	Sulphonamide compounds	300	SU
14	Cefoperazone	30	CF
15	Apramycin	15	APR
16	Colistin	25	CT

Prior to 1996, all *Salmonella* isolates received were tested for antimicrobial susceptibility, but since then only the first isolate from each incident has been tested. The number of cultures received from a farm varies enormously, especially in the case of those from poultry. Some poultry companies have a continuous monitoring programme and large numbers of salmonellas may be received from a particular company. Thus the numbers of a particular serovar and its antimicrobial susceptibility may not

reflect its prevalence in the animal population as a whole but reflect the monitoring programme on a farm or group of farms. Therefore, to better indicate the prevalence of resistance, only the first isolate from each incident has been tested since the start of 1996.

SALMONELLA DUBLIN

Of the 949 *Salmonella* Dublin cultures tested during 2003, 96.4% were susceptible to all 16 antimicrobial drugs (Table 82). The percentage of *S. Dublin* isolates sensitive to all 16 antimicrobials has shown a modest decline over the period 1996-2003, though the majority of isolates remain susceptible and this has been the situation since surveillance began in 1971. Most *S. Dublin* isolates originate from cattle. Resistance to ampicillin, which had been observed for the first time for several years in a very low number of bovine isolates in 2000, was not recorded in 2001 or 2002 and re-appeared in 2003. Resistance to furazolidone and neomycin, which had also not been detected for several years in *S. Dublin*, was observed for the first time in recent years in 2002 and again in 2003. 0.9% of *S. Dublin* isolates were resistant to trimethoprim/sulphonamides in 2002, higher than the figure for 2001, when only 0.2% of isolates were resistant. This figure is of interest, since trimethoprim/sulphonamide resistance also increased in *Salmonella* Typhimurium in 2002, though by a much greater degree. In 2003, resistance to trimethoprim/sulphonamides in *S. Dublin* declined to 0.4%. Resistance to streptomycin increased in 2003, with 1.4% of cultures resistant. These fluctuations are probably related to clonal spread of particular isolates as a result of husbandry and animal movement factors, in addition to variation in the selective pressure exerted by antimicrobial usage.

Table 82: *Salmonella* Dublin: antimicrobial sensitivity monitoring 1999 - 2003

Year	No of cultures	Percentage sensitive to all 16 anti-microbials	Percentage of cultures resistant to:							
			S	SU	T	N	AM	FR	TM	C
1999	357	98.3	1.1	-	-	-	-	-	-	-
2000	863	98.7	0.7	0.7	0.5	-	0.1	-	0.2	0.6
2001	467	98.3	0.2	1.3	-	-	-	-	0.2	0.6
2002	687	97.5	0.3	0.7	0.6	0.4	-	0.6	0.9	0.4
2003	949	96.4	1.4	1.2	0.8	0.2	0.6	0.4	0.4	1.3

SALMONELLA TYPHIMURIUM

The number of cultures of *Salmonella* Typhimurium examined in 2003 was 613, of which 44.5% were Definitive Type (DT) 104, DT104b or U302 (Table 83).

19.6% of the cultures were sensitive to all of the antimicrobials tested, similar to the figure for 2001, when 20.6% of *S. Typhimurium* cultures were fully sensitive and reversing the fluctuation observed in 2002, when 14.5% of cultures were fully sensitive (Table 83). The generally high level of resistance of *Salmonella* Typhimurium isolates is partly a reflection of the numbers of DT104 and its variants DT104b and U302, only 1.5% of which were sensitive to all the antimicrobials tested in 2003. However, the proportion of *Salmonella* Typhimurium isolates comprising DT104 and its variants has declined significantly in recent years and this has been reflected in a decrease in resistance to several antimicrobials, particularly those conferring the pentavalent resistance pattern that is typical of *Salmonella* Typhimurium DT104. In 2003, despite a continuing decline in the proportion of *S. Typhimurium* tested that were DT104 or its variants, the levels of resistance to some antimicrobials in fact increased. This occurred because the definitive types that are replacing DT104 are themselves commonly resistant to one or more antimicrobials. Figure 46 illustrates this fact graphically, showing the percentage of each of the eight commonest definitive types of *S. Typhimurium* that are fully susceptible to all of the antimicrobials tested.

Most *S. Typhimurium* DT104 isolates recovered from cattle had the typical pentavalent resistance pattern AM,C,S,SU,T and were also resistant to CF; this was also the commonest pattern observed in isolates of DT104 from pigs, chickens and sheep. The commonest pattern for isolates from turkeys was AM,C,S,SU,T,CF,NA and this was the third most common pattern in isolates from cattle. All of the isolates that were resistant to cefoperazone were susceptible to ceftazidime and cefoperazone resistance in these isolates is therefore considered to indicate hyperproduction of betalactamase enzymes, rather than possession of extended spectrum betalactamase enzymes. There were no *Salmonella* Typhimurium isolates resistant to ceftazidime recovered in 2003, 2002 or 2001 and no *Salmonella* Typhimurium isolates resistant to cefuroxime were recorded in 1998, 1999 or 2000. This is an important finding, since third generation cephalosporins are one of the important groups of antimicrobials for the treatment of invasive salmonellosis in man.

In 2003, 24.2% of DT104 and 104b isolates were resistant to nalidixic acid and 25.4% resistant to sulphamethoxazole/trimethoprim. This is an increase on the figures for 2002, when 6.3% of DT104 and 104b isolates

were resistant to nalidixic acid and 20.5% resistant to sulphamethoxazole/trimethoprim. In 2001, 19.8% of DT104 and 104b isolates were resistant to nalidixic acid and 12.5% resistant to sulphamethoxazole/trimethoprim. Therefore over this three-year period there have been fluctuations in nalidixic acid resistance, though trimethoprim/sulphonamide resistance has steadily increased. Taking a longer-term view, the figure of 25.4% resistance to sulphamethoxazole/trimethoprim in DT104 and 104b isolates in 2003 can be compared to 17.6% resistance in 1999 and 15.9% in 1998. Nalidixic acid resistance in *S. Typhimurium* DT104 by species of origin is listed in Table 84, which shows that isolates from cattle and turkeys are the main contributors accounting for the observed rise in nalidixic acid resistance. Table 85 gives the equivalent figures for trimethoprim/ sulphonamide resistance by species of origin in *S. Typhimurium* DT104 for 2003.

Considering all definitive types of *S. Typhimurium*, there has also been a marked increase in resistance to sulphamethoxazole/trimethoprim from levels of around 16-24% in 1996-2001 to 44.1% in 2002 and 37.5% in 2003. There is a contribution from DT104 to this overall figure and this has been discussed in the paragraph above. In relation to other phage types of *S. Typhimurium* it is mainly isolates from pigs that account for this rise; a high percentage of many definitive types of *S. Typhimurium* isolated from pigs are resistant to sulphamethoxazole/trimethoprim, a situation that was also observed in 2002. These definitive types of *S. Typhimurium* recovered from pigs include DT193 (47 isolates, 85% resistant to TM in 2002; 38 isolates, 92% resistant to TM in 2003), DT208 (14 isolates, all resistant to TM in 2002; 7 isolates, 43% resistant to TM in 2003), U288 (51 isolates, 94% resistant to TM in 2002; 72 isolates, 90% resistant to TM in 2003) and U308a (59 isolates, 95% resistant to TM in 2002; no isolates in 2003). Three factors have influenced the sulphamethoxazole/trimethoprim resistance figures for *S. Typhimurium* isolates from pigs: (1) The numbers of incidents involving strains which have been highly resistant to TM in previous years, have increased. (2) The proportion of TM-resistant isolates from some definitive types that have previously shown TM resistance has increased (for example in 2000, 53% and 38% of isolates of DT193 and 208 respectively, were resistant to sulphamethoxazole/trimethoprim). (3) There has also been a minor contribution from some definitive types of *S. Typhimurium* from pigs that have not previously shown sulphamethoxazole/trimethoprim resistance in recent years (eg DT12), but which have now shown resistance in either 2002 or 2003.

Table 83: *Salmonella* Typhimurium: antimicrobial sensitivity monitoring 1999 – 2003

Year	No of cultures	Percentage sensitive to all 16 anti-microbials	Percentage of cultures resistant to:									
			S	SU	T	N	AM	FR	TM	C	APR	NA
1999	1177 [†]	18.4	61.2	72.0	78.8	2.0	63.0	0.3	23.1	53.2	1.6	11.3
2000	864 [*]	15.3	63.2	70.8	80.4	2.5	63.8	0.1	23.4	56.5	3.2	7.5
2001	519 ^{††}	20.6	57.8	71.7	75.5	2.9	66.7	0.4	24.3	55.9	2.3	11.9
2002	533 ^{**}	14.5	61.0	77.9	80.1	3.4	70.5	2.6	44.1	62.1	2.4	7.1
2003	613 ^{†††}	19.6	61.7	73.1	74.2	6.2	68.5	0.7	37.5	58.9	3.8	13.5

[†] 620 (52.7%) of these strains were DT104 and its variants.
^{*} 460 (53.2%) of these strains were DT104 and its variants.
^{††} 247 (52.8%) of these strains were DT104 and its variants.
^{**} 239 (44.8%) of these strains were DT104 and its variants.
^{†††} 273 (44.5%) of these strains were DT104 and its variants.

Table 84: Nalidixic acid resistance in *Salmonella* Typhimurium DT104 from domestic livestock. Number of cultures tested (percentage resistant to nalidixic acid) 1999 - 2003

Year	Livestock species					
	Cattle	Sheep	Pigs	Chickens	Turkeys	Ducks
1999	231 (5.2)	35 (2.9)	114 (9.6)	5 (20.0)	24 (66.7)	1 (0)
2000	223 (10.8)	21 (0)	51 (2.0)	7 (14.3)	7 (0)	1 (0)
2001	115 (15.7)	8 (12.5)	19 (21.1)	22 (0)	25 (60.0)	0 (0)
2002	17 (11.8)	32 (0)	0 (0)	67 (7.5)	36 (5.6)	5 (40)
2003	100 (20)	6 (0)	27 (11.1)	12 (8.3)	41 (63.4)	0 (0)

Table 85: Trimethoprim/sulphonamide resistance in *Salmonella* Typhimurium DT104 from domestic livestock in 2003. Number of cultures tested (percentage resistant to trimethoprim/sulphonamide)

Year	Livestock species					
	Cattle	Sheep	Pigs	Chickens	Turkeys	Ducks
2003	100 (33)	6 (0)	27 (14.8)	12 (33.3)	41 (7.3)	0 (0)

1.5% of DT104 and its variants were resistant to neomycin; the main contribution to the overall levels of neomycin resistance seen in *Salmonella* Typhimurium in 2003 came from DT193 of porcine origin (23.7% of 38 isolates tested) and U288 of porcine origin (19.1% of 73 isolates tested). Apramycin resistance levels have increased since 2000 and in 2003, the main phage types involved were *Salmonella* Typhimurium DT193 (11 resistant isolates) and U288 (5 resistant isolates). All of these isolates were of porcine origin and all were also resistant to gentamicin, suggesting that the mechanism involved may be possession of the enzyme aminoglycoside acetyltransferase AAC(3)IV.

Multiple antibiotic resistance (i.e. resistance to four or more antimicrobial agents in the panel of 16) was detected in DTs 12, 104, 104b, 120, 193, 193a, U288 and U302 from cattle; in DTs 41 variant, 104, 193, 193a, U288, U302 from poultry; in DT104 and U288 from sheep and in DTs 12, 12a, 104, 104b, 120, 193, 193a, 195, U288 and U302 from pigs. Of the 34 different definitive types detected, 11 (namely 1, 2a, 30, 40, 49, 56 variant, 85, 96 variant, 99 169 and U317) were fully susceptible to all of the antimicrobials in the test panel.

SEROVARS OTHER THAN *SALMONELLA* DUBLIN AND *SALMONELLA* TYPHIMURIUM

Of the 3652 cultures tested 67.7% were sensitive to all the antimicrobials tested (Table 86), reversing the decline seen in 2002 when 60.3% were sensitive. 124 (3.4%) of the cultures were *S. Enteritidis*, of which 47 were *S. Enteritidis* Phage Type 4 and of these *S. Enteritidis* Phage Type 4 isolates, 91.5% were sensitive to all of the antimicrobials used in the test panel. Levels of resistance to furazolidone and neomycin were higher than those observed in recent years, maintaining the trend observed in 2002. Neomycin resistant isolates originated mainly from poultry (8.5% of isolates from poultry were resistant, with 2541 isolates tested), and most of these neomycin resistant poultry isolates were from ducks, with lower numbers from chickens and turkeys. Furazolidone resistant isolates also originated mainly from poultry (12.4% of isolates from poultry were resistant, of 2541 isolates tested), and many of these isolates were *Salmonella* Indiana and again originated from ducks. This is discussed further below.

Table 86: Salmonellas, other than *Salmonella* Dublin and *Salmonella* Typhimurium: antimicrobial sensitivity monitoring 1999 - 2003

Year	No of cultures	Percentage sensitive to all 16 antimicrobials	Percentage of cultures resistant to:									
			S	SU	T	N	AM	FR	TM	C	APR	NA
1999	2417	73.7	6.8	12.6	16.1	1.0	2.8	0.4	6.6	2.2	0.3	3.1
2000	2877	70.7	5.0	18.0	9.5	0.9	4.8	0.5	13.7	3.5	0.1	5.5
2001	1814	69.8	8.1	20.0	10.0	1.0	5.7	0.6	12.1	6.4	0.2	1.4
2002	2167	60.3	11.2	24.0	13.7	5.2	6.5	3.7	19.5	8.0	0.3	1.9
2003	3652	67.7	10.0	19.0	15.7	6.2	4.4	8.7	12.4	4.5	0.1	2.2

INDIVIDUAL ANTIMICROBIALS

Of the 5214 salmonellas tested in 2003, 67.3% were sensitive to all of the antimicrobials tested – similar to the figure of 61.1% recorded in 2002. This can be compared with figures of 65.5% in 2001 and 59.5% in 1999. Levels of resistance to tetracyclines in isolates from all sources decreased from 33.3% in 1999 to 21.1% in 2000 and further declined to 20.5% in 2001; levels of resistance to tetracyclines were 21.2% in 2002 and 19.9% in 2003. This probably reflects both the proportionate decrease in salmonella isolates of all serovars from pigs and the relative decline in frequency of isolation of *S. Typhimurium* DT104. The level of resistance to neomycin in all salmonella serovars was 5.1% in 2003 and 3.9% in 2002, an increase on the figure of 1.2% recorded in 2001. Levels of resistance to furazolidone remained at 0.3% in 1999 and 2000, though increased slightly to 0.5% in 2001 and increased further in 2002 when levels of 2.9% were recorded. In 2003, 6.3% of all isolates were resistant to furazolidone. The observed increase is considered to reflect increased surveillance of *Salmonella* isolates from ducks rather than a genuine increase in resistance to this antimicrobial, since *Salmonella* Indiana is a frequent salmonella isolate from ducks and is commonly resistant to furazolidone. Numbers of salmonella isolates received from ducks have increased over this period as surveillance of this species has increased. Examination of previous records shows that furazolidone-resistant *Salmonella* Indiana has been present in poultry in England and Wales for many years. In 2002, it is interesting to note that there were no cases of *Salmonella* Indiana infection in humans in England and Wales that were resistant to furazolidone (personal communication, Professor E. J. Threlfall, Laboratory of Enteric Pathogens, HPA Colindale). Resistance of *S. Virchow* isolates to furazolidone declined from 53% in 1998 to 28.5% in 1999, although the numbers of *S. Virchow* isolates tested each year were relatively low at 15

in 1998 and 7 in 1999. Thirty-nine isolates of *S. Virchow* were examined in 2001 and 12.8% were resistant to furazolidone; 59 isolates of *S. Virchow* were examined in 2002 and only 1.7% were resistant to furazolidone. In 2003, 132 isolates of *S. Virchow* were examined and 0.8% were resistant to furazolidone. Resistance to apramycin in all salmonella serovars was 0.5% in 2003, similar to the figure of 0.6% observed in 2002. The overall trend of decreasing resistance to nalidixic acid was not maintained with 3.2% of all *Salmonella* isolates resistant in 2003, compared with 2.4% of all *Salmonella* isolates resistant in 2002, 3.2% in 2001, 4.9% in 2000, 5.3% in 1999, 7.0% in 1998 and 6.5% in 1997.

No resistance was detected to amikacin or ceftazidime.

Table 87: All salmonellas: antimicrobial sensitivity 2003

Origin	No of cultures	Percentage sensitive to all 16 anti-microbials	Percentage of cultures resistant to:									
			S	SU	T	N	AM	FR	TM	C	APR	NA
Cattle	1248	81.2	12.7	14.5	15.3	0.3	13.8	0.3	6.2	13.1	-	2.3
Sheep	274	93.1	2.9	2.7	4.7	-	2.9	0.4	0.4	3.3	-	-
Pigs	297	6.4	56.6	82.2	80.8	11.4	59.9	1.0	59.3	49.5	6.4	8.4
Chicken	1395	64.4	6.7	24.4	8.3	1.9	5.4	6.0	20.1	8.4	-	1.8
Turkey	433	53.6	16.9	33.7	30.7	2.5	17.3	1.2	11.5	9.7	-	15.5
Duck	799	57.2	22.9	15.9	32.3	22.5	1.1	28.2	5.4	1.3	-	0.4
Horse	29	72.4	13.8	20.7	20.7	-	13.8	-	20.7	13.8	3.4	6.7
Other non-avian spp	210	65.7	23.8	29.1	26.7	2.9	24.3	1.9	11.4	17.1	2.4	5.7
Other avian spp	36	69.4	19.4	8.3	11.1	-	13.9	-	5.6	-	-	5.6
Feed	268	91.0	2.1	7.5	4.1	0.6	1.3	0.2	5.8	1.5	-	-
Environment	25	92.0	-	-	-	-	4.0	-	-	4.0	-	4.0
Total	5214	67.3	14.5	22.1	19.9	5.1	11.2	6.3	13.2	10.3	0.5	3.2

Fig 45: Proportion of isolates of *Salmonella* Typhimurium of the ten most frequent types presented to VLA in 2003

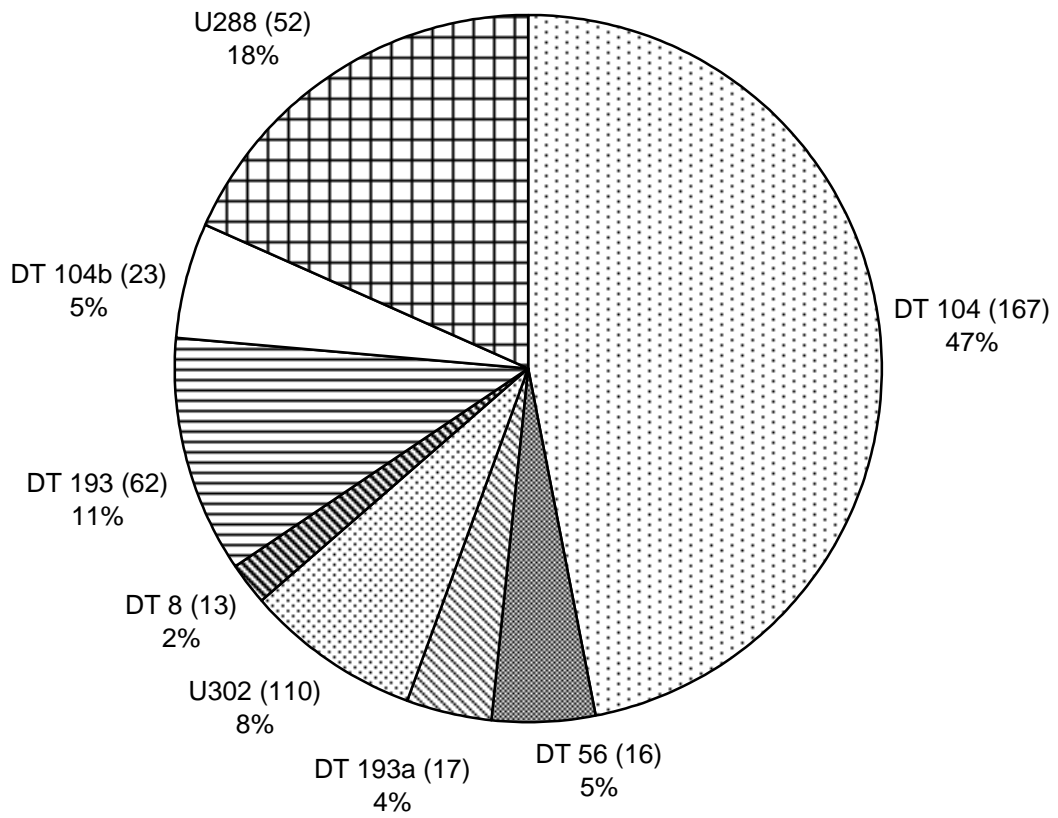


Fig 46: Percentage of the eight most common definitive types of *Salmonella* Typhimurium sensitive to 16 antimicrobial agents in 2003

