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 of these isolates originate 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 30µg cefuroxime disc that had been used in previous years, was replaced with a 30µg ceftazidime disc and in 2004 the 30µg cefoperazone and 25µg colistin discs were replaced with 30µg cefotaxime and 1µg ciprofloxacin discs respectively. These changes were made to enhance the detection of resistance to third generation cephalosporins and fluoroquinolones.

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	Cefotaxime	30	CTX
15	Apramycin	15	APR
16	Ciprofloxacin	25	CIP

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 premises. 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 serotype and its antimicrobial susceptibility may not reflect its prevalence in the animal population as a whole but rather the intensity of 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 516 *Salmonella* Dublin cultures tested during 2004, 97.9% were susceptible to all 16 antimicrobial drugs (Table 81). The percentage of *S.* Dublin isolates sensitive to all 16 antimicrobials has shown only slight fluctuations over the period 1996-2004 and the majority of isolates remain susceptible; 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 then re-appeared in 2003 and 2004. 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, but was not detected in 2004. Since 2000, resistance to trimethoprim/sulphonamides has been observed in *S*. Dublin, a resistance not detected during the period 1996-1999. This is of interest, since trimethoprim/sulphonamide resistance also increased in *Salmonella* Typhimurium in 2002, though by a much greater degree. Resistance to streptomycin increased in 2003, with 1.4% of cultures resistant; 1% of isolates were resistant in 2004. Many of these fluctuations are probably related to clonal spread of particular isolates as a result of husbandry and animal movement factors, in addition to the variation in the selective pressure exerted by antimicrobial usage.

Table 81: *Salmonella* Dublin: antimicrobial sensitivity monitoring 1996 - 2004

Year	No of cultures	Percentage sensitive to all 16 anti-microbials	Percentage of cultures resistant to:							
			S	SU	T	N	AM	FR	TM	C
1996	325	99.7	0.3	0.3	-	-	-	-	-	0.3
1997	284	98.2	0.7	0.7	0.7	-	-	-	-	1.4
1998	281	99.3	0.4	0.4	-	-	-	-	-	0.4
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
2004	516	97.9	1.0	1.2	0.4	-	0.2	-	0.8	0.8

SALMONELLA *TYPHIMURIUM*

The number of cultures of *Salmonella* Typhimurium examined in 2004 was 468, of which 53.8% were definitive type (DT) 104, DT104b or U302 (Table 82). 26.7% of the cultures were sensitive to all of the antimicrobials tested (Table 82). The increase in the proportion of fully susceptible cultures is partly a reflection of the increased contribution of *Salmonella* Typhimurium isolates from birds other than poultry to the overall total. Of 62 cultures examined from these birds, which include isolates from wild birds such as greenfinches, siskins and house sparrows, 57 (92%) were fully susceptible. The definitive types of *Salmonella* Typhimurium involved in these cases were 2, 2a, 40, 56, 56 variant and 99. Although there have been apparent declines in resistance to all of the individual antimicrobials listed when the figures for 2004 are compared with those for 2003, when the contribution of the isolates from birds other than poultry to these values is removed, the resistance levels approximate more closely to those recorded in 2003. The otherwise 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 1.4% of which were sensitive to all the antimicrobials tested in 2004. 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.

The typical pentavalent resistance pattern AM,C,S,SU,T was the commonest pattern seen in *S*. Typhimurium DT104 isolates recovered from cattle, occurring in 48% of 44 isolates examined. This resistance pattern with additional trimethoprim resistance was observed in a further 36% of isolates. The resistance pattern AM,C,S,SU,T was also the commonest resistance pattern observed in DT104 isolates from pigs, whereas the commonest pattern for isolates from turkeys was AM,C,S,SU,T,NA. There were no *Salmonella* Typhimurium isolates resistant to ceftazidime, cefotaxime, amikacin or ciprofloxacin recovered in 2004.

In 2004, 25.4% of DT104 and 104b isolates were resistant to nalidixic acid and 18.3% resistant to sulphamethoxazole/ trimethoprim. 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 nalidixic acid resistance in 2002, when 6.3% of DT104 and 104b isolates were resistant to nalidixic acid (20.5% of isolates were resistant to sulphamethoxazole/trimethoprim in 2002). In 2001, 19.8% of DT104 and 104b isolates were resistant to nalidixic acid and 12.5% resistant to sulphamethoxazole/trimethoprim. Therefore, trimethoprim/ sulphonamide resistance increased in 2001-2003 in *S.* Typhimurium DT 104 and 104b, though has declined in 2004. Taking a longer-term view, the figure of 18.3% resistance to sulphamethoxazole/trimethoprim in DT104 and 104b isolates in 2004 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 83, which shows that isolates from cattle and turkeys have been the main contributors accounting for the observed rise in nalidixic acid resistance in previous years, though all isolates from cattle were susceptible in 2004. Table 84 gives the equivalent figures for trimethoprim/ sulphonamide resistance by species of origin in *S.* Typhimurium DT104 for 2003 and 2004.

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, 37.5% in 2003 and 32.7% in 2004. There is a contribution from DT104 to this overall figure and this is shown in Table 84. In relation to other phage types of *S*.Typhimurium it is mainly isolates of from pigs that account for this rise in sulphamethoxazole/ trimethoprim resistance (Table 85); a high percentage of many definitive types of *S*.Typhimurium isolated from pigs are resistant to sulphamethoxazole/trimethoprim, a sitiuation that was also observed in 2002 and 2003. The definitive and undefined types of *S*.Typhimurium recovered from pigs include DT 193, DT 208, U288 and U308a. The total numbers of isolates of these types and the percentage resistant to trimethoprim/ sulphonamides are shown in Table 86. Three factors have influenced the sulphamethoxazole/ trimethoprim resistance figures for

S. Typhimurium isolates from pigs over the last few years: (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 DT 193 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 82: *Salmonella* Typhimurium: antimicrobial sensitivity monitoring 1995 – 2004

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
1996	2323*	10.8	82.0	87.1	86.5	1.9	81.9	0.4	18.3	78.5	0.6	9.3
1997	1480†	11.4	81.2	85.9	86.1	1.1	79.8	0.3	16.2	75.1	0.7	13.4
1998	1112**	14.7	77.8	82.3	81.7	1.4	77.8	0.2	18.0	73.1	0.8	14.7
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[11]	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
2004	468##	26.7	55.8	63.7	65.6	4.5	58.5	0.6	32.7	49.4	1.5	10.0

* 1717 (73.9%) of these strains were DT104 and its variants
† 1091 (73.7%) of these strains were DT104 and its variants
** 814 (73.2%) of these strains were DT104 and its variants
‡ 620 (52.7%) of these strains were DT104 and its variants
*** 460(53.2%) of these strains were DT104 and its variants
†† 274 (52.8%) of these strains were DT104 and its variants
[11] 239 (44.8%) of these strains were DT 104 and its variants
273 (44.5%) of these strains were DT 104 and its variants
126 (53.8%) of these strains were DT 104 and its variants

Table 83: Nalidixic acid resistance in *Salmonella* Typhimurium DT104 from domestic livestock. Number of cultures tested (percentage resistant to nalidixic acid) 1995 - 2004

Year	Livestock species					
	Cattle	Sheep	Pigs	Chickens	Turkeys	Ducks
1996	1006 (5.3)	89 (9)	102 (6.9)	100 (6)	81 (75.3)	0
1997	597 (11.1)	66 (12.1)	88 (4.5)	31 (16.1)	69 (78.3)	3 (0)
1998	369 (10.3)	53 (5.7)	56 (10.7)	63 (4.8)	80 (71.3)	7 (14.3)
1999	231(5.2)	35(2.9)	114(9.6)	5 (20)	24(66.7)	1(0)
2000	223(10.8)	21(0)	51(2)	7 (14.3)	7(0)	1(0)
2001	115(15.7)	8(12.5)	19(21.1)	22 (0)	25(60)	0(0)
2002	67(7.5)	5(40)	36(5.6)	32 (0)	17(11.8)	0(0)
2003	100(20)	6(0)	27(11.1)	12 (8.3)	41(63.4)	0(0)
2004	44(0)	2(0)	10(10)	6 (0)	39(74.4)	0(0)

Table 84: Trimethoprim/ sulphonamide resistance in *Salmonella* Typhimurium DT104 from domestic livestock in 2003 and 2004. Number of cultures tested (percentage resistant to trimethoprim/ sulphonamide).

Year	Livestock species					
	Cattle	Sheep	Pigs	Chickens	Turkeys	Ducks
2003	100 (33)	6 (0)	27 (15)	12 (33)	41 (7.3)	0 (0)
2004	44 (34)	2 (50)	10 (10)	6 (0)	39 (0)	0 (0)

Table 85: Trimethoprim/ sulphonamide resistance in *Salmonella* Typhimurium (all phage types) from domestic livestock in 2004. Number of cultures tested (percentage resistant to trimethoprim/sulphonamide)

Year	Livestock species					
	Cattle	Sheep	Pigs	Chickens	Turkeys	Ducks
2004	90 (30)	7 (57)	146 (72)	11 (0)	55 (2)	7 (0)

Table 86: Trends in Trimethoprim/ sulphonamide resistance in certain types of *Salmonella* Typhimurium from pigs over the period 2002-2004. Number of cultures tested (percentage resistant to trimethoprim/ sulphonamide)

Year	Determinative type or undefined type			
	DT193	DT208	U288	U308a
2002	47 (85)	14 (100)	51 (94)	59 (95)
2003	38 (92)	7 (43)	72 (90)	0 (0)
2004	19 (79)	1 (100)	71 (97)	3 (0)

1.7% of DT104 and its variants were resistant to neomycin; the main contribution to the overall levels of neomycin resistance seen in *Salmonella* Typhimurium in 2004 came from isolates of porcine origin. Apramycin resistance in *S.* Typhimurium in 2004 declined to levels approximating to those last observed in 1999.

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 and U302 from cattle; in DTs 104 and 120 from poultry; in DTs 104, 193 and U288 from sheep and in DTs 104, 104b, 120, 193, 193a, U288 and U302 from pigs. Of the 26 different definitive and undefined types detected, 10 (namely 2a, 8, 29, 30, 40, 41, 49, 56, 99 and U314) were fully susceptible to all of the antimicrobials in the test panel.

SEROTYPES OTHER THAN SALMONELLA *DUBLIN* AND SALMONELLA *TYPHIMURIUM*

Of the 2942 cultures tested 67.3% were sensitive to all the antimicrobials tested (Table 87), very similar to the figure recorded in 2003, when 67.7% were sensitive. 46 (1.6%) of the cultures were *S.* Enteritidis, of which 14 were *S.* Enteritidis Definitive Type 4 and of these *S.* Enteritidis Definitive Type 4 isolates, 92.9% 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 2001 and earlier, maintaining the trend observed in 2002 and 2003. Neomycin resistant isolates originated mainly from poultry (10.1% of isolates from poultry were resistant of 2035 isolates tested - this can be compared to 2003 when 8.5% of isolates from poultry were resistant, with 2541 isolates tested). The majority of these neomycin resistant poultry isolates were from ducks, with lower numbers from chickens and turkeys. Furazolidone resistant isolates also originated mainly from poultry. In 2004, 11.2% of 2035 isolates from poultry were resistant to furazolidone; in 2003, 12.4% of isolates from poultry were

resistant with 2541 isolates tested. Many of these isolates were *Salmonella* Indiana and again originated from ducks. This is discussed further below.

Table 87: Salmonellas, other than *Salmonella* Dublin and *Salmonella* Typhimurium antimicrobial sensitivity monitoring 1995-2004

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
1995	5085	64.5	6.5	17.0	8.4	1.4	4.1	1.2	10.6	1.6	0.2	1.7
1996	3141	70.6	5.5	21.6	8.9	1.2	5.2	1.0	16.3	1.5	0.2	3.0
1997	2442	74.8	6.3	17.7	10.9	1.1	3.1	0.9	13.7	1.1	0.2	3.0
1998	2227	74.4	6.2	16.3	11.0	0.4	3.3	0.8	11.6	1.8	0.2	4.0
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
2004	2942	67.3	11.6	19.1	17.5	7.2	2.2	7.8	14	1.3	0.3	2.1

INDIVIDUAL ANTIMICROBIALS

Of the 3926 salmonellas tested in 2004, 66.5% were sensitive to all of the antimicrobials tested – similar to the figure of 67.3% recorded in 2003. This can be compared with figures of 61.1% recorded in 2002 and of 65.5% in 2001. 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, 19.9% in 2003 and 21.0% in 2004. This decline in tetracycline resistance over the period 1999-2000 reflects both the proportionate decrease in salmonella isolates of all serotypes recovered from pigs and also the declining numbers of *S*.Typhimurium DT104. The level of resistance to neomycin in all salmonella serotypes was 5.9% in 2004, 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, with 5.9% of isolates resistant in 2004. The observed increase in furazolidone resistance 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 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 records from previous years shows that furazolidone-resistant *Salmonella* Indiana has been present in poultry in England and Wales for many years.

Salmonella Indiana isolates from ducks are also commonly resistant to neomycin. 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 seven in 1999. 39 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, whilst in 2004, 48 isolates were examined and 2.1% were resistant to furazolidone. Resistance to apramycin in all *Salmonella* serotypes was 0.4% in 2004, similar to the figure of 0.5% observed in 2003. In 2004, 2.8% of all *Salmonella* isolates were resistant to nalidixic acid – similar to the figure of 3.2% of all salmonella isolates resistant to nalidixic acid recorded in 2003. This can be compared with 2.4% of all salmonella isolates which were resistant to nalidixic acid 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, cefotaxime or ceftazidime.

Table 88: All salmonellas: antimicrobial sensitivity 2004

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
Cattle	657	85.2	10.4	11.4	12.3	0	9.7	0	4.7	9.0	0.3	0.9
Sheep	239	94.1	2.5	2.9	5.0	0	2.5	0	1.7	2.1	0	0
Pig	209	12.0	59.8	72.7	82.3	7.2	57.9	1.4	56.5	51.7	3.8	4.3
Chicken	958	63.8	8.2	31.2	8.7	1.8	3.3	1.4	26.9	2.1	0.2	2.1
Turkey	374	39.8	23.3	46.8	39.3	2.9	18.2	0	17.6	10.7	0	15.0
Duck	776	61.5	24.6	7.0	28.7	22.9	0.6	27.6	3.7	1.3	0	0.9
Goose	2	100	0	0	0	0	0	0	0	0	0	0
Horse	51	68.6	17.6	21.6	23.5	0	17.6	0	5.9	17.6	0	3.9
Other non-avian spp	204	74.5	12.3	15.7	15.7	4.9	11.8	1.0	9.8	7.4	0.5	3.9
Other avian spp	74	89.2	4.1	2.7	6.8	0	1.4	0	0	2.7	1.4	1.4
Feed	358	79.3	3.9	15.9	15.9	0.3	2.5	0.3	11.7	1.7	0.3	0.6
Environ-ment	24	100	0	0	0	0	0	0	0	0	0	0
Total	3926	66.5	15.5	22.0	21.1	5.9	8.6	5.9	14.5	7.0	0.4	2.8

Fig 45: Number of isolates of *Salmonella* Typhimurium of the eight most frequent definitive or undefined types subjected to susceptibility testing to VLA in 2004

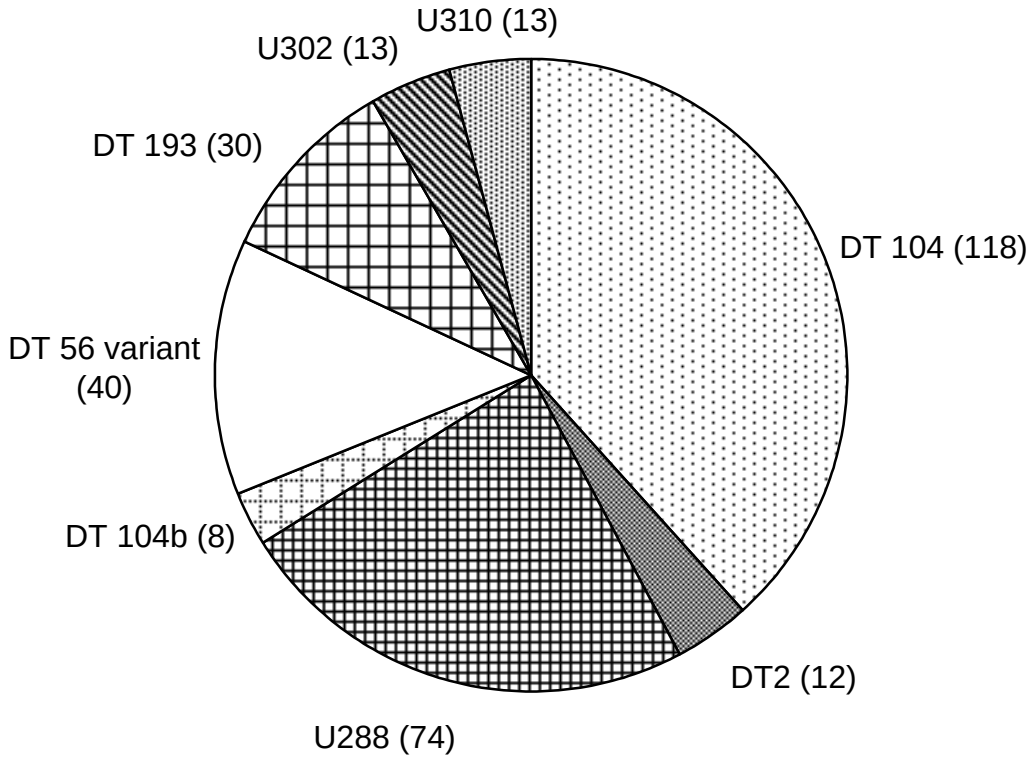

Fig 46: Percentage of the eight most common definitive and undefined types of *Salmonella* Typhimurium sensitive to 16 antimicrobial agents in 2004