

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 majority of isolates originating from cattle, sheep and pigs are from clinical cases. 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 and in 2004 the 30 µg cefoperazone and 25 µg colistin discs were replaced with 30 µg cefotaxime and 1 µg 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	30	CAZ
7	Sulphamethoxazole/trimethoprim	25	TM
8	Chloramphenicol	10	C
9	Amikacin	30	AK
10	Amoxicillin/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	1	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 received 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 365 *Salmonella* Dublin cultures tested during 2005, 98.1% were susceptible to all 16 antimicrobial drugs (Table 80). The percentage of *S. Dublin* isolates sensitive to all 16 antimicrobials has shown only slight fluctuations over the period 1996-2005 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 - 2005. 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 or 2005. Resistance to trimethoprim/sulphonamides was not detected in 2005. Resistance to streptomycin increased in 2003, with 1.4% of cultures resistant; 1.1% of isolates were resistant in 2005. Many of these fluctuations are probably related to the clonal spread of particular strains as a result of husbandry and animal movement factors, in addition to the variation in the selective pressure exerted by antimicrobial usage.

Table 80: *Salmonella* Dublin: antimicrobial sensitivity monitoring 1996 - 2005

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	0.2	0.6
2001	467	98.3	0.2	1.3	-	-	-	-	0.2	0.2	0.6
2002	687	97.5	0.3	0.7	0.6	0.4	-	0.6	0.9	0.4	0.4
2003	949	96.4	1.4	1.2	0.8	0.2	0.6	0.4	0.4	0.4	1.3
2004	516	97.9	1.0	1.2	0.4	-	0.2	-	0.8	0.8	0.8
2005	365	98.1	1.1	0.3	0.3	-	0.8	-	-	-	0.3

***Salmonella* Typhimurium**

The number of cultures of *Salmonella* Typhimurium examined in 2005 was 552; 26.1% of these isolates were definitive type DT104, 104b or U302 (Table 81). 24.1% of all *Salmonella* Typhimurium isolates were sensitive to all of the antimicrobials tested (Table 81). The increase in the proportion of fully susceptible cultures reflects in part the contribution of *Salmonella* Typhimurium isolates from birds other than poultry and horses to the overall total. Of 35 cultures examined from these birds, which include isolates from wild birds, 32 (91%) were fully susceptible and of 27 *S.* Typhimurium isolates from horses, 15 were fully susceptible. One of the most common definitive types of *Salmonella* Typhimurium involved in these cases was 56 variant. The otherwise generally high level of resistance of *Salmonella* Typhimurium isolates is partly a reflection of the contribution of DT104 and its variants DT104b and U302, only 2.1% of which were sensitive to all the antimicrobials tested in 2005. 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 DT 104.

S. Typhimurium U288 and DT193 from pigs comprise 19%(107) and 24% (134) of the total numbers of *S.* Typhimurium isolates; 0.9% of U288 and 27% of DT193 isolates from pigs were fully susceptible. AM,S,Su,T

was the commonest resistance pattern in DT 193 isolates from pigs (36 isolates), followed by AM,C,S,Su,T,Tm (28 isolates) and AM,Su,T,Tm (15 isolates). The commonest resistance pattern in U288 isolates from pigs was AM,C,S,Su,T,Tm (76 isolates).

The typical pentavalent resistance pattern AM,C,S,SU,T was the commonest pattern seen in *S. Typhimurium* DT104 isolates recovered from cattle, occurring for 55% of 40 isolates examined. This resistance pattern with additional trimethoprim resistance was observed in a further 15% of isolates. The commonest resistance pattern for *S. Typhimurium* DT104 isolates from turkeys was AM,C,S,SU,T,NA accounting for 30 of the 32 isolates examined from this species.

There were no *Salmonella* Typhimurium isolates resistant to ceftazidime, cefotaxime or amikacin recovered in 2005.

In 2005, 35.8% of DT104 and 104b isolates were resistant to nalidixic acid and 11.9% resistant to sulphamethoxazole/ trimethoprim and this can be compared to the figures for 2004, when 25.4% of DT104 and 104b isolates were resistant to nalidixic acid and 18.3% 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). Nalidixic acid resistance in *S. Typhimurium* DT104 by species of origin is listed in Table 82, which shows that isolates from cattle and turkeys have been the main contributors accounting for the observed rise in nalidixic acid resistance in recent years. Table 83 gives the equivalent figures for trimethoprim/ sulphonamide resistance by species of origin in *S. Typhimurium* DT104 for 2003 - 2005. Trimethoprim/ sulphonamide resistance increased in 2001-2003 in *S. Typhimurium* DT104 and 104b, though has declined in 2004 and 2005.

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, 32.7% in 2004 and 36.1% in 2005. There is a contribution from DT104 to this overall figure and this is shown in Table 83. 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 84); 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 - 2004. The definitive and undefined types of *S. Typhimurium* resistant to sulphamethoxazole/ trimethoprim and recovered from pigs in 2005 include DT 193 and U288. The total numbers of isolates of these types and the percentage resistant to trimethoprim/

sulphonamides are shown in Table 85. 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. (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 recent years.

Table 81: *Salmonella* Typhimurium: antimicrobial sensitivity monitoring 1996– 2005

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
2005	552 ^{##}	24.1	60.0	71.6	71.0	1.3	67.2	4.2	36.1	53.1	0.7	8.9

- * 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 DT104 and its variants
- # 273 (44.5%) of these strains were DT104 and its variants
- ## 126 (53.8%) of these strains were DT104 and its variants
- ## 144 (26.1%) of these strains were DT104 and its variants

Table 82: Nalidixic acid resistance in *Salmonella* Typhimurium DT104 from domestic livestock. Number of cultures tested (percentage resistant to nalidixic acid) 1995 - 2005

Year	Livestock species					
	Cattle	Sheep	Pigs	Chickens	Turkeys	Ducks
1995	2109 (1.6)	150 (0)	130 (0)	119 (0.8)	120 (63.3)	4 (0)
1996	1006 (5.3)	89 (9)	102 (6.9)	100 (6)	81 (75.3)	0 (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)
2005	40 (12.5)	8 (0)	2 (0)	6 (33.3)	32 (96.9)	0 (0)

Table 83: Trimethoprim/ sulphonamide resistance in *Salmonella* Typhimurium DT104 from domestic livestock in 2003 - 2005. 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)
2005	40 (17.5)	8 (37.5)	2 (0)	6 (33.3)	32 (0)	0 (0)

Table 84: Trimethoprim/ sulphonamide resistance in *Salmonella* Typhimurium (all phage types) from domestic livestock in 2004 and 2005. 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)
2005	71 (14)	13 (31)	317 (56)	10 (20)	37 (3)	13 (0)

Table 85: Trends in Trimethoprim/ sulphonamide resistance in certain types of *Salmonella* Typhimurium from pigs over the period 2002- 2005. 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)
2005	134 (43)	0 (0)	107 (96)	0 (0)

0.7% of DT104 and its variants were resistant to neomycin in 2005; the main contribution to the overall levels of neomycin resistance seen in *Salmonella* Typhimurium in 2005 came from isolates of porcine origin belonging to DT193 and U288. Apramycin resistance in *S. Typhimurium* in 2004 declined to levels approximating to those last observed in 1999 and further declined in 2005. Furazolidone resistance was observed mainly in isolates of *S. Typhimurium* U302, where 19 of 32 isolates from pigs (59%) were resistant. The detection of furazolidone resistance principally in a single undefined phage type may suggest that clonal spread of this phage type is likely to be responsible for the observed level of furazolidone resistance.

Multiple antibiotic resistance (i.e. resistance to four or more antimicrobial agents in the panel of 16) was detected in DTs 12, 49, 104, 104b, 193, 193a and U302 from cattle; in DTs 104, 104b, U288 and U302 from poultry; in DT 104 from sheep and in DTs 104, 104b, 120, 193, 193a, U288, U302, U308 and U310 from pigs. Of the 29 different definitive and undefined types detected, 13 (namely 2, 2a, 30, 36, 40, 41, 56, 85, 93, 99, 135, 170b and 195) were fully susceptible to all of the antimicrobials in the test panel.

Serotypes other than *Salmonella Dublin* and *Salmonella Typhimurium*

Of the 2683 cultures tested 65.6% were sensitive to all the antimicrobials tested (Table 86), similar to the figure recorded in 2004, when 67.3% were fully sensitive. 191 (7.1%) of the cultures were *S. Enteritidis*, of which 31 were *S. Enteritidis* Phage Type 4 and of these *S. Enteritidis* PT 4 isolates, 100% were sensitive to all of the antimicrobials 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 - 2004. Neomycin resistant isolates originated mainly from poultry

(5.7% of isolates from poultry were resistant of 1874 isolates tested in 2005 – this can be compared to 2004 when 10.1% of isolates from poultry were resistant, of 2035 isolates tested). The majority of these neomycin resistant poultry isolates were from ducks, with much lower numbers from chickens and turkeys. Furazolidone resistant isolates also originated mainly from poultry. In 2005, 8.5% of 1874 isolates from poultry were resistant to furazolidone and this is similar to the figures for 2004, when 11.2% of 2035 isolates from poultry were resistant to furazolidone and figures for 2003 when 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 86: Salmonellas, other than *Salmonella* Dublin and *Salmonella* Typhimurium antimicrobial sensitivity monitoring 1995-2005

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
2005	2683	65.6	10.9	23.7	23.6	4.2	4.6	6.2	12.3	2.5	0.1	2.2

Individual Antimicrobials

Of the 3600 salmonellas tested in 2005, 62.5% were sensitive to all of the antimicrobials tested – a slight reduction on the figure of 66.5% recorded in 2004, but similar to the figure of 61.1% recorded in 2002. 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; though increased to 28.5% in 2005. 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 3.3%

in 2005, 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 and 5.2% of isolates resistant in 2005. 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 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 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 7 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. In 2005, there were 22 isolates of *S. Virchow* examined and all were susceptible to furazolidone. As mentioned earlier, there is also a contribution to the overall furazolidone resistance figures from *S. Typhimurium* U302, in which 19 of 32 isolates (59%) from pigs were resistant. Resistance to apramycin in all *Salmonella* serotypes was 0.2% in 2005, similar to the figure of 0.4% observed in 2004. In 2005, 3.0% of all *Salmonella* isolates were resistant to nalidixic acid – similar to the figure of 2.8% of all *Salmonella* isolates resistant to nalidixic acid recorded in 2004. 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 87: All salmonellas: antimicrobial sensitivity 2005

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	499	85.8	10.6	11.6	11.2	0.2	11.6	0	2.4	9.6	0	1.0
Sheep	176	90.3	6.3	8.5	8.5	0	7.4	0	2.8	6.3	0	0
Pig	398	16.6	59.3	75.9	76.1	1.5	64.1	6.3	50.8	49.0	1.0	1.8
Chicken	778	66.7	9.3	26.9	11.6	2.3	4.5	0.5	20.1	3.7	0.3	4.0
Turkey	334	42.2	31.1	51.8	48.5	0.9	19.4	0	15.6	10.8	0	11.4
Duck	822	53.2	15.1	24.7	39.1	10.3	4.6	19.0	7.9	3.3	0	2.6
Horse	44	68.2	18.0	25.0	29.5	4.5	25.0	0	4.5	11.4	0	2.3
Other non-avian spp	169	80.5	9.5	16.0	18.9	0.6	10.1	0	6.5	5.3	0	1.8
Other avian spp	58	86.2	0	8.6	6.9	1.7	0	1.7	0	1.7	0	1.7
Feed	322	88.5	1.2	8.7	9.3	0.9	1.6	0.6	7.1	0.3	0	0
Total	3600	62.5	17.4	28.6	28.5	3.3	13.8	5.2	14.7	10.1	0.2	3.0

Fig 45: Number of isolates of *Salmonella* Typhimurium of the eight most frequent definitive or undefined types subjected to susceptibility testing to VLA in 2005

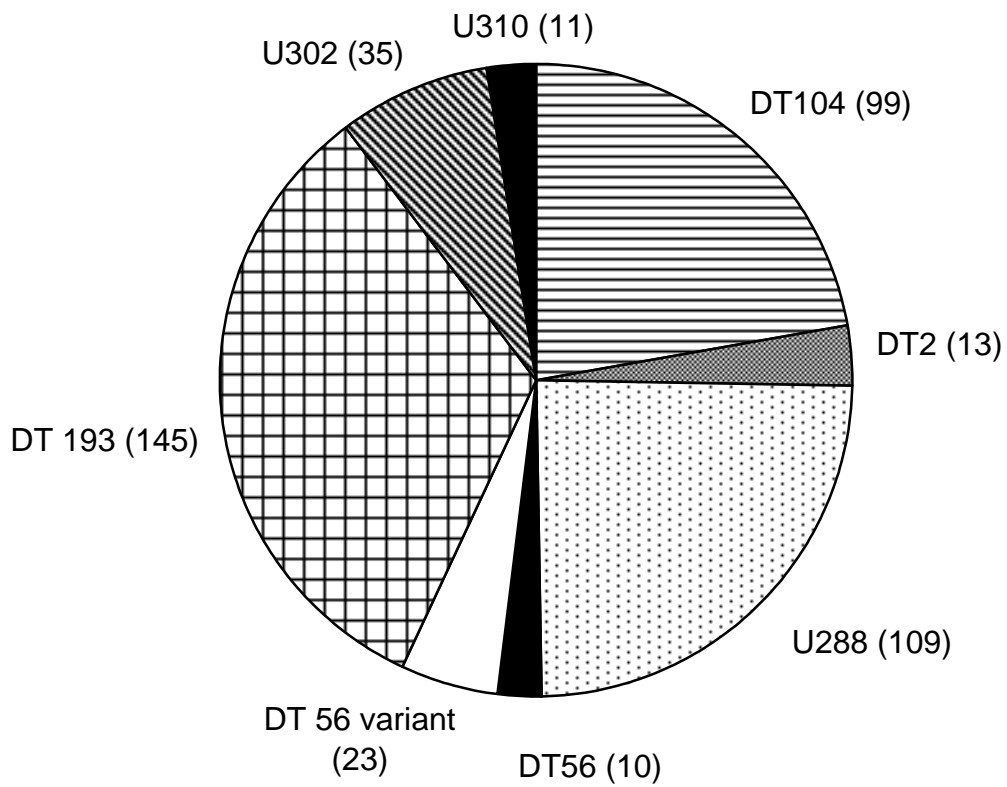


Fig 46: Percentage of the eight most common definitive and undefined types of *Salmonella* Typhimurium sensitive to 16 antimicrobial agents in 2005

