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 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	Code
1	Nalidixic acid	(30 µg)	NA
2	Tetracycline	(10 µg)	T
3	Neomycin	(10 µg)	N
4	Ampicillin	(10 µg)	AM
5	Furazolidone	(15 µg)	FR
6	Ceftazidime	(30 µg)	CAZ
7	Sulphamethoxazole/trimethoprim	(25 µg)	TM
8	Chloramphenicol	(10 µg)	C
9	Amikacin	(30 µg)	AK
10	Amoxicillin/clavulanic acid	(30 µg)	AMC
11	Gentamicin	(10 µg)	CN
12	Streptomycin	(25 µg)	S
13	Sulphonamide compounds	(300 µg)	SU
14	Cefotaxime	(30 µg)	CTX
15	Apramycin	(15 µg)	APR
16	Ciprofloxacin	(1 µg)	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 468 *Salmonella* Dublin cultures tested during 2006, 96.4% were susceptible to all 16 antimicrobial drugs (Table 79). The percentage of *S.* Dublin isolates sensitive to all 16 antimicrobials has shown only slight fluctuations over the period 1999-2006 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 - 2006.

Resistance to furazolidone, 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 - 2006. Resistance to trimethoprim/ sulphonamides was not detected in 2005, though a single resistant isolate was detected in 2006.

Resistance to streptomycin increased in 2003, with 1.4% of cultures resistant, though the prevalence of resistance has subsequently declined. The prevalence of nalidixic acid resistance was 1.5% in 2006, higher than the levels reported since 1999. There was no resistance in 2006 reported to any of the antimicrobials in the panel of 16 which were tested but which are not shown in Table 79.

Many of these fluctuations in the prevalence of resistance 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 79: *Salmonella* Dublin: antimicrobial sensitivity monitoring 1999 - 2006

Year	No of cultures	Percentage sensitive to all 16 anti-microbials	Percentage of cultures resistant to:								
			S	C	SU	T	N	AM	FR	TM	NA
1999	357	98.3	1.1	-	-	-	-	-	-	-	0.6
2000	863	98.7	0.7	0.6	0.7	0.5	-	0.1	-	0.2	0.2
2001	467	98.3	0.2	0.6	1.3	-	-	-	-	0.2	0.2
2002	687	97.5	0.3	0.4	0.7	0.6	0.4	-	0.6	0.9	-
2003	949	96.4	1.4	1.3	1.2	0.8	0.2	0.6	0.4	0.4	0.2
2004	516	97.9	1	0.8	1.2	0.4	-	0.2	-	0.8	0.2
2005	365	98.1	1.1	0.3	0.3	0.3	-	0.8	-	-	-
2006	468	96.4	0.4	0.6	1.3	0.6	0.2	0.9	-	0.2	1.5

SALMONELLA TYPHIMURIUM

The number of cultures of *Salmonella* Typhimurium examined in 2006 was 1136; 27.8% of these isolates were definitive type (DT) 104, DT 104b or U302 (Table 80). 24.2% of all *Salmonella* Typhimurium isolates were sensitive to all of the antimicrobials tested (Table 80). These figures are very similar to those observed in 2005. The 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 1.6% of which were sensitive to all the antimicrobials tested in 2006. 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. This change was particularly marked over the period 1996 - 1998 and has been commented on in previous reports.

S. Typhimurium U288 and DT193 from pigs comprise 20% (229) and 9% (103) of the total numbers of *S*. Typhimurium isolates tested; 0.4% of U288 and 4.9% of DT193 isolates from pigs were fully susceptible. The numbers of fully susceptible isolates of DT193 declined in 2006; 27% of DT193 isolates were fully susceptible in 2005. AM,C,S,Su,T,Tm was the commonest resistance pattern in DT 193 isolates from pigs (27 isolates), followed by AM,S,Su,T (11 isolates) and AM,C,Su,T,Tm (11 isolates). The commonest resistance pattern in U288 isolates from pigs was AM,C,S,Su,T,Tm (155 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 in 46% of 112 isolates examined. This resistance pattern with additional trimethoprim resistance was observed in a further 13% of isolates. The commonest resistance pattern for *S*. Typhimurium DT104 isolates from turkeys was AM,C,S,SU,T,NA accounting for 35 of the 57 isolates examined from this species.

There were no *Salmonella* Typhimurium isolates resistant to ceftazidime, cefotaxime or amikacin recovered in 2006.

In 2006, 16.1% of DT104 and 104b isolates were resistant to nalidixic acid and 18.3% resistant to sulphamethoxazole/ trimethoprim. This can be compared to the figures for 2005, when 35.8% of DT104 and 104b isolates were resistant to nalidixic acid and 11.9% resistant to sulphamethoxazole/ trimethoprim. Nalidixic acid resistance in *S.* Typhimurium DT104 by species of origin is listed in Table 81, which shows that the highest prevalence of resistance to nalidixic acid in 2006

in this phage type occurred in turkeys. Table 82 gives the equivalent figures for trimethoprim/sulphonamide resistance by species of origin in *S.* Typhimurium DT104 for 2003 - 2006.

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, 36.1% in 2005 and 39.8% in 2006. There is a contribution from DT 104 to this overall figure and this is shown in Table 82. In relation to other phage types of *S*.Typhimurium it is mainly isolates from pigs that account for this rise in sulphamethoxazole/ trimethoprim resistance (Table 83); 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 - 2005. The definitive and undefined types of *S*. Typhimurium resistant to sulphamethoxazole/ trimethoprim and recovered from pigs in 2006 include DT193 and U288. The total numbers of isolates of these types and the percentage resistant to trimethoprim/ sulphonamides are shown in Table 84. 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 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.

2.8% of DT104 and its variants were resistant to neomycin in 2006; the main contribution to the overall levels of neomycin resistance seen in *Salmonella* Typhimurium in 2006 came from isolates of porcine origin belonging to DT193 and U288. Furazolidone resistance was observed in isolates of *S*. Typhimurium 104b, where six of 22 isolates from pigs (27%) were resistant. Furazolidone resistance was not detected in the related type U302 in 2006, although it had been seen in 59% of U302 isolates in 2005.

Multiple antibiotic resistance (i.e. resistance to four or more antimicrobial agents in the panel of 16) was detected in DTs 8, 12, 104, 104b, 120, 135, 193 and U302 from cattle; in DTs 12, 104, 104b, 120, 208 and U302 from poultry (ie chickens and/or turkeys); in DT 104 and 104b from sheep and in DTs 56, 104, 104b, 120, 193, 193a, 195, U288, U288 variant, U302, U308 and U310 from pigs. Of the 31 different definitive and undefined types detected, 9 (namely 2a, 7, 40, 41, 49, 93,

99, 170b, U292) were fully susceptible to all of the antimicrobials in the test panel.

Table 80: *Salmonella* Typhimurium: antimicrobial sensitivity monitoring 1999– 2006

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 [a]	18.4	61.2	72	78.8	2	63	0.3	23.1	53.2	1.6	11.3
2000	864 [b]	15.3	63.2	70.8	80.4	2.5	63.8	0.1	23.4	56.5	3.2	7.5
2001	519 [c]	20.6	57.8	71.7	75.5	2.9	66.7	0.4	24.3	55.9	2.3	11.9
2002	533 [d]	14.5	61	77.9	80.1	3.4	70.5	2.6	44.1	62.1	2.4	7.1
2003	613 [e]	19.6	61.7	73.1	74.2	6.2	68.5	0.7	37.5	58.9	3.8	13.5
2004	468 [f]	26.7	55.8	63.7	65.6	4.5	58.5	0.6	32.7	49.4	1.5	10
2005	552 [g]	24.1	60	71.6	71	1.3	67.2	4.2	36.1	53.1	0.7	8.9
2006	1136 [h]	24.2	54.2	70.2	69.2	5.1	65.9	1	39.8	57	1	6.9

[a] 620 (52.7%) of these strains were DT104 and its variants
[b] 460 (53.2%) of these strains were DT104 and its variants
[c] 274 (52.8%) of these strains were DT104 and its variants
[d] 239 (44.8%) of these strains were DT104 and its variants
[e] 73 (44.5%) of these strains were DT104 and its variants
[f] 126 (53.8%) of these strains were DT104 and its variants
[g] 144 (26.1%) of these strains were DT104 and its variants
[h] 316 (27.8%) of these strains were DT104 and its variants

Table 81: Nalidixic acid resistance in *Salmonella* Typhimurium DT104 from domestic livestock. Number of cultures tested (percentage resistant to nalidixic acid) 1999 - 2006

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)
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 (10.0)	6 (0)	39 (74.4)	0 (0)
2005	40 (12.5)	8 (0)	2 (0)	6 (33.3)	32 (96.9)	0 (0)
2006	112 (0)	12 (0)	20 (0)	6 (50)	57 (71.9)	0 (0)

Table 82: Trimethoprim/ sulphonamide resistance in *Salmonella* Typhimurium DT104 from domestic livestock in 2003 - 2006. 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)
2006	112 (22.3)	12 (41.7)	20 (15)	6 (16.7)	57 (8.8)	0 (0)

Table 83: Trimethoprim/sulphonamide resistance in *Salmonella* Typhimurium (all phage types) from domestic livestock in 2004 - 2006. 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)
2006	174 (20)	18 (28)	555 (69)	13 (15)	86 (7)	35 (14)

Table 84: Trends in Trimethoprim/ sulphonamide resistance in certain types of *Salmonella* Typhimurium from pigs over the period 2002- 2006. Number of cultures tested (percentage resistant to trimethoprim/ sulphonamide)

Year	Definitive 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)
2006	103 (72)	16 (25)	229 (96)	0 (0)

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

Of the 2727 cultures tested 58.7% were sensitive to all the antimicrobials tested (Table 85), a slight decline from the figure recorded in 2005, when 65.6% were fully sensitive. 111 (4.1%) of the cultures were *S.* Enteritidis, of which 19 were *S.* Enteritidis PT 4 and of these *S.* Enteritidis PT 4 isolates, 94% were sensitive to all of the antimicrobials in the test panel, with one isolate resistant to streptomycin. *S.* Enteritidis PT1 is a strain which is commonly resistant to nalidixic acid and of 37 isolates of this definitive type tested, all were nalidixic acid resistant. Levels of resistance to furazolidone and neomycin were higher than those observed in 2001 and earlier, maintaining the trend observed in 2002 - 2005. Neomycin resistant isolates originated mainly from poultry (10.3% of 1622 isolates from poultry tested in 2006 were resistant, similar to the figure for 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 2006, 9.4% of 1622 isolates from poultry were resistant and this is similar to the figure for 2005, when 8.5% of 1874 isolates from poultry were resistant to furazolidone. The majority of these isolates were *Salmonella* Indiana and originated from ducks. This is discussed further below.

INDIVIDUAL ANTIMICROBIALS

Of the 4331 salmonellas tested in 2006, 53.7% were sensitive to all of the antimicrobials tested. This is a reduction in comparison with previous years. In 2005, 62.5% of salmonellas were sensitive to all of the antimicrobials tested; the figure was 66.5% for 2004.

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; the prevalence of resistance to tetracyclines was 21.2% in 2002, 19.9% in 2003 and 21.0% in 2004; though increased to 28.5% in 2005 and was 36.4% in 2006. The decline in tetracycline resistance over the period 1999 - 2000 reflected both the proportionate decrease in salmonella isolates of all serotypes recovered from pigs and also the declining numbers of *S*. Typhimurium DT104. The main contribution to tetracycline resistance in 2006 came from *Salmonella* isolates from pigs, turkeys and ducks.

The prevalence of resistance to neomycin in all salmonella serotypes was 5.7% in 2006, 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 prevalence of furazolidone resistance was 3.9% in 2006. 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. The situation was similar in 2006, with 15 *S.* Virchow isolates examined, all of which were susceptible to furazolidone. In 2005, furazolidone resistance was detected in *S*. Typhimurium U302, with 19 of 32 isolates (59%) from pigs resistant; furazolidone resistance was not seen in U302 in 2006 in isolates from any species. *S.* Typhimurium DT104 from cattle (n=1), DT 104b from pigs (n=6) and DT56 variant, were resistant to furazolidone in 2006. Resistance to apramycin in all *Salmonella* serotypes was 0.4% in 2006, similar to the figure of 0.2% observed in 2005.

In 2006, 4.5% of all *Salmonella* isolates were resistant to nalidixic acid – similar to the figure of 3.0% of all *Salmonella* isolates resistant to nalidixic acid recorded in 2005. 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.

0.4% of *Salmonella* isolates from all species were resistant to gentamicin in 2006.

Two *S.* Typhimurium DT104 isolates from chickens were resistant to amoxicillin/ clavulanate, whilst a single rough strain *Salmonella* from chickens was resistant to ciprofloxacin. No resistance was detected to amikacin, cefotaxime or ceftazidime.

Table 85: Salmonellas, other than *Salmonella* Dublin and *Salmonella* Typhimurium antimicrobial sensitivity monitoring 1999-2006

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	2.8	0.4	6.6	2.2	0.3	3.1
2000	2877	70.7	5	18	9.5	0.9	4.8	0.5	13.7	3.5	0.1	5.5
2001	1814	69.8	8.1	20	10	1	5.7	0.6	12.1	6.4	0.2	1.4
2002	2167	60.3	11.2	24	13.7	5.2	6.5	3.7	19.5	8	0.3	1.9
2003	3652	67.7	10	19	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
2006	2727	58.7	15.8	25.1	28.8	6.9	7.2	5.7	14.2	3.2	0.2	4

Table 86: All salmonellas: antimicrobial sensitivity 2006

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	758	77.3	11.7	19.9	19.7	0.5	18.7	0.1	4.9	17.7	-	1.1
Sheep	229	91.7	4.8	7	7.9	-	7.4	0.4	2.6	7	-	-
Pigs	869	11.7	55.6	72.7	81.5	6.6	58.5	0.8	54.8	47	1.3	2.9
Chickens	578	44.6	14.4	33.7	21.6	6.6	5.7	0.7	27.2	8	0.9	10.6
Turkeys	499	50.1	30.7	44.7	39.5	0.4	27.4	-	12	14.6	-	11.8
Ducks	675	55	23	21.6	36.9	18.8	4.7	22.1	8.3	1.8	-	1.3
Geese	14	100	-	-	-	-	-	-	-	-	-	-
Horses	55	78.2	14.5	16.4	12.7	5.4	16.4	-	7.3	10.9	-	5.4
Other avian species	139	66.9	14.4	26.6	28.8	2.2	11.5	0.7	7.2	4.3	-	5.8
Feed	174	89.1	2.9	6.3	6.9	1.7	3.4	-	4	3.4	-	1.1
Total	4331	53.7	24.2	34.4	36.4	5.7	21.9	3.9	19.4	17	0.4	4.5

Fig 48: Number of isolates of *Salmonella* Typhimurium of the eight most frequent definitive or undefined types subjected to susceptibility testing to VLA in 2006

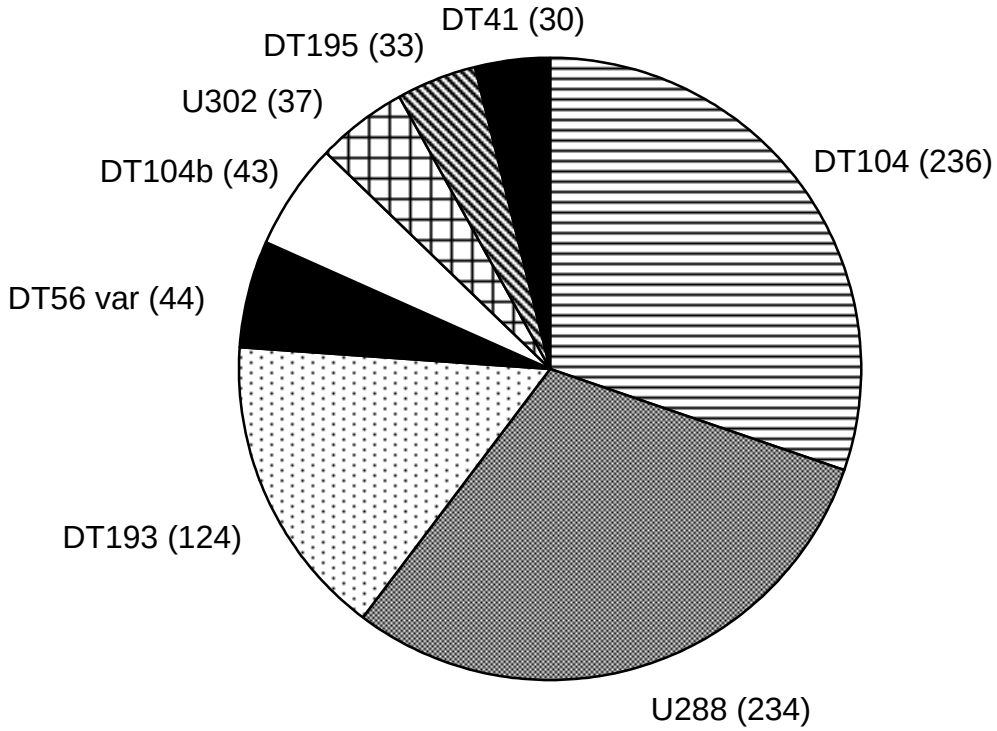
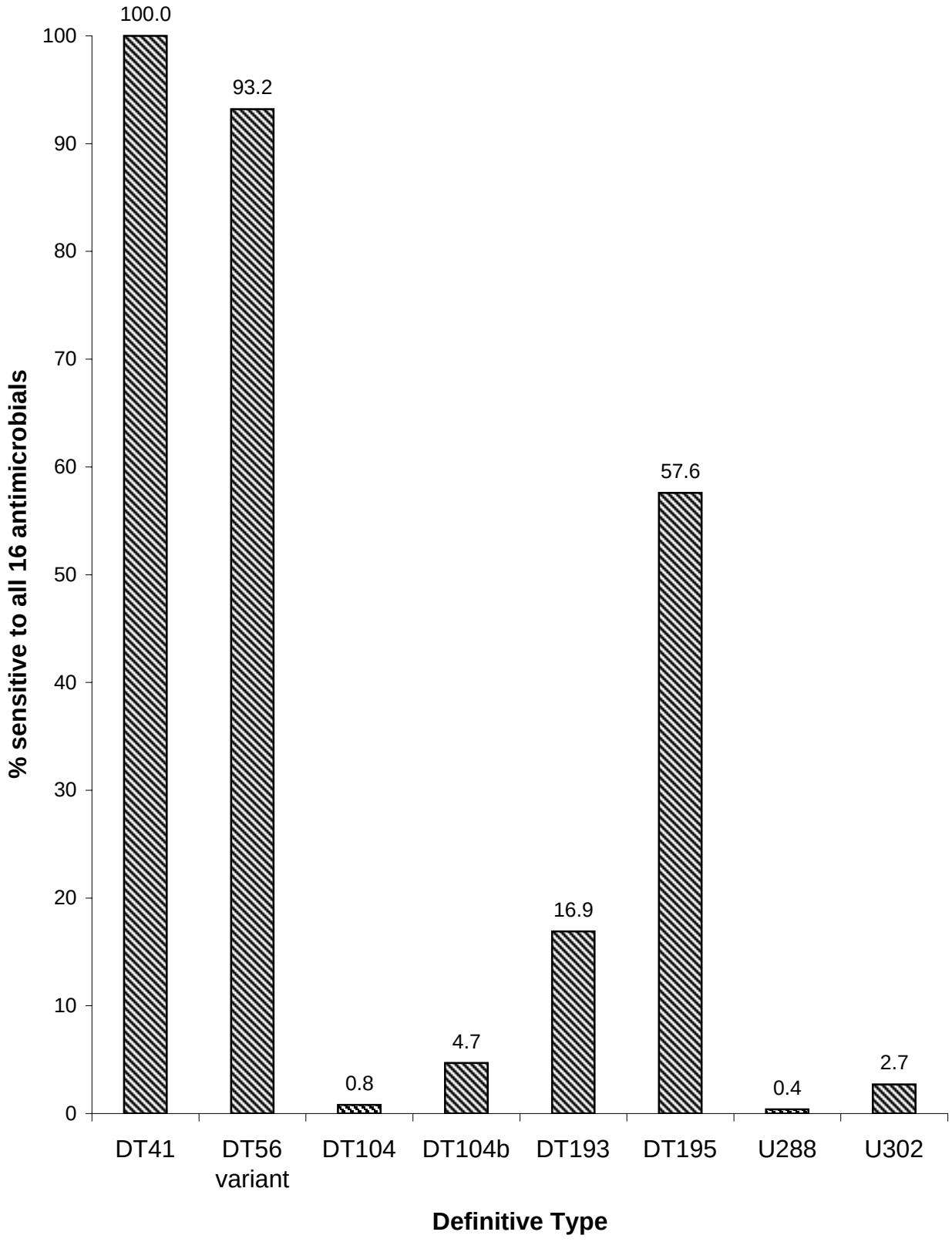
Fig 49: Percentage of the eight most common definitive and undefined types of *Salmonella* Typhimurium sensitive to 16 antimicrobial agents in 2006