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A comparison of multiple drug resistance in salmonellas from humans and food animals in England and Wales, 1981 and 1990

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SUMMARY

For *Salmonella typhimurium* from humans in England and Wales, the incidence of multiple resistance more than doubled over the 8-year period 1981–8 and, over the next 2 years, increased by a further 7%. From 1981 to 1988 both resistance and multiple resistance also increased significantly in *S. virchow* and although multiple resistance did not increase over the next 2 years, the overall incidence of resistance has continued to rise. In 1990 the majority of *S. typhimurium* from cattle were multiply-resistant and the occurrence of such resistance has quadrupled since 1981. Multiple resistance has also increased in *S. typhimurium* from pigs and, to a lesser extent, from poultry. In contrast, multiple resistance has remained uncommon in the poultry-associated serotype *S. enteritidis*. For *S. virchow*, multiple resistance was common in a phage type frequently associated with poultry meat imported from France.

The continuing use of a range of different antimicrobials in calf husbandry has been an important factor in promoting the emergence of multiply-resistant strains of *S. typhimurium* in cattle. In contrast, multiple resistance has remained rare in those serotypes associated with poultry, where the use of such antimicrobials has been less intensive.

It is hoped that recent recommendations discouraging, in veterinary medicine, the prophylactic use of antibiotics with cross resistance to those used in human medicine will result in a reduction in the occurrence of multiresistant strains in food animals and subsequently in humans.

INTRODUCTION

In a previous communication, we reported that from 1981 to 1988 multiple drug resistance doubled for *Salmonella typhimurium* from humans in England and Wales and increased 50-fold for *S. virchow*, but remained constant for *S. enteritidis* [1]. We suggested that for *S. typhimurium* this increase was caused by an upsurge of infections with drug-resistant phage types commonly associated with bovine animals and for *S. virchow*, with phage type 19 (PT 19), a phage type which has been associated with imported poultry meat.

We now describe changes in the incidence of resistance in isolates of these three serotypes from humans over the 2-year period 1989–90, and compare the

occurrence of resistance in isolates made from humans in 1981 and 1990 with isolates from food animals, cattle, pigs and poultry, made in the same years. The implications of the findings are discussed.

METHODS

Bacterial strains: phage typing and screening for resistance to antimicrobial drugs

A total of 30153 human and 7938 food animal isolates of *S. typhimurium*, *S. enteritidis* and *S. virchow* made in 1981 and 1990 were studied. Table 1 refers to these in relation to serotype, year of isolation and source (humans, bovine animals, pigs and poultry). Strains were phage typed by standard methods – Callow [2] and Anderson and colleagues [3] for *S. typhimurium*, Ward and colleagues [4] for *S. enteritidis* and Chambers and colleagues [5] for *S. virchow*.

All isolates were tested for resistance to ampicillin (A), chloramphenicol (C), gentamicin (G), kanamycin (K), streptomycin (S), sulphonamides (Su), tetracyclines (T), trimethoprim (Tm), furazolidone (Fu) and nalidixic acid (Nx) by the methods of Anderson and Threlfall [6] and Ward and colleagues [11]. The levels at which strains were designated resistant were (mg/l): A, 20; C, 10; G, 10; K, 50; S, 10; Su, 100; T, 10; Tm, 2; Fu, 20; Nx, 40.

RESULTS

Incidence of resistance

The incidence of resistance (to one or more drug) and multiple resistance (to four or more drugs) in isolates made from humans and the three food animal groups in 1981 and 1990 is summarized in Table 1.

S. typhimurium

From 1981 to 1988, drug resistance in human isolates of *S. typhimurium* showed no overall increase [1] but over the next 2 years increased by 17%, from 36 to 53%. In contrast, from 1981 to 1988 multiple resistance more than doubled, from 5% in 1981 to 12% in 1988 [1] and over the next 2 years increased by a further 7%, to 19%. In bovine isolates, resistance increased only slightly over the 10-year period 1981–90 but multiple resistance showed an approximate four-fold increase, from less than 20% in 1981 to over 60% in 1990. Isolates from pigs showed a substantial increase in both resistance and multiple resistance, but only a slight increase in the overall incidence was apparent in isolates from poultry. Furthermore, although multiple resistance in poultry isolates showed an eightfold increase, its overall incidence remained low, 8% compared to 66%, in comparison to bovine isolates of *S. typhimurium*.

S. enteritidis

For human isolates, the overall incidence of resistance has remained fairly constant at between 15 and 11% since 1981 and has not increased since 1988. For food animals, only 32 isolates were received in 1981 and all were drug-sensitive.

Table 1. *Incidence of drug resistance in* S. typhimurium, S. enteritidis *and* S. virchow *isolated from humans and food-producing animals in England and Wales in 1981 and 1990*

		Humans			Bovine			Porcine			Poultry		
Serotype	Year	Total	%DR*	%MR†	Total	%DR	%MR	Total	%DR	%MR	Total	%DR	%MR
S. typhimurium	1981	3992	36	5	1157	71	15	49	61	22	177	32	1
	1990	5451	53	19	1178	79	66	144	83	35	1187	37	8
S. enteritidis	1981	1087	15	< 1	8	0	0	2	0	0	22	0	0
	1990	17794‡	11	< 1	74	5	0	9	0	0	3832	14	1
S. virchow	1981	663	16	< 1	2	0	0	0	0	0	9	0	0
	1990	1216	76	11	6	17	0	6	0	0	76	46	9

* DR, drug-resistant (= resistant to one or more antimicrobial).
† MR, multiply-resistant (= resistant to four or more antimicrobials).
‡ 17794 of 18840 strains referred were tested.

Table 2. *Resistance to individual antimicrobial drugs in* S. typhimurium, S enteritidis *and* S. virchow *isolated from humans and food animals in England and Wales in 1981 and 1990 (% resistant)*

	S. typhimurium								*S. enteritidis*			*S. virchow*		
	Humans		Bovines		Porcines		Poultry		Humans		Poultry	Humans		Poultry
Antimicrobial Drug	1981 (3932)*	1990 (5451)	1981 (1157)	1990 (1178)	1981 (49)	1990 (144)	1981 (117)	1990 (1187)	1981 (1087)	1990 (17794)	1990 (3382)	1981 (663)	1990 (1216)	1990 (76)
Ampicillin	5	17	13	66	22	19	< 1	6	1	4	6	1	4	0
Chloramphenicol	5	6	15	45	22	3	< 1	2	< 1	< 1	0	< 1	5	1
Gentamicin	1	1	0	6	0	4	0	< 1	0	< 1	0	< 1	5	0
Kanamycin	6	2	14	2	24	7	< 1	< 1	1	< 1	< 1	< 1	4	1
Streptomycin	16	20	23	66	33	28	13	30	2	3	9	3	8	9
Sulphonamides	30	40	65	75	57	65	32	34	2	2	5	6	13	32
Tetracyclines	22	45	62	76	53	63	12	34	1	2	8	1	10	1
Trimethoprim	8	21	8	53	35	35	0	7	< 1	1	4	0	12	32
Furazolidone	1	2	2	1	0	10	0	2	13	6	3	9	71	24
Nalidixic acid	< 0·1	0·2	< 0·1	0	0	0·7	0	< 0·1	0	0·4	0·2	0	3	0

* Numbers of strains tested.

This low number contrasts with 1990 when 3832 isolates from poultry but only 74 from cattle and 9 from pigs were received. Because of the dichotomy in numbers of isolates received in the respective years, assessment of trends has not been attempted. However, it is noted that of the isolates of *S. enteritidis* studied in 1990, only 5% from cattle and 14% from poultry were drug-resistant and multiple resistance was extremely rare.

S. virchow

For *S. virchow* from humans, both resistance and multiple resistance have shown substantial increases since 1981. From 1981 to 1988 resistance in this serotype increased approximately fourfold, from 16 to 58% and multiple resistance, from 0·2 to 10% [1]. Over the next 2 years resistance has continued to increase and in 1990, 76% of isolates were drug-resistant. In contrast multiple resistance has increased by only 1% since 1988 and in 1990, 11% of isolates were multiresistant. In both 1981 and 1990, isolations of *S. virchow* from cattle and pigs were insignificant in number but in 1990 a substantial number from poultry were received, of which about half were drug-resistant and 10% multiresistant. This contrasts with 1981 when none of the food animal isolates of this serotype was drug-resistant.

Resistance to individual drugs

The incidence of resistance to individual antimicrobial drugs is shown in Table 2.

S. typhimurium

For human isolates of *S. typhimurium*, the most significant increases from 1981 to 1988 were for ampicillin (5 to 12%) and trimethoprim (8 to 11%). In contrast, resistance to kanamycin declined from 6 to 2% [1]. Over the following 2 years the incidence of kanamycin resistance did not change but substantial increases in resistance to ampicillin and trimethoprim, and also to streptomycin, sulphonamides and tetracyclines were observed (Table 2). In contrast, since 1981 the incidence of strains with resistance to chloramphenicol, gentamicin, furazolidone and nalidixic acid has changed little.

In isolates from cattle, there have been substantial increases in resistance to most drugs and in particular to ampicillin, chloramphenicol and trimethoprim. It is also noteworthy that in 1981, none of the isolates was resistant to gentamicin whereas 6% of those isolated in 1990 were gentamicin-resistant. Previous studies have demonstrated that the appearance and spread of strains of *S. typhimurium* PT 204c with resistance to apramycin and gentamicin, mediated by the plasmid-encoded production of the enzyme AAC(3)IV, was responsible for the upsurge in resistance to gentamicin [7]. As in human isolates of *S. typhimurium*, resistance to kanamycin has declined. Of particular note in strains isolated in 1990 is the absence of resistance to furazolidone and the low incidence of resistance to nalidixic acid.

In porcine isolates there have been small increases in resistance to sulphonamides and tetracyclines but a substantial increase in furazolidone resistance. Resistance to ampicillin, streptomycin and trimethoprim has remained essentially

unchanged but like bovine isolates of this serotype, resistance to gentamicin has appeared and kanamycin resistance declined.

In poultry isolates there have been small increases in chloramphenicol, streptomycin and furazolidone resistance and a more noticeable increase in strains resistant to ampicillin, tetracyclines and trimethoprim.

S. enteritidis

For human isolates, the only resistance to increase substantially in incidence since 1981 has been to ampicillin. However, although ampicillin resistance increased from 1 to 10% between 1981 and 1988 [1], by 1990 only 4% of isolates were resistant to this antimicrobial. In contrast the incidence of resistance to high-level furazolidone (MIC: > 20 mg/l) fell from 13 to 2% in 1988 [1] but has subsequently doubled, to 4%, in 1990.

Of isolates made from poultry in 1990, between 3 and 10% were resistant to ampicillin, streptomycin, sulphonamides, tetracyclines, trimethoprim or furazolidone but the incidence of resistance to nalidixic acid has remained at less than 1%.

S. virchow

For *S. virchow* from humans, over the 8-year period 1981–8 there were substantial increases in the incidence of resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, sulphonamides, tetracyclines, trimethoprim and furazolidone [1]. Over the next 2 years, with the exception of furazolidone resistance, the occurrence of resistance to these drugs has altered little. In contrast, resistance to furazolidone has increased by 16%, from 55% in 1988 to 71% in 1990.

A substantial proportion of poultry isolates made in 1990 were resistant to sulphonamides, trimethoprim or furazolidone. However, in contrast to isolates from humans, resistance to tetracyclines was only rarely encountered in poultry isolates and none was resistant to ampicillin.

Patterns of multiple drug resistance and phage types

S. typhimurium

In *S. typhimurium* from humans, from 1981 to 1988 multiple resistance more than doubled, from 5 to 12% and over the next 2 years has increased by a further 7%, to 19%. In 1981 the most common patterns were ACKSSuTTm and ACGKSSuTTm (Table 3) and the majority of strains with these R-types belonged to PT 204c. In both 1988 and 1990 the most common patterns were ASSuT and ACSSuTTm. In both these years the majority of strains of R-type ASSuT belonged to PT 193 and those of R-type ACSSuTTm, to PT 204c.

In bovine and porcine isolates of *S. typhimurium* made in 1981, multiple resistance was almost exclusively restricted to strains of PT 204c. However, in 1990 such resistance had also appeared in PT 193 and the majority of strains of R-type ASSuT from both cattle and pigs belonged to this phage type. PTs 204c and 193 were also identified amongst the few multiresistant strains of *S. typhimurium* from poultry, although in 1990 the majority of *S. typhimurium* of R-type SSuTTm from poultry belonged to PT 8.

Table 3. *Pat of multiple drug resistance in* S. typhimurium, S. enteritidis *and* S. virchow *isolated from hu and food animals in 1981 and 1990*

		1981							
Se	pe	Human		Bovines		Porcines		Poultry	
S.	*imurium*	ACKSSuTTm*	(4)†	CSSuTTm	(10)	ACKSSuTTm	(11)	CSSuT	(1)
		ACGKSSuTTm	(2)	ACKSSuTTm	(9)			KSSuT	(1)
		ASSuT	(1)						
S.	*itidis*	ACKSSuT	(2)	‡		—		—	
		SSuTFu	(1)						
S.	*ow*	CKTFu	(1)	—		—		—	

		1990							
Se	pe	Human		Bovines		Porcines		Poultry	
S.	*imurium*	ASSuT	(8)	ACSSuTTm	(39)	ASSuT	(12)	SSuTTm	(2)
		ACSSuTTm	(2)	ACSSuTTm	(13)	SSuTTmFu	(4)	ASSuT	(1)
				ACGSSuTTm	(5)			ACSSuTTm	(1)
S.	*itidis*	CSSuTTm	(0·2)	—		—		SSuTTm	(3)
		ASTFu	(0·1)	—		—		ASSuTTm	(0·2)
S.	*ow*	ASSuTTmFu	(1)	—		—		SSuTTm	(8)
		CKSSuTTmFu	(1)	—		—		CKSSuTTmFu	(2)
		ACSSuTTmFu	(1)						
		CSSuTTmFu	(1)						

* Drug resistanc bols: A, ampicillin; C, chloramphenicol; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulphonamides etracyclines; Tm, trimethoprir , furazolidone.

† Percentage of i solates of each serotype for the respective years.

‡ —, no multires : isolates.

S. enteritidis

Multiple resistance has remained uncommon amongst human isolates of *S. enteritidis*. In 1988 the most common pattern was that of ASTFu and strains with this R-type comprised 0·8% of isolates [1]. Strains of R-type ASTFu belonged to *S. enteritidis* PT 24, a phage type derived from *S. enteritidis* PT 4 following the acquisition of a drug resistance plasmid coding for AST [8]. In 1990 the occurrence of strains of R-type ASTFu was reduced to 0·1% (Table 3) and the most common pattern in the few multiresistant strains was CSSuTTm. Strains of R-type CSSuTTm belonged to *S. enteritidis* PT 4 and were associated with an outbreak in a sandwich bar in the City of London [9].

In *S. enteritidis* from food animals, multiple drug resistance was extremely rare and was identified only in a small number of isolates made from poultry in 1990. The most common R-type was SSuTTm and the majority of strains with this R-type belonged to *S. enteritidis* PT 5A and had been isolated from ducks.

S. virchow

In *S. virchow* from humans, the incidence of multiple resistance has increased dramatically since 1980. In 1988 the most common patterns were CSTTmFu and CSSuTTmFU [1] and in 1990, ASSuTTmFu, CKSSuTTmFu, ACSSuTTmFu and CSSuTTmFu. The phage type in which multiple resistance was most common in both 1988 and 1990 was PT 19, a phage type frequently associated with poultry meat imported from France [1].

DISCUSSION

These findings demonstrate that since 1981 the incidence of multiple resistance has trebled in human isolates of *S. typhimurium* and has also increased significantly in *S. virchow*. For *S. typhimurium*, multiple resistance was common in bovine and porcine-associated phage types such as PTs 204c and 193 (bovines) and PT 193 (porcines) but was rare in those associated with poultry. Furthermore, in the 2 year period 1988–90, the overall incidence of resistance in these two serotypes has also increased significantly, and in *S. typhimurium*, multiple resistance has also shown a substantial increase.

To a large extent the findings with human isolates of this serotype have paralleled those in food animals. Since 1981 multiple resistance has quadrupled in *S. typhimurium* from cattle and was the norm in strains isolated in 1990. Likewise, since 1981 multiple resistance has almost doubled in isolates from pigs and in 1990 the predominant phage type in which such resistance was encountered was PT 193. These findings are likely to reflect the continuing use of a wide range of antimicrobials in animal husbandry. In contrast, although multiple resistance has increased in poultry isolates of this serotype, the overall incidence has remained at less than 10%. It is however noteworthy that the phage types in which multiple resistance has been identified in poultry are those which are common in bovines.

Both resistance and multiple resistance has remained uncommon in *S. enteritidis* from both humans and food animals. The main reservoir of this serotype is poultry [10] and since 1988 *S. enteritidis* PT 4 has become widely disseminated in poultry

flocks in England and Wales [11]. However it is noteworthy that 6% of human and 3% of poultry isolates made in 1990 were resistant to furazolidone and it is possible that compounds containing this antimicrobial have been increasingly used in poultry in attempts to control infections with *S. enteritidis* PT 4. Multiple resistance was encountered in a few strains of *S. enteritidis* PT 5A from ducks, but we do not have any information about the use of antimicrobials in this food animal.

Like *S. enteritidis*, the main food animal reservoir of *S. virchow* is poultry [10]. The observation that 74% of human and 24% of poultry isolates of this serotype received in 1990 were resistant to furazolidone supports the hypothesis that nitrofurans have been increasingly used in poultry in recent years. The majority of isolates of this serotype with multiple resistance from both humans and poultry meat were found to belong to PT 19 and were frequently associated with poultry meat imported from France [1]. In contrast, multiple resistance was uncommon in *S. virchow* from home-produced poultry.

The results presented above demonstrate that since 1981 there has been a significant increase in multiple resistance in strains of *S. typhimurium*, particularly from bovine animals. The intensive use of a wide range of antimicrobials in calves may well have been an important factor in the increase. Although the use of such antimicrobials in poultry is not as intensive as in cattle, our results demonstrate that strains of *S. enteritidis* and *S. virchow* with high-level resistance to furazolidone are increasing in incidence.

In view of the increasing occurrence of multiple antibiotic resistance in salmonella associated with food animals, it has been reported [12] that the Veterinary Products Committee (VPC) consider that the prophylactic use of antibiotics with cross-resistance to those used in human medicine should be strongly discouraged. It has also been recommended that not only should antibiotics giving cross-resistance to those used in human medicine not be used as growth promotors but also that their prophylactic use in animals should be reconsidered [12]. Although antimicrobials are not recommended for the treatment of uncomplicated salmonellosis in humans, they are essential for the treatment of salmonella septicaemia and focal lesions outside the bowel [13, 14]. In this respect the increasing occurrence in food animals of *S. typhimurium* and *S. virchow* with multiple resistance must be viewed with concern. It is hoped that implementation of the above recommendations regarding the use of antibiotics in food animals will result in a reduction in incidence of such strains in these animals and subsequently in humans.

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