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Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections

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Abstract

Strains of *Salmonella* spp. with resistance to antimicrobial drugs are now widespread in both developed and developing countries. In developed countries it is now increasingly accepted that for the most part such strains are zoonotic in origin and acquire their resistance in the food-animal host before onward transmission to humans through the food chain. Of particular importance since the early 1990s has been a multiresistant strain of *Salmonella typhimurium* definitive phage type (DT) 104, displaying resistance to up to six commonly used antimicrobials, with about 15% of isolates also exhibiting decreased susceptibility to ciprofloxacin. Mutations in the *gyrA* gene in such isolates have been characterised by a PCR LightCycler-based *gyrA* mutation assay, and at least four different mutations have been identified. Multiple resistance (to four or more antimicrobials) is also common in the poultry-associated pathogens *Salmonella virchow* and *Salmonella hadar*, with an increasing number of strains of these serotypes exhibiting decreased susceptibility to ciprofloxacin. Multiple resistance is also being found in other serotypes in several other European countries, and has been associated with treatment failures. For *Salmonella typhi*, multiple drug resistance is now the norm in strains originating in the Indian subcontinent and south–east Asia. Such multiresistant strains have been responsible for several epidemics and some of these have been associated with contaminated water supplies. Furthermore, an increasing number of multiresistant strains of *S. typhi* are now exhibiting decreased susceptibility to ciprofloxacin, with concomitant treatment failures. In developed countries antimicrobial resistance in zoonotic salmonellas has been attributed to the injudicious use of antimicrobials in food-producing animals. It is hoped that the application of Codes of Practice for the use of such agents, which have been prepared by the pharmaceutical industry in response to widespread international concern about the development of drug resistance in bacterial pathogens, will now result in a widespread reduction in the incidence of drug-resistant salmonellas in food production animals and humans on an international scale.

Keywords: Antibiotics resistance; *Salmonella*

Contents

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1. Introduction

In developed countries antimicrobial drug resistance in non-typhoidal salmonella organisms is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals. Such drugs may be used either therapeutically or prophylactically, or for growth promotion (feed additives). Despite legislation targeted at controlling the overall usage of antimicrobials in food-producing animals, in recent years there have been significant increases in developed countries in the occurrence of resistance in non-typhoidal *Salmonella* spp. Such increases have been observed in many countries, not only within Europe but also North America. Of particular concern in such organisms is the development of resistance to key antimicrobials such as the fluoroquinolones [1,2] and, more recently, extended-spectrum β-lactamases [3–5].

There have also been increases in the occurrence of resistance in both non-typhoidal and typhoidal *Salmonella* spp. in developing countries. In contrast to the situation in developed countries such increases have been almost entirely associated with the use of antimicrobials in human medicine, both in hospitals and the community. Examples of increases in resistance in non-typhoidal salmonellas in developing countries, particularly in the Indian subcontinent, south–east Asia, South and Central America and Africa, are exemplified by outbreaks caused by organisms such as *Salmonella wien*, *Salmonella typhimurium*, *Salmonella johannesburg* and *Salmonella oranienburg*, all of which have caused numerous outbreaks of serious disease both in hospitals and the community over wide geographical areas [6]. These serotypes have undergone changes both in their epidemiology and their clinical disease.

The most important serotypes in the UK, Europe and the USA are *Salmonella enteritidis*, *S. typhimurium*, *Salmonella virchow* and *Salmonella hadar*, and the main method of spread is through contaminated food. *S. enteritidis*, *S. virchow* and *S. hadar* are normally associated with poultry and poultry products. In particular *S. enteritidis* and *S. virchow* are associated with chickens and their products, whereas *S. hadar* is turkey-related. In contrast, *S. typhimurium* is a serotype with a more ubiquitous host range, for the most part associated with cattle and pigs but also with poultry and, occasionally, sheep. In general, in developed countries person-to-person transmission is not of major importance in the spread of these serotypes, although some institutional outbreaks have been reported. In most cases the clinical presentation is that of mild to moderate enteritis, the disease is usually self-limiting and antimicrobial therapy is not indicated. In contrast, in developing countries infections caused by strains of the same or similar serotypes are associated with a high incidence of invasive illness, often resulting in septicaemia, with consequent high mortality. An additional feature of these strains has been the possession of plasmid-mediated multiple drug resistance, often with resistance to seven or more antimicrobials. Thus the epidemiology of these strains is also quite different from that of strains of the same serotypes in developed countries. There is no evidence of food animal involvement, either in the spread of the strains, or in the acquisition of drug resistance by such organisms.

For typhoidal organisms, since the late 1980s there have been numerous outbreaks of *Salmonella typhi* with resistance to several key therapeutic antimicrobials in many countries in the Indian subcontinent and south–east Asia [7]. The outbreaks involved are often associated with contaminated water although person-to-person spread is also an important factor. Such strains have also been isolated with increasing frequency in developed countries, particularly from patients with a recent history of return from countries where such strains are endemic [8]. Decreased susceptibility to fluoroquinolone antimicrobials is also becoming increasingly common in isolations of *S. typhi* from patients in the Indian subcontinent and south–east Asia [9,10] and has also been reported in *Salmonella paratyphi* A [11]. As with non-typhoidal salmonellas in developing countries, the strains are frequently transmitted by means of contaminated water supply or by person-to-person spread and there is no evidence of food animal involvement in the acquisition of resistance.

This review discusses resistance to antimicrobial drugs and the implications of such resistance in *Salmonella* spp. which are transmitted to humans by the ingestion of contaminated food or water, with particular emphasis on strains which originate in food-producing animals.

2. Developed countries – non-typhoidal salmonellas

2.1. Historical perspective

Zoonotic pathogenic bacteria with resistance to commonly used antimicrobial agents are now widespread in many developed countries. The problem was first recognised in the UK in *S. typhimurium* in the 1960s. To appreciate the many investigations which have revealed the role of antimicrobials in the development of resistance in *S. typhimurium*, it is important to have an understanding of the methods used for the epidemiological subdivision of this organism. The primary phenotypic method for the subdivision of *S. typhimurium* is 'phage typing', using a method based on that first described by Callow in 1953 [12]. On the basis of this scheme over 200 'definitive phage types' (DTs) of *S. typhimurium* have been defined [13].

Antimicrobial drug resistance and particularly multiple resistance (to four or more drugs) became common in *S. typhimurium* in the mid-1960s but increased dramatically in the 10-year period 1990–1999. Multiple resistance in *S. typhimurium* was first identified in the UK in 1964. In the following 5 years a multiresistant (MR) strain of *S. typhimurium* DT 29 with resistance to ampicillin (A), streptomycin (S), sulfonamides (Su), tetracyclines (T) and

furazolidone (Fu) (= R-type ASSuTFu) caused numerous infections in cattle, particularly in calves, and also in humans [14]. There were several deaths, particularly in vulnerable age groups. As a result of this epidemic, and following widespread concern about the use of antimicrobials as growth promoters (feed additives) in livestock, in 1969 the Swann Committee (The Joint Committee on the use of antibiotics in animal husbandry and veterinary medicine) [15] recommended that feed antibiotics should be available only on the same terms as a scheduled antibiotic and should not be used for growth promotion. Appropriate legislation followed and by 1971 MR *S. typhimurium* DT 29 had disappeared from bovine animals and humans in the UK [6].

Over the next 6 years multiple antibiotic resistance was uncommon in salmonellas in Britain, with only about 3% of strains from humans multiresistant. However, from 1975 to the mid-1980s there was again a substantial upsurge in the incidence of MR *S. typhimurium* from food production animals, particularly cattle, and a concomitant increase in multiresistant isolates from humans. The phage types involved were different from those observed in the 1960s, with the related *S. typhimurium* phage types 204, 193 and 204c predominating [6]. A feature of this outbreak was the sequential acquisition of plasmids and transposons coding for drug resistance to a wide range of antimicrobials: ampicillin, chloramphenicol (C), gentamicin (G), kanamycin (K), streptomycin, sulfonamides, tetracyclines and trimethoprim (Tm) (= R-type ACGKSSuTTm) [6]. The acquisition of antimicrobial drug resistance by these phage types followed the introduction and use, in calf husbandry, of at least some of the antimicrobials or of veterinary analogues with cross-resistance to antibiotics used in human medicine, in calf husbandry. Of particular note was the appearance of resistance to gentamicin, at that time a drug reserved for the treatment of serious invasive disease in humans. This followed the introduction and use of the related aminoglycoside antimicrobial apramycin in attempts to combat infections with strains of *S. typhimurium* DT 204c of R-type ACKSSuTTm [16]. Strains of *S. typhimurium* phage types 204, 193 and 204c became epidemic in calves and humans in the UK, and following the export of infected livestock to several European countries, also caused infections in humans and cattle in Germany, Belgium, France and Italy [17].

Isolations of *S. typhimurium* DT 204 and related strains became progressively less common in cattle and humans in the period 1986–1990. However, from 1991 to 1994 there was a further substantial increase in the incidence of multiple drug resistance in *S. typhimurium* and by 1994 62% of isolates were multiresistant [18]. An important factor in this increase was the epidemic spread, from 1990 to 1995, of a strain of *S. typhimurium* DT 104 with resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines (R-type ACSSuT) [19]. In the early 1990s this MR strain was particularly common in bovine animals and caused infections in cattle in many areas, not only in England and Wales but also in Scotland and the Republic of Ireland. An unusual feature of the strain was that resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines was chromosomally encoded [19]. Also of note in 1994 was a significant increase in both resistance and multiple drug resistance in the poultry-associated serotypes *S. virchow* and *S. hadar*, with a substantial proportion of isolates also exhibiting decreased sensitivity to ciprofloxacin with minimal inhibitory concentrations (MICs) ranging from 0.25 to 1.0 mg l^{-1}) [2]. In contrast, multiple drug resistance in *S. enteritidis* was extremely rare.

2.2. Multiresistant S. typhimurium DT 104 – epidemiological and genetical considerations

MR *S. typhimurium* DT 104 of R-type ACSSuT was first identified in the UK in the early 1980s in isolates of this serotype from exotic birds. With the exception of a small outbreak in humans in Scotland in the mid 1980s there were no isolations in humans until 1989, by which time *S. typhimurium* DT 104 of R-type ACSSuT had started to become distributed in cattle in the UK. Over the next 5 years the strain became epidemic in bovine animals but in contrast to the epidemic multiresistant *S. typhimurium* DTs 29, 204, 193 and 204c (see above), the strain also became common in poultry, particularly turkeys, and also in pigs and sheep [20]. Human infection with MR *S. typhimurium* DT 104 has been associated with the consumption of chicken, beef, pork sausages, and meat paste, and to a lesser extent with contact with food animals. In 1996 infections with *S. typhimurium* DT 104 were recognised in cattle and humans in the USA and over the succeeding 4 years this multiresistant clone has caused infections in food animals and humans in numerous European countries and also in Israel, Canada, Turkey and Japan [21]. In the USA outbreaks of MR *S. typhimurium* DT 104 related to unpasteurised cheese have been responsible for many infections, particularly in states in the south–west of the country [22,23].

Of particular concern has been not only the resistance of *S. typhimurium* DT 104 to a wide range of therapeutic antimicrobials, but also an apparent predilection of the organism to cause serious disease [24,25]. This has been particularly evident in the USA, where over 15% of human isolations of MR *S. typhimurium* DT 104 have been reported to be associated with cases of septicaemia [25]. However, a study made in the UK in 1998 demonstrated that in England and Wales MR *S. typhimurium* DT 104 appeared no more invasive than other common salmonella serotypes and phage types [26].

Preliminary genetic and molecular studies suggested that in *S. typhimurium* DT 104 of R-type ACSSuT the complete spectrum of resistance was chromosomally encoded [19]. This was later confirmed by examining by PCR a

selection of strains from humans and food animals, isolated in several different countries over a 10-year period, for the presence of integrons [27]. Integron hot spots in two copies were observed in all strains conferring resistance to ACSSuT, and were determined by two discrete bands of approximately 1.0 and 1.2 kb. Direct nucleotide sequencing of the individual amplicons indicated that the potential 1.0-kb gene product was ANT (3″)-1a, responsible for resistance to streptomycin (and also to spectinomycin – Sp); the 1.2-kb amplicon contained the gene *bla* P1, encoding the β-lactamase CARB-2 (PSE-1) [27,28]. It was noteworthy that all isolates of *S. typhimurium* DT 104 of the ACSSuT phenotype contained the same gene cassettes irrespective of source (food animal or human), or country of origin [27]. An indistinguishable β-lactamase has also been identified in France in strains designated as '12 atypic', which probably corresponds to MR *S. typhimurium* DT 104 [29]. Subsequent studies have demonstrated that the genes for chloramphenicol and tetracycline resistance are grouped within the two integrons described above [30]. The overall gene complex in MR *S. typhimurium* DT 104 is therefore comprised of a sequence of approximately 14 kb, containing two integrons (A, SSp) and intervening plasmid-derived sequences coding for resistance to chloramphenicol and tetracyclines (for figure showing the arrangement of resistance genes in MR *S. typhimurium* DT 104, see Briggs and Fratamico [30]).

Since 1992 a disturbing feature of MR *S. typhimurium* DT 104 has been the acquisition of additional resistance to trimethoprim and decreased susceptibility to ciprofloxacin such that in 1997 15% of isolates were additionally resistant to trimethoprim (R-type ACSSuTTm) and 13% showed decreased sensitivity to ciprofloxacin (MIC: 0.5–1.0 mg l^{-1}) (= R-type ACSSuTCp_L (Cp, ciprofloxacin) (Table 1) [31]. It has been suggested that the appearance of resistance to trimethoprim may have resulted from the use of trimethoprim-containing compounds in cattle in attempts to combat infection with *S. typhimurium* DT 104 of R-type ACSSuT [31]. The emergence and spread of isolates of multiresistant *S. typhimurium* DT 104 with reduced sensitivity to ciprofloxacin followed the licensing for veterinary use in the UK in November 1993 of the related fluoroquinolone drug enrofloxacin. This antimicrobial was subsequently used for treatment and prophylaxis in both cattle and poultry in the UK. A possible consequence of this has been the rapid development of resistance to nalidixic acid in strains of *S. typhimurium* DT 104 in food-producing animals in the UK, particularly turkeys but also in chickens and cattle [32]. For humans, the clinical significance of decreased sensitivity to ciprofloxacin is controversial. However, a report from Denmark [33], describing a lack of clinical response to fluoroquinolone antibiotics in patients infected in 1998 with MR *S. typhimurium* DT 104 with resistance to nalidixic acid, in an outbreak associated with pork of Danish origin, has demonstrated the importance of such resistance in vulnerable patients.

2.3. Genetic basis of decreased susceptibility to fluoroquinolones in S. typhimurium DT 104

In isolations with decreased susceptibility to ciprofloxacin this property is chromosomally encoded. Amplification and sequencing of the 120-bp quinolone resistance determining region (QRDR) in a panel of MR *S. typhimurium* DT 104 of R-type ACSSuTCp identified two discrete base substitutions at codon aspartate (Asp)-87 and further point mutations at codons serine (Ser)-83 and alanine (Ala)-119 (just outside the QRDR), all giving rise to decreased susceptibility to ciprofloxacin [27]. The most common mutation in Asp-87 involved the change from **G**AC (aspartate) to **A**AC (asparagine). This mutation was identified in 11 of the 15 strains studied, including four from food production animals. An identical mutation, giving rise to decreased susceptibility to ciprofloxacin, was identified in the strain of MR *S. typhimurium* DT 104 responsible for the outbreak in Denmark described above [33]. The second mutation in codon 87 was from G**A**C to G**G**C

Table 1
Predominant patterns of drug resistance in *S. typhimurium* DT 104 from humans in England and Wales, 1990–2000

Year	Total	Antibiogram (%)			
		ACSSuT	ACSSuTTm	ACSSuTCp_L	ACSSuTTmCp_L
1990	259	39	0	0	0
1991	544	44	0	0	0
1992	808	66	1	0.1	0
1993	1526	79	1	0	0
1994	2873	74	12	1	0
1995	2837	54	27	6	0
1996	4006	59	21	13	1
1997	2956	63	17	12	2
1998	2090	61	13	16	2
1999	1030	69	11	11	1
2000	1168	73	10	9	1

Drug resistance symbols: A, ampicillin; C, chloramphenicol; G, gentamicin; K, kanamycin; S, streptomycin; Su, sulfonamides; T, tetracyclines; Tm, trimethoprim; Cp_L ciprofloxacin (MIC: 0.25–1.0 mg l^{-1}).

(glycine) and this mutation was identified only in strains isolated from humans. The mutation at codon 83 was from TCC (Ser) to TTC (phenylalanine) and at codon 119, from GCA (Ala) to GTA (valine). The mutation at codon 83 was observed in a strain of human origin, and that at codon 119 in a strain of porcine origin. Subsequent analysis of a large number of isolates of MR *S. typhimurium* DT 104 with additional decreased susceptibility to ciprofloxacin, using a LightCycler-based PCR-hybridisation *gyrA* mutation assay (GAMA) [34], has confirmed these observations. This work has also demonstrated that strains of MR *S. typhimurium* DT 104 with different mutations in *gyrA* may have arisen independently, either temporally or in different hosts, against a background of clonal spread of *S. typhimurium* DT 104 of R-type ACSSuT.

2.4. Decreased susceptibility to fluoroquinolone antibiotics in non-typhoidal salmonellas

In 1999 the four most common serotypes from humans in England and Wales were *S. enteritidis*, *S. typhimurium*, *S. virchow* and *S. hadar*, comprising 81% of 17 251 isolates identified [35]. All these serotypes have their primary reservoirs in food animals and the primary method of spread to humans is assumed to be through the food chain. For *S. enteritidis* 8% of isolates made in 1999 exhibited decreased susceptibility to ciprofloxacin (MIC: 0.25–0.5 mg l^{-1} ($=Cp_L$)), which is an 8-fold increase in incidence since 1996. For *S. typhimurium* the occurrence of isolates with such resistance decreased from 12% in 1996 to 8% 1999. For *S. virchow* isolates with decreased susceptibility to ciprofloxacin increased from 11% in 1996 to 39% in 1999, and for *S. hadar*, from 60% to 70%. The increasing occurrence of decreased susceptibility to ciprofloxacin in *S. virchow* is particularly concerning because of the propensity of this serotype to cause invasive disease [36] and because ciprofloxacin is now regarded as the first-line drug of choice for such infections.

It has been suggested that the increasing incidence of decreased susceptibility to ciprofloxacin in zoonotic salmonellas such as *S. enteritidis*, *S. virchow* and *S. hadar* in England and Wales may be a consequence of the use of fluoroquinolone antibiotics in poultry since 1993. However, the situation is complex. Like *S. typhimurium*, *S. enteritidis* is also subdivided by phage typing [37] and over 60 'phage types' of this serotype are now recognised. In certain phage types of *S. enteritidis*, for example, phage type (PT) 1, in which the majority of isolates have exhibited decreased susceptibility to ciprofloxacin, there is a strong association between infections in humans and foreign travel [35]. In contrast, in other *S. enteritidis* phage types, which are not necessarily associated with foreign travel, e.g., PT 4, decreased susceptibility to fluoroquinolone antimicrobials is rare [35].

Fluoroquinolone antibiotics have also been used therapeutically in cattle in the UK, and in an extensive milkborne outbreak of MR *S. typhimurium* DT 104 with decreased susceptibility to ciprofloxacin, which occurred in the summer of 1998, the use of the related antimicrobial marbofloxacin may have enhanced the persistence and dissemination and of strains with decreased susceptibility to ciprofloxacin [38]. In this outbreak the use of the LightCycler-based GAMA technology was crucial in demonstrating a link between isolates from cattle, milk and humans.

2.5. Multiple drug resistance in other salmonella serotypes and phage types

In 1999 the incidence of multiple drug resistance in non-typhoidal salmonellas from humans in England and Wales fell in isolations of *S. typhimurium*, *S. virchow* and *S. hadar*, which represent three of the four most common serotypes from cases of salmonella infections in humans. This fall was most noticeable in *S. typhimurium*, where 59% of isolates were multiresistant compared to 81% in 1996. In *S. hadar* 49% of isolates were multiresistant compared to 56% in 1996 and in *S. virchow* the corresponding figures were 14% compared to 19% 3 years earlier [35]. Unfortunately this reduction in multiple resistance has not been maintained and in 2000 67% of *S. typhimurium* and 49% of *S. virchow* were multiresistant (Table 2). Throughout this period multiple drug resistance in *S. enteritidis* has remained very low, with an overall incidence of less than 1%.

Table 2
Incidence of multiple drug resistance in non-typhoidal salmonellas from humans in England and Wales, 1996–2000

Serotype	1996		1999		2000[a]	
	Total	% MR	Total	% MR	Total	% MR
S. enteritidis	18 968	0.4	10 596	0.6	8 468	2
S. typhimurium	5 849	81	2 402	59	2 651	67
S. virchow	1 260	19	524	14	309	49
S. hadar	633	56	519	49	358	36
Other serotypes	3 365	9	3 210	9	2 172	12
	(250 serotypes)		(256 serotypes)		(248 serotypes)	

MR, resistant to four or more antimicrobials.
[a]Provisional figures.

Since 1998 a small number of strains of *S. typhimurium* from humans in England and Wales have exhibited resistance to the third generation cephalosporin ceftriaxone; the majority of such isolates were also resistant to ciprofloxacin [35]. Although only small, the number of ceftriaxone-resistant isolates has doubled since 1998 [39]. In almost all cases the patients from whom the strains had been isolated gave a history of recent return from the Indian subcontinent. It is highly unlikely that these strains were zoonotic in origin, and the use of ceftriaxone in human medicine in developing countries was probably a major factor in the development of resistance. This should be taken into context in a broader sense, since resistance to third generation cephalosporins in *Salmonella* spp. is now being reported in several different countries [3–5] and in the USA has been associated with an outbreak in cattle and humans [5]. This is particularly concerning as third generation cephalosporins such as ceftriaxone are now the drugs of choice in invasive infections caused by strains with resistance to ciprofloxacin.

2.6. Recent developments

Multiple resistance in salmonellas in developed countries is not of necessity confined to *S. typhimurium*, *S. hadar* and *S. virchow*. In Spain emergent multiresistant strains of *Salmonella* spp. with the antigenic structure 4,5,12:i:−,− that is lacking the flagellar 'H' antigen and therefore not classifiable under the Kauffmann–White scheme [40], have been associated with an increasing number of human infections since the mid-1990s [41]. These strains have been referred to as *Salmonella enterica* serotype [4,5,12:i:−] [40]. Although lacking the 'H' antigen these strains react with the *S. typhimurium* typing phages, and on the basis of their phage typing reactions have been designated 'U302' (L.R. Ward, unpublished observations). Such strains of phage type U302 have also caused infections in humans in the UK [42] and Denmark (P. Gerner-Smidt, personal communication). Similarly in Greece multiresistant strains of *Salmonella blockley* have caused numerous infections in humans since 1996 [43]. It should be emphasised that although the most common presentation has been gastroenteritis, in infections caused by both *S. enterica* serotype [4,5,12:i:−] and *S. blockley*, some patients have not responded to antimicrobial treatment, possibly as a consequence of the wide resistance spectrum of the organisms concerned.

Also of concern in recent years has been an increasing association of multiresistant strains of *S. typhimurium* with salad products. In the summer of 2000 there was a national outbreak of *S. typhimurium* DT 104 of R-type ACSSuT in England and Wales epidemiologically associated with the consumption of lettuce [44]. The outbreak was mainly focussed in the West Midlands area of England and over 300 infections were recognised. There was one fatality. Similarly from July to October 2000, patients in five European countries (England, Scotland, Germany, the Netherlands, Iceland) were infected with a strain of *S. typhimurium* DT 204b resistant to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfonamides, tetracyclines, trimethoprim and nalidixic acid, and with decreased susceptibility to ciprofloxacin (R-type ACGKSSuTTmNxCp$_L$). Over 350 laboratory-confirmed cases were recognised. As a result of epidemiological investigations shredded lettuce was implicated as the vehicle of infection. A key aspect of this investigation was the use of harmonised techniques of phage typing and antibiogram analysis coupled with the rapid exchange of molecular fingerprints between laboratories [45]. Although these organisms have not been directly linked to food animals, it is not unlikely that in both outbreaks the epidemiologically implicated vehicle of infection had been contaminated with animal wastes.

3. *S. typhi* and *S. paratyphi* A

S. typhi remains endemic in developing countries in Africa, South and Central America and the Indian subcontinent with an estimated incidence of 33 million cases each year. In contrast, in developed countries such as the UK or the USA the incidence of *S. typhi* is much lower, and the majority of cases are in travellers returning from endemic areas. For patients with typhoid fever administration of an effective antibiotic is essential. Ideally treatment should commence as soon as clinical diagnosis is made without recourse to the results of antimicrobial sensitivity tests. The development of resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim in epidemic strains in developing countries is therefore of major concern [7]. In all multiresistant strains resistance to chloramphenicol, ampicillin and trimethoprim has been encoded by plasmids of approximately 100 MDa belonging to the H_1 incompatibility group (*inc* H_1) [7].

Major epidemics of multiresistant strains spread by contaminated water have been reported in Tajikistan in 1997 [46] and contaminated ice cream was implicated in a major outbreak of multiresistant *S. typhi* in the Philippines in the early 1990s [47]. In the water-borne outbreak in Tajikistan there were over 6000 recorded cases and an alarming development was the appearance of decreased susceptibility to ciprofloxacin in the epidemic strain [46].

In the UK the occurrence of *S. typhi* with decreased susceptibility to ciprofloxacin has increased to 23% in 1999 [9]. The majority of strains with decreased sensitivity to ciprofloxacin have also been resistant to nalidixic acid (MIC: 512 mg l^{-1}). Furthermore, over 50% of isolates with decreased susceptibility to ciprofloxacin were also resistant to chloramphenicol, ampicillin and trimethoprim. Several patients infected with strains with decreased susceptibility to ciprofloxacin did not respond to treatment with fluoroquinolone antimicrobials. In such cases cef-

triaxone was the most frequently used alternative antimicrobial [48]. The majority of patients infected with such strains had recently returned from India, Pakistan or other countries in the Indian subcontinent.

For *S. paratyphi* A, which in developing countries has an epidemiology similar to that of *S. typhi*, there has been an alarming increase in the occurrence of strains with decreased susceptibility to ciprofloxacin since 1999 [11]. A similar increase has also been observed in isolations of *S. paratyphi* A from travellers returning to the UK [49]. However, it should be emphasised that although an important method of transmission of both *S. typhi* and *S. paratyphi* A in developing countries is by contaminated food or water, the acquisition of resistance by these serotypes is a direct consequence of antimicrobial usage in human medicine.

4. Summary and conclusions

Since 1990 there have been dramatic increases in the occurrence of multiply drug-resistant strains of *Salmonella* spp. in many developed countries. Of particular note has been the epidemic spread of multiresistant strains of *S. typhimurium* DT 104, which now appear to have a worldwide distribution. Within *S. typhimurium* DT 104 the increasing spectrum of resistance is of considerable concern, with strains with decreased susceptibility to ciprofloxacin increasing in incidence in the UK and also causing serious disease in humans in other countries.

Drug resistance in zoonotically transmitted salmonellas is an undesirable but almost inevitable consequence of the use of antimicrobials in food animals. For the most part such use is quite legitimate. However, it is regrettable that recommendations propounded in the UK in 1992 by the Expert Group on Animal Feedingstuffs – the Lamming Committee, that any new antibiotics with cross-resistance to those used in human medicine should not be used for prophylaxis in animal husbandry [50], were not accepted.

Although for some salmonellas, e.g., *S. typhi*, the use of antimicrobials in human medicine is important, it is the use of antimicrobials in food animals which has been a major factor in the development of decreased susceptibility to antibiotics such as ciprofloxacin in zoonotically transmitted salmonellas. To combat the development of resistance in zoonotic salmonellas to such important drugs as the fluoroquinolones it is hoped that such antimicrobials will be used judiciously in food animals and that recently introduced Codes of Practice will be followed. In the UK recommendations targeted at the development of a coherent strategy aimed at reducing the veterinary use of antibiotics were published by the Advisory Committee on the Microbiological Safety of Food (ACMSF) in 1999, in their report on Microbial Antibiotic Resistance in Relation to Food Safety [51]. It is hoped that the ACMSF recommendations will now be adopted and that a real and sustained reduction in the incidence of resistance in zoonotic salmonellas in developed countries will soon follow.

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