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Microbial Drug Resistance: Implications for Human Health*

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Introduction

Drug resistance in food-borne bacterial enteric pathogens is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals. Such drugs may be used either therapeutically, metaphylactically or prophylactically, or for growth promotion (feed additives). Despite legislation targeted at controlling the use of antimicrobials in food-producing animals, in recent years there have been significant increases in developed countries in the occurrence of resistance in *Salmonella* spp. and *Campylobacter* spp., and also to a lesser extent in Vero cytotoxin-producing *Escherichia coli* O157 (VTEC O157). There have also been increases in the occurrence of resistance in *Salmonella* spp. in developing countries, but in contrast to the situation in developed countries these increases have been almost entirely associated with the use of antimicrobials in human medicine. This review discusses resistance to antimicrobial drugs and the implications of such resistance to human health in the following food-borne bacteria: *Salmonella* spp., *Campylobacter* spp., and VTEC O157.

Salmonella spp.

In the UK antibiotic resistance and particularly multiple resistance (to four or more drugs) became widespread in *Salmonella enterica,* particularly *S. typhimurium,* from humans and food animals since the mid 1960s. At that time widespread concern about the use of antimicrobials as feed additives for livestock leading to resistance to key therapeutic drugs for the treatment of invasive salmonellosis in humans resulted in the publication, in 1969, of the Swann Report (Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine) (1). Resultant legislation was targeted specifically at feed antibiotics (growth promoters).

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Over the next six years multiple antibiotic resistance was uncommon in salmonellas in Britain, with only about 3 % of strains from humans multiresistant (2). However, from 1975 to the mid 1980s there was again a substantial upsurge in the incidence of multiresistant *S. typhimurium* from food 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 definitive phage types (DTs) 204, 193 and 204c predominating (3). Strains of these phage types 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 (4). A feature of this outbreak was the sequential acquisition of plasmids and transposons coding for drug resistance to a wide range of antimicrobials – ampicillin (A), chloramphenicol (C), gentamicin (G), kanamycin (K), streptomycin (S), sulphonamides (Su), tetracyclines (T) and trimethoprim (Tm) (= ACGKSSuTTm). The acquisition of 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 (5).

Isolations of DT 204 and related strains became progressively less common in cattle and humans in the period 1986 to 1990. However, from 1991–1996 there has been a further substantial increase in the incidence of multiple drug resistance in *S. typhimurium.* An important factor in this increase was the epidemic spread since 1990 of multiresistant *S. typhimurium* DT104 = MR DT104).

Multiresistant *Salmonella typhimurium* DT104

Multiresistant (MR) *S. typhimurium* DT104 with chromosomally-encoded resistance to ampicillin, chloramphenicol, streptomycin, spectinomycin (Sp), sulphonamides and tetracyclines (=R-type ACSSpSuT) (6) became increasingly common in cases of human infection in England and Wales from 1990 to 1996 (7). Isolations from humans increased from about 200 in 1990 to over 4000 in 1996. However, over the last three years there has been a significant decline in isolations, with numbers dropping by 48 % in 1998, to 2090 and to 1030 in 1989 (table 1).

The first isolations of MR DT104 of R-type ACSSpSuT were made in the early 1980s and were from gulls and exotic birds. There were no isolations from humans in England and Wales until 1989, by which time the strain had begun to be isolated from cattle. Over the next five years MR DT104 became epidemic in cattle throughout the UK (8) and since 1992 has also become common in poultry, pigs and sheep (9). The importance of MR DT104 for humans was demonstrated by a case-control study carried out in the early 1990s, in which 36 % of patients were hospitalised and there were several deaths (10). There have subsequently been several reports of an apparent predilection of the organism to cause serious disease (11), although in the UK MR DT104 appears no more invasive than other common serotypes and phage types in terms of isolations from blood culture in comparison to faecal isolations

Table 1
Predominant patterns of drug resistance in *Salmonella typhimurium* DT104 from humans in England and Wales, 1990–99

Year	*Total*	*Antibiogram*			
		ACSSpSuT	*ACSSpSuTTm*	*ACSSpSuTCp$_L$*	*ACSSpSuTTmCp$_L$*
		(%)			
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

Resistance symbols: A = ampicillin; C = chloramphenicol; S = streptomycin; Sp = spectinomycin; Su = sulphonamides; T = tetracyclines; Tm = trimethoprim; Cp$_L$ = ciprofloxacin (MIC: 0.25–1.0 mg/l)

(12). Over the last four years this particular clone has caused outbreaks of infection in food animals and humans in numerous European countries and in North America, both in Canada and the USA (7).

Between 1996 and 1999 there has been a decline of about 75 % in MR DT104 from cases of human infection (table). The reasons for this are not fully understood but it may be significant that since 1995 there has been a substantial decline in isolations of MR DT104 from cattle in the UK. It is possible that the slaughter of beef cattle of greater than 30 months of age since 1996 (the "over 30 month scheme") in response to the BSE crisis may have contributed to this.

Molecular characteristics of MR DT104

Although some variants have been identified, when studied by plasmid typing and pulsed-field gel electrophoresis the majority of MR DT104 are characterised by a distinctive *Xba*I-generated macrorestriction fingerprint and possess a single plasmid of approximately 60 megadaltons (MDa) which does not code for drug resistance (7). In MR DT104 of R-type ACSSpSuT the complete spectrum of antibiotic resistance is chromosomally-encoded and is comprised of a cassette of approximately 13 kb containing two integrons coding the ASu and SSpSu integrons, and intervening plasmid-derived genes coding for resistance to chloramphenicol and tetracyclines (13, 14).

Development of resistance to trimethoprim and ciprofloxacin

Since 1992 a disturbing feature of infections with MR DT104 has been the appearance of additional resistance to trimethoprim and ciprofloxacin (15). In

1998 13% of MR DT104 in England and Wales were additionally resistant to trimethoprim (Tm) (R-type ACSspSuTTm). A further 16 percent showed decreased susceptibility to ciprofloxacin (Cp), with a minimum inhibitory concentration (MIC) ranging from 0.25–1.0 mg/l (=R-type ACSSpSuTCp_L), and 2% were additionally resistant to both trimethoprim and ciprofloxacin (R-type ACSSpSuTTmCp_L). In isolations of R-type ACSSpSuTTm and ACSSpSuTTmCp_L resistance to trimethoprim was encoded by a plasmid of approximately 4.6 megadaltons, which also coded for resistance to sulphonamides. In contrast, in isolations with decreased susceptibility to ciprofloxacin this property is chromosomally encoded. DNA sequencing of the quinolone resistance determining region (QRDR) in isolates of R-type ACSSpSuTCp_L has 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. The most common mutation in Asp-87 involved change from GAC (aspartate) to AAC (asparagine) (13). An identical mutation giving rise to decreased susceptibility to ciprofloxacin has recently been identified in a strain of MR DT104 responsible for an outbreak in Denmark in the summer of 1998 (11). This outbreak was associated with pork of Danish origin. The second mutation in codon 87 was from GAC to GGC (glycine) and this mutation was identified only in strains isolated from humans (13). The mutation at codon 83 was from TCC (Ser) to TTC (phenyl alanine) and at codon 119, from GCA (Ala) to GTA (valine) (13). The significance of these results is that strains of MR DT 104 with decreased susceptibility to ciprofloxacin are not clonal, and it must be considered that such strains may have arisen independently, either temporally or in different hosts.

More recently the mutation in codon 87 from GAC to GGC has been identified in isolations of MR DT104 from humans, cattle, milk and a milk filter in a substantial outbreak in North-west Lancashire in the summer of 1998 (16). This outbreak was associated with a breakdown in on-farm pasteurisation. It is particularly noteworthy that the fluoroquinolone antibiotic marbofloxacin had been in use on the farm in the months preceding the outbreak, and this may have contributed to the persistence and dissemination of the multiresistant strain in the farm environment.

Other salmonella serotypes

Also of note since 1994 has been 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 with decreased sensitivity to ciprofloxacin (MICs: 0.25–1.0 mg/l) (17). Subsequently the occurrence of isolates, not only of *S. virchow* and *S. hadar* but also of *S. enteritidis* with decreased susceptibility to ciprofloxacin has increased substantially in cases of salmonellosis in humans in England and Wales to such an extent that in 1999 eight percent of *S. enteritidis,* 39 percent of *S. virchow* and 70 percent of *S. hadar* showed such resistance (18). For *S. virchow* the increasing occurrence of decreased susceptibility to ciprofloxacin is of

considerable concern because of the invasive potential of this serotype for humans (19).

Campylobacter spp.

For campylobacters, of 5400 strains of *C. jejuni* and 376 strains of *C. coli* isolated from humans in England and Wales in 1997, 11 % were resistant to ciprofloxacin at >8 mg/l. Ciprofloxacin resistance however was most pronounced in *C. coli*, with 22 % of 376 isolates resistant (20, 21). As yet the epidemiology of campylobacter infection in humans is not as clearly defined as that of salmonellas. However it must be considered that a proportion of ciprofloxacin-resistant isolates originated in food-producing animals. This is substantiated by recent experiences in the United States, when it has been demonstrated that a dramatic increase in the occurrence of ciprofloxacin-resistant isolates in Minnesota has been associated both with foreign travel, particularly to Mexico, and also to the increased occurrence of ciprofloxacin-resistant isolates in poultry following the licensing for use of fluoroquinolone antibiotics in chickens in the USA (22).

Vero cytotoxin-producing *Escherichia coli* O157 (VTEC O157)

For O157 VTEC 23 % of 1087 from humans in England and Wales in 1997 were drug-resistant but only 2 % were multiresistant; the most common resistance patterns were streptomycin, sulphonamides and tetracyclines (73 % of resistant strains), and sulphonamides and tetracyclines (14 %) (23). Thus, although multiple resistance remains rare in VTEC O157, resistance to certain antimicrobials and particularly to sulphonamides and tetracyclines, is increasing in incidence. As yet resistance to ciprofloxacin has not been identified in VTEC O157 from humans in infections associated with foods or food animals. For isolates from animals, the overall incidence of resistance has increased since 1994 but multiple resistance remains rare.

Resistance to fluoroquinolone antibiotics: consequences for humans

The emergence and spread in the UK of MR DT104 with decreased susceptibility to ciprofloxacin followed the licensing for veterinary use of the related fluoroquinolone antibiotic enrofloxacin in November 1993. This antimicrobial has subsequently been used extensively in both cattle and poultry for treatment and prophylaxis (24). A consequence of this has been the rapid development of resistance to quinolone antibiotics in strains of MR DT104 from food-producing animals. For humans, the clinical significance of decreased susceptibility to ciprofloxacin is controversial (25). However, in an outbreak in Denmark in 1998, four of 11 hospitalised patients did not respond to treatment with ciprofloxacin and there were two deaths (11). This clearly demonstrates the clinical consequences of decreased susceptibility to ciprofloxacin in this epidemic multiresistant strain.

For campylobacter infections the use of fluoroquinolone antimicrobials in human medicine has also contributed to the emergence of resistant strains (22). However, subsequent to the emergence of indistinguishable strains of fluoroquinolone-resistant campylobacters in poultry and in human infections in the USA following approval of the use of enrofloxacin in poultry in 1995, it would appear that veterinary usage is also an important factor.

Drug resistance in food-borne pathogens is an unfortunate but almost inevitable consequence of the use of antimicrobials in food animals. Although for some pathogens the use of animicrobials in human medicine is also 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 and *Campylobacter* spp. Such use is quite legitimate. However, it is noteworthy that in October 2000 notification has been received that the Food and Drug Administration (FDA) is proposing to withdraw approval of enrofloxacin for usage in poultry in the USA. It is hoped that such measures, coupled with the introduction by pharmaceutical companies of Codes of Practice for the use of fluoroquinolone antibiotics in food animals, will result in a real and sustained reduction in the incidence of resistance to such antibiotics in pathogens such as MR *S. typhimurium* DT 104, *Campylobacter* spp. and other key zoonotic bacterial pathogens.

Summary

Since 1990 there have been dramatic increase in the occurrence multiply drug-resistant strains of zoonotic pathogens causing infections in humans in many developed countries. Of particular note has been the epidemic spread of MR strains of *S. typhimurium* DT104, which now appear to have an almost worldwide distribution. Within DT104 the increasing spectrum of resistance is of considerable concern, with strains with decreased susceptibility to ciprofloxacin increasing in incidence in the United Kingdom and also causing serious disease in humans in other countries. For campylobacters the incidence of ciprofloxacin-resistant organisms is also increasing, with reports of such isolates from numerous countries throughout the world. For both salmonellas and campylobacters the increasing incidence of strains with decreased susceptibility to ciprofloxacin has been linked to the use of related fluoroquinolne antibiotics in animal husbandry. For VTEC O157, although resistance is increasing, multiple resistance and resistance to ciprofloxacin remains rare. It is hoped that Codes of Practice introduced by pharmaceutical companies and by the veterinary profession coupled with such measures as the proposed withdrawal of fluoroquinolone antibiotics for use in poultry in the USA will result in a rapid decline in the occurrence of quinolone resistance in key zoonotic bacterial pathogens on an international scale.

Zusammenfassung

Seit 1990 steigt die Zahl der antibiotikamultiresistenten Stämme bei pathogenen Bakterien aus Tieren dramatisch an. Diese verursachen in vielen zivilisierten Ländern Infektionen bei Menschen. Besonders hervorzuheben ist hier die epidemische Ausbreitung von multiresistenten Stämmen der Gattung *Salmonella typhimurium* DT104, die, wie es scheint, eine weltweite Ausbreitung erfahren haben. Innerhalb von DT104-Stämmen ist das ansteigende Spektrum der Resistenz mit Besorgnis zu sehen bei den Stämmen, die eine herabgesetzte Empfindlichkeit gegenüber Ciprofloxin haben und somit ein vermehrtes Auftreten von Infektionen in Grossbritannien bringen und auch in anderen Ländern schwere Krankheiten beim Menschen hervorrufen. Bei Campylobacter ist die Inzidenz von ciprofloxinresistenten Isolaten ebenfalls steigend, was weltweit belegt ist durch entsprechende Daten aus vielen Ländern. Für Salmonella und Campylobacter ist die Situation der steigenden Inzidenz und verminderten Empfindlichkeit gegenüber Ciprofloxacin gekoppelt mit der Anwendung von verwandten Fluoroquinolon-Antibiotika in der Tierhaltung. Multiresistenz und auch Resistenz gegen Ciprofloxacin ist bei VTEC O157 selten, obwohl hier die Resistenz im Ansteigen begriffen ist. Man hofft, dass durch «Codes of Practice», die von pharmazeutischen Firmen und auch den Tierärzten eingeführt werden, wie etwa die Verbannung von Fluoroquinolon-Antibiotika bei Geflügel in den USA, eine rasche Abnahme der Quinolonresistenz in den Schlüsselträgern, den tierischen bakteriellen Pathogenen, auf internationaler Ebene erreicht werden kann.

Résumé

Depuis 1990, on a observé dans beaucoup de pays industrialisés une augmentation très nette de la présence de souches multi-résistantes aux antibiotiques chez des bactéries pathogènes communes à l'humain et l'animal. Un exemple particulièrement frappant en la matière fut le développement à un niveau presque mondial de souches multi-résistantes de *S. Typhimurium* DT104. Chez DT104, l'élargissement du spectre de la résistance est particulièrement préoccupant car on voit apparaître des souches possédant une sensibilité réduite à la ciprofloxacine. Ces souches, de plus en plus fréquentes au Royaume-Uni, sont également la cause de maladies humaines graves dans d'autres pays. Egalement pour les campylobacters, l'incidence d'organismes résistant à la ciprofloxacine est en augmentation et on les a signalé dans de nombreux pays du monde. Pour les salmonelles et les campylobacters, l'augmentation des cas de souches présentant une sensibilité réduite à la ciprofloxacine a été mise en relation avec l'utilisation d'antibiotiques de la classe des fluoroquinolones dans les élevages. Néanmoins avec VTEC O157, bien que les phénomènes de résistance augmentent, les cas de résistance multiple et de résistance à la ciprofloxacine restent rares. Il est à espérer que les normes d'application introduites par les compagnies pharmaceutiques et les professions vétérinaires, combinées avec le retrait proposé des antibiotiques du type fluoroquinolones pour les élevages de

volailles aux USA, permettent une diminution globale des cas de résistance aux quinolones chez ces bactéries pathogènes.

Key words

Antimicrobial drug resistance, *Salmonella, Campylobacter*, VTEC O157, Fluoroquinolones

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