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Antibiotic resistance of *Campylobacter* in raw retail chickens and imported chicken portions

I. G. WILSON

Northern Ireland Public Health Laboratory, Bacteriology Department, Belfast City Hospital, Lisburn Road, Belfast, BT9 7AD, UK

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SUMMARY

Campylobacter isolates from raw retail chickens ($n=434$) sampled between 1998 and 2000 were tested for resistance to 12 antibiotics. Among 208 campylobacters tested, more than 90% of isolates were susceptible to 4 out of 9 antibiotics (nalidixic acid, erythromycin, chloramphenicol and gentamicin). Most campylobacters were resistant to 3 antibiotics and multiple resistance was found in 4%. Ciprofloxacin resistance was 11%. *Campylobacter* contamination (28%) in imported chickens ($n=150$) was almost half that found in local whole chickens (50%), but the resistance of imported isolates ($n=42$) was similar to that of local campylobacters. Resistance in isolates from imported chicken breasts was generally more common, but to only 4 antibiotics. Resistance patterns of chicken isolates were compared to human clinical isolates ($n=494$), and a greater similarity was found between the clinical and local isolates than with imported campylobacters. Lower chloramphenicol resistance was found in clinical *Campylobacter* isolates than in those from chicken sources.

INTRODUCTION

Human enteric infections with *Campylobacter* are common and are responsible for considerable morbidity and occasional deaths. A large proportion of these infections has been attributed to chickens [1, 2] which are frequently contaminated with these organisms. *Campylobacter* was detected in 57% of 1127 local chickens examined by us between 1995 and 2000 [3]. Antibiotics are used in medicated animal feedingstuffs for growth promotion and prophylaxis, and their increased use has selected for high level resistance in many enteric pathogens [4] and encouraged the persistence of antibiotic-resistance determinants in microbial genomes [5]. The agricultural misuse of antibiotics increases the hazard of multiple resistance to various antibiotic groups [6]. Macrolides and ciprofloxacin may be used in some cases to treat campylobacter infections, and emerging resistance to these is a concern. Resistance to ciprofloxacin arises from the use of enrofloxacin in poultry rearing. Studies of clinical isolates in England showed that ciprofloxacin resistance had risen from 3% in 1991 to 12–19% in 1997 [7].

It is estimated that 1·3 million chickens are killed each week in Northern Ireland (NI), and 500 tons of chicken imported per week. Smaller producers in NI receive substantial quantities of carcasses and portions from the Republic of Ireland (ROI). Chickens and chicken products from NI are mostly exported. Approximately 5% is consumed in NI with the remainder sold through supermarkets and smaller retailers. England, Scotland and Wales receive 85% of exports much of which is sold through major supermarket chains while 10% is sold in the ROI and Europe (Kirsten Dunbar, Food Standards Agency, NI, personal communication).

Table 1. *Resistance of* Campylobacter *spp. from local chickens and imported chicken portions to antimicrobials*

Sample source	Species	Isolates tested	Number resistant to antimicrobial								
			P	E	CFX	C	G	NA	T	TR	CIP
Local whole chickens ($n=412$)	*C. jejuni*	141	141	7 (2 MR)	135 (1 MR)	11	3	9	27	140 (1 n.e.)†	12 (1 MR)
	C. coli	64	64	4	63 (1 MR)	3	2	7	8	63 (1 n.e.)	6 (2 MR, 1 n.e.)
	C. lari	3	3	0	3	1	0	3	0	3	1
	Number (%) resistant*	208	208 (100%)	13 (6%)	203 (98%)	15 (7%)	5 (2%)	19 (9%)	35 (17%)	206 (100%)	23 (11%)
Imported frozen chicken breasts ($n=150$)	*C. jejuni*	33	33	1	31	7	0	6	9	33	6
	C. coli	9	9	0	9	0	0	0	0	9	0
	C. lari	0									
	Number (%) resistant	42	42 (100%)	1 (2%)	40 (95%)	7 (16%)	0 (0%)	6 (14%)	9 (21%)	42 (100%)	6 (14%)

* Includes moderately resistant (MR) isolates.

† n.e., Not examined.

Abbreviations: P, penicillin; E, erythromycin; CFX, cefalexin; C, chloramphenicol; G, gentamicin; NA, nalidixic acid; T, tetracycline; TR, trimethoprim; CIP, ciprofloxacin.

Heavy pathogen contamination and increased or different antibiotic-resistance patterns have potential clinical and public health implications in countries supplied by NI. The study was undertaken in association with the Port Health Authority to assess whether imported chicken portions presented any increased risk to human health.

MATERIALS AND METHODS

A prospective survey of raw retail chickens was undertaken between 1995 and 2000 [3] in which Environmental Health Officers collected wrapped and unwrapped raw chickens from retail premises. Samples were transported to the laboratory in cool boxes at <5 °C. The methods for bacteriological investigation of the raw retail chickens were as previously described [3]. Between 1998 and 2000, all *Campylobacter* isolates were tested for antibiotic sensitivity. Imported frozen chicken portions ($n=150$) from different bags and generally different shipments were collected. These represented shipments originating in third countries that the Port Health Authority required to be tested. Samples were thawed slowly and cut up for examination. Campylobacter enrichments were performed on 25 g portions, and isolates of *Campylobacter* were identified as previously [3].

The susceptibility to antibiotics of *Campylobacter* isolates ($n=250$) from chickens was compared to human clinical isolates ($n=494$) sent to Belfast City Hospital between 1999 and 2000. Isolates were tested by disc susceptibility on Mueller–Hinton agar containing 6% horse blood by standard methods. Discs containing the following antibiotics were used: penicillin (2 μg), erythromycin (5 μg), cefalexin (30 μg), chloramphenicol (10 μg), gentamicin (10 μg), nalidixic acid (30 μg), tetracycline (10 μg), trimethoprim (2·5 μg), and ciprofloxacin (1 μg). Isolates were classified as susceptible, moderately resistant or resistant according to NCCLS guidelines [8].

RESULTS

Campylobacters were isolated from 208/412 (50%) of local chickens examined and from 42/150 (28%) of imported chickens. *C. jejuni* was recovered from 141/412 (34%) of local chickens and was more than twice as frequent as *C. coli*; only 3 isolates of *C. lari* were recovered. The antimicrobial resistance of the isolates is shown in Table 1. All isolates were resistant

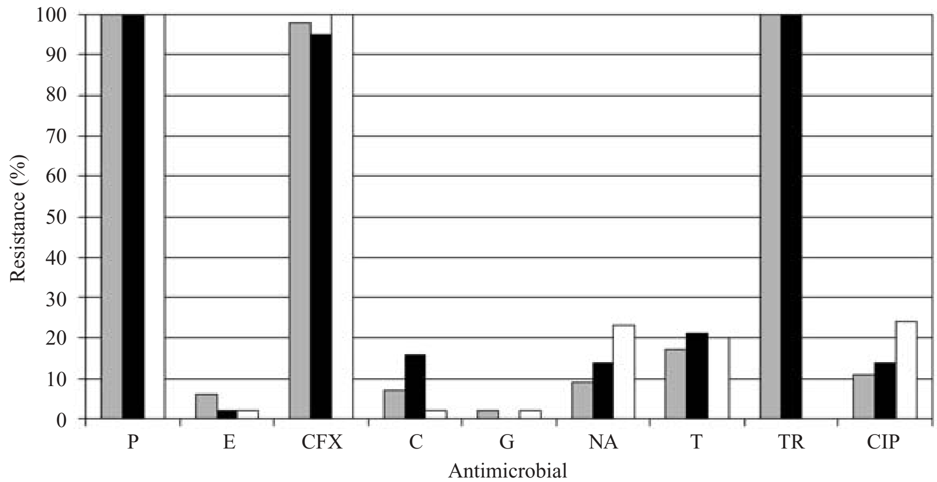

Fig. 1. Comparison of *Campylobacter* antimicrobial resistance from local chickens (▒), imported chicken portions (■) and humans (□). Human isolates not tested for trimethoprim. Antimicrobial abbreviations are as given in Table 1.

to penicillin and trimethoprim. Similar frequencies of resistance were noted to nalidixic acid (9%) and ciprofloxacin (11%). There was a low incidence of resistance to gentamicin (2%), and to erythromycin (6%). Resistance to multiple antibiotics was common and approximately 25% exhibited resistance to 4 or 5 of the agents tested. Nine isolates (4%) were resistant to 6 or more antibiotics.

Clinical *Campylobacter* isolates ($n = 494$, excluding repeat specimens from the same patient) were compared to chicken isolates for antibiotic resistance (Fig. 1). Moderately resistant isolates were counted as resistant and clinical isolates were not tested for trimethoprim resistance. Isolates from the three sources showed similar resistance profiles but more human than chicken isolates were resistant to nalidixic acid and ciprofloxacin, and fewer human isolates were resistant to chloramphenicol.

DISCUSSION

Fifty-seven per cent of chickens were contaminated with *Campylobacter* at retail [3] which is lower than other UK studies have generally reported, but similar to the 56% found in a 1998–2000 study which included campylobacters present on the outer packaging [9]. Sampling at retail probably gives a more realistic estimation of the organisms that present a risk of infecting the consumer than sampling live flocks or at slaughter because of the ability of campylobacters to enter a viable but non-culturable state. It is probable that sampling earlier in the production chain maximizes the recovery of a more diverse *Campylobacter* population which may not be culturable several days later at retail although still capable of causing human infection.

Strains of enteric bacteria isolated before the introduction of antibiotics are generally susceptible to a wide range of antimicrobials [4]. A small proportion of constitutively resistant strains may be present in microbial populations not due to the selective pressure of antibiotics, but the trend in most human pathogens is towards increased resistance. It has been shown that isolates from abattoirs are more resistant than those from retail meats [6], and it is probable that our survey underestimates the frequency of resistance in isolates from live birds. Sampling at the processing stage is preferable for the estimation of antibiotic misuse in animals, while sampling at retail outlets gives more realistic information of the risk to human health.

Although most countries test the resistance of *Salmonella*, generally by disc diffusion, few countries appear to test *Campylobacter* regularly [10]. Large differences in resistance profiles might be expected between countries, reflecting variations in the use of antibiotics. Clinical and poultry isolates of *C. jejuni* from Poland exhibited low-frequency resistance to erythromycin and gentamicin [11] and there were higher levels of resistance among chicken isolates than from clinical sources. We found a low incidence of resistance to gentamicin (2%) and erythromycin (6%), and little difference between *Campylobacter* species. An investigation of clinical isolates from England and Wales found erythromycin resistance in 1% of *C. jejuni* and 13% in *C. coli* [12]. Much higher frequencies of erythromycin resistance in campylobacters have been reported in other countries, and this is of concern since it is useful therapeutically. In Spain, *C. coli* from pigs showed 81% resistance to erythromycin, compared to 29% from humans [13]. However, a study in England which sampled chickens during a 2-month period reported only 1·6% resistant to erythromycin [14] and found lower resistance to chloramphenicol (0·5% *vs.* NI 7%), gentamicin (0% *vs.* NI 2%), similar resistance to fluoroquinolones, and higher resistance to tetracycline (36%; NI 17%). Resistance was greatest in isolates from chicken and *C. coli* was generally more resistant than *C. jejuni*.

There are some practical problems in directly comparing the prevalence of pathogens in imported portions and local chickens to clinical specimens. First, samples in both chicken groups were selected randomly, unlike isolates from clinical specimens which related to the seriousness of each patient's symptoms. Secondly, the skin of local chickens was sampled, but skin was absent from imported portions. This is potentially an important factor in a study of this size. Previous studies have also shown conflicting results, and recovery is higher with skin sampling than carcass rinse [9]. Berrang et al. [15] found variations in *Campylobacter* contamination at different points in the production chain and also between skin and meat. Removal of skin during processing might reduce contamination in the plant, but was not likely to significantly reduce the pathogen exposure of consumers. Another study found a significant, but not major, reduction in contamination of meat with skin removed [16]. The effect of skinning is uncertain, but it may partially account for the lower contamination in imported chicken. Thirdly, freezing and thawing may have affected the counts and resistance of the organisms present.

Since skinning may not reduce the level of contamination substantially, packing multiple breasts into bags prior to freezing would be expected to increase the likelihood of cross-contamination between portions. The lower level of contamination in imported chicken breasts (28%) than local whole birds (57%) was therefore unexpected. Previous work has shown differences in survival of freezing by *Campylobacter* [17], and that survival on meat products is high [18]. Antibiotic-resistance characteristics may also be altered by freezing due to changes of the outer membrane [19]. However, most of the local chickens were fresh, and sufficient numbers of frozen chickens were not available to test the effect of storage temperature on recovery. Bulk packing would also not be expected to substantially change the overall levels of resistance in isolates that are recovered and these differences in resistance between local whole chickens and imported portions should therefore be considered valid.

Fluoroquinolone resistance may be rapidly induced by mutations in the DNA gyrase and topoisomerase IV genes [20]. Fluoroquinolone resistance was 10–11% in our survey which is in keeping with the UK average. Cross-resistance between nalidixic acid and ciprofloxacin was almost total but slightly more campylobacters were resistant to ciprofloxacin than nalidixic acid. A report in 1996 found less than 3% ciprofloxacin resistance in 37 UK chickens compared to 27% from 26 imported poultry [21]. There was an association between ciprofloxacin resistance and increased MICs for tetracycline. We found six ciprofloxacin-resistant imported campylobacters and only 1 of 9 tetracycline-resistant isolates was also resistant to ciprofloxacin. Amongst local isolates, 35 were tetracycline resistant, 23 were ciprofloxacin resistant, and only 3 were resistant to both. Higher ciprofloxacin resistance has been recorded for human isolates in travellers returning from Spain [21] and the Indian subcontinent (J. A. Frost, personal communication). In The Netherlands increasing quinolone resistance in *Campylobacter* from poultry and humans was suggested to be related to the use of enrofloxacin in the poultry industry [22]. A Belgian study reported that 42% of *C. jejuni* and 62% of *C. coli* isolates from broilers were resistant to ciprofloxacin [23] and high levels of *C. jejuni* contamination (81%) were found in chicken meat in Italy, of which 53% were resistant to quinolones. *C. coli* showed greater resistance than *C. jejuni* in this study of animals and meat [24]. Almost all (99%) of campylobacters from pigs and broilers in Spain were resistant to ciprofloxacin [13]. This may reflect the availability and widespread use of fluoroquinolones in Spain, and reinforces the importance of tighter control of prescription policies [25]. Antibiotics that have cross-resistance with those used in human medicine should not be used for animal prophylaxis, but regrettably their use in agriculture continues [26].

All isolates were resistant to penicillin and to trimethoprim. Both of these antibiotics are used to test for constitutive resistance to assist identification of *Campylobacter* and are not used for therapy. Therefore only the 4% of isolates which were resistant to 6 or more antibiotics here were considered multiply resistant to therapeutic antibiotics. This contrasts with an earlier study using a slightly different array of antibiotics and a breakpoint assay in which 13% of chicken isolates proved to be multiply resistant [14].

In conclusion, imported chicken portions compared very favourably with locally produced whole chickens in terms of contamination with *Campylobacter* and antibiotic resistance. Skinning and freezing may have contributed to reduced pathogen contamination. The use of prepared portions may reduce the risk of infection owing to higher initial microbiological quality, and a reduction in manipulation that has the potential to cause cross-contamination. Overall antibiotic resistance in *Campylobacter* isolated from chickens produced in NI is generally of a similar or lower frequency than many other countries, but the use of imported chicken meat from certain countries may further reduce the occurrence of resistant pathogens.

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