

Research Note

Metronidazole Resistance in *Campylobacter jejuni* from Poultry Meat

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ABSTRACT

The occurrence of metronidazole resistance was investigated among *Campylobacter jejuni* in raw poultry meat collected from supermarkets. MICs were determined by the agar dilution procedure in the testing range of 3 to 60 µg/ml metronidazole. The MICs showed a bimodal distribution with a significant proportion of metronidazole-resistant isolates among *C. jejuni* from raw broiler and turkey meat. Metronidazole resistance occurred most frequently among turkey meat isolates ($P < 0.005$). This is the first report of foodborne bacteria carrying metronidazole resistance.

Metronidazole is a 5'-nitroimidazole compound used in veterinary and human medicine to treat a variety of infections caused by anaerobic pathogenic bacteria and protozoa (17). In turkey production, metronidazole has been used for the treatment and prevention of *Histomonas meleagridis* causing blackhead, a life-threatening disease in turkeys (6). Because of its carcinogenic potential, metronidazole was banned from veterinary medicine in Europe in May 2002 (2), and the related compounds ronidazole and ipronidazole had been previously withdrawn as feed additives for turkeys in Europe in 1998 and 1999, respectively. Metronidazole has not been applied in commercial broiler production in Europe. In humans, metronidazole is applied for the treatment of *Helicobacter pylori* infections, gynecological infections, chronic periodontitis, amoeba, and other infectious diseases (3). Resistance to metronidazole plays an important role in the failure to eradicate *H. pylori* (15), probably as a consequence of the widespread clinical use of metronidazole.

It has been suggested that metronidazole resistance is inherent in microaerophilic *H. pylori* and therefore would be present in other microaerophilic organisms such as *Campylobacter* (19). According to some authors, the oxygen status of the cell is a major contributor to the metronidazole susceptibility, and it has been suggested that scavenging of oxygen from the intracellular environment may, at least in part, account for the sensitivity of microaerophilic *Campylobacter* to metronidazole (12, 18).

For the treatment of *Campylobacter* infections, metronidazole is not considered relevant. However, the exact mechanisms behind the cause and spread of metronidazole

resistance remain unclear, and the molecular basis of metronidazole resistance in microaerophilic bacteria is not fully understood (9, 11). It is therefore probable that metronidazole resistance may spread between microorganisms, but this has not been investigated. Some reports on the occurrence of metronidazole resistance in the *Campylobacter* genus exist (4–8, 10, 16, 20, 22), but none of these have investigated *Campylobacter* from foods.

The aims of the present study were to investigate the occurrence of metronidazole resistance among *C. jejuni* in raw poultry meat by determination of MICs and to compare metronidazole resistance levels in *C. jejuni* isolated from raw turkey meat and raw chicken meat.

MATERIALS AND METHODS

Raw poultry meat samples were collected randomly from local retail establishments in Denmark according to the national *Campylobacter* surveillance program. The procedure for isolation of thermotolerant *Campylobacter* spp. was nonselective for metronidazole and other antibiotics (13). Verification of colonies as *Campylobacter jejuni* was performed by phase-contrast microscopy for characteristic morphology and motility, Gram staining, catalase and oxidase production, and hydrolysis of hippurate and indoxyl acetate.

Only isolates belonging to the species *C. jejuni* were included, and only one isolate per food sample was included in the study. Agar dilution on Müller-Hinton II agar (BBL, Becton Dickinson, Sparks, Md.) was applied for the susceptibility testing of isolates to metronidazole in the concentrations of 3, 7.5, 15, 30, and 60 µg/ml. This testing range was selected in order to facilitate comparability with previous reports. Inoculum was prepared as previously described (1), and plates were inoculated with approximately 10⁴ CFU per spot by a multipoint inoculator (Applied Quality Services Ltd., Horsham, UK), placing 21 spots on each 90-mm agar plate. Plates without antibiotics were included for inoculation control as the first and the last plate in a series of inoculations. Plates were allowed to dry before they were placed

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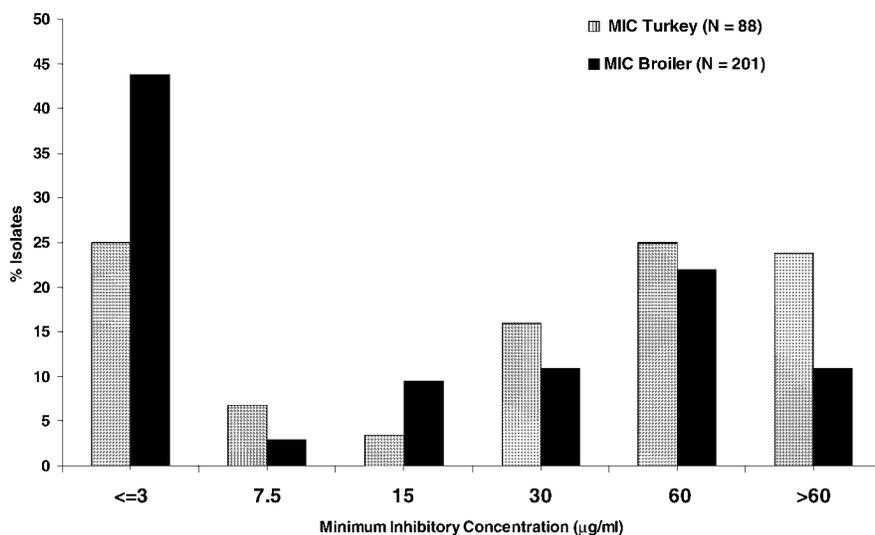


FIGURE 1. Distribution of *Campylobacter jejuni* MICs of metronidazole among isolates from raw broiler and turkey meat.

in jars and incubated under microaerophilic conditions at 37°C for 48 h. Microaerophilic conditions (6% O₂, 7% CO₂, 7% H₂, 80% N₂) were generated by the Mart Anoxomat-System (Mart Microbiology BV, Lichtenvoorde, The Netherlands). The MIC was defined as the lowest concentration of metronidazole, which did not allow visible growth. The strain *C. jejuni* ATCC 33560, used as standard and tested five times independently, produced MICs of metronidazole of either 7.5 or 15 µg/ml. This strain performed as expected for antibiotics other than metronidazole under the same testing conditions.

RESULTS AND DISCUSSION

Two hundred one isolates from broiler meat and 88 isolates from turkey meat were tested for susceptibility to metronidazole. The distributions of MICs for *C. jejuni* from broiler meat and turkey meat are presented in Figure 1. Among broiler meat isolates, MIC₅₀ (the MIC for 50% of the strains) was 15 µg/ml and MIC₉₀ was >60 µg/ml, whereas among turkey meat isolates, MIC₅₀ was 30 µg/ml and MIC₉₀ was >60 µg/ml. Isolates were distributed in two subpopulations, one with low MIC (<3 up to 7.5 µg/ml) and another with elevated MIC. There is no internationally established breakpoint for the interpretation of metronidazole resistance in *C. jejuni*. By introducing a tentative metronidazole breakpoint of ≥30 µg/ml, the percentage of metronidazole resistance among isolates from raw broiler meat was calculated to 44%, whereas the percentage of metronidazole resistance among isolates from raw turkey meat was 65%. Statistical analysis of the occurrence of metronidazole resistance in broiler meat and turkey meat indicates significant difference for $\chi^2 = 7.88$; $P < 0.005$ with 1 df. These high frequencies of metronidazole resistance are probably not due to a direct selective pressure, for metronidazole has not been applied in Denmark for commercial turkey production throughout the last 10 years. Additionally, the control of veterinary drug residues, performed by the Danish Veterinary and Food Administration, has not found residues of metronidazole in either Danish or imported products. As regards coresistance of metronidazole with other antibiotic resistances, all isolates in this study were tested for resistance to seven antibiotics: tetracycline, chloramphenicol, erythromycin, gentamicin, streptomycin,

ciprofloxacin, and nalidixic acid, and there was no indication of coresistance between metronidazole and any of these seven antibiotics. Finally, to date there is no scientific evidence of metronidazole resistance being horizontally transferred to or from *C. jejuni*, whereas transformation of metronidazole resistance has been documented within *H. pylori* and related species (14, 21, 23). Instead, as suggested by Stanley and Jones (20), it is possible that a host-phenotype relationship exists, based on the fact that high rates of metronidazole resistance, between 82 and 100%, have been observed among strains of avian origin compared with strains of *C. jejuni* from ruminant hosts, which were generally more susceptible to metronidazole (20). Most strains in the above-mentioned study were only tested at a metronidazole concentration of 5 µg/ml, but MIC determinations performed on 29 strains of broilers, turkeys, and starlings in the testing range of 3 to 60 µg/ml produced average (mode) MICs of 15 µg/ml (20). We observed a similar mode MIC among *C. jejuni* from raw broiler meat and an even higher mode MIC of metronidazole resistance among *C. jejuni* from raw turkey meat. These findings indicate that metronidazole resistance may be a useful epidemiological tool for the tracing of sources in *C. jejuni* infections, although data are needed from other raw meat sources in order to validate the hypothesis.

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