ABSTRACT

A 55 Samples of chicken meat were collected from slaughterhouse in Baghdad city, these samples consisted of skin, breast, thigh and retail which were investigated for Campylobacter jejuni. The C. jejuni were recovered from 85.45% of the samples and 85.10% of these isolates were found to be resistant to nalidixic acid by the disc diffusion and agar dilution methods (MICs 64-256 μg/ml), they were also resistant to the other fluoroquinolones; norfloxacin (MICs 128-256 μg/ml), ofloxacin (MICs 64-128 μg/ml), ciprofloxacin (MICs 32-64 μg/ml), and all forty chicken isolates of C. jejuni resistant to fluoroquinolones were resistant to tetracycline (MICs ≥64 μg/ml).

INTRODUCTION

Campylobacter jejuni are the commonest enteropathogens in developed countries and cause an acute diarrhal disease in both animals and humans world wide (1,2). The normal microflora in intestine of poultry is the main source of C. jejuni infection (3), so the important risk factors for the acquisition include the handling or eating of chicken meats (4).

Campylobacter enteritis is treated by antimicrobial treatment and fluoroquinolones is considered the drug of choice (5), but since 1990s there is a rapid increase of Campylobacter resistance to fluoroquinolones and has been recognized in many countries (6,7). In Iraq there, s no literatures indicating the rate of occurance of Campylobacter jejuni in poultry or animal products.

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The resistance to fluoroquinolones has been suggested to be due to the use of quinolones in animal production industries especially poultry (8), but recently rapid and persistent resistance was actually shown to develop in chickens treated with fluoroquinolones (9).

The resistance of Campylobacter jejuni to fluoroquinolones due to spontaneous or induced chromosomal mutation has been reported (10).

The aim of the study was to determine the incidence of Campylobacter jejuni in chicken meat from traditional slaughterhouse, and to determine in vitro susceptibility of isolates to fluoroquinolones by determination of minimum inhibitory concentration since nalidixic acid – resistant Campylobacter jejuni strains were observed in the laboratory in the preliminary disc diffusion test.

**Experimental**

**Sampling procedure**

A 55 Samples of chicken meat were purchased from slaughterhouse in Baghdad city, these samples consisted of skin, breast, thigh and retail which were examined for Campylobacter. All samples were collected aseptically and placed in an ice chest transported for analysis to postgraduate laboratory of microbiology in the college of sciences at Al-Mustansiriyah University in the period between April and August 2002.

Preston broth medium was used as first enrichment broth consisted for 1 litter of nutrient broth NO.2 (Oxoid Ltd; England), trimethoprim 10 mg; polymyxin B 5000 I.U.; cycloheximide 100mg and rifampicin 10mg (Samaraa Drug, Iraq) (11).

The Campylobacter selective medium used was Blair-Wang medium consisted of 1 litter of Columbia agar base (M144 Hi-media laboratories Ltd. Bombay-India) supplemented with antibiotic supplement (FD006 Hi-media) that are recommended for the isolation of Campylobacter spp. per vial, sufficient for 500ml of medium; vancomycin 5mg, polymyxin B 2500 I.U., trimethoprim 2.5mg, amphotericin B 1mg and cephalosporin 7.5mg. For enhanced growth of Campylobacter growth supplement (FD009, Hi-media) was added per vial, sufficient for 500ml of medium; sodium pyruvate 0.125g, sodium metabisulphite 0.125g and ferrous sulphate 0.125g (12).

**Isolation and Identification**

10 grams samples of chicken meat were placed in 90ml of preston broth supplement with 5% horse blood and then incubated at 42oC for 24hrs. in anaerobic jar containing 5% O2, 10% CO2 and 85% N2 (gas generating kit) BBL Campy-pak microaerophilic system Envelopes (Becton, Dickinson and Campany Sparks, MD USA) (13). Suspect
colonies were Gram-stained and examined by phase-contrast microscopy for typical morphology, suspect colonies were further characterized by the following tests: Oxidase and catalase production, hippurate hydrolysis, growth temperature, nitrate reduction, H2S production, failure to growth in 3.5% sodium chloride, and their Susceptibility to cephalothin (30 µg/disc, BBL,USA) and nalidixic acid (30 µg/disc, BBL,USA) (14).

Antimicrobials susceptibility test
The susceptibility of forty nalidixic acid resistant Campylobacter jejuni isolates to the fluoroquinolones; ciprofloxacin, norfloxacin, ofloxacin (Aventis pharma, Tunisia), tetracycline (Samaraa Drug, Iraq), was performed by using disc diffusion method, briefly, overnight culture of isolates were grown on campylobacter blood agar under microaerophilic condition. Colonies were suspended to approximately 0.5 McFarland standard in Mueller-Hinton broth and inoculated onto Mueller-Hinton agar supplemented with 5% sheep blood (15,16).

Minimum inhibitory concentration
MIC was done by using agar dilution method as recommended by NCCLS (17) for nalidixic acid resistant Campylobacter jejuni strains. The organisms that overnight cultured on blood agar plates were suspended to approximately 0.5 McFarland standard in Mueller-Hinton broth (Oxoid, England) and inoculated onto Mueller-Hinton agar plate supplemented with 5% sheep blood and containing serial dilutions of antimicrobial agents. The Mueller-Hinton agar plates were incubated at 420°C for 24 hrs. in a microaerophilic atmosphere by microaerophilic system envelope (BBL, USA) (16). Quality control was done by using Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922. The results were interpreted according to the criteria of the NCCLS (18).

RESULTS AND DISCUSSION
The percentage and occurrence of Campylobacter jejuni in traditional abattoirs were recovered in 85.45%(47/55) of chicken meat samples with the different sites from 55 samples.

Fluoroquinolones Susceptibility
Fourty of the 47 (85.10%) Campylobacter jejuni isolates were found to be resistant to nalidixic acid in the disk diffusion test and the agar dilution methods (MICs: 64-256) µg/ml, they were also resistant to the other fluoroquinolones; norfloxacin (MICs: 128-512) µg/ml, ofloxacin (MICs: 32-128) µg/ml, ciprofloxacin (MICs 32-64) µg/ml, a
well as all forty isolates of *C. jejuni* that resistant to fluoroquinolones were resistant to tetracycline (MICs $\geq 64$ µg/ml), results are summarized in table 1.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>MICs(µg/ml)</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic acid (16 µg/ml)*</td>
<td>(64-256)</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>256</td>
<td>4</td>
</tr>
<tr>
<td>Norfloxacin (4 µg/ml)*</td>
<td>(128-512)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>256</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>512</td>
<td>4</td>
</tr>
<tr>
<td>Ofloxacin (2 µg/ml)*</td>
<td>(32-128)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin (1 µg/ml)*</td>
<td>(32-64)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>18</td>
</tr>
<tr>
<td>Tetracycline (4µg/ml)*</td>
<td>$\geq 64$</td>
<td>40</td>
</tr>
</tbody>
</table>

* = MIC breakpoints suggested as breakpoints for aerobic bacteria of antimicrobials according to NCCLS(18).

The resistant rate of *C. jejuni* to fluoroquinolones become very high in different countries(6,7,10). In our study the resistance rate found to be near to many studies reported in the world ,and these quinolone resistant *C. jejuni* isolates reported in our study were the first documented resistant isolates in Baghdad.

The emergence of fluoroquinolones resistant isolates appeared at the bigning of the 1990s (19).The origin of resistance to fluoroquinolones probably reflects the use of fluoroquinolones in veterinary medicine in some infectious diseases (20), and the emergence of fluoroquinolones resistance among *Campylobacter* was previously proposed to be due to the frequent use of fluoroquinolones in the poultry production industry (21),in United States the first fluoroquinolones resistance appeared after the use of the antimicrobial agents in the poultry production industry (22), and it has now also been shown that the development of fluoroquinolones resistance develops rapidly in *Campylobacter* infected broiler treated with quinolones (9), and a bright rates of resistance to fluoroquinolones have been demonstrated in *Campylobacter* strains isolated from food animals (23). In this study the isolates found to be resistant to tetracycline and the
high rate of tetracycline resistance in chicken meat isolates might be attributed to the use of tetracycline as a growth promoter in poultry feed. The use of tetracyclines such as doxycycline in agriculture is likely responsible for the large number of tetracycline-resistant C. jejuni isolates. Avrian et al. suggested that the tet(o) gene may be rapidly and spontaneously transferred in vivo without antimicrobial selection between C. jejuni strains in the digestive tracts of chickens (24). Surveillance of tetracycline resistance is important due to the potential for plasmid-mediated transfer of the tet(o) gene (25), as well as genes encoding resistance to other antimicrobials for other potential pathogens (26). Charvalos et al. (27) showed that capable of isolation of a fluoroquinolones-resistant strain which isolated from pefloxacin-containing agar was also resistant to tetracycline, erythromycin, chloramphenicol, and beta-lactams. Also Li et al. (28) reported that concomitant resistance rates among nalidixic acid-resistant C. jejuni isolates from their patients (exclusively children) were as follows: gentamicin 2%, erythromycin 12%, tetracycline 97%, and ciprofloxacin 66%. The cross resistance between nalidixic acid and fluoroquinolones was also observed in our study. Similar results have been reported by Rautelin et al. but in clinical isolates (29). Fluoroquinolones resistance in Campylobacter from food animal is now recognized as an emerging public health. Smith et al. (30) found that patients infected with resistant C. jejuni had longer duration of diarrhea than patients with fluoroquinolones-sensitive isolates. As Campylobacter infections can be serious in immunocompromised patients, the identified treatment failure raises the concern that fluoroquinolones-resistant strains may increase Campylobacter-associated death in this group of patients.

The primarily source of Campylobacter jejuni infections in human is believed to be the handling and/or consumption of contaminated meat, especially poultry meat. The control of Campylobacter in the food chain has now become a major target of agencies responsible for food safety world-wide.

The susceptibility of Campylobacter jejuni to fluoroquinolones is of great importance regarding the treatment of bacterial gastro-enteritis caused by these isolates as the resistance rate of Campylobacter jejuni to fluoroquinolones appears to be increased.
REFERENCES


