Full Length Research

Identification of *Campylobacter* species and their Antibiotic Resistance Patterns from Raw Bovine Meat in Addis Ababa, Ethiopia

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Campylobacter is considered mainly as food-borne pathogen, with raw or undercooked meat. A cross sectional study was conducted to identify Campylobacter species and their antimicrobial resistance patterns from raw bovine meat samples in Addis Ababa Yeka sub city from January to March 2013. A total of 384 meat samples were collected using sterile method and analyzed following standard methods. Campylobacter species were isolated in 36 (9.4%) of the samples. Subsequent identification of the isolates showed that *Campylobacter jejuni* was the most dominant species accounted for 28(78%) of the total isolates followed by *Campylobacter coli* 8(22%). Significant (P ≤ 0.05) difference was observed between the two Campylobacter species identified. The antibiotic susceptibility tests were done on a total of 36 isolates of the two species. Isolates showed 100%, 91.7%, 86.1% and 77.8% sensitivity to chloramphenicol, Ciprofloxacin, Nalidixic acid and Erythromycin, respectively. The resistance to ampicillin cephalothin, amoxycillin and trimethoprim-sulfamethoxazole is 97.2%, 88.8%, 83.3% and 72.2%, respectively. Raw meat could serve as a potential vehicle for transmitting campylobacter species. Implementation of hygienic practices from slaughter house to the retailers, proper handling and cooking of foods of meat are very important in preventing Campylobacter infections.

Key words: Campylobacter, Antibiotic resistance, food-borne diseases and raw meat.

INTRODUCTION

Food-borne diseases occur as a result of consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria (Rosef et al., 2008; Wonget al., 2009). They are currently considered as the leading cause of sporadic bacterial gastroenteritis (Gilbert and Slavik, 2004; WHO, 2001; Anonymous, 2003). These species can be found in the reproductive organs, intestinal tracts, and oral cavity of animals and humans. Fecal matter is a major source of contamination and could reach carcasses through direct deposition (Abdalla et al., 2009). Animal food products can become contaminated by this pathogen during slaughtering and carcass dressing (Caprioli et al., 2005).

The most important *Campylobacter* species associated with human illness are *Campylobacter jejuni* and *Campylobacter coli*. These organisms have frequently been associated with poultry, which are considered as the primary source. However, other meats such as lamb and beef have also been implicated as source of contamination (Stanley and Jones, 2003; Hussain et al., 2007). Several countries have reported the epidemiology of different *Campylobacter* species in bovine (Stanley and Jones, 2003; Kassa et al., 2005). However, little is known about the presence of *Campylobacter* species in bovine to suspect them as possible sources of infection in humans in Ethiopia.

Antibiotics are extensively used worldwide in human and veterinary medicine for treatment or prevention of microbial diseases and as a feed additive for growth promotion (Philips et al., 2008). This has contributed to the development of antimicrobial and encouraged the persistence and transfer of antimicrobial resistance. In zoonotic *Campylobacter* bacteria, the use of antimicrobial agents in animals can enhance the development of drug-resistant bacterial populations that may pose a potential threat to the consumer (Kurincic et al., 2005). Thus, this research was conducted to identify *Campylobacter*
species and determine antibiotic resistance patterns from bovine meat of retailers in Addis Ababa, Yeka sub city.

MATERIALS AND METHODS

Study area

Addis Ababa is a capital city of Ethiopia. The city is located at 9°1'48"N 38°44'24"E coordinates. It has an average altitude of 2,300 meters above sea level. The city lies at the foot of Mount Entoto. Yeka sub city by which this study was conducted is found to the northeastern suburb of the city.

Study design

A cross sectional laboratory-based survey study was employed to determine the prevalence and antimicrobial sensitivity patterns of Campylobacter species isolated from bovine meat samples in Addis Ababa, Yeka sub city retailers between January and March 2013.

Sample size

The sample size was determined using an appropriate formula based on the 95% confidence limits and 5% sampling error.

\[ n = \left( \frac{Z_{\alpha/2}}{2} \right)^2 \frac{p \times (1-p)}{d^2} \]

In determining the sample size, the p value was taken as 50% (maximum value) because of the absence of recent data on prevalence of Campylobacter species in the study area. Thus, the total sample size used in this study, as determined by the aforementioned formula, was 384.

Sample collection

A total of 384 raw meat samples were collected from 192 retailers in the sub city twice within two weeks difference (n=384). Approximately forty five grams of meat samples were collected randomly from different surface sites of carcass. All samples were placed in separate plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory in a cooler box with ice packs.

Sample preparation

Samples collected from each carcass were placed in a plastic bag containing 225 ml of buffered peptone water and homogenized using a stomacher for two minutes.

The resulting suspension was used for isolation of Campylobacter species.

Isolation of Campylobacter species

Isolation of Campylobacter species was made following standard procedures. In brief, 20ml of a meat sample rinse solution was mixed with the same volume of Preston broth supplemented with FBP (iron, bisulphate, and pyruvate) and incubated at 42°C for 24 hours in a micro aerophilic atmosphere. After incubation, the universal bottles with enriched samples were properly shaken and sub-cultured onto modified charcoal cefoperazone deoxycholate agar (mCCDA) for primary isolation. The anaerobic jar containing the inoculated plates was then incubated at 42°C for 48 hours.

After 48 hours incubation, the growth was provisionally evaluated by colonial morphology. Typical mucoid, spreading and convex colonies were identified. Colonies on each mCCDA plate were separately subcultured on Colombia Base Agar (Oxoid Ltd) with 5% sheep blood and incubated in a micro aerophilic environment at 37°C for 24 to 36 hours. Suspected colonies were noted for absence of hemolysis on blood agar, shiny, convex, and colorless to grayish colony characteristics with irregular or round edged nature.

Suspected Campylobacter species colonies from pure culture were picked-up using wire loop and smeared on glass slides for gram staining. The colonies were tentatively identified as Campylobacter species when a curved or S-shaped Gram-negative rods showing “seagull winged” appearance were observed under the microscope.

Identification of Campylobacter species

The test used to differentiate C. jejuni and C. coli is sodium hippurate hydrolysis test. For this test a loop-full of colony was suspended in 400 µl of 1% sodium hippurate solution. The suspension was incubated at 37°C for 2 hours, and then slowly 200 µl of 3.5% ninhydrin solution was added to the side of the tube to form an overlay. After re-incubation at 37°C for 10 minutes, observation was made for color change. Positive reaction was indicated by dark purple/blue color while negative reaction was shown by clear or grey suspension. The isolates with a positive reaction were considered as C. jejuni and those isolates with negative reactions were considered as C. coli.

Antimicrobial susceptibility test for Campylobacter species

The antimicrobial susceptibility test was performed for all
36 isolates of both *C. jejuni* (28) and *C. coli* (8) species following the standard agar disk diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2003) using commercial antibiotic disks (Oxoid).

**Data Analysis**

The data collected were entered and managed in MS Excel program. SPSS version 12 for windows was used for data analysis. A p-value <0.05 was considered indicative of a statistical significant difference.

**RESULTS**

**Prevalence of Campylobacter Species**

A total of 384 bovine carcass samples were investigated for thermophilic Campylobacter species during the study period. Of these, 36 (9.4%) were positive for Campylobacter species. This study revealed that among the Campylobacter species isolated from bovine carcass samples, *C. jejuni* accounted for 28 (78%) isolates and the rest 8 (22%) isolates were *C. coli*. A statistically significant difference (p<0.05) was observed between the prevalence of the two identified Campylobacter species. The ratio of the frequency of occurrence of *C. jejuni* to *C. coli* sample was 4:1 (Table 1).

**Antibiotic susceptibility patterns of Campylobacter Isolates**

The antibiotic susceptibility tests were made to all Campylobacter species. The overall percentage of resistance pattern is shown in (Table 2). The antimicrobial sensitivity testing result showed that isolates were highly sensitive to Ciprofloxacin, 100% and Chloramphenicol, 91.7%. Isolates also showed higher sensitivity in percentages, 86.1% and 77.8% for Nalidixic acid and Erythromycin, respectively. In this study, the resistance pattern to Ampicillin was very high, 97.2%. Higher resistance rates were also found for Chloramtholin and Amoxycillin, 88.8% and 83.3%, respectively.

In this study, the antimicrobial susceptibility testing of both Campylobacter species was done independently (Table 3). All (100%) *C. jejuni* species were sensitive to Erythromycin and Nalidixic acid. They also showed 92% sensitive to both Chloramphenicol and Ciprofloxacin. *C. jejuni* isolates were 100%, 85.7% 78.6% and 75% resistance to Ampicillin, Amoxycillin and Cephalothin and Trimethoprim-Sulphamethoxazole, respectively.

The antimicrobial susceptibility test for *Campylobacter coli* isolates showed that 100% sensitive to Ciprofloxacin and Nalidixic acid, and 87.5% sensitive to Chloramphenicol. The high resistance to tetracycline and ampicillin in this study could be due to frequent use of antibiotics (Allos, 2001). Higher resistance rates, 100%, 87.5% and 75% were also observed for Cephalothin, Ampicillin, and Amoxycillin respectively in *C. coli* isolates.

**DISCUSSION**

The prevalence of *C. jejuni* in this study is slightly lower than that reported by Desalegne and Adane (2010) in Ethiopia, where 94.1% and 5.9% of the isolates belonged to *C. jejuni* and *C. coli*, respectively. The finding in this study is higher than the findings of the previous prevalence studies done by Dadi and Asrat (2008) (6.5%)
Table 3. The antimicrobial susceptibility of each identified *Campylobacter* species.

<table>
<thead>
<tr>
<th>Species name and No.</th>
<th>Susceptibility</th>
<th>AMX No. (%)</th>
<th>AM No. (%)</th>
<th>CE No. (%)</th>
<th>Cl No. (%)</th>
<th>CH No. (%)</th>
<th>E No. (%)</th>
<th>NA No. (%)</th>
<th>SXT No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni (n = 28)</td>
<td>S</td>
<td>0 (100)</td>
<td>0 (100)</td>
<td>0 (100)</td>
<td>26(92.8)</td>
<td>26(92.8)</td>
<td>28(100)</td>
<td>28(100)</td>
<td>6(21.4)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4(14.3)</td>
<td>0 (100)</td>
<td>6(21.4)</td>
<td>2(7.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1(3.6)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>24(85.7)</td>
<td>28(100)</td>
<td>22(78.6)</td>
<td>0 (0.0)</td>
<td>2 (7.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>21(75)</td>
</tr>
<tr>
<td>C. coli (n = 8)</td>
<td>S</td>
<td>0 (100)</td>
<td>0 (100)</td>
<td>0 (100)</td>
<td>8(100)</td>
<td>7(87.5)</td>
<td>4 (50)</td>
<td>8 (100)</td>
<td>4(50)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2(25)</td>
<td>1(12.5)</td>
<td>0 (0.0)</td>
<td>4 (50)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>6 (75)</td>
<td>7 (87.5)</td>
<td>8 (100)</td>
<td>0 (0.0)</td>
<td>1(12.5)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (37.5)</td>
</tr>
</tbody>
</table>

AMX: Amoxycillin; AM: Ampicilline; CE: Cephalothin; CH: Chloramphenicol; CI: Ciprofloxacin; NA: Nalidixic acid; E: Erythromycin; SXT: Trimethoprim-sulfamethoxazol; S: Sensitive; I: Intermediate; R: Resistant.

in Ethiopia and Nonga et al. (2010) (5.6%) in Morogoro, Tanzania, McNamara, (1995) (4%) in USA and Vanderlinde et al., (1998) (0.8%) in Australia. Foods of animal origin have been incriminated for being the main sources for Campylobacter infection in humans (Oberhelman and Taylor, 2000). Since raw meat from beef is widely consumed in the country, the occurrence of Campylobacter in meat increases the likelihood of the pathogen's transmission to humans. However, it is lower than reported prevalence by Salihu et al., (2009), 12.9% in Nigeria and Taremi et al., (2005), 10% in Iran. These variations could be due to type of samples collected and the method of sample collection, differences in sample size, and the difference in isolation and identification procedures.

Similar antibiotic susceptibility patterns have been observed in a previous study conducted in Ethiopia where 80-100% of isolates from food animals were sensitive to these antimicrobial agents (Dadi and Asrat, 2008; Kassa et al., 2005). The proportions of resistant *Campylobacter* isolates to ampicillin and tetracycline in the current study were lower than those reported by earlier studies from Iran where 13.6% and 63.6%, respectively (Rahimi, 2010). This could be due to the fact that this antibiotics were prescribed less frequently in treating diarrheal cases around the study area due to fear of its side effect compared to other drugs since it causes damage to bone marrow (Smeltzer and Bare, 1995).

In conclusion, raw bovine meat could serve as potential vehicles for thermophilic Campylobacter infection to humans through consumption of raw/undercooked bovine meat. Raw meats originating from animals grown for human consumption using a traditional slaughter system are often contaminated with thermophilic Campylobacter species which represent a high risk of contamination of the carcass.

**Recommendations**

The importance of proper handling and cooking of foods of animal origin are very important in preventing Campylobacter and other potential pathogens. Coordinated actions are needed to reduce or eliminate the risks posed by these pathogens at various stages in the food chain. More epidemiological studies are needed in order to determine the possible role of bovine as a source of reservoir of the pathogen. Public education is crucial not to eat raw meat or any undercooked animal origin foods.

**ACKNOWLEDGMENTS**

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