Campylobacter in sheep, calves and broiler chickens in the central region of Algeria: Phenotypic and antimicrobial resistance profiles

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Received 28 July, 2016; Accepted 6 September, 2016

The study was conducted in four slaughterhouses in Bouira Province, central region of Algeria. Campylobacter in the main food animals (sheep, calves and broilers) were studied to evaluate the prevalence, phenotypic characteristics and antibiotic susceptibility of isolated strains. Out of 200 sheep, 200 calves and 100 broilers swab samples collected. 150 strains were isolated and identified. A study of sensitivity to 14 antibiotics by the disc diffusion method was performed. This finding shows that, Campylobacter species are very common in avian samples isolates of (96%) but less frequent in sheep and calves (13 and 14% respectively). On the entire isolated strains, Campylobacter jejuni was the most common with an isolation rate of 58% followed by C. coli and C. lari. The majority of isolated Campylobacter strains showed as multidrug resistant. High rates of resistance to different antibiotics tested were observed in broilers, mainly to Nalidixic acid (96.8%), Ciprofloxacin (91.6%) and Erythromycin (88.54%); the lowest level of resistance was found to the Tetracycline (44.7%). The high frequency of digestive portage noted in food animals and the high rate of antibiotic resistance constitutes a real threat to public health in study area. In conclusion, significant Campylobacter isolation rate and multiple drug resistance should be at acceptable level so as to increase productivity livestock rearing off the study sites.

Key words: Campylobacter, sheep, calves, broiler, frequency, antibiotic resistance, Algeria.

INTRODUCTION

Campylobacter germs are a leading cause of enteric zoonotic infections (OIE, 2005; WHO, 2012). Poultry is generally considered to be the most important single reservoir for Campylobacter, mainly Campylobacter jejuni (Hakkinen et al., 2007). Contamination of chicken carcasses by this germ often occurs during the

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slaughtered process and consumption of chicken meat is a significant source of human Campylobacter infections (Lupo et al., 2014; Dasti et al., 2010; Humphrey et al., 2007).

Other animals are also common carriers of Campylobacters (Inglis et al., 2004; Hakkinen et al., 2007). Many studies have reported an association with cattle and sheep (Wesley et al., 2000; Nielsen, 2002; Stanley and Jones, 2003). Direct-contact exposure to bovine feces and ingestion of unpasteurized milk are well-documented causes of outbreaks of Campylobacteriosis (Baeg et al., 2005).

Most Campylobacter infections in humans are self-limiting and do not require antimicrobial therapy. However, in systemic infections or in immunocompromised individuals, erythromycin and fluoroquinolones are used as the drugs of choice. Many studies have reported an increase in the resistance of Campylobacter to various antimicrobials, including the drugs of choice (Chen et al., 2010; Cody et al., 2010).

Development and transmission of antibiotic-resistant Campylobacter are complicated by the fact that Campylobacter is a zoonotic pathogen and is therefore exposed to antibiotics in animal production (Bogaard and Stobberingh, 2000; McEwen and Fedorka-Cray, 2002).

In Algeria, there are few studies regarding Campylobacteriosis in humans (Guechi, 1984; Megraud et al., 1999) and food animals (El amir et al., 2013; Messad et al., 2013). Besides, no studies have been carried out on sheep and calves yet.

The present study was performed on food animals: sheep, calves and broilers in three slaughterhouses Bouira Province, central region of Algeria. Therefore, the objective of this study is: isolation and identification of Campylobacter germs, phenotypic characterization and the study of antibiotic resistance of the isolated strain.

MATERIALS AND METHODS

Samples collection

The study was conducted in two periods: the first from June 2009 to February 2010, and the second from August 2013 to January 2014.

The study targeted 200 sheep, 200 calves and 100 broilers randomly selected from a population of 1200 sheep, 2400 calves and 10 lots of broilers slaughtered in three rural slaughterhouses and a bird slaughterhouse located at Bouira Province.

Sheep samples were taken from different regions namely: Bouira, Oued Souf, Saida and Boumerdes. Avian and bovine samples come from the same region (Bouira).

Isolation and identification of Campylobacter

Stool samples were taken by rectal swab immediately after slaughter. The isolation in Petri dishes was performed on a Karmali medium, maximum one hour later (Oxoid France; CM0935) containing a selective supplement (Oxoid France; SR0069E).

The inoculated dishes were instantly placed in jars (AnaeroJar Tm; Oxoid, AG0025A) containing Gas-pack bags for microaerophilic (bioMérieux, France) were used. The jars were incubated for 48 h at 37°C.

All bacterial colonies with a macroscopic appearance of Campylobacter, Gram stain and oxidase tests were performed. Strains were stored at -80°C in BHIB (brain heart infusion broth) supplemented with 20% glycerol.

The strains belonging to the genus Campylobacter have been subjected to complete biochemical identification (catalase research by gallery species identification Api Campy "bioMérieux, France") and an antibiotic sensitivity test.

Antimicrobial susceptibility testing of Campylobacter isolates

We tested antibiotics in the list of CA-SFM (CA-SFM, 2012), namely: Amoxicillin (AM, 10 µg), amoxicillin + clavulanic acid (AMC, 10 µg), cephalothin (CF, 30 µg), cefotaxime (CTX, 15 µg), gentamicin (GM, 15 µg), tobramycin (TM, 10 µg), erythromycin (E, 15 UI), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE, 30 UI), chloramphenicol (C, 30 µg).

Spiramycin (SP, 100 µg) and metronidazole (MTR, 16 µg) were added to the above list because of their use in veterinary medicine in Algeria.

The antimicrobial susceptibility test was performed by disk spray on Muller Hinton medium (Pasteur Institute of Algeria) with 5% blood with dishes incubation for 48 h at 37°C, in jars under a microaerophilic atmosphere at 37°C. In parallel, Campylobacter jejuni (ATCC 33560) was used as reference strain. The interpretation of the antibiogram was made according to the standards of CASFM (CA-SFM, 2012).

Data analysis

The results were analyzed using SPSS statistical software using the X2 test. Significant differences were considered when probability (p) was equal to or less than a risk (p ≤0.05).

RESULTS

Prevalence of Campylobacter in sheep, calves and broiler chickens

Out of a total of 500 samples, 30% positivity rate (152/500) was observed. Depending on the species, the isolation rate was 13% (26/200), 14% (28/200) and 96% (152/500) was observed. For sheep, calves and broilers respectively (Table 1).

Campylobacter isolated species

In various animal species, we noted a predominance of the C. jejuni species with a frequency of 58% followed by C. coli (21%), C. lari (10%), C. fetus (7%) and C. sputorum (4%) (Figure 1).

The study of isolation frequency of different Campylobacter species depending on the animal species showed that C. jejuni is the most common regardless of the animal species (Figure 2).
Table 1. *Campylobacter* isolation rates of different animal species (P <0.05).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Number of samples</th>
<th>Number positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>200</td>
<td>26 (13%)</td>
</tr>
<tr>
<td>Calves</td>
<td>200</td>
<td>28 (14%)</td>
</tr>
<tr>
<td>Broiler chickens</td>
<td>100</td>
<td>96 (96%)</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>150 (41%)</td>
</tr>
</tbody>
</table>

**Figure 1.** Total frequency of isolation of different species of *Campylobacter*.

**Figure 2.** *Campylobacter* species isolated in different animal species.

Isolation rate depending on the region of sheep origin

In sheep, the isolation rate of *Campylobacter* strains differs depending on the regional origin of animals. Indeed, a significant difference was recorded between the positivity rates of the different regions (Table 2).

Antimicrobial susceptibility

The strains isolated from the broiler chickens had
Table 2. Isolation rate strains in sheep by region.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oued Souf (n=50)</th>
<th>Bouira (n=50)</th>
<th>Saida (n=50)</th>
<th>Boumerdes (n=50)</th>
<th>Total N=200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of isolated strains</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Percentage of positives</td>
<td>26.92</td>
<td>26.92</td>
<td>34.62</td>
<td>11.54</td>
<td>13</td>
</tr>
</tbody>
</table>

(P₁-value =0.000<0.05). P₁-value: Value for the isolation rate of the sheep’s strains isolated from different regions.

Table 3. Campylobacter resistance rates among different animal species.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sheep (N=26)</th>
<th>Calves (N=28)</th>
<th>Broiler chickens (N=98)</th>
<th>P₂-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>9 (34.62%)</td>
<td>13 (50.00%)</td>
<td>78 (81.25%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AMC</td>
<td>4 (15.38%)</td>
<td>3 (11.54%)</td>
<td>72 (75.00%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>CF</td>
<td>8 (30.77%)</td>
<td>6 (23.08%)</td>
<td>49 (51.04%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>CTX</td>
<td>3 (11.54%)</td>
<td>9 (34.62%)</td>
<td>38 (39.58%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>GM</td>
<td>8 (30.77%)</td>
<td>7 (26.92%)</td>
<td>45 (46.88%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>K</td>
<td>4 (15.38%)</td>
<td>2 (7.69%)</td>
<td>10 (10.42%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>TM</td>
<td>4 (15.38%)</td>
<td>2 (7.69%)</td>
<td>10 (10.42%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>E</td>
<td>15 (57.69%)</td>
<td>10 (38.46%)</td>
<td>85 (88.54%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>SP</td>
<td>10 (38.46%)</td>
<td>16 (51.54%)</td>
<td>35 (36.46%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>TE</td>
<td>11 (42.31%)</td>
<td>6 (21.42%)</td>
<td>43 (44.79%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>8 (30.77%)</td>
<td>2 (7.69%)</td>
<td>10 (10.42%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>MTR</td>
<td>16 (61.54%)</td>
<td>27 (85%)</td>
<td>86 (89.58%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>AN</td>
<td>16 (61.54%)</td>
<td>27 (85%)</td>
<td>93 (96.88%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>CIP</td>
<td>1 (3.85%)</td>
<td>2 (7.69%)</td>
<td>88 (91.67%)</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

N: Number of strains, n: Number of strains resistance to antibiotic. P₂-Value: Value for the antimicrobial resistance difference between the strains isolated from sheep, calves content and those isolated from broiler chickens samples to the same antibiotic.

significantly greater rates of resistance compared to Campylobacter strains isolated in sheep and calves.

The highest levels of resistance broiler strains were noted to ciprofloxacin (91.6%), nalidixic acid (96.8%) and Erythromycin (88.5%).

In this study, different strains isolated had varying levels of resistance depending on the antibiotic and animal species (Table 3).

DISCUSSION

A variation in the prevalence of Campylobacter was found between the different studies in different parts of the world in sheep, cattle (Stanley and Jones, 2003) and in broilers (Jorgensen et al., 2011).

In our present work, we noted 26 and 28% positivity rates for sheep and calves, respectively, and 96% positivity rate for broilers. This result is consistent with some studies (Gouali et al., 2005; Dadi et al., 2008; Salihu et al., 2009a, b; Messad et al., 2013) but is different from others (Desmonts et al., 2004; Igimi et al., 2008; Bae et al., 2005; Garcia et al., 2010).

According to certain authors, the variation in Campylobacter isolation frequency observed between different studies would be linked to a number of factors such as: the season (Hannon et al., 2009), geographic location (Berrang et al., 2000) as well as the sample size (Jeffrey et al., 2001).

The choice of research method and use of enrichment media can also play an important role. Indeed, Garcia et al. (2010) and Hakkinen et al. (2007) showed an improvement of 15 and 30% respectively due to the use of a medium enrichment.

In various animal species, we noted a prevalence of C. jejuni (62, 53 and 59% in sheep, calves and broiler respectively), which is consistent with most studies in the world. Such as 79.6% for Nigeria according to Salihu et al. (2009a), 79.6% for China according to Chen et al. (2010), 47.7% for Italy according to Parisi et al. (2007), 87 and 34.1% for Canada according to Hakkinen et al. (2007) and Hannon et al. (2009), respectively. The reasons for the variations are unknown and could be attributable to differences in production practices and
environments (Chen et al., 2010).

From the viewpoint of sensitivity to antibiotics, *Campylobacter* strains isolated from the broiler had significantly higher rates of resistance compared to the ovine and bovine strains.

It is noteworthy that several studies which have dealt with the antibiotic susceptibility of *Campylobacter* strains isolated from broiler samples reported similar rates in our vis-à-vis ciprofloxacin (91.6%) (Kang et al., 2006; Chen et al., 2010; Kovalenko et al., 2014) and nalidixic acid (96.8%) (Kang et al., 2006; Rahimi et al., 2010; Chen et al., 2010; Kovalenko et al., 2014).

The high fluoroquinolone-resistance rates of *Campylobacter* may be attributed to the widespread use of fluoroquinolones in poultry production in Algeria. This class of antibiotics is used for both prevention and control of poultry diseases. It is well known that the use of fluoroquinolones in poultry selects fluoroquinolone resistant mutants and leads to the emergence of fluoroquinolone-resistant *Campylobacter* in the treated birds.

Teuber suggested that the use of enrofloxacin (derivates close to the fluoroquinolones used in human medicine) in animals flocks has probably exerted a selection pressure in animal reservoirs (Teuber, 2001).

About Erythromycin, we noted in our study a high level of resistance to this antibiotic in broilers (88.5%). According to a WHO (2008) report, macrolides are widely used in farms, and this practice is known to promote the selection of resistant *Campylobacter* strains in animals. Lin et al showed that the use of Erythromycin in low dose for a long time selects *Campylobacter* strains resistant to this antibiotic (Lin et al., 2007).

In this study, a high proportion of *Campylobacter* avian resisted to metronidazole (89%). According to the study of Stanley and Jones (2003), already 80 to 100% of *Campylobacter* strains were resistant to this antibiotic in 1998.

About tetracycline, the resistance rate (44.7%) we got for all avian strains is much higher than reported by Kuana et al. (2008) in Brazil that varies between 15 to 16% and by Ronner et al. noted in a study in four European countries (35.4%). This rate is similar to the rate of resistance noted by Beatriz et al. in Spain (Oporto et al., 2009).

In our series of *Campylobacter* strains, low percentages of resistance to chloramphenicol were found, in accordance with the results presented in previous publications conducted in Algeria (Messaad et al., 2013). High susceptibility to chloramphenicol could be explained by none or moderate use of this antibiotic due to its non-registration in Algeria since 2006 (WHO, 2008).

**Conclusion**

The prevalence of *Campylobacter* in calves and sheep slaughter was low, indicating that calves and sheep can be considered as a minor source of *Campylobacter* infection to consumers.

Unlike that of broilers, which showed a high isolation rate (96%). High levels of resistance to certain antibiotics in strains isolated from different animal species, and multi detected resistance can be explained by the use of these antimicrobial agents in animals. Therefore, proper strategies have to be designed and implemented to minimize its effect on broilers, and also calves and sheep as well, besides this, correct drug usage should be recommended to minimize resistance so as to increase livestock production in the studied district.

**Conflict of interests**

The authors have not declared any conflict of interest.

**REFERENCES**


