Isolation of *Campylobacter jejuni* from Cloaca and Cecum Content of Chicken Broilers Bred in Intensive Systems in the Western Part of Romania

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Abstract

*Campylobacter* spp., belongs to the group of thermo-tolerant bacteria, and is the most frequent cause of gastrointestinal diseases in humans following consumption of poorly cooked chicken meat. The aim of our study was to test the common methodology for isolation of *Campylobacter jejuni* species from cloaca and cecum content of chicken broilers breed in intensive systems in Western part of Romania. The experiments were conducted during July –September 2013. As biological material we used chicken broilers from 6 intensive breeding facilities from the West part of Romania, from which cloaca swabs and cecum content were recovered as samples. Bacteria isolation was performed by inseminating Petri dish with Muller Hinton Agar media, after bacterial growth, they were subculture on Muller-Hinton Agar with Skirrow. The bacteria were tested by Gram staining and Oxidase test. Bacterial growth was detected from all samples when grown on Mueller-Hinton Agar, but when the bacteria was passed on Muller Hinton Agar with selective supplement (Skirrow) 27 out of 36 samples remained positive (75.0%). With respect to the sample origin 13 (72.2%) samples from cloaca swab and 14 (77.7%) from cecum content grown on campylobacter selective media. All samples from Muller-Hinton supplemented with Skirrow tested negative for Gram staining and positive for oxidase test. We have successfully isolated *Campylobacter* spp., strains from farms and private producers in the western part of Romania.

Keywords: *Campylobacter jejuni*, chicken broilers, intensive breeding facilities

1. Introduction

*Campylobacter* spp., belongs to the group of thermo-tolerant bacteria, and is the most frequent cause of gastrointestinal diseases in humans following consumption of poorly cooked chicken meat [1]. Campylobacter is a leading cause of enteric illness in many the western world and in the developing countries [2]. Human campylobacteriosis is the most common cause of food poisoning in most of the industrialized world, and the reduction and/or elimination of *C. jejuni* in the food chain, particularly from chicken products, represents the primary strategy used to control this disease [3].

Campylobacter can break through biosecurity barriers and enter poultry houses, colonizing the chicken intestine and quickly multiplying in the intestinal mucosa. However, Campylobacter does not induce health or welfare problems in chickens. Campylobacter spreads fast within broiler flocks and almost all birds will be infected within one week [4]. In chickens, *C. jejuni* colonizes the mucus overlying the epithelial cells primarily in the ceca and the small intestine but may also be recovered from elsewhere in the gut and from the spleen and liver. Once colonization is established, campylobacters can rapidly reach extremely high numbers in the cecal contents, as high as $10^9$ CFU
in experimentally challenged birds although this level may be lower in naturally colonized birds [3]. The aim of our study was to test the common methodology for isolation of *Campylobacter jejuni* species from cloaca and cecum content of chicken broilers breed in intensive systems in Western part of Romania.

2. Materials and methods

The experiments were conducted during July – September 2013

**Biological material:** Chicken broilers from 6 intensive breeding facilities from the West part of Romania. From each breeding unit 3 animals were taken for study, a total of 18 animals were used for this study.

**Sample collection:** Cloaca swabs were recovered using a sterile cotton swab, after collection the sample was sealed into a sterile plastic tube and transported to the laboratory. The fecal samples were collected from cecum directly into the laboratory, under sterile conditions, for this the cecum was recovered from the animals into a sterile petri dish and transported in the laboratory. Within 15-20 minutes from collection the samples were processed.

**Bacterial isolation:** The sample from cloaca swab was dissolved into 900 µl sterile water, after that serial dilutions were performed until 10^5 and 100 µl of each dilution was plated on to 9.5 cm Petri dish with Muller Hinton Agar media (CM0337, Thermo scientific), prepared according to the producer instructions. For the feces sample approximately 100 µl of the cecum content was diluted into 900 µl of sterile water, and serial dilutions was performed from this. Dilutions from 10^2 to 10^6 were plated on the same media as swab samples. After 72-96 hours of incubation at 37°C, in microaerophilic conditions, colonies with *Campylobacter sp.* aspect were picked and inseminated on Muller-Hinton agar media supplemented with campylobacter selective supplement (Skirrow, SR0069, Thermo scientific). All plates were incubated at 37°C in microaerophilic conditions using CampyGen AGS (CN0025A, Thermo scientific).

**Gram Staining:** Evaluation of the results was performed using Gram Staining Kit (77730 Fluka Analytical). The Gram staining method is one of the most important staining techniques in microbiology. It is almost always the first test performed for the identification of bacteria. The gram positive bacteria retain the crystal violet-iodine complex and appear purple brown under microscopic examination. Gram negative bacteria are not stained by crystal violet. The staining was performed according to manufacturer instruction. Briefly, the slide smear was flooded with Gram's crystal violet Solution, than Gram's iodine Solution and Gram's Decolorizer Solution, at the end the smears were counter stained with Gram's safranin Solution. All samples were examined under microscope with oil immersion objective.

**Oxidase test:** was performed using Oxidase reagent (55 635, Biomerieux). Briefly: 2 - 3 drops of reagent to the center of a filter paper and allow a few seconds for absorption, a loopful of test colony onto the filter paper, the result appear within 5-10 seconds. Positive oxidase colonies produce of a dark purple color, negative colonies remain colorless. Oxidase test was performed on all samples that were recovered from campylobacter selective media.

3. Results and discussion

Gram staining was performed also for the samples cultured on Muller-Hinton agar, with no Campylobactor selective supplement and we noticed a mixture of Gram positive and Gram negative bacteria (figure 1). When subculture of the isolated bacteria was performed on Muller-Hinton supplemented with Skirrow Gram staining showed little or no Gram positive bacteria (figure 2).

Bacterial growth was detected from all samples when grown on Mueller-Hinton agar, but when the bacteria was passed on Muller Hinton Agar with selective supplement (Skirrow) 27 out of 36 samples remained positive (75,0%) (table 1). With respect to the sample origin 13 (72.2%) samples from cloaca swab and 14 (77.7%) from cecum content grown on campylobacter selective media. From location 6, only one sample grown on Muller Hinton agar, supplemented with Skirrow. On this farm there was a particularity, in respect to biosecurity measures, since it was a family farm; only one person was entering into the poultry breeding facility. This may lead to an improved hygiene and a high level of biosecurity on this farm. A high level of biosecurity on the farm could protect against Campylobacter. Some correlations were found [5], but even an extremely
A high level of biosecurity does not guarantee a Campylobacter-free flock at the time of slaughter. Improved disease prevention measures and hygiene may lead to a lower prevalence of Campylobacter. [6].

**Table 1.** Occurrence of *Campylobacter* species in samples tested

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Total number of samples recovered</th>
<th>No. of samples grown on Muller Hinton</th>
<th>No. of samples grown on Muller Hinton Skirrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloaca swab</td>
<td>18</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Cecum content</td>
<td>18</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>36</td>
<td>27</td>
</tr>
</tbody>
</table>

**Figure 1.** Gram staining of the bacteria cultured on Muller-Hinton media

**Figure 2.** Gram staining of the bacteria cultured of Muller-Hinton media with Skirrow

**Figure 3.** Aspect of Gram staining of the samples isolated from location 1, age of the animals 42 days. S - samples isolated from cloaca swab, F - samples isolated from cecum content. Samples with the same indices are from the same animal.

**Figure 4.** Aspect of Gram staining of the samples isolated from location 2, age of the animals 45 days. S - samples isolated from cloaca swab, F - samples isolated from cecum content. Samples with the same indices are from the same animal.
Figure 6. Aspect of Gram staining of the samples isolated from location 4, age of the animals 26 days. S - samples isolated from cloaca swab, F - samples isolated from cecum content. Samples with the same indices are from the same animal.

Figure 8. Aspect of Gram staining of the samples isolated from location 5, age of the animals 37 days. From this location only one sample was isolated on Muller–Hinton agar supplemented with Skirrow.
intensive poultry breeding facilities West part of Romania.

4. Conclusion

We have successfully isolated Campylobacter spp., strains from farms and private producers in the western part of Romania. We are next investigating the virulence of these strains by comparing their pathogenicity with a well-known human pathogenic strain (*Campylobacter jejuni* 81-176) using *in vitro* virulence assay.

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References