Virulent genes of Campylobacter jejuni contaminating chicken cuts and giblets

Saad M. Saad1, Mohamed A. Hassan1, Nahla A. bou El Roos2, Eman F. Ahmed2

1 Department of Food Hygiene and control, Faculty of Veterinary Medicine, Benha University
2 Animal Health Research Institute, Shebin El Koom Branch.

ARTICLE INFO

Keywords
Campylobacter jejuni
Chicken meat
Liver
PCR

ABSTRACT

The objective of this examination was to decide the predominance of Campylobacter spp. in some chicken cuts and giblets items by both ordinary and sub-atomic techniques. 120 chicken item tests (25g each) were gathered from different general stores at Menofia legislature of chicken breast, thigh, liver and gizzard. Most examples were defiled with Campylobacter species and chicken liver demonstrated the most elevated pollution (56.67%) trailed by gizzard (53.33%), thigh (30%) and breast (23.33%). Campylobacter jejuni was the most segregated serotype (6.67%), (13.33%),(16.67%) and (23.33%) in breasts, thigh, gizzard and liver ,individually. hipO quality was identified in sechded C. jejuni. Harmfulness qualities cdtA, cdtB and cdt C. cdtA and cdtB, cdtC, CdtA, CdB and CdtC were 30%, 15%, 25%, 10%, 15% and 5% of the detached C. jejuni strains, separately. At long last, the use of atomic techniques is better than regular strategies in identification of Campylobacter serotypes.

1. INTRODUCTION

Chicken meat industry is the greatest provider of worthy creature protein with high meat yield, low shrinkage in cooking and extraordinary wellspring of amino acids, nutrients and minerals (Oulkeiret al., 2017). Campylobacter has been recuperated from chicken remains, poultry meat parts and supplies in handling plants overall (García-Sánchez et al., 2017). Campylobacter is a zoonotic microbe and is the primary driver of human bacterial gastroenteritis on the planet (Humphrey and O’Brien, 2007 and Tam and Rodrigues, 2012). The regularly detailed pathogenic species is C. jejuni representing over 90% of the cases, trailed by C. coli speaking to 7% of the diseases, with the remainder of cases being principally C. lari and C. fetus (Moore and Corcoran, 2005). Human C. jejuni and C. coli contaminations don’t contrast with respect to clinical indications and length of ailment. Notwithstanding, patients tainted with C. coli are in general more seasoned than those with C. Jejuni (Karenlampi and Rautelin, 2007). The incubation period is 2 to 5days, and the disease brings about an intense self-restricting gastrointestinal sickness normally settled in multi weeks, described by mellow to extreme watery/wicked loose bowels, fever, nausea, discomfort and stomach torment (Blaser, 1997). In created nations the greater part of the human cases happen from pre-summer until summer (Kovats and Edwards, 2005). Death rate is inadequately characterized however low, with passing regularly restricted to immuno-traded off patients or those experiencing another extreme sickness, for example, entrails malignant growth (Allos, 2001). There is impressive epidemiological proof that the main danger factor related with human Campylobacter contamination is the presence of this living being in chicken (Sheppard-Dallas et al., 2009). Notwithstanding immediate corpse pollution, intestinal substance sully machines, working surfaces, defensive apparel and worker’s hands expanding the open door for cross-tainting of without campylobacter cadavers (FAO and WHO, 2002).

Campylobacter species are overall significant reason for bacterial gastroenteritis (Moore et al., 2005). As a matter of fact, C. jejuni and C. coli are liable for 90% and 10% of human enteric disease cases, individually (Lastovica, 2006). Campylobacter contaminations in people are typically described without help from anyone else restricting watery/bleeding the runs, abdominal cramps, queasiness and fever; in any case, extreme neurological sequelae, bacteremia and other extra intestinal complexities may grow rarely (Blaser and Engberg, 2008). The recognizable proof and segregation of C. jejuni and C. coli is viewed as risky on the grounds that it just relies upon a solitariness test dependent on the hydrolysis of hippurate (Steinhauserova et al., 2001). Subsequently, atomic distinguishing proof strategies have been portrayed as an option to the off base, tedious, biochemical phenotypic techniques (LaGier et al., 2004). Various traditional PCR measures focusing on an assortment of qualities, for example, hipO, glyA, cadI, ceuE and mapA have been recorded (On and Jordan, 2003). Along these lines, the objective of this investigation is recognizable proof of Campylobacter species by sub-atomic and regular strategies in some chicken cuts and offal's.

2. MATERIAL AND METHODS

2.1. Collection and preparation of samples:
An aggregate of 120 random examples of fresh chicken cut and giblets were gathered from chicken butchering shops
with various disinfection levels in Menofia government. The gathered examples were characterized into Fresh chicken meat was comprised of 30 chicken breast, 30 chicken thigh. Chicken giblets included 30 chicken livers and 30 chicken gizzards. The examples were acquired in their packaging as offered to the shoppers and moved straightforwardly to the lab in a fridge without undue delay where they were ready for bacteriological assessment.

2.2. Appraisal of campylobacter tally:
2.2.1. Arrangement of tests in enhancement stock: Accurately 25 grams of each example were aseptically put to sterile stomacher sack containing 225ml Bolton particular advancement stock for homogenization at that point enhanced (sallam, 2001).

2.2.2. Confinement on particular plating media (Bolton et al., 1984)
Loopfulls from the recently hatched stock societies were streaked on adjusted Campylobacter Charcoal Deoxycholate Agar (CCDA, Biolite Italiana) base plates enhanced with 10 mg/L of amphotericin B and 32 mg/L of cefoperazone (Biolite, Italiana). The immunized plates were hatched for 48 h at 42°C under microaerophillic condition (5% O2, 10% CO2 and 85% N2) utilizing CampyGen sachets (Vandepitte and Verhaegen, 2003).

2.3. Identification of hypothetical settlements:
2.3.1. Microscopically assessment (ISO, 1995): By a stage contrast magnifying instrument, for trademark, twisting or bended slim poles with a wine tool like motility.
2.3.2. Gram staining according to ISO, (1995):
2.3.3. Biochemical distinguishing proof following OIE, (2008): Catalase test, Oxidase test, Triple sugar iron test, Lead acetate acid derivation strip, Growth within the sight of 1% glycine, Nitrate decrease, Hippurate hydrolysis test, Nalidixic corrosive opposition and Growth temperature resistance were done.
2.3.4. Serological recognizable proof of Campylobacter species by Latex Agglutination Kit:It was done by Oyarzabal et al., (2007).

2.4. Polymerase Chain Reaction (PCR):
2.4.1. Groundwork groupings utilized for PCR ID framework: Utilization of PCR for 23S rRNA quality as corroborative apparatus for location and recognizable proof of Campylobacter species was done. Besides, multiplex PCR was utilized for ID and portrayal of cytological distending poisons spoke to by cdtA, cdtB and cdtC as harmfulness qualities of Campylobacter jejuni
2.4.2. DNA Extraction utilizing QIA amp pack (Ehsannejad et al., 2015).

2.4.3. DNA intensification.
2.4.3.1. Intensification response of 23S rRNA (Wang et al., 2004).The intensification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany).
2.4.3.2. Amplification of the chose destructiveness qualities (Carvalho et al., 2013).Precisely, 40 μl of PCR combination were utilized. All responses contained appropriate concentrations of 3 preliminary sets, 0.2 mM every one of dATP, dCTP, dGTP and dTTP, 1 × Ex Taq DNA polymerase cradle, and 1.0 U of Ex Taq DNA polymerase in a 40-mL response volume.

3. RESULTS
As shown in table (1) results revealed that the incidence of Campylobacter spp. was positive for all samples. The highest incidence was found in chicken liver and gizzard (56.67%), (53.33%) followed by thigh and breast (30%), (23.33%), respectively. It is evident from results recorded in table (2) that the incidence of C. jejuni was (6.67%), (13.33%), (16.67%) and (23.33%) in breast, thigh, gizzard and liver, respectively. Table (3) showed Latex Agglutination test for confirmatory identification of C. jejuni was (23.33), (16.67), (13.33) and (3.33) breast, thigh, gizzard and liver, respectively. Table (4) and photo (1 & 2) showed the occurrence of virulence genes of C. jejuni strains isolated from the examined samples of chicken cuts and giblets. Virulence genes cdtA, cdtB and cdtC were present at a rate of 44.4%, 11.1%, 16.7%, 27.8% of the examined strain of C. jejuni, respectively.

4. DISCUSSION
Chickens having up to 100% asymptomatic transporters of Campylobacter in their intestinal lots and may hold up to 109 microorganisms for each 25 g, which quickly spread among different chickens. This much surpasses the human irresistible portion (Humphrey et al., 2007).

Table 1 Incidence of Campylobacter species in the examined samples of chicken cuts and giblets.

<table>
<thead>
<tr>
<th>Chicken cuts and giblets</th>
<th>No. of ex. samples</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>30</td>
<td>7</td>
<td>23.33</td>
</tr>
<tr>
<td>Thigh</td>
<td>30</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>Gizzard</td>
<td>30</td>
<td>16</td>
<td>53.33</td>
</tr>
<tr>
<td>Liver</td>
<td>30</td>
<td>17</td>
<td>56.67</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>49</td>
<td>40.83</td>
</tr>
</tbody>
</table>

Table 2 Incidence of Campylobacter jejuni isolated from the examined samples of chicken cuts and giblets (n=30).

<table>
<thead>
<tr>
<th>Identified strains</th>
<th>Breast</th>
<th>Thigh</th>
<th>Gizzard</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>6.67</td>
<td>13.33</td>
<td>16.67</td>
<td>23.33</td>
</tr>
</tbody>
</table>

Table 3 Latex Agglutination test for confirmatory identification of Campylobacter jejuni isolated from the examined samples of chicken cuts and giblets (n=30).

<table>
<thead>
<tr>
<th>Chicken cuts and giblets</th>
<th>Latex Agglutinating Kit Observation</th>
<th>Latex Agglutinating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breasts</td>
<td>+ve agglutination clumps</td>
<td>7</td>
</tr>
<tr>
<td>Thighs</td>
<td>+ve agglutination clumps</td>
<td>5</td>
</tr>
<tr>
<td>Gizzards</td>
<td>+ve agglutination clumps</td>
<td>4</td>
</tr>
<tr>
<td>Livers</td>
<td>+ve agglutination clumps</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>15</td>
</tr>
</tbody>
</table>

N.B. % was calculated according to total number of samples

Table 4 Occurrence of virulence genes of C. jejuni strains isolated from the examined samples of chicken cuts and giblets (n=18).

<table>
<thead>
<tr>
<th>Virulence genes</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>cdtA</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>cdtB</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>cdtC</td>
<td>3</td>
<td>16.7</td>
</tr>
<tr>
<td>cdtD</td>
<td>5</td>
<td>27.8</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>
As depicted in Table (1) results delineated that liver and gizzard indicated the higher occurrence of Campylobacter contamination than different examples. It may allude to the first intestinal pollution during fowl destruction (Moore et al., 2005). Our outcome was higher than that of Khalifa et al. (2013) (36%) and Ei-Tras et al. (2015) (23.5%); the pervasiveness contrasts could be credited to confinement strategies, test types and size not withstanding occasional and provincial varieties (Allos, 2001; Omara et al., 2015). Results in Table (2) the distinguishing proof of Campylobacter strains confined from inspected tests indicated that C. jejuni, C. coli and C. butzleri were distinguished in the pace of 28%, 20% and 12% in breast separately. C. jejuni, C. coli and C. lari were distinguished in pace of 24% 16% and 8% separately in breast. In thigh the pace of disengagement of C. jejuni, C. coli, C. lari and C. cinaedi were 40 %16%, 8 % and 8% individually. Concerning thighs the pace of disconnection of C. jejuni,C. coli, C.lari and C. upsaliensis were 36%, 12% and 8% individually. Livers were debased with C. jejuni, C. coli, C. lari and C. cinaedi in pace of 48 %, 20 %, 8%and 4 % separately. Gizzards were contaminated with C. jejuni, C. coli and C. lari in rate, of 44 %, 24 % and 12% individually. Among the zoonotic Campylobacter species, for example C. jejuni, C. coli, C. lari and C. upsaliensis, the previous two species are answerable for by far most of the human food borne contaminations, representing 90% and 5-10% of cases (Mikučiūtė et al., 2016). The zoonotic C. jejuni is perhaps the most poultry holding microorganisms, with high general wellbeing risk ordinarily connected with chicken, arrangement to the prevalent degrees of human utilization (Humphrey et al., 2007). Photograph (1) demonstrated the presence of hipO gen in separated C. jejuni. Polymerase chain response (PCR) focusing on hipO quality was utilized already for ID of C. jejuni in chickens; meat and human examples (Khalifa et al., 2013). It is the primary Campylobacter genome to be sequenced was C. jejuni by Parkhill et al. (2000). Utilization of sub-atomic instruments, for example, PCR may assist with maintaining a strategic distance from a portion of the constraints of current techniques, where the hipO quality is explicit for C. jejuni strains (Sinha et al., 2004). Table (3) and photographs (1&2) indicated the Occurrence of destructiveness qualities of C. jejuni strains confined from the inspected tests of chicken meat and giblets as cdA, cdB and cdC, cdA and cdB, cdB and cdC were available in 44.4% , 11.1%, 16.7%, 27.8% separately. These qualities are included essentially in attachment and intrusion and they alluded to as harmfulness factors starting here onwards (Chansiripornchai and Sasipreeyaoyan, 2009). All in all, the most noteworthy frequency of Campylobacter strains was established in chicken.

5. REFERENCES


