Comparison between Different Methods for Detection of Salmonella Species in Imported and Local Duckling

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**Abstract**
Two hundred freshly dead and apparently healthy ducklings from 160 imported and 40 local species (Muller, pekin, Muscovy and Baladi) were bacteriologically examined for isolation and identification of Salmonella species. Only 10 samples were positive with prevalence 5% in all collected samples. These isolates were further characterized by polymerase chain reaction. The result revealed 10 different servore as following S. Jedburgh, S. Harrisonburg, S. Braenderup, S. Southbank, S. Sekondi II, S. Sinchew, S. Brandenburg from imported duckling and S. Ruzizi, S. Give and S. Entertidis from local duckling with 0.5% for each. Salmonella isolates were tested for antimicrobial sensitivity and the most resistance rate was for trimethoprim with 80%, intermediate resistance for Penicillin, amoxicillin with 40% and 100% sensitive for norfloxacin. Based on PCR, all examined salmonella were positive 100% (7/7) for stn virulence gene, while 42.85% (3/7) of the tested salmonella isolates were positive to aadB antibiotic resistance gene.

**Introduction**
Ducks are frequently used by human populations throughout the world for a variety of reasons; duck meat and duck eggs are consumed for protein-specific dietary purposes, raised as pets for children Loharikar et al. (2012). Unfortunately, disease risks are associated with contact with ducks and may contribute to adverse health effects in people. Aside from food- infections, a cluster of non-typhoid Salmonella (NTS) human infections has also been associated with ducklings Gaffga et al. (2012). Outbreaks of human Salmonellosis caused by contact with ducks have been reported in some countries, such as Australia, United States, United Kingdom and Denmark Merritt and Herlihy (2003). Even though clinical disease has occasionally been described in very young ducklings, infection is usually subclinical Fedorka-Cray et al. (2000). Although ducks are very
resistant to systemic infection caused by *Salmonella*, they are potential reservoirs of this organism and may shed it in the feces, contaminating the environment *Barrow et al.* (1999). *Salmonella* enteric serovars, their virulence genes combinations and antibiotic resistance, garner attention for their potentiality to contribute to the adverse health effects on populations throughout the world *Osman et al.* (2014).

This study attempted to address this outstanding issue on whether genetic determinants for both antibiotic resistance and virulence genes could be harbored by the same transferable element and further confirm the association between antibiotic resistance and virulence in duckling.

**Material and Methods**

**Sample:**
A total examined 200 apparently healthy and freshly dead duckling including 160 imported one day old duckling and 40 local ducklings with ages of 10 and 14 days. The collected samples were liver, cecal tonsils, spleen, and yolk sac if found.

**Bacteriological isolation and identification of *Salmonella***:
The procedure for isolation and identification of *Salmonella* were conducted according to *ISO 6579 (2002)* procedure.

**Serotyping of *Salmonella* isolates:**
Two diagnostic *Salmonella* antisera sets were used, (*Denka Seiken co., LTD*) for polyvalent (O) I, II, III antisera and monovalent *Salmonella* O and (*Pro- lab diagnostic, U.K*) for flagellar H for both phase I and phase II.

The disk diffusion test technique was applied according to *Bauer et al.* (1966). Eight types of antibiotic from different groups Gentamicin, Ciprofloxacin, Amoxicillin, Doxycycline, Trimethoprim, Nalidixic acid, Norfloxacin and Penicillin. The interpretation of inhibition zone of tested culture was according to CLSI, (2011).

**Molecular Identification of *Salmonella* Isolates:**
A total of 7 presumptive samples of *Salmonella* species by cultural, morphology and biochemical characteristics, were tested by specific primer employing PCR assay which was more sensitive in the confirmation of the isolates.

**Extraction of DNA:** It was done according to QIAamp DNA mini kit (Qiagen – Germany) instructions.

**Preparation of PCR Master Mix used for cPCR**
Oligonucleotide primers used in cPCR

Oligonucleotide Primers used to amplify *Salmonella* and its virulence and antibiotic resistance genes are listed in Table (1).
Table (1): Oligonucleotide primers sequences Source:

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer Sequence 5' - 3'</th>
<th>Amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>aadB</td>
<td>F.GAGCGAAATCTGCGGCTCTGG</td>
<td>319 bP</td>
<td>Frana et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>R.CTGTTACAACGGACTGGCCGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stn</td>
<td>F. TTG TGT CGC TAT CAC TGG CAA CC</td>
<td>617 bP</td>
<td>Murugkar et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>R.ATT CGT AAC CCG CTC TCG TCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.5. Cycling conditions of cPCR: Temperature and time conditions of the primers during PCR are shown in Table (2).

Table (2): Cycling conditions of the different primers during cPCR:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primary denaturation</th>
<th>Secondary denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>No. of cycles</th>
<th>Final extension</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>aadB</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec.</td>
<td>58°C 30 sec.</td>
<td>72°C 30 sec.</td>
<td>35</td>
<td>72°C 10 min.</td>
<td>Frana et al., 2001</td>
</tr>
<tr>
<td>stn</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec.</td>
<td>59°C 45 sec.</td>
<td>72°C 45 sec.</td>
<td>35</td>
<td>72°C 10 min.</td>
<td>Murugkar et al., 2003</td>
</tr>
</tbody>
</table>

DNA Molecular weight marker: 100-1000 bp
Agarose gel electrophoresis: Sambrook J (1989) with modification

Results

Prevalence of Salmonella sp. isolated from duckling:
Salmonella sp. was recovered with total prevalence 5% (10/200) from them 7 were recovered from 160 imported duckling with 4.37% while 3 isolates from 40 local duckling with percent 7.5%.

Serotyping of Salmonella sp. recovered from duckling:
The result revealed 10 different serovare as S.Jedburgh, S.Harrisonburg, S.Braenderup, S.Southbank, S.SekondiII, S.Sinchew, S.Brandenburg from imported duckling and S.Ruzizi, S. Give and S.Entertidis from local duckling with 0.5% for each.

Antimicrobial sensitivity test among the isolates:
The most resistance rate was for trimethoprim with 80% (8/10), intermediate resistance for Penicillin, amoxicillin with 40% (4/10) for each and gentamycin with 30% (3/10). The isolates were highly sensitive with 100% for norfloxacin followed by doxycycline, nalidixic acid and ciprofloxacin with 90%.

Among Salmonella serotypes S.Braenderup, S.Brandenburg and S. Givewere the most multidrug resistant serotypes with 50% followed by S.Harrisonburg with 37% while S.Entertidis was sensitive for all 8 antimicrobial agents.
Detection of *stn* virulence gene and *aadB* resistance genes by Conventional polymerase chain reaction:
The results showing that *stn* virulence gene was positive in all *Salmonella* serovars while *aadB* antibiotic resistance gene specific for Gentamycin was positive in only 3 serovare are *S. Braenderup*, *S. Brandenburg* and *S. Give with* (42.85%).

**Photo (1):** Agarose gel electrophoresis with positive PCR amplification of (319bp) fragment of antibiotic resistance *aadB* gene from DNA of positive(1,6,7) *Salmonella* isolates.

**Photo (2):** Agarose gel electrophoresis with positive PCR amplification of (617 bp) fragment of virulence gene *stn* from DNA of 7 positive *Salmonella* isolates.

**Discussion**
There has long been an association for long between ducks and *Salmonella*, largely through the consumption of duck eggs, which historically was associated with a high probability of ‘food poisoning’.(Shivaprasad and Barrow (2013).
In the present study the prevalence of *Salmonella* among the tested duckling was 5%. Nearly the same rates were obtained by Osman et al. (2010) who isolated *Salmonella* from 150 1-day-old ducklings with 6.6% from lung samples and 8.6% from cecum samples, Hui and Das (2001), Gong et al. (2014) and Badr et al. (2015) who recovered *Salmonella* with 5.36%, 6.8% and 6.45% of the tested samples respectively while lower rates were recorded by Dilmaghani et al. (2011) and Abdallah et al. (2015) with 0.6% and 2.8% respectively and higher rates were recorded by Binh et al. (2000) Jamali et al. (2014) with 24.8%, 78.5% respectively. Tsai and Hsiang (2005) demonstrated that ducklings younger than 2 weeks of age had a significantly higher *Salmonella* prevalence rate than other age groups.

The high frequency of *Salmonella* recovery from imported day-old ducklings causes great concern because of the zoonotic potential of this pathogen and its economic importance to commercial poultry breeding Ribeiro et al. (2006). In this study the prevalence of *Salmonella* in imported one-day old duckling was low comparing with other researchers results as Ribeiro et al. (2006) and Myint (2004). While the prevalence rate in local duckling with 7.5% which was higher than the imported duckling, this result is agree with that of El-Tawab et al. (2015) who isolated *Salmonella* from local duckling with 9.6% and differed from results of Osman et al. (2014) in which prevalence of imported was 18.5% and in local was 12%.

The present study revealed that there were 10 different serovars recovered from 10 *Salmonella* isolates as following S.Jedburgh, S.Harrisonburg, S.Braenderup, S.Southbank, S.Sekondi II, S.Sincler, S.Brandenburg from imported duckling and S.Ruzizi, S.Give and S.Entertidis from local duckling with 0.5% for each. Most of serotypes isolated by other researchers were S.Braenderup and S. Enteritidis. First S. Enteritidis was nearly the same as reported Osman et al. (2014) with 2.2% (3/135) from imported ducklings and 2.7% (2/75) from domestic duckling, Abdallah et al. (2015) with 0.31% and higher as mentioned by Gong et al. (2014) with 13.4, Doosti et al. (2016) with 43.6%. Second S.Braenderup which was higher results in Adzitey et al. (2012) with 12% and Nor Faiza et al. (2013) with 50%.

The most resistance rate was for trimethoprim with 80% and highly sensitive with 100% for norfloxacin followed by doxycycline, nalidixic acid and ciprofloxacin with 90%. Among *Salmonella* serotypes S.Braenderup and S.Brandenburg were the most multidrug resistant serotype with 50% followed by S.Harrisonburg and S.Give with 37% while S.Entertidis was sensitive for all 8 antimicrobial
agents. The results agree with other researcher’s results as Mondal et al. (2008) who found that duck isolates were highly sensitive for ciprofloxacin and nalidixic acid and Badr et al. (2015), who reported that Salmonella isolates were highly sensitive to gentamycin, amoxicillin clavulanic acid, norfloxacin with 100% and disagree with Doosti et al. (2016), who found Salmonella isolates sensitive to sulfa-methoxazole trimethoprim (77.6%) and high resistance to amoxicillin clavulanic acid (67.4%) and for nalidixic acid with (87.0%) and Carraminana et al. (2004) found that no isolates were resistant to trimethoprim-sulfamethoxazole, ciprofloxacin. The high levels of resistant isolates reported in many publications may be due to the worldwide overuse of antimicrobials in different fields, which has placed enormous pressure on the selection of antimicrobial resistance among bacterial pathogens and endogenous microflora (Capita et al. (2007).

The data recorded in this study revealed that stn gene is detected in all tested Salmonella strains with 100% and this result agree with Murugkar et al. (2003) who found that stn gene is widely distributed among Salmonella irrespective of the serovars and the source of isolation. It is a target gene to explore the possibility of direct detection of Salmonella from samples from biological sources. The data recorded in this study revealed that aadB gene was detected only in 3 Salmonella strains which were S. Braenderup, S. Ruzizi and S. Give and was absent in other Salmonella strains. The result was higher as reported by Ahmed et al. (2009) and Ibraheem (2015) with 90% and 91.7% respectively. In This study the prevalence of aadB resistance gene in tested Salmonella isolates genotypically correlated with the phenotypic resistance of all isolates phenotypic resistant for gentamicin and this result disagree with Ibraheem (2015), who found that 12 Salmonella isolates from chicken have aadB gene and 8 of them were phenotypic resistance against gentamicin and agree with Randall et al. (2004) who found that 2 gentamicin-resistant strains contained the aadB gene. It was concluded that ten different Salmonella species were recovered with total prevalence 5% and the most resistance rate was for trimethoprim and the lowest for enrofloxacin and stn was found in all sample while aad B only found in 3 serotype.

References


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الملخص العربي

مقارنة بين الطرق المختلفة للكشف عن ميكروب السالمونيلا في البط المستورد والمحللي

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** العمل المرجعي للرقابة البيطرية على المنتج الداجنى بالدقى *** طبيبة بيطري

تم تجميع 200عينة من صغار البط المستورد والبط المحلي من مزارع مختلفة لفحصها بباكتريولوجيا للكشف عن السالمونيلا تضمنت 160 عينة من البط المستورد بعمر يوم واحد و40 عينة من البط المحلي باعمار تتراوح بين 10 ايام و14 يوم. وكانت نسبة العزل بعد استخدام الطريقة التقليدية للعزل وتاكيدها بالاختبارات البيوكيميائية كانت نسبة العزل الكلية 5% بنسبة 4.375% من البط المستورد و 7.5% من البط المحلي. كما أظهرت النتائج السيرولوجية عشرين عطرات مختلفة من ميكروب السالمونيلا وكانت كالاتي: S.Jedburgh، S.Harrisonburg، S.Brandenburg، S.Sinew، S.Sekondi، S.Southbank، S.Braenderup، S.Give، S.Ruzizi السالمونيلا إنترتيديس من البط المحلي بنسبة 0.5% لكل عطرة.

تم دراسة العزلات التي تم الحصول عليها في المختبر لأنماط الحساسية المضادة للميكروبات من خلال طريقة الإفزاع. وقد وجد أن كل عزلات السالمونيلا كانت حساسة للنورفلوكساسين بنسبة 100% والتي يمكن استخدامها كدواء مفضل لعلاج وفي الوقت نفسه كانت 80% من عزلات السالمونيلا مقاومة للميثوبريم وبسبب أن جين stn هو جين متواجد في كل أنواع سالمونيلا إنترتيديس بغض النظر عن نوع العطرة المراد الكشف عنها وبالتالي تم استخدامه كطريقة للكشف عن وجود السالمونيلا في العينات المختبرة باستخدام تقنية الجينية الحيوية كجانب الضرورة في جميع عزلات السالمونيلا. وباستخدام تقنية الجينية الحيوية للكشف الحجيني الخاص مقاومة المضاد S.Braenderup الجيني جنتاميسين aadB الحيوي. كانت إيجابيا فقط في ثلاث عزلات S.Give و S.Brandenburg.

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