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. Only10 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.*SekondiII, .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 % and100% sensitive for norfloxacin. Based on PCR, all examined *salmonella* were positive100 %(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 humanSalmonellosis caused by contact with ducks have been reported in some countries, suchas Australia, United States, UnitedKingdom 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:***

Target gene	Primer Sequence 5`- 3`	Amplified product	Reference
aadB	**F.**GAGCGAAATCTGCCGCTCTGG	319 bP	**Frana *et al.* (2001)**
	R.CTGTTACAACGGACTGGCCGC		
stn	F. TTG TGT CGC TAT CAC TGG CAA CC	617 bP	**Murugkar *et al.* (2003)**
	R.ATT CGT AAC CCG CTC TCG TCC		

2.5. 4.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:***

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension	Reference
aadB	94˚C 5 min.	94˚C 30 sec.	58˚C 30 sec.	72˚C 30 sec.	35	72˚C 10 min.	Frana *et al*., 2001
stn	94˚C 5 min.	94˚C 30 sec.	59˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.	Murugkar *et al*., 2003

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%).

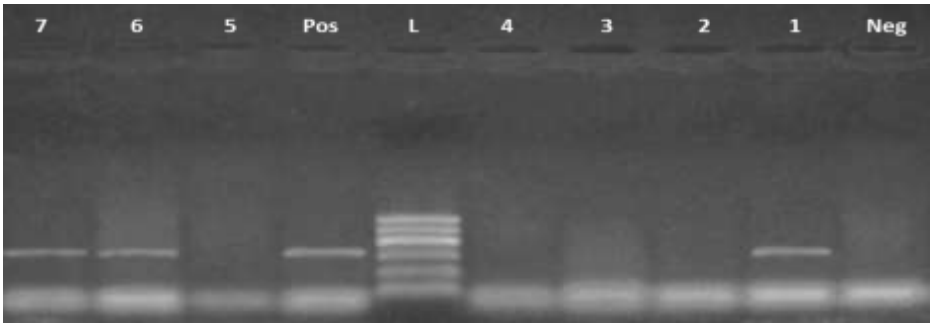

photo (1): Agarose gel electrophoresis with positive PCR amplification of (**319bp**) fragment of antibiotic resistance ***aad*B** gene from DNA of positive**(1,6,7)** *Salmonella* isolates

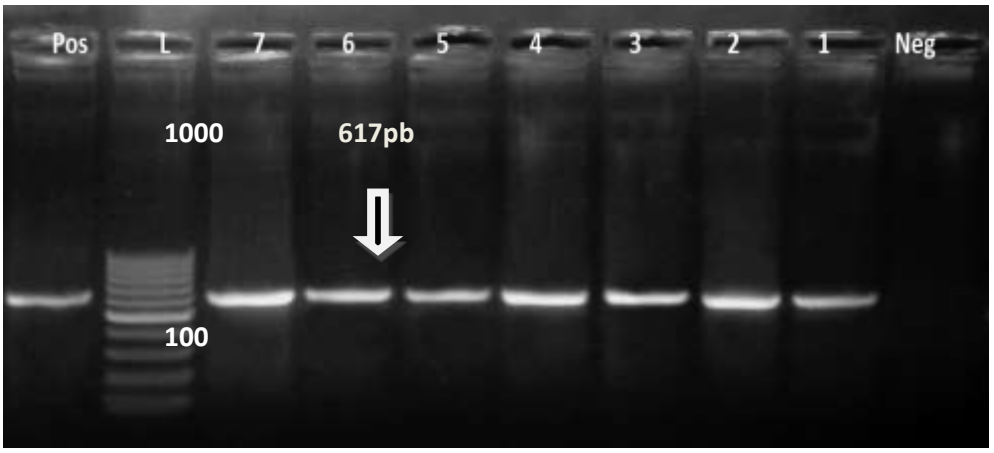

Photo (2): Agarose gel electrophoresis with positive PCR amplification of (**617 bp**) fragment of virulence gene ***stn*** from DNA of 7 positive *Salmonella* isolates.

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 Abdallahet 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 serovares recovered from 10 *Salmonella* isolates 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.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, ***Abdallahet 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 *aad*B 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 *aad*B 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 *aad*B 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 trimethoprime and the lowest for enrofloxacin and stn was found in all sample while *aad* B only found in 3 serotype.

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الملخص العربي

مقارنه بين الطرق المختلفه للكشف عن ميكروب السالمونيلا في البط المستورد والمحلي

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** المعمل المرجعي للرقابة البيطرية على الانتاج الداجنى بالدقى-*** طبيبةبيطرية

تم تجميع 200عينة من صغار البط المستورد والبط المحلي من مزارع مختلفة لفحصها بكتيريولوجيا للكشف عن السالمونيلا تضمنت 160 عينه من البط المستورد بعمر يوم واحد و40 عينه من البط المحلي باعمار تتراوح مابين 10 ايام و14 يوم . وكانت نسبه العزل بعد استخدام الطريقه التقليديه للعزل وتاكيدها بالاختبارات البيوكيميائيه كانت نسبه العزل الكليه 5% بنسبه 4.375% من البط المستورد و7.5 % من البط المحلي .كما أظهرت النتائج السيرولوجيه عشره عترات مختلفه من ميكروب السالمونيلا وكانت كالاتيS.Jedburgh:، S.Harrisonburg، S.Braenderup، S.Southbank، S.Sekondi الثاني، S.Sinchew، S.Brandenburg من البط المستورد و S.Ruzizi، S.Giveوسالمونيلاإنتيرتيديس من البط المحلي بنسبه 0.5٪ لكل عتره .تم دراسة العزلات التي تم الحصول عليها في المختبر لأنماط الحساسية المضادة للميكروبات من خلال طريقة الاقراص .وقد وجد أن كل عزلات السالمونيلا كانت حساسه للنورفلوكساسين بنسبه 100 ٪ والتي يمكن استخدامها كأدوية مفضلة للعلاج وفي الوقت نفسه كانت 80٪ من عزلات السالمونيلا مقاومة للميثوبريم وبسبب ان جين *stn*هو جين متواجدفي كل انواع سالمونيلا إنتيريكا بغض النظر عن نوع العتره المراد الكشف عنها وبالتالي تم استخدامه كطريقه للكشف عن وجود السالمونيلا في العينات المختبره باستخدام تقنية الجزيئية الحيويه كان الجين الضراوة *stn* إيجابية في جميع عترات السالمونيلا .وباستخدام تقنية الجزيئية الحيويه للكشف الجين الخاص بمقاومه المضاد الحيوي جنتاميسين *aad*B كان إيجابيا فقط في ثلاث عتراتS.Braenderup وS.Brandenburg وS.Give. وهذا توافق لاختبار الحساسيه للمعزولات في المعمل.