Bacteriological studies on native and imported apparently healthy one day old chicks

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Abstract

One thousands and eighty one day old chicks were examined for isolation of different bacteria. *Salmonella* was detected in 11.7% in native chicks while 5.2% among imported chicks, *E. coli* was isolated from 17.6% in native chicks while 23.6% in imported, *Staph aureus* was present in 29.4% in native and 5.2% in imported chicks. Serological typing of *Salmonella* was *Salmonella* Norwich, *Salmonella* Wilhelburg in native chicks while in imported chicks were *Salmonella* Brancoster, *Salmonella* Sekondi II. *E .coli* sero-typing reveled O125, O153, O86a in native chicks while in imported chicks were O26, O78, O36, O15, O124, O169, O6, O28 and one un-typeable strain. Seven *Staph aureus* isolates five from native and two from imported. Antibiogram of isolated bacteria was done. All *Salmonella* strains were sensitive to Gentamycin while all *E. coli* strains were sensitive to Amoxicillin + Clavulanic acid and Nitrofurantoin. All *Staph aureus* isolates were sensitive to Amoxicillin + Clavulanic acid.

Key words: one day chicks, *Staph aureus, Salmonella, E. coli*

Introduction

Salmonellosis is one of the most widespread food-borne zoonoses in industrialized as well as developing countries ***(Molla et al, 2003).*** Bacteria of genus *Salmonella* are members of the family *Enterobacteriaceae*. They are Gram-negative, facultative anaerobes and inhabit the intestinal tract of animals and may be recovered from a wide variety of hosts, specially poultry and swine, human, foods and environment. Besides, these bacteria may be pathogenic to wild and domestic animals and humans ***(Holt et al, 1994).*** Conventional culture methods used for the detection and isolation of *Salmonella* include, nonselective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspected colonies are then confirmed biochemically and serologically ***(Fakhr et al, 2006).***

Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic diseases in poultry. These diseases continue to cause economically

significant losses in many nations and absorb a large investment of resources in testing and control efforts in others. Infected poultry flocks are also among the most important reservoirs of *Salmonellae* that can be transmitted through the food chain to humans. ***(Gast, 2008)*** *Escherichia coli* is bacteria of genus *Escherichia* which is member of the family *Enterobacteriaceae*, which is composed of organisms that can grow aerobically or anaerobically and utilize simple carbon and nitrogen sources, Gram-negative, non-acid-fast, uniform staining, non-spore-forming bacillus, usually 2–3 0.6 µm. Organisms grown in culture are more variable in size and shape. ***(Barnes et al, 2008).*** It is the most important agent causing secondary bacterial infection in poultry and may also be a primary pathogen ***(Gross, 1994).*** Colibacillosis is the most frequently reported disease in surveys of poultry diseases or condemnations at processing ***(Barnes et al, 2008).***
Staphylococcal infections are a worldwide problem in chickens and turkeys and cause economic losses due to decreased weight gain, decreased egg production, and condemnation of carcasses at slaughter. The general term *staphylococcus* refers to the morphology of these microorganisms; in stained smears, gram-positive, coccoid in shape, and it often found resemble clusters of grapes when grown on solid media. ***(Andreasen, 2008).*** They may occur in short chains bacterial antimicrobial drug resistance is a worldwide problem that is exacerbated by the diminishing number of new antimicrobial drugs in the pharmaceutical pipeline ***(Talbot et al, 2006 and Okonkoet al, 2009)*** and the effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections ***(Nawaz et al, 2009).*** This work was carried out in order to assess the occurrence of *Salmonella, E. coli* and *Staph aureus* in apparently healthy normal one day old chicks.

Materials and methods

Bacterial Isolation

A total of 1080 different samples from (internal organs (liver, heart, lung, yolk) and paper lining chick boxes) were collected 570 from imported chicks and 510 from native chicks which were submitted to reference laboratory for veterinary quality control on poultry production from 2013- 2014. Samples which were taken from chicks were (internal organs and paper lining chicks' boxes) All samples were examined bacteriologically for presence of *Salmonella, E .coli and Staphylococcus.* Isolation and Identification of *Salmonella, E .coli* and *Staphylococcus* was done according to standard methods ***(ISO 6579:2002; Lee and Arp 1998 and ISO 6888-1:2003)*** respectively.

Serological identification of *Salmonella* was done according to ***(Popoff, 2001)*** and serological typing of E.coli was carried out according to ***(Lee et al, 2009)*** by using Known antisera for each organism (Sifin).

Antibiotic sensitivity test

The antibiogram of Bacterial Isolates were done by disc-diffusion test for isolates *Salmonella* and *E. coli* against 10 antibiotics Amoxicillin + Claviolinic acid, Tetracycline, Streptomycin, Ciprofloxacin, Norfloxacin, Trimethoprim - sulfonamethoxazole (SXT), Gentamycin, Nalidixiic acid, Nitroforintin and Chloramphenicol while *Staph aureus* tested against 11 antibiotics (Oxoid) Penicillin, Amoxicillin + Claviolinic acid, Tetracycline, Ciprofloxacin, Norfloxacin, trimethoprim - sulfonamethoxazole (SXT), Doxycycline, Gentamycin, Chloramphenicol, Amikin, Ofloxacin and Erythromycin, according to the Clinical and Laboratory Standards Institute/ Formerly National Committee for Clinical Laboratory Standard ***(CLSI/NCCLS, 2009)***. Briefly, 3-5 pure bacterial colonies were selected and put on 2 mL Muller Hinton broth in test tube. The test tubes were incubated at 37°C for slight turbidity compared against 0.5 McFarland tube. Muller Hinton agar plate was inoculated with previously prepared culture using sterile bacterial cotton swab in three different directions. Then the antibiotic disks were distributed on the inoculated plate. The plate was incubated in 37°C for 24 hrs as previously described. Inhibition zones were measured to detect the resistant strain.

Table (1) *the break point of Enterobacteriacae according to (CLSI/NCCLS, 2009).*

Antimicrobial Discs	code	Disc Potency Mg/disc	Interpretation		
			Zone diameter (mm)		
			Sensitive ≥	Intermediate	Resistant ≤
Amoxicillin + Clavulinic acid	Am+CL	10-20 µg	18	14-17	13
Chloramphenicol	C^{30}	30 µg	18	13-17	12
Ciprofloxacin	CF^{5}	5 µg	21	16-20	15
Gentamicin	G^{10}	10 µg	15	13-14	12
Nalidixic acid	NA^{30}	30 µg	19	14-18	13
Nitrofurantoin	F300	300 µg	17	15-16	14
Norfloxacin	NX^{10}	gµ10 µg	17	13-16	12
Streptomycin	S^{10}	10 µg	15	12-14	11
Trimethoprim-sulfamethoxazole	SXT	23.75 µg	16	11-15	10
Tetracycline	T^{30}	30 µg	15	13-15	11

Table (2) *the break point of Staph. aureus (CLSI/NCCLS, 2009).*

Antimicrobial Discs	code	Disc Potency Mg/disc	Interpretation		
			Zone diameter (mm)		
			Sensitive ≥	Intermediate	Resistant ≤
Amikacin	Ak^{10}	30 µg	17	15-16	14
Amoxicillin + Clavulinic acid	Am+CL	10-20 µg	20	15-18	19
Chloramphenicol.	C^{30}	30 µg	18	13-17	12
Ciprofloxacin.	CF^{5}	5 µg	21	16-20	15
Doxycycline.	DO^{30}	30 µg	16	13-15	12
Erythromycin	E^{15}	15 µg	23	14-22	13
Gentamicin.	G^{10}	10 µg	15	13-14	12
Norfloxacin.	NX^{10}	10 µg	17	13-16	12
Penicillin	P^{10}	10 I.U.	29	-	28
Tetracycline.	T^{30}	30	19	15-18	14
Trimethoprim-sulfamethoxazole	SXT	23.75 µg	16	11-15	10

Results

Salmonella were isolated from native chicks 11.7% (2 isolates) while 5.2% (2 isolates) among imported chicks.

E. coli by 17.6% (3 isolates) in native chicks while 23.6% (9 isolates) in imported.

Seven samples were coagulase positive *Staph aureus* by 29.4% (5 isolates) in native and 5.2% (2 isolates) in imported chicks.

Serological identification:

Serological identification revealed different serovars of *Salmonellae* as *Salmonella* Norwich and *Salmonella* Wilhelmburg were isolated from native chicks and among imported chicks were *Salmonella* Brancaster *and Salmonella* Sekondi II. (Table 3)

E. coli serogroups from native chicks were O125, O153, O86a, while O26, O78, O36, O15, O124 O169, O6, O28 and one un-typeable strain were obtained from imported chicks.

Antibiotic sensitivity test

Salmonella

sensitivity of 4 *Salmonella* strains to Norfloxacin, Gentamycin, Amoxicillin + Clavulanic acid, Chloramephenicol, Tetracycline and Trimethoprim-sulfamethoxazole, Nitrofurantoin, Ciprofloxacin and Nalidixic acid were 100%, 100%, 75%, 75%,50%, 50%, 50%, 25% and 25% respectively. Theses strains showed intermediate resistance to Ciprofloxacin and Amoxicillin + Clavulanic acid by 25% for each of both. The resistance was 100%, 75%, 50%, 50%, 50%, 50% and 25% to Streptomycin, Nalidixic acid, Ciprofloxacin, Tetracycline, Trimethoprim-sulfamethoxazole, Nitrofurantoin and Chloramephenicol respectively. (Table 4)

E. coli

Sensitivity of 12 *E. coli* strains to Amoxicillin + Clavulanic acid, Nitrofurantoin, Chloramephenicol, Norfloxacin, Gentamycin, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Nalidixic acid, Tetracycline and Streptomycin by 100%, 100%, 66.6%, 66.6%, 58.4%, 50%, 50%, 41.6%, 25% and 25% respectively. While showed intermediate resistance to Tetracycline, Ciprofloxacin, Nalidixic acid, Streptomycin and Gentamycin by 33.3%, 16.6%, 16.6%, 16.6% and 8.3% respectively. Resistance was to Streptomycin, Trimethoprim-sulfamethoxazole, Nalidixic acid, Tetracycline, Ciprofloxacin, Chloramephenicol, Gentamycin, Norfloxacin, by 58.3%, 50%, and 41.6%, 41.6%, 33.3%, 33.3%, 33.3% and 33.3% respectively. (Table 5)

Staph aureus

Sensitivity of 7 *Staph aureus* strains to Amoxicillin + Clavulanic acid, Ofloxacin, Norfloxacin, Amikin, Ciprofloxacin, Gentamycin, Doxycycline, Penicillin, Tetracycline, Trimethoprim-sulfamethoxazole and Erythromycin by 100%, 85.7%, 85.7%, 85.7%, 85.7%, 85.7%, 57.15%, 57.15%, 57.15%, 42.85% and 14.3% respectively. Strains produced intermediate resistance to Doxycycline, Gentamycin, Tetracycline, Ciprofloxacin, Trimethoprim-sulfamethoxazole and Norfloxacinby 28.55%, 14.3%, 14.3%, 14.3%, 14.3% and 14.3%. The strains revealed resistance to Erythromycin, Trimethoprim-sulfamethoxazole, Penicillin, Tetracycline, Amikin, Doxycycline and Ofloxacin by 85.7%, 42.85%, 42.85%, 28.55%, 14.3%, 14.3% and 14.3%. (Table 6)

Table (3): *Serotyping of Salmonella Sp. (Popoff 2001)*

Salmonella Sp.	Key
Salmonella Norwich	Somatic : O6, O7 Flagler first : e , h Second: 1 , 6
Salmonella Wilhelmburg	Somatic : O1, O4, [O5], O12, O27 Flagler first : z_{38} Second: [e,n,z_{15}]
Salmonella Brancaster	Somatic : O3, O10 Flagler first : e, n, x Second: 1, 7
Salmonella Sekondi II.	Somatic : O1, O4, O12,O27 Flagler first : z_{29} Second: ----

Table (4) *Results of antibiotic sensitivity test of Salmonella isolates*

antimicrobial Discs	Sensitivity of *Salmonella* isolates n = 4					
	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Norfloxacin	0	0%	0	0%	4	100%
Gentamicin	0	0%	0	0%	4	100%
Amoxicillin + Clavulinic acid	0	0%	1	25%	3	75%
Chloramphenicol	1	25%	0	0%	3	75%
Tetracycline.	2	50%	0	0%	2	50%
Trimethoprim-sulfamethoxazole	2	50%	0	0%	2	50%
Nitrofurantoin	2	50%	0	0%	2	50%
Ciprofloxacin	2	50%	1	25%	1	25%
Nalidixic acid	3	75%	0	0%	1	25%
Streptomycin	4	100%	0	0%	0	0%

Table (5) *Results of antibiotic sensitivity test of E. coli*

antimicrobial Discs	Sensitivity of *E. coli* isolates n = 12					
	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Amoxicillin + Clavulinic acid	0	0%	0	0%	12	100%
Nitrofurantoin	0	0%	0	0%	12	100%
Chloramphenicol	4	33.3%	0	0%	8	66.6%
Norfloxacin	4	33.3%	0	0%	8	66.6%
Gentamicin	4	33.3%	1	8.3%	7	58.4%
Ciprofloxacin	4	33.3%	2	16.6%	6	50%
Trimethoprim-sulfamethoxazole	6	50%	0	0%	6	50%
Nalidixic acid	5	41.6%	2	16.6%	5	41.6%
Tetracycline.	5	41.6%	4	33.3%	3	25%
Streptomycin	7	58.3%	2	16.6%	3	25%

Table (6) *Results of antibiotic sensitivity test of Staph aureus*

Antimicrobial Discs	Sensitivity of *Staph aureus* isolates n = 7					
	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Amoxicillin + Clavulanic acid	0	0%	0	0%	7	100%
Ofloxacin	1	14.3%	0	0%	6	85.7%
Norfloxacin	0	0%	1	14.3%	6	85.7%
Amikin	1	14.3%	0	0%	6	85.7%
Ciprofloxacin	0	0%	1	14.3%	6	85.7%
Gentamycin	0	0%	1	14.3%	6	85.7%
Doxycycline	1	14.3%	2	28.55%	4	57.15%
Penicillin	3	42.85%	0	0%	4	57.15%
Tetracycline	2	28.55%	1	14.3%	4	57.15%
Trimethoprim-sulfamethoxazole	3	42.85%	1	14.3%	3	42.85%
Erythromycin	6	85.7%	0	0%	1	14.3%

4. Discussion

Salmonellosis is the most important foodborne disease, in both animals and man ***(Brenner et al, 2000),*** causing over 1400 human cases a year in the USA ***(Mead et al, 1999).*** *Salmonella enterica* is a zoonotic species that can acquire its resistance in livestock that resulting animal food products are important vectors for the transfer of resistant bacteria from animals to humans ***(Majtaá-novaá et al, 2010).***

Both *E. coli* and *Staph aureus* are of the most important avian bacterial diseases ***(RAJI et al. 2003 and MAMZA et al, 2010)*** in Nigeria. *E. coli* is the most common avian bacteria in the intestine and it causes huge economic losses in poultry industry. Bacterial organisms of the genus *Staphylococcus* are one of the most prevalent pathogens in both humans and animals ***(Casey et al, 2007 and Suleiman et al, 2013).***

Salmonella, E. coli and *Staph. aureus* were isolated from apparently healthy one day old chicks in this study which was similar to ***Liu et al (2010)*** who collect 550 samples from five chicken farms in Shanghai during March 2005 to October 2006.

In this study *E. coli, Salmonella, Staph. aureus* were isolated from internal organs of one day old chicks (yolk , heart , lung and liver) and *E. coli* was the most predominant isolate followed by *Staphylococcus aureus* then *Salmonella* this was comply with ***(Amare et al, 2013)***.

In this study *Salmonella* Spp. was isolated by 11.7% and this disagree with ***(Osman et al, 2010)*** who

Isolate the *Salmonella* Spp. from one day old chicks by 23.3%. The four *Salmonella* strains were isolated from the internal organs and paper-lining chick boxes of one day old chicks which corresponds with ***(Osman et al, 2010)*** who isolated *Salmonella* isolated from the same sites. The four strains were *Salmonella* Wilhelmbur, *Salmonella* Norwich, *Salmonella* Sekondi Π and *Salmonella* Brancaster) were differ from the *Salmonella* strains which isolated by ***(Osman et al, 2010)*** who isolated *Salmonella* Newport, *Salmonella* Kentucky, *Salmonella* Enteritidis. *Salmonella* Shubra, *Salmonella* Saintpaul and *Salmonella* Agona were isolated. Also ***(Volkova et al,2011)*** reported that the flocks were hatched at sever broiler hatcheries, the mean within flock prevalence of *Salmonella* positive samples was 6.5% and ranged from 0% to 86.7% of the 65 flocks studied 25 (38.5%) had at least one *Salmonella* positive sample.

In the present investigation all *Salmonella* strains were sensitive to Gentamyicin and Norfloxacin by 100% similar results as ***(Balala et al, 2006)*** and Amoxaicillin + Clavinilic acid (75%) which is in agreement with ***(Habrun et al, 2010).*** In addition to Chloramphenicol (75%), while was resistant to Streptomycin (100%), Naladixic acid 75%, Tetracycline (50%), Ciprofloxacin (25%), and Nitrofurntion (25%) which is in accordance with ***(Anyanwu et al, 2010)*** who reported that the pure isolate of *Salmonella* paratypi was sensitive to Ciprofloxacin , Kanamycin, Chloramphenicol , Gentamycin, also resembling to ***(Liu et al, 2010)*** who reported that all *Salmonella* isolates were sensitive to Gentamycin.

In this study *E. coli* was isolated by 17.6% from one day old chick and this results was nearly agree with ***(Khalil and Einas El-Shamy, 2012)*** who isolate *E. coli* by 19% from one day old chicks and not compatible with the result obtained with ***(Roshdy et al, 2012)*** who isolated *E. coli* by 28.7% from one day old chicks**.** Different isolates of *E. coli* strain were isolated in this work including (O125, O153, O86a) from native chicks while (O26, O78, O36, O6, O28, O124, O15, O169, untypeable) were isolated from one day old imported chicks, which is in accordance with previously reviewed by ***(Roshdy et al, 2012)*** who isolated O78 and O125 from chicken but also they demonstrated different strains such as (O44,O158,O114,O111,O103,O142 ,O26,O127 and O164)

Moreover *E. coli* strains in this work showed 100% sensitivity to Nitrofurntion which is as recorded by ***(Fatma et al, 2012),*** Amoxaicillin + Clavinilic acid (100%) which is nearly similar to ***(Csaba et al, 2008)***, highest percent of resistance is to Streptomycin (58.3%), Trimethoprim-

sulfamethoxazole (50%) which is in accordance with ***(Salehi and Bonab 2006; Habrun et al, 2010)*** and also high percent of sensitivity to Chloramphenicol 66.6% to Gentamyicin(58.3%) and Norfloxacin 66.6%, Ciprofloxacin (50%), Trimethoprim-sulfamethoxazole (50%), Naldixic acid (41.6%) which is complying with was resistant to Tetracycline and Streptomycin (25%),which nearly similar to **(Rashid et al, 2013)** isolated *E. coli* from One day old chicks of selected Breeder farm in Bangladesh and this isolates were sensitive to Enrofloxacin and Ciprofloxacin but resistant to Cloxacillin , Nalidaxic acid and Erythromycin .but. isolated *E. coli* strain in this work was sensitive to Ciprofloxacin (50%) and Naldixic acid (41.6%). Also agree with **(Anyanwuetal, 2010)** who reported that the pure isolate of *E. coli* was sensitive to Ciprofloxacin, Kanamycin, Chloramphenicol, and Gentamycin**.**

In this study Coagulase positive *Staph. aureus* was isolated by 29.4% and this results was nearly agree with**(Khalil and Einas El-Shamy, 2012)** who isolate *Staph. aureus* by 20% from one day old chicks. Coagulase positive *Staph. aureus* strain in this study was sensitive to Amoxaicillin + Clavinilic acid (100%), and Gentamicin, Amikin, Ciprofloxacin, Ofloxacin, Norfloxacin all are 85.7% for each one, Trimethoprim-sulfamethoxazole was (42.8%) which is complying with (**Suleiman et al, 2013)** who reported that *Staph. aureus* strains were susceptible to Ciprofloxacin and Gentamycin but disagree with our study in mentioned that *Staph. aureus* was resistant to Gentamycin. Higher percent of sensitivity to Ciprofloxacin and Gentamicin which resembles to **(Otalu et al, 2011)**. Higher percent of resistance to Erythromycin and Penicillin has been found which is in accordance with who reported that large proportion of *Staph aureus* isolates from the transportation container were resistant to, Penicillin G and Erythromycin **(Daka et al, 2012)**.

In this investigation all Coagulase positive *Staph. aureus* strains were sensitive to Amoxaicillin + Clavinilic acid which agree with **Losito et al, 2005** while highest percent of resistance to Erythromycin and Penicillin similar to (**Otalu et al, 2011).**

In conclusion *Salmonella, E.coli* and *Staph aureus* can be isolated from apparently health one day old chicks and antibiotic resistance strains can transmit from parents to chicks through eggs.

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الملخص العربي

دراسات بكتريولوجيه على الكتاكيت المحليه و المستورده السليمه ظاهريا سن يوم

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تم فحص ١٠٨١ كتكوت عمر يوم و عزل انواع مختلفه من البكتريا . و قد تم عزل ميكروب السالمونيلا فى الكتاكيت المحليه بنسبه ١١,٧% فى حين ان كانت نسه العزل فى الكناكيت المستورده هى ٥,٢% و ايضا تم عزل ميكروب الايشيريشيا كولاى بنسبه ١٧,٦% فى الكتاكيت المحليه و بنسبه ٢٣,٦% فى الكتاكيت الستورده و تم عزل ميكروب العنقودي الذهيبى بنسبه ٢٩,٤% في الكتاكيت المحليه و ٥,٢% فى الكتاكيت المستورده . وكانت نتيجه التصنيف السيرولوجي للسالمونيلا فى الكتاكيت المستورده هى *Salmonella* Brancoster, *Salmonella* Sekondi II بينما فى الكتاكيت المحليه كانت *Salmonella* Norwich, *Salmonella* Wilhelburg . و كانت نتيجه التصنيف السيرولوجى لعترات الاشيرشيا كولاي هى O125, O153, O86a فى الكتاكيت المحليه بينما كانت فى الكتاكيت المستورده O26, O78, O36, O15, O124, O169, O6, O28 و عتره واحده غير مصنفه. ايضا تم عزل سبع عترات من الميكروب العنقودي الذهبى خمسه من الكتاكيت المحليه و اثنين من الكتاكيت المستورده وقد تم اجراء اختبار الحساسيه للمضادات الحيويه و كانت جميع عترات السالمونيلا حساسه للجنتاميسين فى حين كانت جميع عترات الاشيريشيا كولاي حساسه للاموكسيسيلين +حمض الكلافولينك و نيتروفورانتين. و كانت جميع عترات الميكروب العنقودى الذهبى حساسه للاموكسيسيلين +حمض الكلافولينك.
بى حساسه للاموكسيسيلين +حمض الكلافولينك.