Research Article

Wide Prevalence of Critically Important Antibiotic Resistance in Egyptian Quail Farms with Mixed Infections

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Abstract | Quail meat and egg production represent a promising source to cover the deficit in animal protein in developing countries including Egypt. However little is known about the prevalence and antibiotic resistance of major bacterial pathogens such as *Escherichia Coli, Staphylococcus aureus Salmonella* and *Pasteurella spp*. in Egyptian quail farms. Such information is important for drug choice and success of treatment as well as spotting the light on emerging antimicrobial resistance that represent major concern for public health. A total one hundred swabs and 500 organ samples were collected from apparently healthy and freshly dead quails respectively. Bacterial isolation and characterization were performed in accordance with the clinical laboratory standards and confirmed by PCR. In life birds, only *E.coli* and *Salmonella* could be recovered from Cloacal swabs, while in freshly dead birds all four pathogens disseminated in various organs with higher incidence of mixed compared to single infection. Different serotypes of *E.coli* and *Salmonella* could be recovered from dead birds however *E.coli* (O78) and *S.enteritidis* were recovered mainly from heart and liver. The recovered *E.coli*, *S.aureus* and *P. haemolytica* isolates recovered from mixed infection cases showed (57.1-100%) resistance to highly important antibiotic group (Doxycycline, Tetracycline, Trimethoprim sulfa methoxazole and Chloramphenicol) and showed dissimilar pattern of resistance to critical important antibiotic group. *Salmonella* isolates showed antibiotic resistance to Nalidixic acid (100%) and Nitrofurantoin (42.9%). Strict biosecurity measures are required to reduce the incidence of mixed bacterial infection and subsequently reduce the spread of antibiotic resistance genes between bacterial spp.

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Introduction

Quails are small birds that belong to the family of Phasianidae. Commercially, they are reared for meat and egg production. Generally, it has similar nutritional value as broilers but, interestingly, the layers quail breeds (either after finish the laying periods or male sexed wrongly) of higher rating in term of taste (Da Cunha, 2009). Globally, the commercial farming of quails is increasing day by day as it requires low investment for rearing and maintainsrapid growth rate (marketing within 4-5weeks), early onset of laying (6-7 weeks), relatively low feed intake and high reproduction rate (Albino and Barreto, 2003; Murakami and Ariki, 1998; Santos et al., 2011).

Currently, China, France, Spain, Italy, and USA are the major player in Quail production. Howev-

er, Egypt has one of the fast growing quail industry worldwide (Da Cunha, 2009). During the last decade, quail meat production has been experiencing growth in the Egyptian poultry sector to satisfy the growing demand for animal protein in Egypt (El Nagar and Ibrahim, 2007; Yusuf et al., 2016).

Quails are susceptible to most infections that affect domestic poultry but comparatively more resistant to infection than chickens with proper management However more deaths recorded are due to managerial errors (Fraqueza et al., 2016) considering the fact that quail droppings produce far more ammonia than other poultry.

Several reports took into account isolation and characterization of bacterial pathogens such as *Pasteurella spp., E. coli, Streptococci, Staphylococci* , *Salmonella*, *Mycoplasma gallisepticum* from quail and quail products (Burns et al., 2003; Edwards, 1936; Kumar et al., 2001; Roy et al., 2006; Turgay et al., 2002; Wang et al., 2010). However, few reports referred to mixed infection with other microorganisms (Murakami et al., 2002) and their antibiotic sensitivity assay (Helm et al., 1999; Sultana et al., 2013).

It is widely accepted that any suggested program designed to prevent food borne diseases and/or dissemination of pathogens to the environment should start from the farm where the birds grow up. However, quail production is growing at moderate but steady pace in Egypt based on the growing demand for quail products (meat and eggs) among Egyptian consumers (El Nagar and Ibrahim, 2007) .Therefore, the aim of the present study was to investigate the prevalence of *Salmonella*, *E. coli, Pasteurella and Staphylococcus* pathogens in quail farms and their natural dissemination in various organs. In addition, the documentation of the presence and extent of antibiotic resistance in these pathogens which represent a potential hazard to human and other animal species.

Material and Methods

Samples collection

A total 600 samples were collected from 150 quails during early 2015 and early 2016 from 13 farms distributed in: Giza (n =3), PortSaid (n =4), Kafer El Sheikh (n =5),and Cairo (n =1). Cloacal and tracheal swabs were collected from 50 apparently healthy quails (100 swabs). While heart, liver, lung, intestine and bone marrow were aseptically and separately collected from 100 freshly dead birds (500 organs).

Present study was conducted in strict accordance with the recommendations of Ministry of agriculture and land reclamation and ministry of environment, Egypt for Experimental animal infection and sampling. The protocol of the study was reviewed and approved by committee of laboratory biosafety of reference laboratory for quality control on poultry production (RLQP). All bacterial isolation and characterization were performed in bacteriology unit at RLQP under strict control measures.

Bacterial isolation and confirmation

All collected samples were screened for the presence of *Pasteurella, Staphylococcus, Salmonella* and *E.coli.* Bacterial colonies were confirmed bacteriologically according to standard procedures previously described (Holt et al., 1994; ISO 6888-2:1999; ISO 6579:2002 and Lee and Arp (1998) genetically using conventional PCR. Briefly, DNA extracted from enriched suspected single colony using QIAamp DNA Mini Kit (Qiagen) according to manufacturer's instruction and amplified using EmeraldAmp Max PCR Master Mix (Takara, Japan), primers sequences and thermal profile as previously described by Hu et al. (2011) for *E .coli,* Mason et al. (2001) for *S. Aureus,* Oliveira et al. (2003) for *Salmonella* and Deressa et al. (2010) for *Pasteurella***.** Furthermore, *Pasteurella sp.* Isolates were confirmed by pathogenicity testing in mice as previously described by Holt et al. (1994). Briefly, 0.2 mL of overnight incubated broth of the suspected colony injected intra-peritoneal into 2 mice and heart blood from dead mice was smeared and stained with crystal violet for detection of *Pasteurella* bipolarity and re-isolation and identification of *Pasteurella sp*.

Bacterial serogrouping

Serogrouping of the *Salmonella* and *E. coli* confirmed isolates was done as previously described by Popoff and Le Minor (2001) and Lee et al. (2009) respectively.

Antimicrobial susceptibility testing

The susceptibility of the confirmed isolates to antimicrobial agents was determined via standard disk-diffusion method as described by Bauer et al. (1966) using the following antibiotic discs: Amoxicillin + Clavulanic acid. (Am+CL), Ciprofloxacin (CF), Norfloxacin(NX), Gentamicin (G), Tetracycline (T),

Table 3: *Prevalence of E.Coli serotypes in life and dead Quails, and dissemination in various organs.*

Birds	Samples	Isolates No.	Serogrouping	
			serotyped	Unserotyped
Life	Cloacal swabs	3	O 55	0
	Tracheal swabs	0	0	0
Dead	Liver	20	10(O125), 1(O20), 7(O127), 2(O78)	0
	Heart	15	4(O125), 1(O127), 2(O78), 6(O44)	2
	Lung	1	O125	0
	Intestine	10	8(O20)	2
	Bone marrow	1	O78	0

Table 1: *Prevalence of bacterial pathogens in life and freshly dead Quails, and the dissemination in the tested samples.*

birds	Samples	Number of isolates			
		Salmonella	*E. Coli*	Staph. aureus	pasteurella
Life	Cloacal swabs	11	3	0	0
	Tracheal swabs	0	0	0	0
Dead	Liver	23	20	30	25
	Heart	23	15	15	25
	Lung	0	1	20	13
	Intestine	3	10	0	0
	Bone marrow	5	1	5	2
total		65	50	70	65

Table 2: *Prevalence of single and mixed infection in the tested samples.*

Bacterial isolates	Single		mixed		Total	
	No.	%*	No.	%*	No.	%**
Salmonella sp.	35	53.4	30	46.6	65	26
E.coli	0	0	50	100	50	20
Staph. aureus	20	28.6	50	71.4	70	28
Pasteurella haemolytica	20	30.8	45	69.2	65	26

Trimethoprim sulfamethoxazole. (SXT), Chloramphenicol (C), Nalidixic acid (NA), Nitrofurantoin (F), Streptomycin (S), Penicillin (P), Doxycycline (DO), Erythromycin (E) and Amikacin (Ak) (Oxoid, Basingstoke, UK). The zone of inhibition measured and interpreted according to the recommendation of the disc manufacturer.

Statistical analysis

Data generated on the prevalence of different pathogens and their dissemination in various organs as a single or mixed infection and their resistance to different antibiotics analyzed using a chi-squared test to determine whether there were significant differences between single and mixed infection and resistance to various antibiotics ($P < 0{\cdot}05$).

Results and Discussion

Prevalence of bacterial pathogens in quail farms

Bacterial pathogens identified from the present study were recovered from 14 out of 100 collected samples from apparently healthy birds and 236 out of 500 samples collected from freshly dead birds. In details, in life birds only *Salmonella* and *E.coli* were recovered from 11 and 3 cloacal swabs respectively and no pathogens could be recovered from tracheal swabs (Table 1). While in freshly dead birds, *Salmonella*, *E.coli, S. aureus* and *Pasteurella haemolytica* were recovered from 54, 47, 70 and 65 samples respectively, with higher prevalence for all pathogens in liver and heart. From the lungs, mostly *S. Aureus* and *P. haemolytica* were recovered. Mostly *E.coli* followed by *Salmonella* was recovered from intestine (Table 2). Furthermore, our results revealed a higher incidence of mixed infection rather than single infection as mixed cases represent 100, 71.4 and 69.2% of the positive cases of *E.coli*, *S. aureus* and *P. haemolytica* respectively. While in cases of *Salmonella*, mixed infection represent 46.6% of the total positive cases with *Salmonella* (Table 2).

Prevalence of *Salmonella* and *E.coli* serotypes

Regarding *E.coli* serotyping; only O55 were recovered from life bird's cloacal swabs. On the other hand, O125, O20, O44, O127 and O78 were recovered from dead quail with presence of O78 only in liver and heart (Table 3).In contrast, 3 *Salmonella* serotypes were recovered from life birds namely *S. Agona, S. Senftenberg* and *S. Emek*. However, *S.enteritidis* were recovered only from dead bird's liver, heart and bone marrow (Table 4).

Table 4: *Prevalence of salmonella serotypes in life and dead Quails, and dissemination in various organs.*

Bird		Isolates No.	Serogroup (no. type)
Life	Cloacal swabs	11	3(S. Agona),5(S. Senftenberg), 3(S. Emek)
	Trachealswabs	0	0
Dead	Liver	23	5(S. Agona), 4(S.Senftenberg),7(S. Magherafelt, 2(S.Enteritidis), 2(S. Emek), 3(S. Kouka)
	Heart	23	7 (S. Agona), 5(S.Senftenberg), 5(S. Magherafelt), 2(S.Enteritidis), 1(S. Kouka), 3(S.Atakpam)
	Lung	0	0
	Intestine	3	3 (S. Agona)
	Bone marrow	5	2 (S. Agona),1 (S.Senftenberg), 2(S.Enteritidis)
Total		65	

Table 5: *Antimicrobial resistance percentages of Salmonella, E.coli, S.aureus and P. haemolytica isolates detected in life and freshly dead quails.*

	Antimicrobial Discs	Salmonella (%)	*E.coli* (%)	S.aureus (%)	P. haemolytica (%)
Critically Important	Amoxicillin + Clavulinic acid (Am+-CL $^{20-10}$)	28.6	71.4	N/A	58.5
	Ciprofloxacin (CF^{5})	14.3	57.1	0	41.5
	Norfloxacin (NX^{10})	0	14.3	0	27.7
	Gentamicin (G^{10})	0	14.3	57.1	27.7
	Nalidixic acid (NA^{30})	100	57.1	N/A	72.3
	Nitrofurantoin (F^{300})	42.9	28.6	N/A	73.8
	Streptomycin (S^{10})	14.3	71.4	N/A	84.6
	Penicillin (P^{10})	N/A	N/A	100	N/A
	Erythromycin (E^{15})	N/A	N/A	100	N/A
	Amikacin (Ak^{30})	N/A	N/A	71.4	N/A
Highly Important	Doxycycline (DO^{30})	N/A	N/A	100	N/A
	Tetracycline (T^{30})	0	71.4	57.1	100
	Trimethoprimsulfameth- oxazole. SXT$^{1.25-23.75}$)	14.3	100	85.7	100
	Chloramphenicol. C^{30}	28.6	85.7	57.1	100

Prevalence of antibiotic resistance in the recovered isolates

According to disc diffusion test, *Salmonella* isolates showed significant resistance to NA (100%) and F (42,9%). *E.coli* isolates showed resistance to Am+CL (71,4%), to CF (57,1%), T (71,4%), SXT (100%), C (85,7%), NA (57,1%) and S (71,4%). *S. aureus* isolates, showed 100 % resistance to P, E and DO. SXT (85,7%). 57,1% to G, T and C. 100% of *P. haemolytica* isolates were resistant to T, SXT and C, 84,6% to S, about 72,5% to NA and F and 58,5% to Amoxicillin and Clavulanic. (Table 5).

In light of the importance of quail production for the developing country as a measure of decreasing the problem of animal protein shortage (Shanaway, 1994) and the implemention of strategies for intensive quail production. The present study was designed to survey the incidence of four major bacterial pathogens in life and dead quails, their dissemination and antibiotic resistance profiles and to assess the risk for human consumers as well as in the choice of drugs and treatment.

In the present study, the dissemination of bacteria in several organs especially in liver and heart in the freshly dead birds suggest the onset of acute septicemic disease (Kabir, 2010) that could be the cause of the death. This agrees with the gross lesion findings in freshly dead birds which reveal the presence of septicemia, fibrinous pericarditis, perihepatitis, airsacculitis and in some cases showed enteritis. From the life

and apparently healthy quails, *Salmonella* and *E.coli* were isolated from the cloacal swabs without apparent clinical signs. Noteworthy, this asymptomatic carriers represent a potential source of human salmonellosis and colibacillosis (Behravesh et al., 2014; Kabir, 2010) due to an increasing infection pressure in the environment (Kabir, 2010). This has a correlation with the spread of antibiotic resistant bacteria and genes within the environment (Pruden et al., 2013).The relative higher incidence of *Salmonella* in apparently healthy quail is alarming comparing to the findings reported by McCrea et al. (2006) in the US and Dipineto et al. (2014) in Italy who found no *Salmonella* in life quail tested.

Modern housing facilities allow for a high density quail rearing but with inadequate biosecurity and poor hygienic measures on farms predisposes to the entrance, persistence and subsequently dissemination of various bacterial species as well as mixed infections (Burkholder et al., 2008; De Vylder et al., 2011; Kabir, 2010). Similarly, our finding showed that most of the positive cases were of mixed infections which highlighted the critical importance of the enhancement of hygienic and biosecurity measures in quail farms in Egypt.

E.coli O serotypes: O1, O2, O35 and O78 are reported to be frequently associated with colibacillosis therefore, suggesting that these serotypes harbor certain characteristic genetic features required for virulence mechanisms such as lipopolysaccharide, temperature-sensitive hemagglutination (Tsh), and increased serum survival factor (ISS) (Cloud et al., 1985; La Ragione and Woodward, 2002; Mellata et al., 2003; Vidotto et al., 1990). However, this suggestion was fundamentally flawed, because of the likelihood of colibacillosis to be associated with a wide range *of E.coli* rather than a single avian pathogenic *E.coli* (Collingwood et al., 2014). The correlation between O serogroup and their pathogenicity appear to vary widely geographically and temporally (Blanco et al., 1998; Frydendahl, 2002). This is in agreement with our findings , as there is no predominant serogroup recovered in both life nor dead birds. In the present study, the highest prevalent serotype was O125, O20, O127, O44 and O78 respectively. It was noteworthy that O125, O127 and O44 serogroups were included in Enteropathogenic *E. coli* (EPEC) that is associated with infantile diarrhea in many developing countries (Doyle, 1990), while O20 was included in Enterotoxigenic *E. coli* (ETEC) that represent the common cause of traveler's diarrhea for peoples who travel from areas with good hygiene to to areas with lower hygienic standards (Doyle, 1989).

Different serotypes of *Salmonella* could be recovered from life and dead birds. However, *Salmonella enteritidis* was recovered only from dead bird's liver, heart and bone marrow and likely associated with systemic infection that represent a potential source for contamination of carcasses especially in life birds markets widely distributed in Egypt. *S. Enteritidis* considered one of the most important human pathogens that is associated with the consumption of contaminated poultry meat and egg (Suresh et al., 2006).

The excessive and massive usage of antibiotics on intensive food animal production especially poultry and pork production represent the cornerstone for emergence, persistence and spread of the resistant bacteria that not only threatens animal health but also represent a major threat to human health globally (WHO, 2014). The resistance bacteria in food animals can transmit to humans directly via consuming contaminated animal food and contact with the animal (occupational) or indirectly from environment that receives these bacteria from infected animals and fecal materials (FAO, 2011). Antimicrobials agents have been classified based on their importance to human into three categories, critically important, highly important and important antimicrobials (WHO, 2011). In the present study, nine antibiotics considered as the critical important group and four that represents the highly important group (Table 5) have been used to test the recovered isolates for antibiotic resistance using disc diffusion test. *E.coli* isolates showed resistance to four antibiotics that belongs to the critically important group (57.1-71.4%) and three that belong to the highly important group (71.4-100%). *S.aureus* isolates showed resistance to four antibiotics that belong to critical important group and highly important group (57.1-100%). *P.heamolytica* isolates showed resistance to five antibiotics belong to critical group (41.5-84.6%) and highly important group (100%). Interesting, *Salmonella* isolates showed significant antibiotic resistance to NA (100%) and F (42.9%). It is worth noting that, bacterial species recovered from the present study showed different pattern of resistance to critically important group of antibiotics and comparable pattern of resistance to highly important group of antibiotics. The antibiotic resistance genes

are transmissible to other bacteria of the same and/or different bacterial species (FAO, 2011) this highlight the problematic of presence mixed bacterial infection with different antibiotic resistance pattern that was the case in the present study, raising serious concerns about public health. Altogether, the majority of bacterial isolates in the present study, except salmonella, showed resistance to the highly important antimicrobial group (DO, T, SXT and C). However, no predominant pattern against critically important group.

Conclusions

The present study highlight the correlation between prevalence of antibiotic resistance and the incidence of mixed infection. Altogether, much more attention should be paid to biosecurity measures in quail farms in Egypt and prevent the abuse of antibiotic so as to reduce the incidence of mixed infections and subsequently reduce the spread of antibiotic resistance genes between different bacterial populations.

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Authors' Contribution

EF and HR designed the work,conducted the field work and collected the samples, EF AS and HR conducted the laboratory investigations, EF and AS wrote the manuscript and all read and approved the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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