

ISOLATION AND DETECTION OF BACTERIAL SPECIES FROM VISCERAL ORGANS OF QUAILS

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Abstract: A total of thirty normal quail birds were bought from local market. Quail birds were slaughtered and samples taken aseptically from internal organs of each bird for bacteriological investigation. The result showed isolation of 154 bacterial isolates from different organs of quail birds. Among the 30 quail birds 22 birds were showed positive for microbial identification (73.33%). The isolates ranged from *E. coli* 20.77% (32 isolates), as a high percent, then *Corynebacterium spp.* 19.48 % (30 isolates), *Staphylococcus aureus* 16.23 % (25 isolates), *Bacillus spp.* 16.23% (25 isolates), *Enterococcus faecalis* 11.68% (18 isolates), *Klebsiella pneumoniae* 9.09% (14 isolates), *Proteus spp.* 2.59% (4 isolates), *Pasteurella multocida* 2.59% (4 isolates) and *Coagulase-ve staphyloco-ccus* 0.6% (1 isolate). This study showed *E. coli* and *Corynebacterium spp.* were dominant bacteria in the internal organs of quail birds. Many studies reported that quail birds were resistant to many bacterial diseases, so that these birds may act as mechanical transporting for different bacterial species to humans and animals with the risky of transporting of resistance bacterial species for many antibiotics.

Keywords: Wild birds; Bacterial isolates; Internal organs.

Introduction

Japanese quails are members of the pheasant family phasianidae (Naveen, 1997). Japan is the native place of this species of quail which was domesticated as long ago as the 12th century, at the beginning of this century these birds have been bred in large numbers for dual-purpose of meat and eggs production (Abd El-Gawad et al., 2008). Quail meat in some countries considered as a good food for all ages due to its high meat yield, little shrinkage during cooking, fast cooking and serving and also due to their delicacy and low level of cholesterol. In addition, quail meat is tender and fortified with nutrients (Mounteny, 1981). Because it is a perfect source of vitamin B6, niacin, thiamin, pantothenic acid and riboflavin, so quail meat favorite more than other species of poultry meat (Paulillo, 2009). Considerable quantities of quail meat are used for human consumption due to their easily adapt to commercial management conditions, with good performance in term of meat and egg production (Edris,

1997). Today a considerable numbers of live quail birds are sold in local birds markets for human consumption, so the present study aimed to detect the bacterial types that may be found in the organs of these birds.

Materials and methods

Specimens

A total of thirty normally quail birds were bought from local birds markets and transported directly to laboratory, then slaughtered and samples taken aseptically from internal organs of each bird that included liver, lung, gizzard and intestine. The samples were put in sterile nutrient broths and incubated at 37°C for 24 hours (Quinn et al., 2003).

Culturing

Each broth was inoculated on three media included nutrient agar, sheep blood agar and MacConkeyagar and incubated aerobically at 37°C for 24-48 hours (Koneman et al., 1998).

Identification of Bacterial isolates

Purification was done and colonial characteristics and blood hemolysis were studied. After that smears from specific colonies were prepared and stained by Gram's stain to study the shape, arrangement and staining reaction (Quinn et al., 2004). Also some selective media were used like mannitol salt agar for staphylococci growth, Edward's medium for streptococci, Hoyle's medium for *Corynebacterium* and MacConkey agar for enterobacteriaceae. Biochemical tests were applied for each specific bacterial isolates and included catalase, oxidase, indole production, methyl red, VP, citrate utilization, gelatin hydrolysis, urease, triple sugar iron, nitrate reduction (Barrow, 2003).

Results

Results of the study revealed isolation of 154 bacterial isolates from different organs of quail birds. Among the 30 quail birds 22 birds were showed positive for microbial identification (73.33%). The isolates ranged from *E. coli* 20.77% (32 isolates), as a high percent, then *Corynebacterium spp.* 19.48 % (30 isolates), *Staphylococcus aureus* 16.23 %(25 isolates), *Bacillus spp.* 16.23% (25 isolates), *Enterococcus faecalis* 11.68% (18 isolates), *Klebsiella pneumoniae* 9.09% (14 isolates), *Proteus spp.* 2.59% (4 isolates), *Pasteurella multocida* 2.59% (4 isolates) and *Coagulase -ve staphyloco-cus* 0.6% (1 isolate). These bacterial types were classified according to the bird organs as follows;

Liver specimens

Seventeen liver specimens were positive for bacterial isolation (56.66%). Six bacterial types (34 isolates) were isolated from specimens of livers (Table 1). *E.coli* appeared in a high

percent (26.47%) between the total isolates from liver, and also as a mixed isolate along with other bacterial types (Table 2).

Table (1): Isolated bacterial types from liver specimens

Bacterial type	Numbers of isolates	Percentage (%)
<i>Corynebacterium spp.</i>	7	20.58
<i>E. coli</i>	9	26.47
<i>Bacillus spp.</i>	8	23.52
<i>Staphylococcus aureus</i>	3	8.82
<i>Klebsiella pneumoniae</i>	1	2.94
<i>Enterococcus faecalis</i>	6	17.64
Total		34
100.00		

Table (2): Mixed and pure bacterial types isolated from liver specimens

Bacterial type	Number of liver specimens	Percentage (%)
<i>Corynebacterium spp.</i>	4	23.52
<i>E. coli</i>	3	17.64
<i>Corynebacterium spp. + Bacillus spp.</i>	2	11.76
<i>Corynebacterium spp. + Staphylococcus aureus</i>	1	5.88
+ <i>E. coli</i>	2	11.76
<i>Bacillus spp. + E. coli + Enterococcus</i>	2	11.76
<i>Bacillus spp. + E. coli + Staphylococcus aureus</i>	1	5.88
<i>Bacillus spp. + E. coli</i>	1	5.88
<i>Klebsiella + Enterococcus</i>	1	5.88
<i>Bacillus spp.</i>		
Total		17
100.00		

Lung specimens

Among the lung specimens, only twenty specimens were positive for bacterial isolation. Eight bacterial types (44 isolates) were isolated from specimens of lungs (Table 3). *Corynebacterium spp.* appeared predominant bacterial type from lungs as a pure or mixed culture, followed by *Staphylococcus aureus* (Table 4).

Table (3): Isolated bacterial types from lung specimens

Bacterial type	Number of isolates	Percentage (%)
<i>Corynebacterium spp.</i>	10	22.72
<i>Staphylococcus aureus</i>	9	20.45

<i>E. coli</i>	7	15.90
<i>Klebsiella pneumoniae</i>	6	13.63
<i>Bacillus spp.</i>	6	13.63
<i>Pasteurella multocida</i>	4	9.09
<i>Enterococcus faecalis</i>	1	2.27
<i>Coagulase -ve Staph.</i>	1	2.27
Total	44	
100.00		

Table (4): Mixed and pure bacterial types isolated from lung specimens

Number of lung specimens	Bacterial type	Percentage (%)
3	<i>Coryne. + Staph. aureus</i>	13.63
3	<i>Corynebacterium spp.</i>	13.63
4	<i>Staphylococcus aureus</i>	18.18
4	<i>E. coli+ Klebsiella pneumonia + Bacillus spp.</i>	18.18
1	<i>Coryne. + Enterococcus+ E. coli</i>	4.54
1	<i>Coryne. + E. coli + Coagulase -ve</i>	4.54
2	<i>Staphylococcus</i>	9.09
2	<i>Pasteurellamultocida + Coryne.</i>	9.09
1	<i>Pasteurella multocida + Klebsiella pneumonia +</i>	4.54
1	<i>Bacillus spp.</i>	4.54
	<i>Coryne. + Staph.aureus+ Proteus</i>	
	<i>Coryne. + E. coli + Staph. aureus</i>	
22	Total	
100.00		

Gizzards specimens

In gizzard specimens six bacterial types (36 isolates) were isolated and the Gram positive bacteria were dominant, particularly *Bacillus spp.* and *Staphylococcus aureus* (table 5). More frequently mixed isolation from these specimens included *Bacillus spp.* with *Enterococcus faecalis* (table 6).

Table (5): Isolated bacterial types from gizzards specimens

Bacterial type	Number of isolates	Percentage (%)
<i>Corynebacterium spp</i>	5	13.88
<i>Bacillus spp.</i>	10	27.77
<i>Staphylococcus aureus</i>	10	27.77
<i>Enterococcus faecalis</i>	4	11.11
<i>E. coli</i>	4	11.11
<i>Klebsiella pneumoniae</i>	3	8.33
Total	36	
100.00		

Table (6): Mixed and pure bacterial types isolated from gizzards specimens

Number of gizzard specimens	Bacterial type	Percentage %
2	<i>Corynebacterium spp.</i>	9.09
3	<i>Bacillus spp. + Enterococcus faecalis</i>	13.63
4	<i>Bacillus spp.</i>	18.18
4	<i>Staphylococcus aureus</i>	18.18
3	<i>Staph. aureus + Bacillus spp.</i>	13.63
2	<i>E. coli + Klebsiella pneumonia</i>	9.09
1	<i>Klebsiella pneumonia + Enterococcus</i>	4.54
1	<i>faecalis</i>	4.54
2	<i>Coryne. + Staph. aureus</i>	9.09
	<i>Coryne. + E. coli+ Staph. aureus</i>	
22	Total	100.00

Intestinal specimens

Between seven bacterial types (40 isolates) isolated from intestines of quail birds, *E. coli* and *Corynebacterium spp.* represented in a high percent (table 7), also these two types isolated together more frequently as a mixed culture (table 8).

Table (7): Isolated bacterial types from intestines specimens

Bacterial type	Number of isolates	Percentage (%)
<i>Corynebacterium spp.</i>	8	20.00
<i>E. coli</i>	12	30.00
<i>Enterococcus faecalis</i>	7	17.50
<i>Staphylococcus aureus</i>	4	10.00
<i>Klebsiella pneumoniae</i>	4	10.00
<i>Proteus spp.</i>	4	10.00
<i>Bacillus spp.</i>	1	2.50
Total	40	
	100.00	

Table (8): Mixed and pure bacterial types isolated from intestines specimens

Number of intestine specimens	Bacterial type	Percentage %
2	<i>Corynebacterium spp.</i>	10.00
3	<i>E. coli</i>	15.00
2	<i>Coryne. + E. coli</i>	10.00
4	<i>E. coli + Enterococcus faecalis</i>	20.00
1	<i>Coryne. + Enterococcus faecalis + Proteus</i>	5.00
1	<i>spp.</i>	5.00
2	<i>Coryne.+Staph. aureus + Bacillus spp.</i>	10.00
2	<i>Klebsiella pneumonia + Enterococcus</i>	10.00
3	<i>faecalis</i>	15.00

	<i>Coryne. + Klebsiella pneumoniae</i>	
	<i>Proteus + E. coli + Staph.aureus</i>	
20	Total	100.00

Discussion

According to the results of this study many bacterial types were isolated from different organs of quail bird's involved *Corynebacterium spp.*, *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Bacillus spp.*, *Proteus spp.*, *Pasteurella multocida* and *Coagulase -ve Staphylococcus*. These results were agreed to the results of previous studies about the isolation of same bacterial types from quail, but differed from them in rates of isolates (Roy et al., 2006). The differences between the results could be attributed to the variations in climate and environment of husbandry regions especially temperatures variation that effect on the bacterial growth (Dhasarathan, 2006).

Results of the liver samples revealed isolation of six bacterial types (Table 1) included *Corynebacterium spp.*, *E. coli*, *Bacillus spp.*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Enterococcus faecalis*. Many studies referred to the isolation of one or more of these bacterial species from livers particularly *E. coli*, *Enterococci* and *Staphylococci* (Lauková, 2009), while *Corynebacterium spp.* isolation was not referred previously; and this will confirm the environmental changes (Dhasarathan, 2006). The bacterial types isolated from liver specimens were similar to those isolated from intestine and this conclude that livers bacteria might passed from intestine to liver or may reach the liver by extension from adjacent air sacs or from less frequently, by extension up the biliary tree (Schmidt, 2003).

Eight bacterial types (44 isolates) were isolated from specimens of lungs (Table 3). *E.coli* appeared predominant bacterial type from lungs as a pure or mixed culture, followed by *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumonia*, *Bacillus spp.*, *Pasteurella multocida*, *Enterococcus faecalis*, *Coagulase -ve Staphylococci* (Table 4). Many researches were observed isolation of these bacterial species from lungs of diseased quails (Thenmozhi et al., 2010).

Gizzard specimens were appeared positive for bacterial isolation and this study showed isolation of six bacterial types (36 isolates) and also revealed that Gram positive bacteria were dominant, particularly *Bacillus spp.* and *Staphylococcus aureus* (Table 5) as mentioned by some studies (Burns, 2003). More frequently mixed isolation from these specimens included *Bacillus spp.* with *Enterococcus faecalis* (Table 6). The results of gizzard's specimens referred that these bacterial types may come from environment through

contaminated food and water, and then when passed to intestine definitely they spread to the other organs (Thomas, 2007).

Seven bacterial types (included 40 isolates) isolated from intestines of quail birds, *Corynebacterium spp.* and *E.coli* represented in a high percent (Table 7), also these two types isolated together more frequently as a mixed culture (Table 8). Other isolated bacteria from intestines were involved *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus spp.* and *Bacillus spp.* These results accepted with the previous studies in the same line which referred that the intestinal bacteria may be the main source for contamination of other internal organs (Hong-lin et al., 2010).

This study showed that *E.coli* and *Corynebacterium spp.* were dominant in the organs of quail birds, and some studies related these bacteria with many infections in humans (Collins, 1986). On the other hand the Japanese quail are reported to be resistant to many diseases and in addition many bacterial isolates obtained from Japanese quail and their environment showed high resistance to multiple drugs (Barnes, 1997), so that these birds may act as mechanical transporting for different bacterial species to humans and animals with the risky of transporting of resistance bacterial species.

References

- [1] Abd El-Gawad AH, Hemid AEA, El-Wardany I. Alleviating the effect of some environmental stress factors on productive performance in Japanese quail. W J Agricul Sci 2008; 4 (5): 605-611.
- [2] Barnes H J, Gross WB. Colibacillosis: in Diseases of Poultry. 10th ed. B.W. Calneked. Mosby-Wolf PubLtd., London, UK. 1997: p. 131-139.
- [3] Barrow GI, Feltham RKA. Cowan and Steel's manual for the identification of medical bacteria. University of Cambridge, Cambridge, United Kingdom. 2003:p.28-48.
- [4] Burns KE, Otalora R, Glisson JR, Hofacre CL. Cellulitis in Japanese quail (*Coturnixcoturnix japonica*). Avian Dis. 2003; 47(1):211-214.
- [5] Collins MD, Cummins CS. Genus *Corynebacterium* In: Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's Manual of Systematic Bacteriology. William & Wilkins, Baltimore.1986:p.1293-1386.
- [6] Dhasarathan P, Uma GG, Rajkumar Seasonal variations in microbial population in sivakasi soil with reference to the influence of temperature. Poul Res. 2006; 25(1): 114-118.

- [7] Edris AM, Shaltout FA, Arab WS. Bacterial evaluation of quail Meat. Benha Vet Med J. 1997; 16 (1):1-14.
- [8] Hong-lin W, Jun Y, Hua-bin S, Ling L, Di-yun A, Qing-ping L, Guoyuan W, Rong-rong Z, Lin Z. Isolation and identification of Enteropathogenic *E. coli* and Salmonella from quail and their drug sensitivity test. Vet Rec.2010;166:147-148.
- [9] Koneman EW, Allen SD, Dowell VR, Janda WM,Sommers HM, Winn WC. Color Atlas of Diagnostic Microbiology 3rd ed., Lippincott Co.1988:p. 87-92, 24, 115.
- [10] Lauková A, Michlovcová G. Enterococci isolated from Japanese quails exposed to microgravity conditions and stability of their properties. ActaVet Brno.2009; 78:253–258.
- [11] Mounteny GJ. Poultry products technology, second ed., The Avi pub.co. Inc. Westport Connecticut.1981:p. 67-69.
- [12] Naveen KA, Arun ES. Diseases of quail. Poultry adviser.1997; 25(8): 43-48.
- [13] Paulillo AC, Schmidt EM. Experiential vaccination against Newcastle disease in japanese quails (*Coturnixcoturnix japonica*): clinical and immunological parameters. Inter J Poult Sci. 2009; 8(1):52-54.
- [14] Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology 6th ed. Mosby. Edinburgh, New-york.2004:p. 191-208.
- [15] Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC. Veterinary Microbiology and Microbial Diseases. Blackwellpub Co.USA.2003 :p. 84 – 96.
- [16] Roy P, Purushothaman V, Koteeswaran A, Dhillon AS. Isolation, Character-ization, and antimicrobial drug resistance pattern of *Escherichia coli* isolated from japans equail and their environment. J Appl Poult Res. 2006;15:442–446.
- [17] Schmidt RE, Reavill DR, Phalen DN. Pathology of pet and aviary birds. A Blackwell Pub Com.1st ed. 2003:p.17-23,56-58, 74.
- [18] Thenmozhi V, Malmarugan S, Suresh P, Jeyanthi C. Isolation and identification of bacterial respiratory pathogens in japanese quails. Ind J Field Veterin. 2010; 6(1): 54- 56).
- [19] Thomas NJ, Hunter DB, Atkinson CT. Infectious Diseases of Wild Birds. Blackwell Pub. 1st ed. 2007:P. 237-245, 265, 284.