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Concurrent bacterial and Mycoplasma co-infection with the infectious bronchitis virus disease in broilers

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Abstract

Co-circulation of many bacterial and viral pathogens were frequently observed among commercial broiler flocks causing severe economic losses. The aim of this study was to determine the prevalence of various bacterial poultry diseases associated with infectious bronchitis virus (IBV) disease in broilers chickens in Egypt. The study was conducted in 41 poultry farms and the incidence of IBV was found in 78% poultry farms. The dominants-circulated bacterial agents with positive IBV examined broiler farms were *Escherichia coli* (94%) followed by *Staphylococcus aureus* (81%), Confections with Mycoplasma or Salmonella with IBV infected chickens were (12 %) for each. Antibiotic sensitivity test for isolated *E. coli* and *Staphylococcus aureus* were shown multidrug resistant. The obtained results provided insights into the widespread of bacterial and viral diseases in Egyptian poultry commercial flocks and concluded that clinical diagnosis is not always accurate as laboratory diagnosis due to the complex nature of diseases etiologies and co-existence of more than one pathogen. Hence, we recommend continuous monitoring and characterization of such infectious pathogens that will provide substantial information for better understanding of the pathogenesis of bacterial and viral pathogens in poultry. Moreover, it is important to emphasize on the awareness of biosafety and biosecurity measures.

***Key Words**: Bacterial pathogens, viral infection, poultry, prevalence, Egypt.*

Introduction

Reports from past decades showed that natural co-infections are expected to occur, and mixed infections of bacterial pathogens (*Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Ornithobacterium rhinotracheale*, *Escherichia coli* and *Staphylococcus aureus*) were reported associated with other viral disease as avian influenza (AI), Newcastle disease virus (ND) and /or infectious bronchitis (IB) disease **Haghighat-Jahromi *et al.*, (2008); Roussan *et al.*, (2008); Pan *et al.*, (2012)** and **Franca *et al.*, (2014)**. It was well known that viral respiratory infection predisposes the secondary bacterial infection including the commensal bacteria through mechanical damage of ciliated and goblet cells that in turn facilitate the bacterial attachment and colonization (**Bakaletz, 1995; Wilson *et al.*, 1996 and El Ahmer *et al.*, 1999)**. It is worth mentioning that the worse effect of the secondary bacterial infection not only exaggerating the pathogenesis but also increase the bacterial density that decrease the antibiotic efficiency (**Tan *et al.*, 2012**) which commonly observed in the field. Bacterial pre-infection could be of benefit to viral pathogenesis (**Tashiro *et al.*, 1987; Kishida *et al.*, 2004)**. In contrast bacterial infection may limit the viral pathogenicity either through augmentation of the immune response to viral pathogens and/or prevent or decrease the viral attachment to the susceptible cells (**Sid *et al.*, 2016)**. Diseases of the respiratory tract are a significant component of the overall

disease incidence in poultry. In many cases, respiratory disease observed in a flock may be a component of a multi systemic disease or it may be the predominant disease with lesser involvement of other organ systems (**Glisson, 1998**).

In Egypt, poultry sectors were distinguished by farm sizes ranging from the farms having <1,000 to ≥10,000 birds. Poultry farms are endemic with many viral and bacterial pathogens which possessa serious threats for the survival of poultry population especially at small scale level, and causing huge economic losses to the poultry industry in the country. The major poultry disease threats include Infectious bronchitis (IB), Avian Influenza (AI), Newcastle disease (ND), *Escherichia coli* infection, Mycoplasma, Staphylococcus, and Salmonellosis (**Abd El-Ghany *et al.,* 2012; Arafa *et al.,* 2012; Hussein *et al.,* 2013; and Hassan *et al.,* 2016)**.

Despite of intensive vaccines programs, IBV continues to circulate and possesses diverse effects on the poultry industry in Egypt. Previous studies reported field co-infection and co-circulation of IBV with other respiratory viral diseases as AI H5N1 and H9N2 as well as NDV (**Hassan *et al.,* 2016)**. However, so far, most of the viral-bacterial interference studies don't entirely reflect the field situation where the poultry populations are exposed to more than one infectious and/or non-infectious disease agents.

With this background, the aim of the current study was undertaken to investigate the prevalence of some avian bacterial pathogens and Mycoplasma associated with infectious bronchitis virus in the Egyptian broiler chicken farms during the period between 2015 to 2016.

Materials and Methods

1- Samples collection:

Samples were collected from representing 41 commercial farms showing respiratory distress, dysnea, wet droppings and varied mortalities in 10 governorates during the period of 2015 to 2016. Samples were collected by the Reference Laboratory for Veterinary Quality Control on Poultry Production.

The collected samples were different organs (liver, yolk sacs, lungs) and bone marrow of both recently died and slaughtered chickens for bacteriological examination and organs (trachea, lungs and kidneys) for histopathological examination. Oral swabs were collected for virological examination using appropriate transport media to avoid dryness and transported at 4°C under a standard biosafety measure. Samples collected from each farms sector were pooled together.

2- Bacterial isolation and identification:

2.1. Isolation and identification of *E. coli*: it was performed according to **Quinn *et al.,* (2002**); on Mac Conkey's agar and Eosin Methylene blue agar (EMB).

2.2. The standard conventional culture method was used in the isolation of *Salmonella spp.* According to **ISO 6579, (2002)**. Presumptive Salmonella colonies were confirmed by using API 20E (bioMérieux 20100, Marcy L' Etoile, France). *S. aureus* was isolated and identified based on **Geidam *et al.,* (2012)** method through cultivation on mannitol salt agar (Oxoid, UK). Confirmation was done by gram staining, microscopy, catalase, coagulase, oxidase and sugar fermentation tests.

3- Mycoplasma was isolated and identified according to **Kleven *et al.* (1996).**

4- Antibiotic sensitivity test:

The antibiogram of Bacterial Isolates were done by disc-diffusion test for Salmonella, *E. coli*, *Staphylococcus aureus* & Mycoplasma against 16 antimicrobial (oxoid) (**table 1, 2**) Amoxicillin+clavulinic acid Am+CL, Nalidixic acid, nitrofurantoin, norofloxacin, streptomycin, Trimethoprim-sulfamethoxazole, tetracyclin, Levofloxacin, Ampicillin, Deoxycycline, Colistinsulphate, Pencillin, enroflox-

acin, Tylosin, ciprofloxacin, Gentamycin according to the Clinical and Laboratory Standards Institute/ Formerly National Committee for Clinical Laboratory Standard **(CLSI/ NCCLS, 2009).**

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

Antimicrobial Discs	Disc Potency Mg/disc	Interpretation		
		Zone diameter (mm)		
		Sensitive ≤	Intermediate	Resistant ≥
Amoxicillin-clavulanic	20/10 µg	≥18	14–17	17 ≤13
Streptomycin	10 µg	≥15	12–14	≤11
Nalidixic acid	30 µg	≥19	14–18	≤13
Nitrofurantoin	300 µg	≥17	15–16	≤14
Ampicillin	10 µg	17	14-16	13
Ciprofloxacin	5 µg	21	16-20	15
Colistin (MethanSulphate)	10 µg	11	9-10	8
Doxycycline hydrochloride.	30 g µ	14	11-13	10
Gentamicin	10µg	15	13-14	12
Levofloxacin	5µg	17	14-16	13
Norfloxacin	10 µg	17	13-16	12
Trimethoprim-sulfamethoxazole	23.75µg	16	11-15	10
Tetracycline	30 µg	15	13-15	11

Table (2). The break point of *Staphylococcus* **(CLSI/NCCLS, 2009).**

Antimicrobial Discs	Disc Potency Mg/disc	Interpretation		
		Zone diameter (mm)		
		Sensitive ≤	Intermediate	Resistant ≥
Amoxicillin-clavulanic	**20/10 µg**	≥ 20	–	≤ 19
Penicillin	**10 units**	≥ 29	–	≤ 28
Nitrofurantoin	**300 µg**	17	15–16	≤ 14
Ampicillin	**10 µg**	29	-	28
Ciprofloxacin.	**5 µg**	21	16-20	15
Doxycycline.	**30µg**	16	13-15	12
Gentamicin.	**10 µg**	15	13-14	12
Levofloxacin	**5 µg**	19	16-18	15
Norfloxacin.	**10 µg**	17	13-16	12
Tetracycline.	**30**	19	15-18	14
Trimethoprim-sulfamethoxazole	**23.75 µg**	16	11-15	10

5- Molecular diagnosis of IBV:

RNA extraction from pooled swabs was done by using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA) according to the manufacturing instructions as procedures, Briefly, the samples were first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA, Buffering conditions were then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the samples were loaded onto the QIAamp Mini spin column, the

RNA bind to the membrane and contaminants were efficiently washed away in two steps using two different wash buffers, High-quality RNA was eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA was free of protein, nucleases, and other contaminants and inhibitors.

RT-PCR was done by using Prime Script ™ one step RT-PCR kit (Takara Bio USA, Inc.) by using specific primersfor S1 gene of IBV (**table 3**), the reactions were performed in a thermal cycler (Applied Biosystems) (ABI, USA) and The RT-PCR conditions were as follows: Reverse transcription (50ºC/ 30 min.), initial denaturation and enzyme activation (94ºC/ 2 min.), followed by 40 cycles of denaturation (94ºC/ 30 sec.), annealing (50ºC/ 30 sec.), and extension (72ºC/ 1 min.) then final extension (72ºC/ 10 min.).

The PCR product was separated by gel electrophoresis using 1.5% agarose gel stained with ethidium bromide and the bands were detected and visualized on ultraviolet (UV) light transilluminator Gel Documentation analyzer.

Table (3). Primers used in IBV amplification

Primer	Sequence (5′-3′)	Reference
IBV-S1-F	5-CAC TGG TAA TTT TTC AGA TGG-3	**(Adzhar *et al.*,(1997))**
IBV-S1-R	5-C AGA TTG CTT ACA ACC ACC-3	

6- Histopathological examination:
Samples of trachea, lungs and kidneys were collected and fixed in 10% buffered neutral formalin. The fixed tissues were embedded in paraffin, sectioned at 4 µm thick and stained with haematoxylin and eosin H&E (**Bancroft and Gamble, 2008).**

Results

1. Bacterial identification:
In the prevalence carried out in the current study, out of total 41 poultry farms, 37 farms were found positive for *E. coli.* Among the tested farms, 33 farms were confirmed positive for isolation *S. aureus*. Only four commercial farms were found positive for Salmonella with the help of standard conventional culture method. four farms were found positive for detection of Mycoplasma.
The findings of the field study and the laboratory tests of 41 broiler chicken flocks with respiratory signs are summarized in **(table 4)** and figure 1.

Table (4). Bacteria/viruses from 41 broilers examined farms 2015-2016 identified in the frams of the current study, (-) indicates negative results while (+) indicates a positive result.

No.	Results				
	Staphylococcus aureus	***Salmonella***	***E. coli***	***Mycoplasma***	**IBV**
1	+*	-**	+	-	+
2	+	-	+	-	+
3	+	-	+	-	+
4	+	-	+	-	+
5	-	+	+	-	+
6	+	-	+	-	-
7	+	-	+	-	-
8	-	+	+	+	+
9	+	-	+	-	-
10	+	+	+	-	+
11	+	-	+	-	+
12	+	-	+	-	+
13	+	-	+	-	-
14	+	-	+	-	-
15	+	-	+	-	+
16	+	-	+	-	+
17	+	-	+	-	+
18	+	-	+	-	+
19	-	-	+	-	+
20	-	-	-	-	-
21	+	-	+	+	-
22	+	-	+	+	+
23	+	-	+	-	+
24	-	-	-	-	+
25	+	-	+	-	+
26	+	-	+	-	+
27	+	-	+	-	+
28	-	-	-	+	-
29	+	-	+	-	-
30	+	-	+	-	+
31	-	-	+	-	+
32	+	-	+	-	+
33	-	-	+	-	+
34	+	-	-	-	+
35	+	-	+	-	+
36	+	-	+	-	+
37	+	-	+	-	+
38	+	+	+	-	+
39	+	-	+	-	+
40	+	-	+	-	+
41	+	-	+	-	+
Total	**33**	**4**	**37**	**4**	**32**
%	**80.4%**	**9.7%**	**90%**	**9.7%**	**78%**

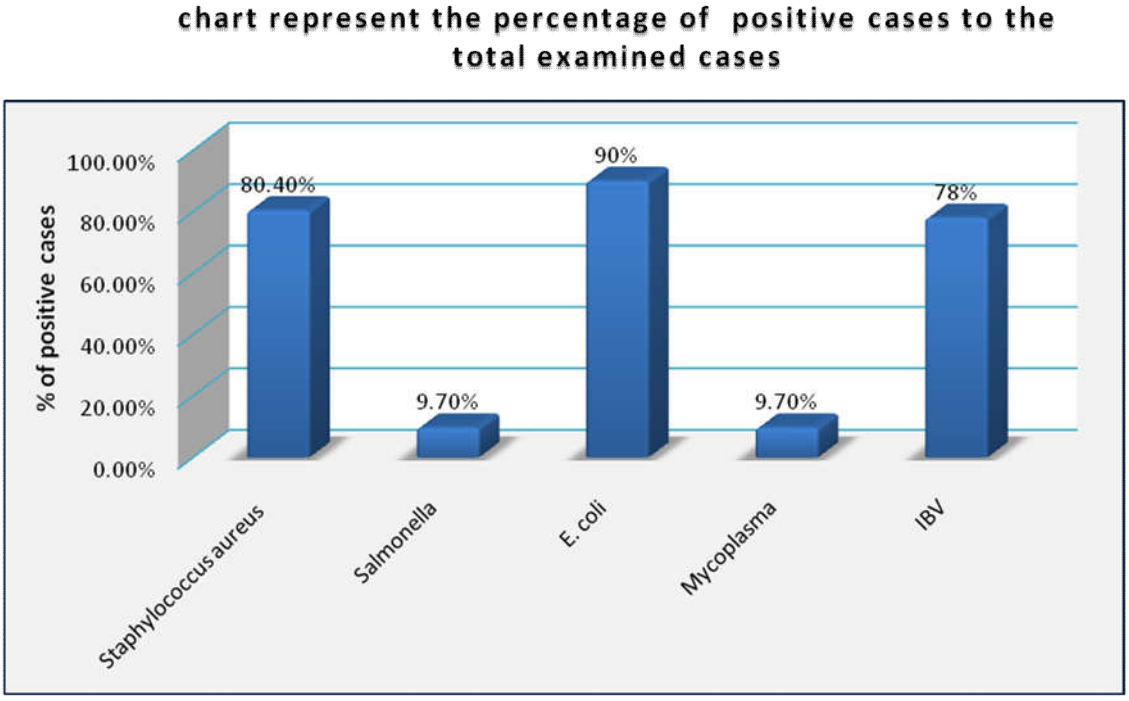

Figure (1). Excel chart indicating the percentage of positive cases identified in the frams of this study.

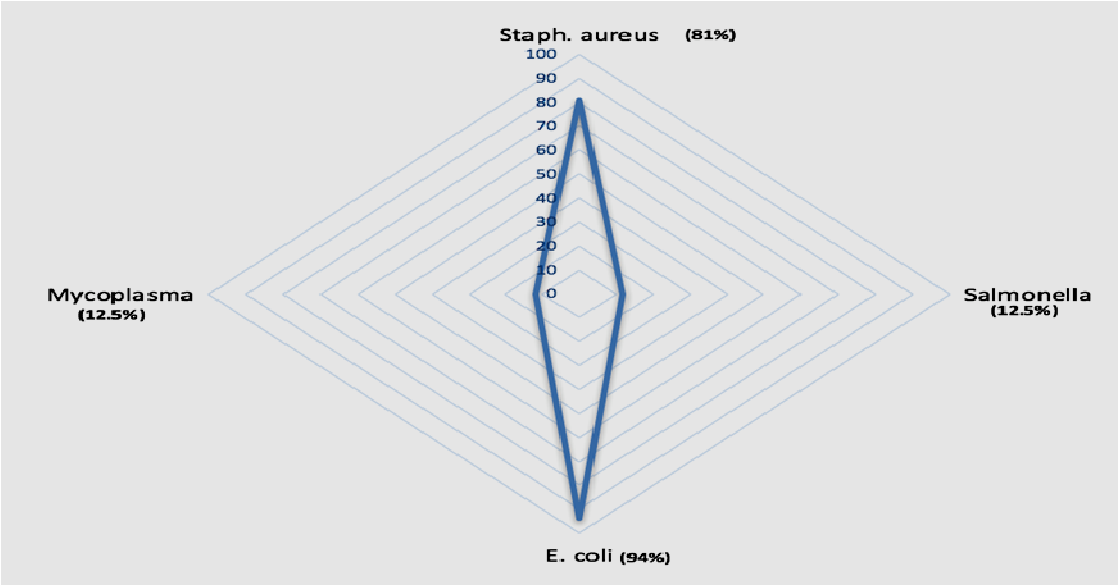

Figure (2). Radar chart showing the percent of positive cases of different bacterial pathogens associated with IB viral infection positive cases.

2. Sensitivity test:

By using disc diffusion method showed that salmonella isolate were totally resistant 100% to (Amoxicillin+clavulinic acid, Ampicillin, Pencillin and Deoxycycline). While *Escherichia coli* isolates showed highest resistant to nitrofurantoin and enrofloxacin (97.3%) followed by Deoxycycline 94.6 %, Sixteen % *Escherichia coli* isolates showed total resistance to antibiotics used in sensitivity test. Mycoplasma isolates showed that 100% sensitive to enrofloxacin, Tylosin, ciprofloxacin and Nalidixic acid**.** There was a significant resistance of *Staphylococcus aureus*to most antibiotics, highest resistance to Deoxycycline (96.97%), followed by nitrofurantoin (90.91%) and 87.88% for Amoxicillin+clavulinic acid Trimethoprim-sulfamethoxazole, Pencillin,

enrofloxacin and ciprofloxacin**,** 18% *Staphylococcus aureus* isolates showed total resistance to antibiotics used in sensitivity test **(table 5).**

3. Histopathology results

Collected organs from farms suffering of high morbidity and mortality (15-20%) were showing (**Figure 3**) predominate severe pneumonia with profuse cellular exudate in pulmonary air capillaries and par bronchial lumen. Tracheal mucosa showed prominent hyperplasia of glandular epithelium with lymphocytic submucosal infiltration. Kidneys showed characteristic renal cast and focal necrosis of renal tubules. Necrosis of the proximal convoluted tubule and distension of distal convoluted tubule. In addition, necrotic foci, heterophils, and lymphocytes are observed in the interstitial spaces.

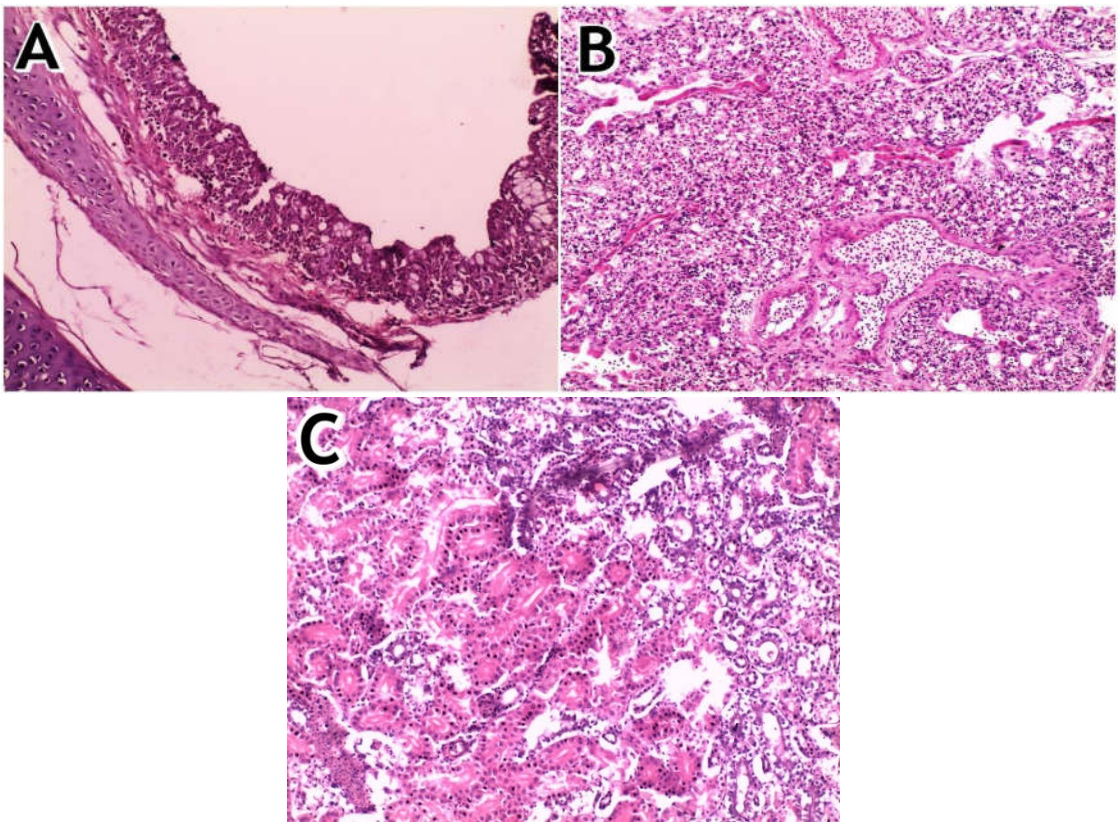

Figure (3). The histopathological examination of trachea, lung, and kidney samples revealed severe inflammatory and necrotic reaction in response to infection. Briefly, (A) Trachea showed hyperplasia of tracheal glands (arrows) (x20). (B) Air capillaries were filled with cellular exudates (arrows) (x20). (C) Kidney showed renal tubules were filled with esinophillic renal cast with focal necrosis of neighboring tubules (arrows) (x20) all stained with H&E.

4. Infectious bronchitis identification:

Based on the reverse transcription-PCR described in the earlier section, the results suggest an acute infection with IBV among 32 poultry cases from 41 collected samples which reprent 78% of total cases. Co-infection (s) was/were recorded with other bacterial pathogens as *Staphylococcus aureus, Salmonella*, *E. coli* and *Mycoplasma* (**Table 4**). The highest prevalence was observed associated with *E. coli* and *Staphylococcus aureus* infections with percent of 90% and 80.4% respectively. The lowest incidence of co-infection with IBV was shown to be in associated with *Salmonella* and *Mycoplasma* was 12.5% (**Figure 4**).

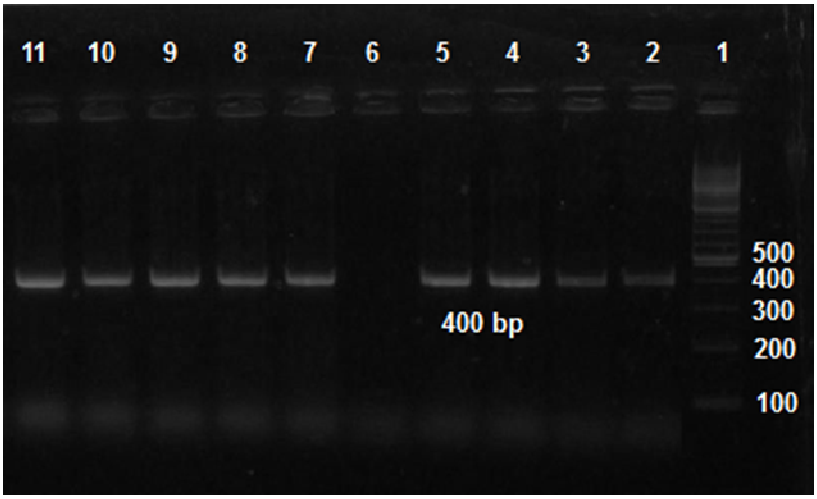

Figure (4). Agarose gel electrophoresis showing the result of amplification of s1 gene of IB, lane 1 is 100 bp ladder, lanes 2, 3, 4, 5, 7, 8, 9, 10 are positive samples of 400 bp, lane 6 is the negative control and lane 11 is positive control

Table (5). Result of antibiotics resistance of bacterial isolates by disc diffusion method

	Salmonella			***Escherichia coli***			**Mycoplasma**			***Staphylococcus aureus***		
	S%	**I%**	**R %**	**S%**	**I %**	**R%**	**S%**	**I %**	**R%**	**S%**	**I%**	**R%**
Amoxicillin+clavulinic acidAm+CL	0	0	100	-	-	-	-	-	-	9.09	3.03	87.88
Nalidixic acid	-	-	-	-	-	-	100	0	0	-	-	-
nitrofurantoin	25	0	75	2.7		97.3				9.09	0	90.91
norofloxacin	25	0	75	-	-	-	-	-	-	21.22	0	78.78
streptomycin	25	50	25	-	-	-	-	-	-	-	-	-
Trimethoprim-sulfamethoxazole	25	0	75	13.52		86.5	-	-		12.12	0	87.8
tetracyclin	25	0	75	-	-	-	-	-	-	-	-	-
Levofloxacin	50	0	50	-	-	-	-	-	-	9.09	6.06	84.8
Ampicillin	0	0	100	-	-	-	-	-	-	-	-	-
Deoxycycline	0	0	100	2.7	2.7	94.6	-	-	-	3.03	0	96.9
Colistinsulphate	50	0	50	-	-	-	-	-	-	-	-	-
Pencillin	0	0	100	-	-	-	-	-	-	12.2	0	87.8
enrofloxacin	-	0	-	2.7	-	97.3	100	0	0	0	0	87.8
Tylosin	-	-	-	-	-	-	100	0	0	0	0	0
ciprofloxacin	75	0	25	21.6	0	78.4	100	0	0	9.1	3.03	87.8
Gentamycin	50	0	50	37.8	5.4	67.5	100	0	0	21.2	3.03	75.7

(S) susceptible (I) intermediate (R) resistant,
Figures were represented by percentage (%) in table

Discussion

Infectious bronchitis virus, in addition to *Staphylococcus aureus,* Salmonella, *Escherichia coli* and Mycoplasma are considered as significant poultry pathogens which cause heavy economic losses all over the world. Other factors may promote the respiratory distress cases including poor sanitation, overcrowding and poor management (**Chanie *et al.,* 2009**).

Poultry industry in Egypt is being hampered by a number of pathogens (bacterial and viral diseases) as well as many other non pathogen related factors. Over the last 10 years, and despite of the application of different vaccination strategies in poultry farms in different regions in Egypt, infectious bronchitis virus has been isolated from outbreaks in poultry and pose a threat for the Egyptian poultry industry, and has been reported in both vaccinated and non-vaccinated flocks. Moreover, different causes of bacterial diseases are heavily prevalent among the Egyptian poultry populations including *Staphylococcus aureus,* Salmonella, *Escherichia coli* and Mycoplasma (**Ammar *et al.,* 2016).**

The diffuse massive respiratory lesions shown in the examined broiler chickens in the study provide evidence of field IBV infection rather than postvaccine over reaction (**Ignjatovic and Sapats, 2000; Cavanagh and Naqi, 2003)** Pathological lesions were shown due to IBV strains characterized by hyperplasia of tracheal epithelium and moderate lesions of interstitial pneumonia but, confection with bacterial pathogens led to severe pneumonia associated with high morbidity and mortality (**Sid *et al.,* 2015**). IBV could induceciliostasis in the tracheal epithelium (**Cook *et al.,* 1976; Hassan *et al.,* 2016)** and may, therefore, the opportunity of bacterial confection is provoked and the patogenecity is exaggerated **(Haghighat-Jahromi *et al.,* 2008).**

Tracheitis and progressive congestion was observed with the presence of mucoid plug at the tracheal lumen could play a role in severty of pathogenesis through potential impairment of clearance of bacterial pathogens in respiratory tract (**Dwars *et al.*, 2009).** Presence of decilliation and inflammatory infiltration help confection with bacterial pathogens that may explain the severity of pneumonia in lungs (**Dwars *et al.*, 2009).**

Nephropathogenic IBV strains cause nephritis characterized by swelling and congestion of the kidney, sometimes with pallor of ureters that contain urate deposits. Coinfection with bacterial pathogens such as *E. coli* may lead to a more complex outcome, usually associated with high mortality (**Bande *et al.,* 2016**).

Based on the results obtained in our study, multiple respiratory infections might be the cause of high mortality in investigated flocks. All identified pathogens were present in different combinations as multiple infections. The widespread presence of those infectious diseases reflect the poor vaccination practices and poor biosecurity measures. In addition, most of the sampled poultry farms lack proper disinfectants and physical barriers, which favor transmission of infectious diseases. Suboptimal biosecurity with bad conditions of buildings and equipment may lead to exposure of commercial poultry to pathogens circulating in bird populations.

Indeed, further investigation and characterization of additional respiratory pathogens are required in order to adapt appropriate control strategies in the future. Moreover, education of farmers will be also an important measure to improve poultry health in Egypt.

Conclusion

The study has concluded that the high mortalities observed in IBV infected flocks under field conditions in Egypt might be attributed to confection with bacterial pathogens and their acquired nature of antibiotics resistance. The current study results indicate that concurrent bacterial and viral infection required very substantial accurate laboratory diagnosis and hard to be confirmed only by clinical diagnosis.

References

Abd El-Ghany, A.W.; El-Shafii, S.; M.E.H. (2012). A Survey on Salmonella Species Isolated from Chicken Flocks in Egypt. Asian Journal of Animal and Veterinary Advances 7, 489-501.

Adzhar, A.; R.E. Gough; D. Haydon; K. Shaw; P. Britton and D. Cavanagh (1997). Molecular analysis of the 793/B serotype of infectious bronchitis virus in Great Britain, Avian Pathol., 26, pp. 625–640

Ammar, A.M.; Abd El-Aziz, N.; Abd El Wanis, S. and Bakry, N. (2016). Molecular versus conventional culture for detection of respiratory bacterial pathogens in poultry. Cellular and molecular biology. 62. 52-56. 10.14715/cmb/2016.62.2.9.

Arafa, A.S.; Hagag, N.M.; Yehia, N.; Zanaty, A.M.; Naguib, M.M. and Nasef, S.A. (2012). Effect of cocirculation of highly pathogenic avian influenza H5N1 subtype with low pathogenic H9N2 subtype on the spread of infections. Avian Dis. 56, 849-857.

Bakaletz, L.O. (1995). Viral potentiation of bacterial super infection of the respiratory tract. Trends in microbiology 3, 110-114.

Bancroft, J. and Gamble, M. (2008). Theory and Practice of Histological Techniques, 6th ed. Churchill Livingstone Elsevier, Philadelphia, PA.

Bande, F.; Siti Suri Arshad, Omar, A.; Bejo H.M.; Abubakar, S.M. and Abba, Y. (2016). Pathogenesis and Diagnostic Approaches of Avian Infectious Bronchitis. Advances in Virology Volume 2016, 4621659-70.

Cavanagh, D. and Naqi, S. (2003). Infectious bronchitis, In diseases of poultry, YM. SAIF, H. J. BARNES, J. R. GLISSON, A.M. FADLY, L.R. McDOUGALD., and D.E. Swayne, eds. (Ames, Iowa: Iowa state university Press), pp 101-119.

Chanie, M.; Negash, T. and Tilahun, S.B. (2009). Occurrence of concurrent infectious diseases in broiler chickens is a threat to commercial poultry farms in Central Ethiopia. Trop Anim Health Prod 41, 1309-1317.

CLSI (NCCLS) (Clinical and Laboratory Standards Institute), (2009). Quality Control for Commercially Prepared Microbiological Media; Approved Standard - Third Edition, 2009. Vol. 24 , No. 19.

Cook, J.K.; Darbyshire, J.H. and Peters, R.W. (1976). The use of chicken tracheal organ cultures for the isolation and assay of avian infectious bronchitis virus. Archives of virology 50, 109-118.

Dwars, **R.M.; Matthijs, M.G.; Daemen, A.J.; van Eck, J.H.; Vervelde, L. and Landman**, **W.J. (2009).** Progression of lesions in the respiratory tract of broilers after single infection with *Escherichia coli* compared to super infection with E. coli after infection with infectious bronchitis virus. Veterinary immunology and immunopathology 127, 65-76.

El Ahmer, O.R.; Raza, M.W.; Ogilvie, M.M.; Weir, D.M. and Blackwell, C.C. (1999). Binding of bacteria to HEp-2 cells infected with influenza A virus. FEMS Immunology & Medical Microbiology 23, 331-341.

Franca, M.; Howerth, E.W.; Carter, D.; Byas, A.; Poulson, R.; Afonso, C.L. and Stallknecht, D.E. (2014). Co-infection of mallards with low-virulence Newcastle disease virus and low-pathogenic avian influenza virus. Avian Pathol 43, 96-104.

Geidam, Y.A.; Z. Zakaria; S.A. Aziz; S.K. Bejo and J. Abu (2012). High prevalence of multidrug resistant bacteria in selected poultry farms in Selangor, Malaysia. Asian J. Anim. Vet. Adv., 7: 891-897.

Glisson, J.R. (1998). Bacterial respiratory disease of poultry. Poultry science 77, 1139-1142.

Haghighat-Jahromi, M.; Asasi, K.; Nili, H.; Dadras, H. and Shooshtari, A.H. (2008). Confection of avian influenza virus (H9N2 subtype) with infectious bronchitis live vaccine. Arch Virol 153, 651-655.

Hassan, K.E.; Shany, S.A.; Ali, A.; Dahshan, A.H.; El-Sawah, A.A. and El-Kady, M.F. (2016). Prevalence of avian respiratory viruses in broiler flocks in Egypt. Poult Sci 95, 1271-1280.

Hussein, A.H.; Ghanem, I.A.; Eid, A.A.; Ali, M.A.; Sherwood, J.S.; Li, G.; Nolan, L.K. and Logue, C.M. (2013). Molecular and phenotypic characterization of *Escherichia coli* isolated from broiler chicken flocks in Egypt. Avian Dis., 57, 602-611.

Ignjatovic, J. and Sapats, S. (2000). Avian infectious bronchitis virus. Rev. Sci. Off. Int. Epiz. 19: 493-508.

ISO 6579 (2002). Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella spp.*

Kishida, N.; Sakoda, Y.; Eto, M.; Sunaga, Y. and Kida, H. (2004). Co-infection of *Staphylococcus aureus* or Haemophilus paragallinarum exacerbates H9N2 influenza A virus infection in chickens. Archives of virology 149, 2095-2104.

Kleven, S.H.; Jordan, F.T.W. and Bradbury, J.M. (1996). Avian mycoplasmosis (Mycoplasma gallisepticum). In Manual of standards for diagnostic tests and vaccines, 3rd Ed. Office International des Epizooties, Paris, 512-521.

Pan, Q.; Liu, A.; Zhang, F.; Ling, Y.; Ou, C.; Hou, N. and He, C. (2012). Co-infection of broilers with *Ornithobacterium rhinotracheale* and H9N2 avian influenza virus. BMC veterinary research 8, 104.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C. and Leonard, F.C. (2002). Blackwell Scientific Publications; Oxford, London: Veterinary Microbiology and Microbial Diseases. (pp. 240–245)

Roussan, D.A.; Haddad, R. and Khawaldeh, G. (2008). Molecular survey of avian respiratory pathogens in commercial broiler chicken flocks with respiratory diseases in Jordan. Poult Sci 87, 444-448.

Sid, H.; Benachour, K. and Rautenschlein, S. (2015). Co-infection with Multiple Respiratory Pathogens Contributes to Increased Mortality Rates in Algerian Poultry Flocks. Avian diseases 59, 440-446.

Sid, H.; Hartmann, S.; Petersen, H.; Ryll, M. and Rautenschlein, S. (2016). *Mycoplasma gallisepticum* modifies the pathogenesis of influenza A virus in the avian tracheal epithelium. International Journal of Medical Microbiology 306, 174-186.

Tan, C.; Smith, R.P.; Srimani, J.K.; Riccione, K.A.; Prasada, S.; Kuehn, M. and You, L. (2012). The inoculums effect and band pass bacterial response to periodic antibiotic treatment. Molecular systems biology 8, 617.

Tashiro, M.; Ciborowski, P.; Reinacher, M.; Pulverer, G.; Klenk, H.D. and Rott, R. (1987). Synergistic role of staphylococcal proteases in the induction of influenza virus pathogenicity. Virology 157, 421-430.

Wilson, R.; Dowling, R. and Jackson, A. (1996). The biology of bacterial colonization and invasion of the respiratory mucosa. European Respiratory Journal 9, 1523-1530.