KLEBSIELLA PNEUMONIAE INFECTION IN BROILER CHICKENS

Marwa. A.Tantawy¹⁾, Amer, H.A.²⁾, El-Khyate, F. F. ³⁾, and Moshira, A. El-Abasy³⁾

ABSTRACT

The epidemiology, Pathogencity and the possible ways of treatment of Klebsiella pneumoniae (K. pneumoniae) infection as a respiratory pathogen in broiler chicken farms in different places in Gharbia 2014/2017.For this purpose Goernorate were investigated during 150 samples were collected from lung, trachea, liver and intestine samples of 150 diseased, 1-5-week-old commercial broiler chickens suffered from respiratory disorders, diarrhea, growth retardation and mortality with pneumonia, pericarditis, airsaculitis and enlarged liver. Bacteriological examination of swabs from trachea, lung, liver and intestine revealed Gram negative, non-motile rod-shaped organisms which were commonly isolated from lung, liver, intestine and trachea. The isolation trials revealed 10 K. pneumoniae positive cases with an incidence (6.6%). Identification of the isolated strains Biochemically by API 20 E and molecularly by polymerase chain reaction (PCR) revealed that the isolates belonged to K. pneumoniae. The K. Pneumoniae isolate was inoculated experimentally in 7 dayold chicks and clinical signs, lesion were reproduced. Clinical signs& P.M lesions characteristic in K.Pneumoniae infection was reproduced when the K. Pneumoniae isolates was inoculated in 7 day old chicks. K.Pneumoniae was also reisolated. Clinicopathological features and

¹⁾ Researcher in Kafrelsheikh University.
²⁾ Department of Pathology, A.R.R.I, Giza.

³⁾ Poultry Disease Department, Faculty of Veterinary Medicine, Kafrelsheikh University.

re-isolation of the organism from experimentally infected chicks were recorded. The results of the in vitro antibiotic sensitivity test revealed that the isolated strains were highly sensitive to gentamycin, amikacin, sulphamethoxazole—trimethoprime, ciprofloxacin and chloramphenicol, considerably sensitive to kanamycin, norfloxacin, oxytetracyclin and neomycin but resistant to Ampicillin and Erythromycin.

The clinical signs, post-mortem findings and the histopathological lesions of tissue sections from different organs of experimentally infected chickens were less severe after treatment with gentamycin, amikacin combined with sulphamethoxazole—trimethoprime in drinking water for 3 consecutive days.

INTRODUCTION

Poultry diseases is one of the most important segment of the agriculture sector in Egypt , Where investment in this industry is about 20 billion Egyptian pounds .It contributes a large part of the country's supply of animal protein (White meat and eggs). This sector has faced serious challenges through the respiratory infection.

Enterobacteriacae infection is still causing severe losses especially in young birds. *Klebsiella pneumoniae* is one of more than 40 genera that comprise the Enterobacteriacae family which plays as human and animal respiratory pathogen .*Klebsiella* infection in poultry has been recently reported to cause high mortality in balady chicks (*Sarakby*, 1979 and *Karaman*, 1980).

Klebsiella pneumoniae infection is conveyed to the developing and very young chicks after hatching but adult birds act as a carrier (*Sekariah and Seth*, 1957).

The upper respiratory tract of healthy birds can harbor *Klebsiella* microorganism which can behave as opportunistic pathogens (*Sandra and Duarte carreia 1998*) K. pneumoniae can cause localized or systemic infection in poultry and other birds (*Shivaprasad*, 1998), celliultitis in turkey and could be associated with primary contact dermatitis and skin abrasions. (*Gomis et al.*,2001).

K.pneumoniae infections in growing broiler chickens did not receive much care in our country in spite of considerable importance which are found to be implicated in poultry diseases resulting in significant losses, therefore the work reported in this study was undertaken to investigate the role of *K. pneumoniae* organism in causing disease in growing broiler chickens at Gharbia Governorate, and confirming the pathogenicity by experimental infections using the isolated organism in 3 day old chicks further more studying the in vitro sensitivity test of the isolated organism against different antibiotics followed by treatment trial of experimentally infected chicks.

MATERIALS AND METHODS

1. Collected samples:

Specimens were collected from 150 diseased, freshly dead and sacrificed broiler chicks suffering from diarrhea and sudden death obtained from 35 farms at different localities in El-Gharbia Governorate from 2014-2017 and individual cases subjected to clinical examination. Samples were subjected to clinical, post-mortem and bacteriological

examinations. Under complete aseptic condition, loopfuls from liver, lungs, trachea and intestine were streaked on MacConkey's agar and XLD agar. The inoculated plates were incubated micro-aerobically (10% CO₂) at 37° C for 48 hrs. The suspected colonies were picked up, purified and identified morphologically and biochemically using (API 20 E Micro Test Strip Bio-Merieux, France) according to *Nawaz et al.*, (2006) and *Kamran et al.*, (2014). Molecular identification of *K. pneumonia* suspected colonies were carried out using PCR.

2. Bacterial isolation:

Field strains of *K. pneumonia* isolated from diseased broilers were used for pathogenicity test by oral, intramuscular and subcutaneous injection *(Wilki et al, 2000)*. The *K. pneumonia* isolates were grow on MacConkey,s agar plates for 48 hrs at 37° C micro-arobically. A loopful of colonies was inoculated into brain heart infusion broth for serial ten-fold dilution of the organisms. Growth was adjusted by turbidity 10° CFU/ml (Tatum *et al.*, 2012).

3. Biochemical identification:

The biochemical identification of the isolated strains was performed using API 20 E strip (Bio-Merieux, Lyon, France).

4. Molecular detection (PCR):

The molecular detection of the isolated strains was performed by using PCR and the Amplification of DNA was performed on thermal cycler (Master cycler, Eppendorf, Hamburg, Germany) according to **Townsend et al.** (1998). Primers were designed against (16S-23S rDNA) as previously described by **Liu et al.** (2008). Expected product size was 130 bp.

5. Experimental infection:

Atotal of 80, 1- day – old, White Lohman chicks, free from Klebsiella pneumoniae infection were floor reared and fed on antibiotic-free ration. Feed and water were added ad *libitum*. Chicks were kept for one week before infection to insure that they were free from Klebsiella infection. Birds were randomly divided into 5 group.

- **Group (1):** of 10 chicks inoculated orally with 1 ml of sterile saline and kept as non infected non treated control group.
- **Group(2):** of 10 chicks inoculated orally with 1 ml sterile saline (non infected) and divided into 3 subgroups A,B, and C which treated with Gentamycine 0.2mg/chick/day for consecutive days, Amikacin 15 mg/kg/day for 3 consecutive days and sulpha-Trimethoprime 10/kg/day for 10 days, respectively
- **Group (3):** of 20 chicks were inoculated orally with 1ml of broth culture containing 5×10^9 colony forming unit per ml (CFU/ml)and subdivided into 3 subgroups(A,B and (C)treated with gentamycin, Amikacin and Sulphatrimethoprim, respectively with similar doses to group (2).
- **Group (4):** of 20 chicks were intramuscularly infected with 1 ml broth culture contain 5×10 9 CFU/ml and subdivided into 3 subgroups (A, B and C) and treated similar to group (3).

Group (5): Of 20 chicks were subcutaneously infected with 1 ml broth culture contain 5 ×10 9 CFU and divided into 3 subgroup (A, B and C) and treated with gentamycin, Amikacin combined with Sulphmethoprime similar to group (4)

All chickens were kept under close observation for 3 weeks during which clinical signs and mortality were recorded. Dead and sacrificed chickens were subjected to postmortem and bacteriological examinations for re-isolation of the inoculated organism.

Table (1): experimental infection

Group codes	Infection and treatment	Breed	NO of birds	Inoculation and dose
1	Non infected –non treated	White Lohman	10	Inoculated orally with 1ml of sterial saline (Blank control)
2A	Non infected treated with Uccmagent	White Lohman	3	Inoculated injectable with 1 ml of sterial saline then give Uccmagent 0.2 mg /chicks once for 3 days (control -ve)3
2B	Non –infected treated with Amikin	White Lohman	3	Inculated injectable with 1 ml of sterial saline then give Amikin 15mg/kg/day for 3 days (control –ve)
2C	Non – infrcted treated with Septazole	White Lohman	4	Iculated orally with I ml of sterile saline then give Septazole40 mg/kg/day for 10 days (control –ve)
3A	Infected orally and treated with Uccmagent		6	Iculated orally with 1 ml of 5×109after two weeks divided into 3 groups and NO 1treated with Uccmagent,
3B	Infected orally and treated with Amikin	White Lohman	6	NO. 2treated with Amikin and
3C	Infected orally and treated with septazole		8	NO.3 treated with Septazole.
4A	Infected I/Mand treated with Uccmangent		6	Inculated I/M with 0.25 ml of 5×109 after two weeks divided into 3 groups and NO1 treated with Uccmagent,
4B	Infected I/M and treated with Amikin	White Lohman	6	NO 2 treated with Amikin and
4C	Infected I//M and treated with Septazole	Lomman	8	NO 3 treated with Septazole.
5A	Infected S/C and treated with Uccmagent	XX /1 *.	6	Iculated S/C with 0.25 ml 5×109 after two weeks divided into 3 groups and NO1treated with Uccmagent ,
5B	Infected S/Cand treated with Amikin	White Lohman	6	NO 2 treated with Amikin and
5C	Infected S/C and treated with Septazole		8	NO 3 treated with Septazole

6. Antibiogrmme:

The antibiotic sensitivity test of the isolates was investigated against 20 antimicrobial agents using the disc diffusion technique according to *Cruick-shank et al.*, (1975). The test procedure was that recommended by the National Committee for Clinical Laboratory Standards (1990).

7. Treatment trials:

Uccmagent (Gentamycin) (Uccma-pharm) 2-4mg/kgbody weight. every 8 hours for 3 days, Amikin (each vial contain 250 mg amikacin sulfate) (Smithklin Beecham - Pharm), 15-20 mg/kg body weight for 3 days. and Septazole suspension (Sulfamethoxazole+ Trimethoprim) (Alexandria Pharm), 4--20 mg/kg body weight were used in drinking water for 5 consecutive days for treatment of experimentally challenged chickens with *K.Pneumoniae*.

8. Histopathology:

Specimens of lung, liver and intestine were taken from experimentally infected birds fixed in 10% neutral buffered formalin, washed, dehydrated in different concentrations of alcohols, cleared in xylol and embedded in paraffin wax by routine methods. They were then Sectioned at 4 μ m, stained with haematoxylin and eosin (HE) stain, and examined by light microscopy (*Bancroft and Gamble*, 2007).

9. Statistical analysis:

Statistical analysis of the obtained data was carried out according to *Petri and Watson* (1999).

RESULTS AND DISCUSSION

Ten isolates suspected to be *K. Pneumoniae* were isolated from 150 diseased broiler chickens with (6.6) isolation rate. Table (2).

Examined chickens suffered from respiratory disorder, septicamiemia, peritonitis, salpingitis, air sac diseases, omphalitis, artheritis, panophthalmitis and intestinal disturbances. Such diseases cause great economic losses in poultry industry not only due to high mortality rate in young bird, slow growth and poor feed conversion rates in growing birds but also due to decrease in egg production and hatchability of the infected eggs. *Plesser et al.*, (1975); *Mahalingam et al.*, (1988) and Rennie et al., (1990).

The prevalence of K. Pneumoniae isolated from various internal organs of examined chichs was described in Table (3). The isolation rate was higher in lungs (60%) than the liver (40%) and the lowest rate of isolation was from the intestine (10%). Similar results were reported by Gylstorff and Gerlach (1974) and Buxton and Fraser (1977).

K. Pneumoniae isolated from internal organs of diseased chickens on MacConkey agar, were Gram negative bacilli, non-spore forming, capsulated, non-motile and arranged singly. Colonies appear lactose fermenting, dome shaped, 3-4 mm diameter after overnight incubation at 37° C. Fig. (1). Similar results were obtained by *Mona Mohammed Aly* (2014).

The biochemical activity of the recovered isolates were typed to that of K. *Pneumoniae* and described in Table (4). The isolates were negative in motility, Indole test was variable, Methyle red and H_2s production were negative, Vogas proskuaer, Citrate utilization, Urease

and Sugar fermentation were positive, Similar results were reported by *Kawakib Ibraheem Al Zubaid*(2009).

Molecular identification by using PCR revealed that 10 isolates were positive and amplified at 130 bp Fig. (2).similar findings were obtained by Fang et al. 2004; Ku et al. 2008 and Cheng et al. 2010). Also Yu et al. (2007).

The experimentally infected. 7-day-old broiler chickens suffered from decreased body weight gain, ruffling of the feather, progressive weakness and prostration. Respiratory symptoms appeared on the second day after infection and were manifested by gasping, abnormal breathing while intestinal troubles were represent by yellow- greenish diarrhea. The mortality rate ranged from 15-20%. Table (5). Similar results were reported to *Abd-Alla* (1981).

The post-mortem lesions of dead and sacrificed birds revealed hyperemia of the lunge, congestion of the liver, spleen, enlargement of the gall bladder and sometimes enteritis. Fig. (2). the organisms were reisolated from lung and liver. These results were similar to that reported by *Ann Moursy et al* (1982) and *Dessouky et al*. (1982).

The results of the in vitro antibiotic sensitivity test revealed that all tested *K.pneumoniae* isolates were highly sensitive to gentamycin, Amikacin and Sulphamethoxazal, moderate sensitive Ciprofloxacine and Chloramphenicol and low sensitive to Noreflaxacine, Neomycin and Oxytetracycline but resistant to Cefotaxim, Amoxacillin and Cephalothin. Table (6), (7). These results were similar to that obtained by *Sundaresan et al.* (2007).

Treatment trials:

The results of treatment trial of experimentally infected chicks were described in table (8). The clinical signs and post-mortum lesion were improved and mortality were disappeared in experimentally infected chicks and treated with Gentamycin 2-4 mg/kg. Body weight for three days, Amikacin 15/20 mg/kg. Body weight for three days and Sulphamethoxazol-trimethoprim 40mg/kg+ 8 mg/kg for three days.

In this study, Klebsiella isolates showing 20% resistance to amikacin. A low prevalence of amikacin resistance (7%) to Klebsiella spp. isolated from meat samples were also reported by *Gundogan et al.* On the other hand, *Ullah et al.* (2009) reported that 63. 04% of Klebsiella isolates were susceptible to amikacin.

Klebsiella strains recorded high antibiotic resistance with multiple antibiotic resistance (MAR). Rate of multiple antibiotic resistances was extremely high which may be due to the hazard routinely use antibiotics for treatment and control of bacterial diseases in poultry farms. When these antibiotics are administered to the birds at low levels for a long period, certain bacterial species become resistant (*Kilonzo-Nthenge et al.* 2007). These antibiotic-resistant bacteria can reach to human through consumption of food products from animal origin and by direct contact (*Van den and Stobberingh, 2000*). Our finding is in accordance with a previous literature reported by *Davies et al.* (2016) who reported a 25% multiple drug resistance of K. pneumoniae isolates from p psittacines. Also *Bonnedahl et al.* (2014) detected 13% MDR strains from the samples they collected from ageese and free-living gulls in Alaska, USA.

Histopathological examination of lungs of experimentally infected chickens revealed diffuse interstitial pneumoniae characterized by infiltration of monocular cells and hyperplastic bronchial epithelium Fig. (a), the liver of experimentally infected chickens showed vacuolar degeneration and sporadic necrotized hepatocytes Fig. (b) and the intestine showed necrosis, destruction and infiltration of mononuclear cells in the intestinal lumen Fig.(g).

The lung of experimentally infected chicken with *K. Pneumoniae* and treated with Gentamycin showed few areas with mild interstitial pneumonia and congestion of the large blood vessels. **Fig.** (d). The liver of experimentally Infected chicken with *K. Pneumoniae* and treated with Amikacin showed necrotic foci and mild mononuclear cell infiltration in the parenchyma **Fig.** (h). Similar results were obtained by *Eman* (1998).

The treatment improved the infected cases by different degrees, the best results obtained by gentamicin in which all organs seemed to be normal followed by Amikacin and sulphamethoxazol which leave mild effect on tissues on infected birds neither clinically nor histopathologically.

Table (2): Results of *Klebsiella Pneuonmiae* isolation

Total No. of chicks samples	Klebsiella	infection	Non klebsiella infection		
	No.	%	No.	%	
150	10	6.6	140	93	

Table (3): Prevalence of *Klebsiella Pneuonmiae* isolation in various organs of the examined chicks

Organs	Total NO. of examined	NO. of the isolates	Precentage of the isolation (%)
Lungs	150	6	4%
Livers	150	3	2%
Intestine	150	2	1.3%

Fig. (1): Klebsiella Pneumoniae on MacConkey agar (lactose fermenter colonies).



Table (4): Biochemical tests for identification of *K.pneumoniae*:

Biochemical test	K.pneumoniae
Motility	-
Indole	V
Methyle red	-
VogesProskuaer	+
Citrate utilization	+
Urease	+
H2S	-
Nitrate reduction	V
Gelatin liquefaction	-

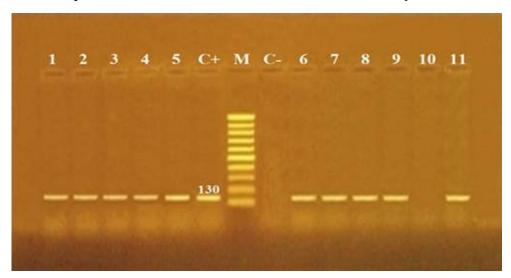
Biochemical test	K.pneumoniae
ODC	-
LDC	+
Arginine dihydrolase	-
ONPG	V
Sugar fermentation	
Lactose	+
Sucrose	+
Dulcitol	V
Salicin	+
Arabinose	+
Inositol	+
Xylose	+

(+): Most strain positive.

(-): Most strains negative.

(v): Some strains positive, other negative, Variable.

Fig. (2): Agarose gel electrophoresis of PCR of 16S-23S rDNA "ITS" (130 bp) specific for detection andidentification of *Klebsiella pneumoniae*



Lane~M~: 100 bp~ladder~as~molecular~size~DNA~marker

Lane C+ : Control positiveK. pneumoniae for 16S-23S ITS.

Lane C- : Control negative.

Lanes from 1 to 9 & 11: Positive K. pneumoniaestrains.

Lane 10 : Negative K. pneumoniaestrain.

Table (5): Result of experimental infection of 7 - day - old broilers with *K.pneumoniae*.

	No. of	Death time						Mort	ality					
Group No.	No. of dead chicks at different intervals post-infection/ day						More	anty						
	birds	2	3	4	7	9	13	14	16	18	20	21	No.	%
1	10	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	0	0	0	0	0	0	0	0	0	0	0	0	0
3	20	2	0	0	0	1	0	1	0	0	1	1	6	30
4	20	2	2	1	1	0	1	1	0	1	1	2	12	60
5	20	3	0	1	1	1	0	1	0	0	1	1	9	45

Fig. (3): Experimentally infected chickens with *K.Pneumoniae* showing the clinicopathological features of the disease, diarrhea and past vent were observed in chicks and congestion of the lung







B-Pasty Diarrhea



C-Hemorragic lung

Table (6): Results of in vitro antibiotic sensitivity test of *K. pneumoniae* isolates against different 20 antimicrobial agents.

Antimicrobial agent	S		I		R	
Antiniici obiai agent	NO	%	NO	%	NO	%
Cephalothin (CN)	-	-	-	-	10	100
Amoxicillin(AMX)	-	-	1	10	9	90
Erythromycin (E)	1	10	2	20	7	70

Cefotaxim (CF)	-	-	3	30	7	70
Ampicillin (AM)	1	10	3	30	6	60
Oxytetracycline (T)	2	20	2	20	6	60
Norfloxacin (NOR)	3	30	1	10	6	60
Chloramphenicol (C)	4	40	1	10	5	50
Neomycin (N)	2	20	3	30	5	50
Ciprofloxacin (CP)	5	50	1	10	4	40
Kanamycin (K)	3	30	4	40	3	30
Sulphamethoxazol (SXT)	6	60	1	10	3	30
Amikacin (AK)	7	70	1	10	2	20
Gentamicin (G)	9	90	-	-	1	10

Table (7): Antimicrobial resistance profile of *K.pneumoniae* strains (n=10).

NO	Strain	Antimicrobial resistance profile	MAR index
1	K.pneumoniae	CN, AMX, E, CF, AM, T, NOR, C, N, CP, K, SXT, AK, G	1
2	K.pneumoniae	CN, AMX, E, CF, AM, T, NOR, C, N, CP, K, SXT, AK	0.928
3	K.pneumoniae	CN, AMX, E, CF, AM, T, NOR, C, N, CP, K, SXT	0.857
4	K.pneumoniae	CN, AMX, E, CF, AM, T, NOR, C, N, CP	0.714
5	K.pneumoniae	CN, AMX, E, CF, AM, T, NOR, C, N	0.643
6	K.pneumoniae	CN, AMX, E, CF, AM, T, NOR	0.500
7	K.pneumoniae	CN, AMX, E, CF	0.286
8	K.pneumoniae	CN, AMX	0.143
9	K.pneumoniae	CN, AMX	0.143
10	K.pneumoniae	CN	0.071
		Average 0.529	

CN: Cephalothin	AMX: Amoxicillin	E: Erythromycin	CF: Cefotaxim
AM: Ampicillin	T: Oxytetracycline	NOR: Norfloxacin	C: Chloramphenicol
N: Neomycin	CP: Ciprofloxacin	K: Kanamycin	SXT: Sulphamethoxazol
AV. Amileosin	C. Contamioin		

Table(8): Treatment trials of experimentally infected broilers with *K.Pneumoniae*.

Group	No. of birds	Treatment	Drug used	Mortality	
No.	No. of birds	Treatment	Di ug useu	No.	%
1	10	Non Infected +Non treated	-	0	0
2	10	Non Infected + treated	Uccmagent+ Amikin+ Septazole	0	0
3	20	Non Infected + treated	Uccmagent+ Amikin+ Septazole	6/20	30%
4	20	Non Infected+ treated	Uccmagent+ Amikin+ Septazole	13/20	65%
5	20	Infected + treated	Uccmagent+ Amikin+ Septazole	9/20	45%

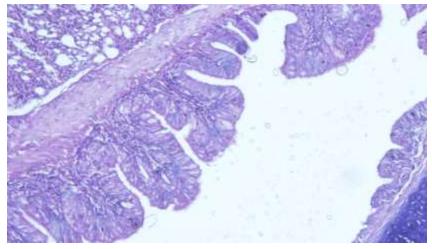


Fig. (a): Lung of a chick Infected orally with 1ml broth culture contain 5×10^9 CFU/ml. of K.

Pneumoniae (group3) showing hyperplastic bronchial epithelium (H&E, X100).

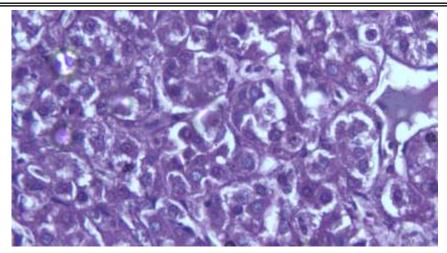


Fig. (b): Liver of a chick Infected orally with 1ml broth culture of k. *Pneumoniae* contained 5×10^9

CFU/ml. (group3) showing vacuolar degeneration and sporadic necrotized hepatocytes (H&E, X400).

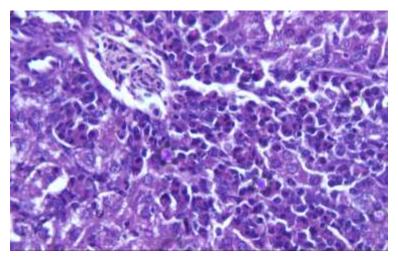


Fig. (c): Liver of a chick Infected orally with 1ml broth culture of K.

Pneumoniae contained 5×10^9

CFU/ml. (group3) showing infiltration of monocular cells and sometimes neutrophils in the portal areas (H&E, X400).

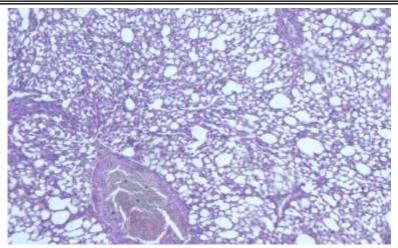


Fig. (d): Lung of a chick orally Infected with 1ml broth culture of K. *Pneumoniae* contained 5×10^9

CFU/ml. and treated with Gentamycin (group 3A) showing few areas with mild interstitial pneumonia and congestion of the large blood vessels (H&E, X40).

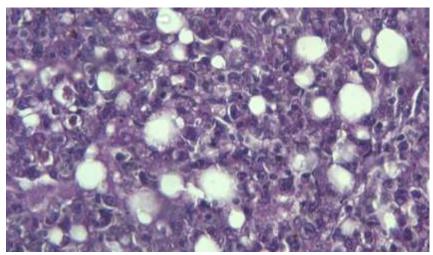


Fig. (e): Lung of a chick intramuscularly Infected with 1ml broth culture of K. *Pneumoniae* contained 5×10^9 CFU/ml. (group 4) showing diffuse interstitial pneumoniae characterized by infiltration of monocular cells in the interstitial tissue (H&E, X100).

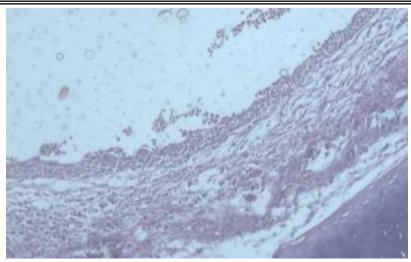


Fig. (f): Trachea of a chick infected intramuscularly with 1ml broth culture of *K. Pneumoniae* contained 5×10^9 CFU/ml.(group4) showing trachitis, desquamation of tracheal epithelium with sub-epithelial edema and infiltration of mononuclear cells (H&E, X40).

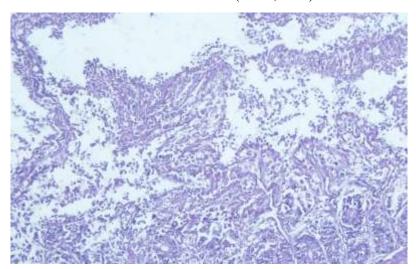


Fig. (g): Intestine of a Chick intramuscularly infected with 1ml broth culture of *K. Pneumoniae* contained 5×10⁹ CFU/ml. (group4) Showing necrosis, destruction and infiltration of mononuclear cells in the intestinal lumen (H&E, X40).

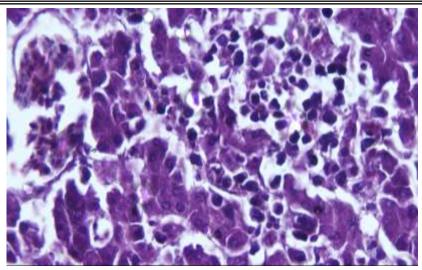


Fig. (h): Liver of a chick intramuscularly Infected with 1ml broth culture of K. *Pneumoniae* contained 5×10^9 CFU/ml and treated with Amikacin. (group 4B) showing necrotic foci and mononuclear cell infiltration in the parenchyma (H&E, X400).

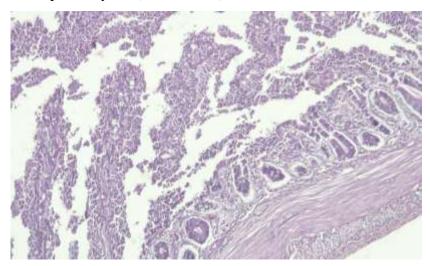


Fig. (i): Intestinal glands of a chick intramuscularly infected with 1ml broth culture of *K. Pneumoniae* contained 5×10^9 CFU/ml and treated with Amikacin (group 4B) showing destructed, degenerated and shrinked

intestinal glands with mononuclear cell infiltration in the lamina propria (H&E, X40).

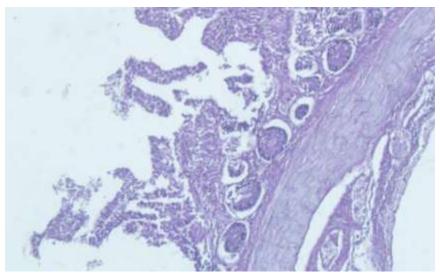


Fig. (j): Intestine of a Chick subcutaneously Infected with 1ml broth culture of *K. Pneumoniae* contained 5×10^9 CFU/ml (group 5) showing necrosed, destructed and shrinked intestinal villi (H&E, X40).

CONCLUSION

This study spotlight the prevalence and incidence of *Klebsiella Pneumoniae* in clinically diseased chicken suffered from respiratory manifestation. The study confirmed the pathogenic potential of the isolated strains and their association with clinical manifestations in respiratory tract infections of broiler chicken. Antimicrobial susceptibility pattern showed high multiple antibiotic resistances which require strict regulations of the use of antibiotics in veterinary therapy to minimize the emergence of resistant bacteria in chickens which may increase the public health problem.

REFERENCES

- *Abd Alla. O. A. M. (1981):* Histopathological and clinic pathological studies on poultry following artificial infection by Klebsiella. M. V. Sc. Thesis, Fac. Vet. Med., Cairo Univ.
- Ann, Mours; Zeinab, M. Niazi and Osaman, MA. (1982): Some pathological and bacteriological aspects of Klebsiella infection J.Egypt .Vet. Med. Ass., 42 (2):125-136.
- *Bancroft, J. D. and Gamble, M. (2007):* Theory and Practice of Histological Techniques. ^{5th} Ed. Churchill Livingstone, London, UK, pp: 125-138.
- Bonnedahl J, Hernandez J, Stedt J, Waldenström J, Olsen B & Drobni M (2014): Extended-Spectrum β-lactamases in Escherichia coli and Klebsiellapneumoniae in gulls, Alaska, USA.Emerging Infectious Diseases, 20, 897-899.
- Buxton, A. and Fraser, G. (1997): Animal Microbiology, Vol. 1Blackwell Scientific Publications, Oxford.
- Cheng HY, Chen YS, Wu CY, Chang HY, Lai YC. And Peng HL (2010): rmpA regulation of capsular polysaccharide biosynthesis in Klebsiella pneumoniae CG43. J Bacteriol. 192:3144–3158.
- *Cruickshank*, *R.*, *Dugaid*, *J. P.*, *Marmion*, *B. P. and Swain*, *R. H.* (1975): Medical Microbiology, 12th Ed, 522-35. Living stone L.T.D, Edinburgh, London, New York.
- *Eman*, *M*. *Z*. (1998): Microbiology and serologe on avien Klebsiellae M.V.Sc. Thesis, Fac. Vet. Med., Cairo Univ.

- Fang CT, Chuang YP, Shun CT, Chang SC and Wang JT. (2004):
 A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications. J Exp Med. 199:697–705.
- Gomis, S. K. Amoako. M, Ngeleka, L. Belanger, B. Althouse, L, kumor, E. Waters, s. Stephens, CRiddell, A.Potter and B.Allan (2001): Histopathologic and bacteriologic evaluations of cellulitis detected in legs and caudal abdominal regions of turkeys Avian diseases 46: 192-197.
- *Gundogan N, Cıtak S, Yalcin E (2011):* Virulence properties of extended spectrum beta-lactamase-producing Klebsiella species in meat samples. J. Food Prot. 74:559-564.
- *Gylstorff, I. and Gerlach, H. (1974):* Klebsiella aerogenes infection in turkey poults and therapy experiment with polymyxin B.Deut. Tierarzt. Woch., 81 (13): 298-299.
- Jeffrey, J. S., Shivaprasad, H. L., Duran, L., Cardona, C. J. and Charlton, B. R. (1993): Facial cellulitis associated with fowl cholera in commercial turkeys. Avian diseases, 1121-1129.
- Kamran, M., Ahmad, M. U. D., Anjum, A. A., Maqbool, A., Muhammad, K., Nawaz, M., and Ali, M. A. (2014): Studies on the antibiotic sensitivity pattern of isolates of P. multocida from baffaloes JAPS, Journal of Animal and Plant Sciences, 24: 1565-1568.

- *Karaman*, *R*. (1980): Studies on some bacterial diseases of poultry casusing high mortality in balady hateries (in Monofia Province).M.v.Sc., thesis Fac. Vet Med .Cairo Univ.
- Kawakib Ibraheem AL Zubaidi (Thi-Qar University -College of Science) (2009): Identification of the most bacterial isolates from the bile of broiler Chicks by API 20E system.
- *Kilonzo-Nthenge*, S. N. Nahashon, 1 F. Chen, and N. Adefope, (2007): Prevalence and Antimicrobial Resistance of Pathogenic Bacteria in chicken and guinea fowel. Poultry Science 87:1841–1848.
- *Ku YH*, *Chuang YC and Yu WL*. (2008): Clinical spectrum and molecular characteristics of Klebsiella pneumoniae causing community- acquired extrahepatic abscess. J Microbiol Immunol Infect. 41: 311–317.
- Liu, Y.; Liu, C.; Zheng, W.; Zhang, X.; Yu, J.; Gao, Q.; Hou, Y. and Huang, X. (2008): PCR detection of Klebsiella pneumoniae in infant formula based on 16S-23S internal transcribed spacer. Inter. J. Food Microbiol., 125: 230-235.
- Mahalingam, P.; Masillamony, P. R.; Palanismuni, K.S. and Venugopalan, V. T. (1988): Virulence attributes of E. Coli wdKlebsiella isolates from hatcheries. Ind. Vet J., 65 (4): 283-287.
- Nawaz, I., Munir, R., Farooq, U., Kausar, R. and Khanum, A. (2006): Whole cell protein profiling of Pasteurella multocida field isolates in Pakistan. Pakistan Veterinary Journal, 26(4): 157-160.

- *Petri and Watson* (1999): Statistics for Veterinary and Animal Science, 1st Ed., U.S.A. Prevalence of antibodies against ORT in broilers and breeders in Southeren Brazil. Avian Diseases, 47:731-737.
- Plesser, O.; Even-Shoshan, A. and Bendheim, U.(1975): The isolation of Klebsiella Pneumoniae from poultry and hatcheries. Refuah Vet., 32 (3):99-105.
- Rennie, R. P.; Anderson, C.M.; Weinsley, B.C.; Albritton, W.I. and mahony, D.E. (1990): Klebsiella Pneumoniae gastroenteritis masked by clostridium perfringens. J. Cline. Microbio., 28 (2):216-219.
- Sandra, O.; Jesas and J,H.,Duarte carreia (1998): Potential pathogens recovered from upper respiratory tract of Psittacine birds. Department of morphology antMinical Facu. Of Vet. Rua Gomes. Freler 1199 Lisboa Codex. Portugal.
- *Sarakby, T.M.B.* (1979): Studies on the epidemiology of K. infection in poultry, M.V. Sc. Thesis Fac. Vet. Med. Cairo Unv.
- Sekariah, P. C. and Seth, R. N. (1957): Studies on Klebsiella pneumoniae infection in chicks Ind. Vet, J., 34, 315-320.
- Shivaprasad, H.L. (1998): An overview of anatomy, Physiology and Pathology of urinary system in birds, AV proceedings, PP.
- Sundaresan, N.R.; Sukumar, K.; Rajukumar, K.; Nagarajan, K.; Jaganathan, S. and Dorairajan, N. (2007): Antimicrobial susceptility of bacterial isolates from sheep different experimental patterns and ELISA. J. Egypt. Vet. Med. Assos. 62:57-71.

- Tatum, F. M., Tabatabai, L. B. and Briggs, R. E. (2012): Cross-protection against fowl cholera disease with the use of recombinant *Pasteurella multocida* FHAB2 peptides Vaccine. Avian Dis. 56 (3): 589-91.
- *Ullah*, *F Malik SA and Ahmed J (2009)*: Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. Afr. J. Microbiol. Res. 3:676–680.
- Van den Bogaard AE, Stobberingh EE (2000): Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents. 14: 327-35.
- Wilkie, I. W., Grimes, S. E., Boyle, D. and Frost, A. J. (2000): The virulence and protective efficacy for chickens of *Pasteurella multocida* administered by different routes. Vet. Microbiol. 72 (1-2):57-68.
- Yu VL, Hansen DS, Ko WC, Sagnimeni A, Klugman KP, von Gottberg A, (2007): Virulence characteristics of Klebsiella and clinical manifestations of K. pneumoniae blood stream infections. Emerg Infect Dis. 13:986–93.

استهدفت الدراسة مغرفة وبائية المرض وطرق العلاج المناسب لعدوى الكيبسيلا نيمونى كمسبب رئيسى لأمراض الجهاز التنفسي على دجاج التسمين. لهذا الغرض تم فحص مزارع دجاج التسمين في أماكن مختلفة في محافظة الغربية في الفترة من 2014–2017م.

تم الفحص البكتيريولوجي لعينات من الرئتين والقصبة الهوائية والكبد والأمعاء مجمعة من 20 مزرعة تسمين مصابة عند عمر 3–40 يوم كان تعانى من ضعف وصعوبة في التنفس وإفراز سائل مخاطي والتهاب في العشاء المخاطي والتهاب في الحويصلات الهوائية ونقص في إنتاج البيض وحدوث نافق في بعض الأحيان. ونفوق وأسفرت الصفة التشريحية عن وجود التهابات رئوية وتضخم في الكبد والطحال. وكانت نتائج الفحص البكتيريولوجي لمسحات من الرئتين والقصبة الهوائية والكبد والأمعاء أن الميكروبات المعزولة كانت سالبة الجرام غير متحركة عصوية الشكل لها خاصية تجمع الصبغة عند طرفي الميكروب.

أسفرت النتائج عن عزل عدد 10عترات من الكيبسيلا نيموني بنسبة 6.6%.

تم عمل العدوى الاصطناعية لمعزولات الكيبسيلا وتم تسجيل الأعراض الإكلينيكية والصفة التشريحية ونسبة النفوق واعادة عزل الميكروب المحقون.

أوضحت النتائج أن العترات المعزولة كانت شديدة الحساسية لكل من (الجنتاميبسين، الأميكاسين سلفاميزاكزول)، ومتوسط الحساسية السيبروفلوكساسين والكلوراميفيكول وقليل الحساسية للنيوميسين ،نورفلوكساسين و الأوكسى تتراسيكلين ولكنه مقاوم اموكسيسيلين ، سيفالوسين النيوميسين وسيفوتكسيم.

حينما تم علاج الدجاج المعدي اصطناعيا بحقن جنتاميسين والامكين لمدة ثلاث أيام متتالية وإعطاء سلفاميزاكزول في ماء الشرب في نفس الوقت. لوحظ اختفاء الأعراض الإكلينيكية والصفة التشريحية المميزة للمرض.