Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 55 (2): 98-106. Oct. 2017
DOI: 10.5455/ajvs.249636

Molecular Characterization of Some Bacteria Isolated from Broiler Chickens Showing Respiratory Manifestations

Eman M.M. Elghazaly[1], **Eman k. Sedeek**[1], **Samy A. Khalil**[2]
[1]Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University, Matrouh branch
[2]Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University

Key words:
E. coli, P. aeruginosa, virulence genes, antibiogram, plasmid profile

Correspondence to:
emanmoneer1@gmail.com

ABSTRACT

Fermented wheat germ extract (FWGE) is a multisubstance composition contains 2-methoxy benzoquinone and 2, 6-dimethoxy benzoquinone which are likely to exert some of its biological effects as well as it is a concentrated source of vitamins, minerals, and protein. An experimental trial of FWGE supplementation to broiler feed from one day old with a rate of 0.5, 1.5 and 3 g/kg feed was tried. Results revealed that all doses of FWGE increased body weight significantly ($p \leq 0.05$), especially with the dose of 3 g/kg feed. Also, FCR values decreased significantly ($p \leq 0.05$) in the FWGE treated groups. Regarding biochemical analysis at 35 days old, the most significant results obtained with the doses of 1.5 and 3 g FWGE/kg feed especially in SGOT, SGPT, creatinine, uric acid, total protein, glucose, and triglycerides levels. Physiologically, FWGE only increased hemoglobin concentration significantly ($p \leq 0.05$) without alteration of red blood and white blood cells counts. There was also a significant increase ($p \leq 0.05$) in the intestinal weight in relation to carcass weight% and a significant decrease ($p \leq 0.05$) in the liver and total body fat weights in relation to carcass weight%. At 45 days (10 days after vNDV challenge), the mortality rates were 60% in the non-treated non-vaccinated challenged chicken group 5 and 4% in the non-treated vaccinated challenged chicken group 4 without appearance of any mortality in the 3 FWGE treated groups. Also, addition of FWGE had a positive effect on HI titers for NDV in the collected serum samples at 45 days old. Finally, it was concluded that FWGE improved the general health condition of broilers regarding biochemical and physiological parameters and immune response to NDV vaccination.

1. INTRODUCTION

Diseases of the respiratory tract have an important role in poultry. Among the systemic disease, respiratory system diseases generally ranked first (Glisson, 1998). A mixed infection is the main causative agent of chronic respiratory diseases in chickens (Nunoya et al., 1999).These diseases may result as either primary or multi system diseases. Respiratory system diseases may result from bacteria, virus, parasite, fungi or nutritional environmental factors (Glisson, 1998)

Escherichia coli is considered one of the most serious bacteria that affecting poultry and other animals and causing significant economic losses in poultry industry and also affecting humans (Moulin scholeur et al., 2007). *E. coli* has an important role in lower-respiratory-tract infections in poultry. Although in severe cases mortality can be over 20%. It is associated with high morbidity and loss in productivity resulting in greatest economic loss (Gross, 1994).

Temperature-sensitive haemagglutinin (tsh) of *E. coli* plays a significant role in the colonization of air sacs (Dozois et al., 2000); P-fimbriae (pap) are responsible for the adhesion to internal organs in later stage (Dho-Moulin and Fairbrother, 1999).

Pseudomonas aeruginosa primarily affects the upper respiratory tract of poultry, causing rhinitis, sinusitis and laryngitis (Bailey et al., 2000).Pathogenicity of *P. aeruginosa* in birds is mainly associated with septicemic and respiratory infections and sinusitis (Hai-ping, 2009).

P. aeruginosa are able to survive in variable environmental conditions due to its versatile nutritional abilities and its ability to resist high concentrations of common antibiotics (Aumeran et

al., 2007). Resistance to antibiotic has been occurred due to combination of restriction of antibiotic uptake through the outer membrane and a variety of energy-dependent mechanisms (Presteri et al., 2007).

P. aeruginosa is a classic opportunistic pathogen because it has the ability to resist many antibiotics and disinfectants; and also due to it has a large number of putative virulence factors and acquired resistance due to plasmids (Shahid and Malik, 2004).

Staphylococcus aureus is considered to be a normal resident of the chicken, located on the skin and feathers and in the respiratory and intestinal tracts. Avian Salmonellosis caused by *Salmonella* species is related to economic problems that occur in all stages of poultry industry from production to marketing. It resulted in drop of egg production, fertility, hatchability and increased early chick mortality (Abd - Ellatef, 1995).

The aim of the study was planned for isolation and identification of bacteria from broiler chickens showing respiratory manifestations, Confirmation of biochemically identified *E. coli* isolates by PCR as an accurate and sensitive technique, detection of virulence genes of *E. coli* associated with respiratory system infection, detection of sensitivity of *P. aeruginosa* to different antibacterial agents and Plasmid profile of *P. aeruginosa.*

2. MATERIALS AND METHODS:

2.1. Sampling:

A total of 41samples (16 nasal swabs, 11tracheal swabs and14lung samples) were collected. The collected swabs were immersed into nutrient or trypticase soya broth and transferred to the laboratory of Microbiology Department, Faculty of Veterinary Medicine, Alexandria University in an insulated ice box without delay to be subjected for bacteriological examination.

2.2. Isolation and identification of bacteria from broiler chickens with respiratory manifestations:

The collected samples were immersed into tryptic soya broth or nutrient broth and incubated at 37 ˚C for 18hrs then subcultured into 5% blood agar base, nutrient agar, Mannitol salt agar, MacConkey`s agar, Eosin methylene blue agar medium, Cetrimide agar medium and Muller-Hinton agar medium and incubated at 37 ˚C for 24-48hrs.suspected colony from different media were picked up and subjected to morphological and biochemical identification (Quinn et al., 2011).

2.3. Antibacterial sensitivity test for isolated *P. aeruginosa*:

After preparation of McFarland's No.1 from *P. aeruginosa* isolates, a sterile Pasteur pipette was used to inoculate the suspension on the surface of Muller Hinton agar plate. Excess fluid was removed by the pipette then the plate was incubated for about 30 min. The chosen antibiotics discs were applied to adequate spacing so that two discs shouldn`t closer than 24 mm from one center to the other center and no more than 15 mm from the edges of Petri dish by using sterile fine pointed forceps. The discs were pressed gently to ensure full contact of discs to the medium, and then plates were incubated at 37 ˚C for 24 hours. After incubation, the degree of sensitivity was determined by measuring the easily visible and clear zone of inhibition of growth produced by diffusion of antibiotics from disc into the surrounding medium. The results were interpreted according to NCCLS (1990).

2.4. Plasmid profile of *isolated P. aeruginosa*:

The isolated and biochemically identified *P. aeruginosa* isolates were tested for presence or absence of plasmid using alkaline lysis method (Sambrook et al., 1989). For detection of plasmid DNA expression after Subculture (five times) of *P. aeruginosa* in presence of half Minimum Inhibitory Concentration (MIC) of ofloxacin antibiotic; one disc of ofloxacin was dissolved (200 mg / L) in falcon tube containing 10 ml distilled water. About, 25 µl from antibiotic suspension containing 0.5 MIC were added to one litter of sterile nutrient agar medium after its cooling to 50˚C to avoid destruction of antibiotic. The medium containing half MIC of ofloxacin antibiotic (0.5 mg /L) was poured into sterile Petri dishes and left to dry. Bacterial isolates having plasmid DNA were streaked into nutrient agar medium containing half MIC of ofloxacin antibiotic. Plates were incubated at 37˚C for 24hrs. The previous steps were repeated five times to detect effect of half MIC of ofloxacin antibiotic (0.5 mg /L) on expression of plasmid DNA by alkaline lysis method (Sambrook et al., 1989). For detection of plasmid DNA expression after Subculture of *P. aeruginosa* in absence of antibiotic; a sterile nutrient agar medium without antibiotic was poured into sterile Petri dishes and left to dry. Bacterial isolates having plasmid DNA were streaked into nutrient agar medium without antibiotic. The plates were incubated at 37˚C for 24 hrs. The previous steps were repeated (5, 10 and 15 times) to detect effect of subculture of bacteria containing plasmid DNA in absence of antibiotic on expression of plasmid DNA by alkaline lysis method (Sambrook et al., 1989).

2.5. Molecular identification of isolated *E. coli:*

a. DNA extraction:

DNA Extraction was carried out for 21 biochemically identified *E. coli* isolates by boiling method (Sambrook and Russell, 2001).

B. DNA Molecular weight marker:

The ladder was mixed gently by pipetting up and down. Six µl of the required ladder were directly loaded.

C. Agarose gel electrophoresis (Sambrook and Russell, 2001).

1. RESULTS AND DISCUSSION

In recent years respiratory diseases become the main hazards to the poultry industry causing significant economic losses. When chicks are exposed to stress, bacteria often penetrate protective barriers of the respiratory tract and causing severe damage to the heart and lungs by causing chronic respiratory disease (Lin et al., 1993), so the aim of this study to identify bacteria causing respiratory manifestations in broiler chickens.

In this study the results of identification of bacteria isolated from respiratory system of broiler chickens suffering from respiratory manifestations revealed that the most commonly isolated bacteria were *E. coli, P. aeruginosa, Corynebacterium spp,* Coagulase negative *Staphylococcus, Salmonella Entritidis, Staphylococcus aureus and Pasteurella multocida* which were isolated at a percentage of 33.3%, 26.9%, 15.9%, 11.1%, 6.4%, 4.8% and 1.6%, respectively as shown in table (4). The obtained results were similar to that reported by Mamza et al. (2010) who isolated *E. coli* from lung (15.5%) and trachea (15.3%) at a total percentage of 30.3%. these results were lower than that reported by Murthy et al. (2008) who isolated *E. coli and Pasteurella multocida* at a percentage of 51.9% and 9.6% , respectively.

Table (1): Primer sequences for amplification of virulence genes of ***E. coli***.

Target gene	Primer used	Primer sequence	Amplified Product (bp)	Reference
PhoA	phoA-F phoA-R	*CGATTCTGGAAATGGCAAAAG* *CGTGATCAGCGGTGACTATGAC*	720	*Hu et al., 2011*
Papc	Papc-F papc-R	*GACGGCTGTACTGCAGGGTGTGGCG* *ATATCCTTTCTGCAGGGATGCAATA*	328	*Tiba et al., 2008*
TSH	TSH-F TSH-R	*GGTGGTGCACTGGAGTGG* *AGTCCAGCGTGATAGTGG*	620	*Provence and curtiss, 1994*
FimH	*fimH-f* *FimH–R*	*TGCAGAACGGATAAGCCGTGG* *GCAGTCACCTGCCCTCCGGTA*	*508*	*Tiba et al., 2008*

Table (2): Components of PCR reaction used for detection of virulence genes of *E. coli*

Component	Volume/reaction
Emerald Amp GT PCR master mix (2x premix)	12.5μl
Forward primer(20 pmol)	1.25μl
Reverse primer (20 pmol)	1.25μl
Template DNA	10μl
Total	25 μl

Table (3): Cycling conditions of the primers used for amplification of genes of virulence of ***E. coli***

Gene	Initial denaturation	Denaturation	Annealing	Extension	No. of cycles	Final extension
Papc	94˚C 5 min.	94˚C 1min.	63˚C 1min.	72˚C 2 min.	30	72˚C 10 min.
Tsh	94˚C 5 min.	94˚C 1min.	55˚C 1min.	72˚C 2 min.	30	72˚C 10 min.
*Pho*A	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
Fimh	95˚C 2 min.	94˚C 30 sec.	58˚C 30 sec.	72˚C 1min.	33	72˚C 7 min.

Table (4): Bacteria isolated from broiler chickens suffering from respiratory manifestations.

Isolated bacteria	No. of isolates	%

E. coli	21	33.3
P. aeruginosa	17	26.9
Salmonella enteritidis	4	6.4
Pasteurella multocida	1	1.6
Staphylococcus aureus	3	4.8
Coagulase negative Staphylococcus	7	11.1
Corynebacterium species	10	15.9
Total	63	100

%: according to total number of isolated bacteria

Table (5): Incidence of bacteria isolated as a single culture from broiler chickens suffering from respiratory manifestations:

Suspected isolates	NO. of single culture	%
E. coli	14	34.1%
P. aeruginosa	6	14.6%
Coagulase negative ***Staphylococcus***	4	9.8%
Staphylococcus aureus	1	2.4%
Corynebacterium species	4	9.8%
Salmonella Enteritidis	2	4.9%
Pasteurella multocida	0	0%
Total	31	75.6%

No. of examined samples = 41 % was calculated according to total number of samples.

Table (6): Incidence of bacteria isolated as mixed culture from broiler chickens suffering from respiratory manifestations:

Suspected isolates	NO. of mixed culture	%
E. coli and *Corynebacterium species*	1	2.4%
E. coli and *Staphylococcus aureus*	1	2.4%
E. coli and *Salmonella Enteritidis*	1	2.4%
E. coli and *P. aeruginosa*	2	4.9%
P. aeruginosa and *Corynebacterium species*	1	2.4%
Salmonella Enteritidis and *Staphylococcus aureus*	1	2.4%
Pasteurella multocida and *Corynebacterium species*	1	2.4%
Coagulase negative *Staphylococcus* and *Corynebacterium species*	2	4.9%
Total	10	24.4%

No. of examined samples = 41 % was calculated according to total number of samples

This disagreement may be due to samples collected under complete aseptic condition which reduce the chance for environmental bacteria to grow. These results were higher than that reported by Berag and Elhassan (1987) who isolated *E. coli and P. aeruginosa* at a percentage of 17.92% and 10.4%, respectively and this dis agreement may be due to using different breed, age or season.

Results of amplification of *E. coli* (phoA) coding gene by using PCR. Twenty one isolates of biochemically identified *E. coli* were randomly studied for detection of phoA gene using PCR technique. The specificity of the primers was confirmed by positive amplification of fragment with the extracted DNA of the bacterial isolate. Out of 21 tested isolates, ten isolates (47.6%) were positive for the phoA gene as shown in figure (1, 2, and 3). The PCR assay yielded amplified products of 720bp specific for (phoA) gene.

Results of amplification of *E. coli* virulence genes associated with respiratory system by PCR (tsH, fimH and papC): out of ten detected *E. coli* isolates by PCR, only 3(30%) *E. coli* isolates were positive for tsh gene (specific band at 620bp) as shown in figure (4), 7(70%) *E. coli* isolates were positive for fimH gene (specific band at 508bp) as shown in figure (5), only 1(10%) *E. coli* isolate was positive for papc gene (specific band at 328bp) as shown in figure (6).

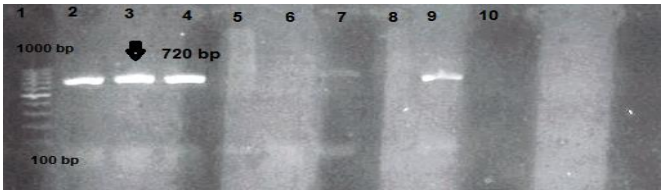

Figure (1): Agarose gel electrophoresis (1.5 %) of the amplified pho A gene of the isolated *E. coli*: Lane (1): DNA molecular weight ladder (100bp ladder). Lanes (2, 3, 4 and 7) indicate positive results for phoA gene (specific band of 720bp). Lane (9): control positive for phoA gene. Lanes (5, 6 and 8): are negative results for phoA gene.

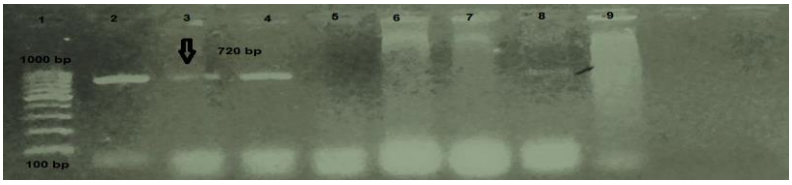

Figure (2): Agarose gel electrophoresis (1.5 %) of the amplified phoA gene of the isolated *E. coli*. Lane (1): DNA molecular weight ladder (100bp ladder). Lane (2): control positive for phoA gene. Lanes (3, 4 and 8): Positive results for phoA gene (specific band of 720bp). Lanes (5, 6, 7 and 9): negative results for phoA gene.

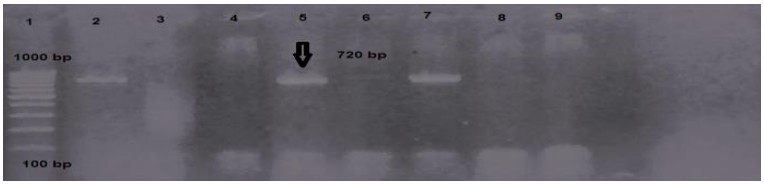

Figure (3): Agarose gel electrophoresis (1.5 %) of the amplified phoA gene of the isolated *E. coli*. Lane (1): DNA molecular weight ladder (100bp ladder). Lane (2): control positive for phoA gene. Lanes (5, 6 and 7): Positive results for phoA gene (specific band of 720bp). Lanes (3, 4, 8 and 9): negative results for phoA gene.

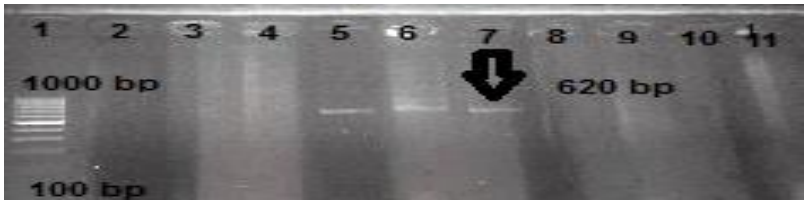

Figure (4): Agarose gel electrophoresis (1.5 %) of the amplified tsh gene of the isolated *E. coli*. Lane (1): DNA molecular weight ladder (100bp ladder). Lanes (5, 6and7): Positive results for tsh gene (specific band of 620bp). Lanes (2, 3, 4, 8, 9, 10 and 11): negative results for tsh gene**.**

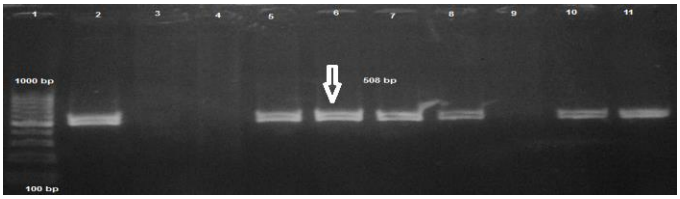

Figure (5): Agarose gel electrophoresis (1.5 %) of the amplified fim H gene of the isolated *E. coli*. Lane (1): DNA molecular weight ladder (100bp ladder). Lanes (2, 5, 6, 7, 8, 10 and 11): Positive results for fimH gene (specific band of 508bp). Lane (3, 4and 9): negative results of fim H gene.

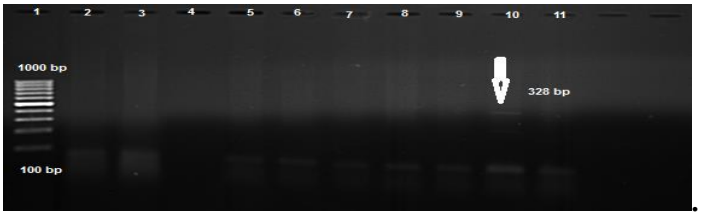

Figure (6): Agarose gel electrophoresis (1.5 %) of the amplified papC gene of the isolated *E. coli*. Lane (1): DNA molecular weight ladder (100bp ladders). Lane (10): Positive results for papc gene (specific band of 328bp). Lanes (2, 3, 4, 5, 6, 7, 8, 9 and 11): negative results for papC gene.

Table (7): Results of antibiotic sensitivity of ***P. aeruginosa*** and some ***E. coli*** isolates:

Antibiotics	No. of isolates		S				I				R			
	P. aeruginosa	***E. coli***	***P. aeruginosa***		***E. coli***		***P. aeruginosa***		***E. coli***		***P. aeruginosa***		***E. coli***	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Levofloxacin	17	3	1	5.9	1	33.3	0	0	0	0	16	94.1	2	66.7
Erythromycin			0	0	0	0	1	5.9	2	66.7	16	94.1	1	33.3
Streptomycin			1	5.9	1	33.3	0	0	0	0	16	94.1	2	66.7
Norfloxacin			1	5.9	1	33.3	3	17.7	0	0	13	76.5	2	66.7
Rifampin			0	0	0	0	0	0	0	0	17	100	3	100
Chloramphenicol			0	0	0	0	1	5.9	1	33.3	16	94.1	2	66.7
Neomycin			0	0	0	0	1	5.9	2	66.7	16	94.1	1	33.3
Ciprofloxacin			1	5.9	1	33.3	0	0	0	0	16	94.1	2	66.7
Ofloxacin			1	5.9	1	33.3	0	0	0	0	16	94.1	2	66.7

%: according to number of isolates, **R:** resistant**, S:** susceptible**, I:** intermediate susceptible.

The obtained results showed that presence of phoA gene in *E. coli* isolated from broiler chickens with respiratory manifestations at a percentage of 47.6% that higher than the result obtained by (Rasha et al.,2015) who recorded that PCR for amplification of phoA gene of *E. coli* at a percentage of 37.5%. This disagreement may be due to difference in the site of isolation from broiler chickens.

The recovered results showed that presence of tsh in *E. coli* isolated from broiler chickens with respiratory manifestations at a percentage of 30% that agree with the result reported by Oh et al., (2011) who found that PCR for amplification of tsh gene of *E. coli* at a percentage of34.48% and lower than the result obtained by (Roussan et al., 2014) who identified tsh gene with a percentage (66%) by using PCR.

This study detected presence of fimH gene in *E. coli* isolated from respiratory system of broiler chicken at a percentage of 70%. This result is higher than the result that said by(Mbanga and Nyararai, 2015) who said that percent of PCR for amplification of fimH gene of *E. coli* was (33.3%) of APEC isolates and lower than the result obtained by (Roussan et al., 2014) who identified fimH gene with a percentage (94%) by using PCR.

In this study, the percentage of papC gene was 10% which is lower than the result obtained by (Rocha et al., 2008) who found that papC gene with percentage of 24.3%.

As shown in table (7), the antibiotic resistance pattern showed that *P. aeruginosa* showed high resistance to levofloxacin (94.1%), streptomycin (94.1%), ciprofloxacin (94.1%), ofloxacin (94.1%), erythromycin (94.1%), chloramphenicol (94.1%), neomycin (94.1%) and norfloxacin (76.5%) but intermediate susceptible to erythromycin (5.9%), chloramphenicol (5.9%) and neomycin (5.9%) and norfloxacin (17.7 %) and susceptible to levofloxacin (5.9%), streptomycin (5.9%), ciprofloxacin (5.9%), ofloxacin (5.9%) and norfloxacin (5.9%). This result agreed with that conducted by (Olayinka et al., 2009) who reported that *P. aeruginosa* showed resistance to ciprofloxacin and chloramphenicol at a percentage of 90.2% and 97.8%, respectively but higher than that reported by (Akingbade et al., 2012) who found that P. aeruginosa showed resistance to erythromycin, streptomycin, ofloxacin, ciprofloxacin at a percentage 72.7%, 65.5%, 60% and 35.5%, respectively. This disagreement may be due to difference in strains of bacteria. *E. coli* isolates showed high resistance to rifampin (100%), levofloxacin (66.7%), streptomycin(66.7%) , norfloxacin (66.7%), chloramphenicol (66.7%), ciprofloxacin (66.7%), erythromycin (33.3%) and neomycin (33.3%) but intermediate susceptible to erythromycin (66.7%), neomycin (66.7%) and chloramphenicol (33.3%) but susceptible to levofloxacin (33.3%), streptomycin (33.3%), norfloxacin (33.3%), ciprofloxacin (33.3%) and ofloxacin (33.3%). This results agreed with that revealed by (Moniri and dastehgoli, 2007) who reported that *E. coli* showed resistance to ciprofloxacin at a percentage of 69.7% but higher than that reported by (Miles et al., 2006) who found that *E. coli* showed resistance to ciprofloxacin, norfloxacin and and ofloxacin at a percentage of 11.8%, 20.6% and 14.7%, respectively and this higher resistance may be due to frequent using of the same antibiotic in treatment which lead to resistance to this antibiotic but lower than that reported by (Jahantigh and Reza, 2015) who said that *E. coli* isolates were resistant to ciprofloxacin and norfloxacin at a percentage of 91% and 88%, respectively.

Plasmid DNA profile of extracted plasmid DNA from *P. aeruginosa* and some *E. coli* isolates: Out of 17 *P. aeruginosa* isolates, 15(88, 2%) isolates showed plasmid DNA profile (specific band

at 25kbp) and out of 3 *E. coli* isolates, 2 (66.7%) isolates also showed plasmid DNA profile at the same size as shown in figure (7).

Results of plasmid DNA profile of plasmid DNA extracted from *P. aeruginosa* and some *E. coli* isolates after subculture with 0.5 minimal inhibitory concentrations (MIC) of ofloxacin. All positive plasmid DNA profile of *P. aeruginosa* isolates (15 isolates) and *E. coli* isolates (2 isolates) showed plasmid DNA profile (specific band at 125kbp) after subculture with 0.5 MIC of ofloxacin as shown in figure (8).

Results of plasmid DNA profile of plasmid DNA extracted from *P. aeruginosa* and some *E. coli* isolates after (5, 10 and 15) subculture without antibiotic: All positive plasmid DNA profile of *P. aeruginosa isolates* (15 isolates) and *E. coli* isolates (2 isolates) showed plasmid DNA profile (specific band at 25kbp) after (5, 10 and 15) subculture without antibiotics shown in figure (9, 10 and 11).

In this study plasmids were not disappear by (5,10 and15) subculture and this disagree with that reported by Chadfield et al. (2001) who reported that disadvantage of plasmid profiling are not associated with epidemiological identical strains and lost by repeated subculture of bacteria over long period of times.

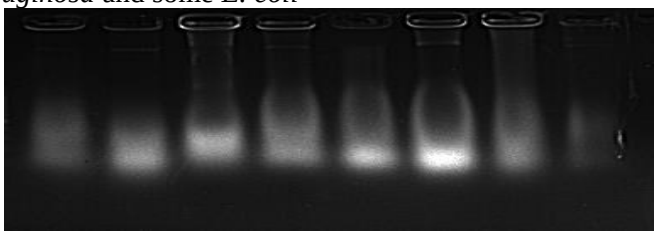

Figure (7): Agarose gel electrophoresis (1.5 %) of plasmid DNA extracted from *P. aeruginosa* and some *E. coli* isolates (specific band at 25kbp)

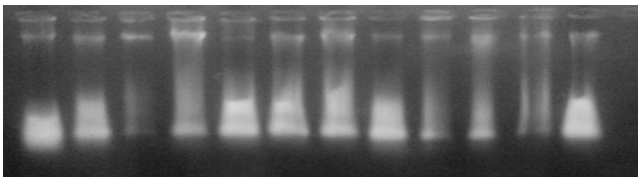

Figure (8): Agarose gel electrophoresis (1.5 %) of plasmid DNA extracted from *P. aeruginosa* and some *E. coli* isolates (specific band at 25kbp) after 5 subcultures with 0.5 MIC of ofloxacin.

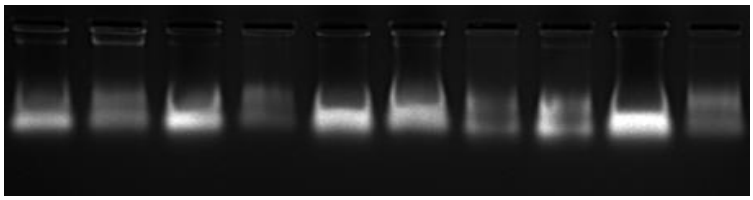

Figure (9): Agarose gel electrophoresis (1.5 %) of plasmid DNA extracted from ***P. aeruginosa*** and some ***E. coli*** isolates (specific band at 25kbp) after 5 subcultures without antibiotic.

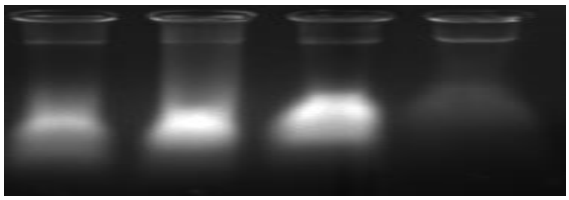

Figure (10): Agarose gel electrophoresis (1.5 %) of plasmid DNA extracted from *P. aeruginosa* and some *E. coli* isolates (specific band at 25kbp) after 10 subcultures without antibiotic.

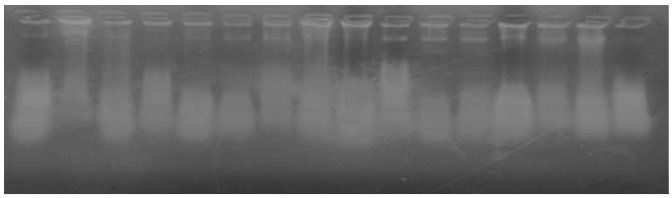

Figure (11): Agarose gel electrophoresis (1.5 %) of plasmid DNA extracted from *P. aeruginosa* and some *E. coli* isolates (specific band at 25kbp) after 15 subcultures without antibiotic.

REFERENCE

Abd EL-Latif, M.M., 1995. Bacterial causes of lowering fertility, hatchability and early embryonic deaths in balady hatcheries in Dakahlia Governorate. M. V. Sc. Thesis (Bacteriology) Fac. Vet. Zag. Univ.

Akingbade, O.A., Balogun, S.A., Ojo, D.A., Afolabi, R.O., Motayo, B.O., Okerentugba, P.O., Okonko, I.O., 2012. Plasmid Profile Analysis of Multidrug Resistant P. aeruginosa isolated from Wound Infections in South West, Nigeria: World Applied Sci. J. 20 (6): 766-775.

Aumeran, C., Paillard, C., Robin, F., 2007. P. aeruginosa and Pseudomonas putida outbreak associated with contaminated water outlets in an oncohaematology pediatric unit. J. Hosp. Infect. 65:47-53.

Bailey, T. A., Silvanose, C. D., Naldo, J. N., Howlett, J. H., 2000. P. aeruginosa infections in kori bustards (Ardeotiskori). Avian Pathol. 29: 41–44.

Berag, N. M. S., El Hassan, S. M., 1987. Bacterial respiratory tract infections of chicken in Kasala area, thesis, Khartoom University.

Chadfield, M., Skov, M., Christensen, J., Madsen, J.M., Bisgaard, M., 2001. An epidemiological study of Salmonella enterica serovar4, 12: b: in broiler chicken in Denmark. Vet. Microbiol. 82, 233-247.

Dho-Moulin, M., Fairbrother, J. M., 1999. Avian pathogenic Escherichia coli (APEC), Vet. Res., vol. 30 (2-3): 299–316.

Dozois, C.M., Dho-Moulin, M., Bree, A., Fairbrother, J.M., Desautels, C., Curtiss, R. III. 2000. Relationship between the Tsh autotransporter and pathogenicity of avian Escherichia coli and localization and analysis of the TSH genetic region, Infection and Immunity, vol. 68, no. 7, pp. 4145–4154.

Glisson, J. R., 1998. Bacterial respiratory diseases of poultry. Poult. Sci. 77(8): 1139-1142.

Gross, W. G., 1994. Diseases due to Escherichia coli in poultry, p. 237–259. In C. L. Gyles (ed.), Escherichia coli in domestic animals and humans-1994.CAB International, Wallingford, United Kingdom.

Hai-ping HE., 2009. Isolation and identification of P. aeruginosa in chicken dead-embryos. Chinese Qinghai J Anim. Vet. Sci. 3: 25-27.

Hu, Q., Tu, J., Han, X., Zhu, Y., Ding, C., Yu, S., 2011. Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer, Escherichia coli, and Salmonella enterica simultaneously from ducks. J. Microbiol. Methods 87:64-69.

Jahantigh, M., Reza, E. D., 2015. Antimicrobial Drug Resistance Pattern of Escherichia coli isolated from Chickens Farms with Colibacillosis Infection: Open J. Med.Microbiol. 5: 159-162.

Lin, M. Y., Cheng, M. C., Huang, K.J., Tsai, W. C., 1993. Classification, pathogenicity, and drug susceptibility of hemolytic gram-negative bacteria isolated from sick or dead chickens. Avian Disease 3, 6±9.

Mamza, S. A., Egwu, G.O., Mshila, G. D. 2010. Antibiotic susceptibility patterns of beta lactamase producing Escherichia Coli and Staphylococcus aureus isolated from chickens in Maiduguri (Arid zone), Nigeria. Vet. Arthiv. 80: 283-297.

Mbanga, J., Nyararai, Y.O., 2015. Virulence gene profiles of avian pathogenic Escherichia coli isolated from chickens with colibacillosis in Bulawayo, Zimbabwe: Onderstepoort J. Vet. Res. 82(1), 850: 8.

Miles, T. D., McLaughlin, W., Brown, P. D., 2006. Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans: BMC Vet. Res. 2:7.

Moniri, R., Dastehgoli, K., 2007. Antimicrobial Resistance among Escherichia coli strains Isolated from Healthy and Septicemic Chickens. Pakistan J. Biol. Sci. 10: 2984-2987.

Moulin–Schouleur, M., Reperant, M., Laurent, S., Bree, A., Mignon-Grasteau, S., Germon, p., Rasschaert, D., Scholeur, C., 2007. Extra intestinal pathogenic Escherichia coli strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. 1-J. clin. Microbiol., 45 (10): 3366-76.

Murthy, T. R. G. K., Dorairajan, N., Balasubramaniam, G. A., Dinakaran, A.M., Saravanabava, K., 2008. Pathogenic bacteria related to respiratory diseases in poultry with reference to Ornithobacterium rhinotracheale isolated in India:Veterinarski Arhiv 78 (2): 131-140.

NCCLS (National Committee for Clinical Laboratory Studies) (1990): Performance standard for antimicrobial disc susceptibility test for edition NCCLS documents M2-A4, Villanova, PA, 19085, USA.

Nunoya, T. T., Yagihashi, M., Tarima, Nagasawa, Y. 1999. Occurrence of kerato conjunctivitis apparently caused by Mycoplasma gallisrpticum in layer chickens. Vet. Pathol. 32:11-18.

Oh, J. Y., Kang, M.S., Yoon, H., Choi, H.W., An, B.K., Shin, E.G., Kim, Y.J.M., Kim, J. et al., 2011. The embryo lethality of E. coli isolates and its relationship to the presence of virulence associated gene. Poult. Sci. 91: 370-375.

Olayinka, A. T., Olayinka, B. O., Onile B. A., 2009. Antibiotic susceptibility and plasmid pattern of P. aeruginosa from the surgical unit of a university teaching hospital in north central Nigeria: Int. J. Med. Med.Sci. (3): 079-083.

Presteri, E., Suchomel, M., Eder, M., Reichmann, S., Lassnigg, A., Graninger, W., Rotter, M., 2007. Effects of alcohols, povidoneiodine, and hydrogen peroxide on biofilms of Staphylococcus epidermidis. J. Antimicrob. Chemother. 60:417-420.

Provence, D. L., Curtiss, R. III., 1994. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic Escherichia coli strain. Infect. Immun. 62:1369–1380.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., FitzPatrick, E.S., 2011. Vet. Microbiol. Microbial Dis.Wiley.

Rasha, G. T., Khalil, S. A., Ellakany, H. F., Torky, H. A., 2016. Mycoplasma Synoviae and other Associated Bacteria Causing Arthritis in Chicken: Alex. J. Vet. Sci. 49 (2): 163-169.

Rocha, A. C.G.P., Rocha, S. L.S., Lima-Rosa, C.A.V., Souza,G.F., Moraes H. L.S., Salle,F. O., Moraes, L. B., Salle, C. T.P., 2008. Genes associated with pathogenicity of avian Escherichia coli (APEC) isolated from respiratory cases of poultry: Pesq. Vet. Bras. 28(3):183-186.

Roussan, D. A., Hana, Z., Khawaldeh,G., Shaheen, I., 2014. Differentiation of Avian Pathogenic Escherichia coli strains from Broiler Chickens by Multiplex Polymerase Chain Reaction (PCR) and Random Amplified Polymorphic (RAPD) DNA. Open J. Vet. Med. 4: 211-219.

Sambrook, J., Fritsh, E.F., Maniatis, T., 1989. Molecular cloning a laboratoryManual.2nd ed., Cold Spring HarborLaboratory Cold Spring. New York.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press.

Shahid, M., Malik, A., 2004. Plasmid mediated amikacin resistance in clinical isolates of P. aeruginosa. Indian J. Med. Microbiol. 22(3): 182-184.

Tiba, MR., Yano, T., da Siva, D. 2008. Genotypic characterization of virulence factors in Escherichia coli strains from patients with cyst Rev. Inst. Med. Trop. Sao Paulo. 50(5): 255-260.