



Phenotypic and Genotypic Characterization of Some Bacterial Isolates (*Escherichia Coli*, *Klebsiella Oxytoca*) From Chickens

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ABSTRACT

The present study was performed on a total of 104 recently dead chickens from Gharbia Governorate that inspected for *Enterobacteriaceae* from internal organs after clinical and postmortem examination. The results revealed that 95 samples were positive, *Escherichia coli* (53), where *Salmonella.spp.* (31), *Citrobacter freundii* (14), *Enterobacter cloacae* (6), *Klebsiella oxytoca* (5), *Proteus vulgaris* (4) , *Acinetobacter lowoffii* , *Edwardsiella tarda* respectively, and *Enterobacter gergoviae*, *Enterobacter pantoe* (2) respectively. Amikacin and gentamicin were the highest efficiency antimicrobial agents used in vitro with variation in sensitivity to others except *Proteus vulgaris* was resist to all. Seven *E.coli* isolates were serotyped and identified as O₁, O₆, O₁₂₅ and O₁₄₆. PCR results for *E.coli* showed that *tspE4c2* and *yjaA* pathotypic genes(at 152 bp and 211 bp) were detected in all, but only O₁₂₅ and O₁₄₆ have *chuA* gene (at 279 bp). While *hlyA* (at 450 bp) was detected in O₆, *eaeA* (at 248bp) and *stx1*(at 614 bp) gene was detected in 1 strain of (O₁₂₅), *iss* gene (at 323 bp) was detected in another isolate of O₁₂₅ and *stx2* (at 779 bp) was absent in all. All that indicate that *E. coli* strains isolated were extra-intestinal pathogenic strains. PCR results for 5studied *K. oxytoca* strains showed that all of them leak *iroN* ,but all have *pehX* gene.

Key words: *Escherichia coli* , *Klebsiella oxytoca*, chickens , PCR .

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1. INTRODUCTION

Poultry industry plays an important role in the national income .They are domesticated birds raised for their meat ,eggs and their edible offals. Poultry is the second most widely eaten type of meat in the world especially chickens. Bacteria are responsible for important group of naturally occurring diseases of chickens. These diseases cause substantial morbidity and mortality which mostly involve the respiratory and digestive

systems. Although conferring many health-associated benefits, gastrointestinal tract bacteria shows some drawbacks rather than pathogenesis. They compete with the host for nutrients within the GIT and may secrete toxic and anti-nutritional compounds that induce a continuous inflammatory response in the GIT and stimulate fast turnover of epithelial cells at the expense of bird performance (Yegani and Korver 2008)

Enterobacteriaceae are gram negative bacteria which their members are widely distributed throughout the environment in soil, water, on plants as well as in the intestines of animals . However, *Salmonella typhi* , that causes typhoid fever in man and is found only in humans. *Enterobacteriaceae* can cause disease by attacking their host in a number of ways. The most important factors are motility, colonization factors, endotoxin and enterotoxin (Markey et.al., (2013). The family *Enterobacteriaceae* consists of many strains that colonize the small and large intestine, and includes members of commensal microbiota as well as pathogens. Several pathogenic *Enterobacteriaceae*, specially *Escherichia coli* strains, cause diarrhoea, urinary tract infections, mastitis, arthritis and meningitis in both humans and animals (Fairbrother et al., 2005 and Nagy and Fekete 2005). *Salmonella* spp. cause bacillary white diarrhea, enteritis and septicaemia in chicks and fowl typhoid in all ages. Fimbrial gene clusters isolated from *Salmonella* genomes may be associated with tissue tropism due to host specificity or receptor specificity (Kisiela et al., 2012 and Yue et al., 2012). *Enterobacteriaceae* can be divided into three groups based on their pathogenicity into : Major pathogens of animals such as *Salmonella* species, *Escherichia coli* and 3 of the *Yersinia* species and the other side is opportunistic pathogens that are occasionally cause infections as *Proteus*, *Klebsiella* , *Enterobacter* , *Serratia*, *Edwardsiella*, *Citrobacter*, *Morganella* and *Shigella* and organisms of uncertain significance for animals (Markey et.al., 2013).

Colibacillosis is the most frequently reported live poultry diseases or at processing. Healthy and sick broilers, layers, and breeders showed the incidence of avian pathogenic *E.coli* infection to be 17.7%, 38.6%, and 26.9% with high percent of multi-drug resistance .

Salmonellosis can transmitted by vertical , horizontal or mechanical transmission which cause variation in mortality from PD , maximize during the second week after hatching, with a rapid decline between 3 – 4 week of age and mortality from FT ranging from 10 – 93 % . It's mortality rate is often higher than morbidity rate . So the aim of the work was studing the phenotypic and the genotypic characters of some bacteria isolated from chickens.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 104, recently dead chickens , from different farms at Gharbia Governorate for bacteriological examination. The examined chickens were of different ages. Each examined organ was taken alone in sterile packet in ice bag with minimum of delay for bacteriological examination from liver, gizzard, heart, lung and spleen from all ages and both sexes.

2.2. Bacteriological examination

A small piece of the organ was inoculated in the Macconkey broth and then incubated at 37°C for 24 hours. Loopfuls from Macconkey broth was inoculated on Macconkey agar then nutrient agar incubated at 37°C for 24-48 hours. Cultivation on selective media as XLD, Brilliant green, EMB for *Enterobacteriaceae*. Suspected colonies were picked up and sub cultured on brain heart broth +50% glycerol and incubated at 37°C for 24 hours, then kept in freezer for further studies (Markey et.al., 2013).

2.3. In-Vitro anti-microbial sensitivity test:

Subculture from the isolated strains were prepared and subjected to the sensitivity test against different antimicrobial discs (Markey et.al., 2013) (table,3).

2.4. Biofilm of *E. coli* isolated :

A loopful of *E. coli* was inoculated in 10 mL of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37°C for 24 hr. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube.

2.5. Serological identification of *E. coli* and *K. oxytoca* isolates

E. coli isolated were identified serologically using *E. coli* polyvalent and monovalent O antisera by slide agglutination technique. The tested strains were picked up from an 24 hours old colony on nutrient agar was emulsified with a loop in the drop of the

3. RESULTS

The postmortem lesions of diseased chickens included fibrinous pericarditis, perihepatitis, congestion with petechial hemorrhages in internal organs as liver, lung, spleen, kidneys, heart and intestine. The results of isolation (Table,1-2) showed 95/104 (91.35%) were positive for *Enterobacteriaceae* isolation, where 10 different bacterial isolates. *E. coli* isolated from 53 samples , *K. oxytoca* isolated from 5 samples , *S. spp* isolated from 31samples, *Citrobacter freundii* isolated from 14 samples , *Enterobacter cloacae* isolated from 6 samples , *Enterobacter gergoviae* isolated from 2 samples , *Enterobacter pantoe* isolated from 2 samples , *Acinetobacter lowoffii* isolated from 4 samples , *Proteus vulgaris* isolated from 4

physiological saline on the slide and mixed thoroughly with a drop of antiserum for one minutes at El dokki institute according to Markey et.al., (2013).

2.6. Virulence genes of *E.coli* detection by PCR

PCR was applied by using eight sets of primers for detection of 8 pathotyping and virulence genes that may play a role in virulence of *E.coli* (table,7). These genes were hemeutilization protein A gene (*chuA*) , Specific DNA fragment (*tspE4c2*) and Inner membrane protein (*yjaA*) , intimin gene (*eae A*) , increased serum survival gene (*iss*), haemolysin (*hlyA*), shiga toxin 1 gene (*stx1*) and shiga toxin 2 gene (*stx2*). Tested genes used for *K. oxytoca* were (*pehX*) polygalacturonase gene and *iroN* .It was applied on 7 random isolates of *E.coli* and 2 isolates of *K.oxytoca* following QIAamp® DNA Mini Kit instructions (Catalogue no. 51304);Emerald Amp GT PCR mastermix (Takara) Code No.RR310A and agarose gel electrophoreses (Sambrook et al.,1989).

samples and *Edwardsiella tarda* isolated from 4 samples.

The in- vitro sensitivity tests showed that the isolated all *E.coli* strains, *Salmonellae* species , *klebsiella oxytoca*, *Edwadsiella tarda* and *Enterobacter spp.* showed high sensitivity to amikacin , gentamicin , variation in sensitivity to apramycin, spiramycin , trimethoprim and resist to neomycin, kanamycin,

spectinomycin, lincomycin, oxytetracycline. *Citrobacter freundii* revealed high sensitivity to amikacin , gentamicin , neomycin , apramycin and resistant to lincomycin , kanamycin , oxytetracycline, spiramycin, trimethoprim, spectinomycin. *Acinetobacter lowoffii* isolates showed high sensitivity to apramycin, moderate sensitivity to amikacin, gentamicin, trimethoprim, spectinomycin,

spiramycin and resistant to oxytetracycline. *Proteus vulgaris* isolates showed high resistance to all antimicrobial agents used.

The result of *E.coli* biofilm (table,4) was 46.5% strong, 23.3% weak and 30.2% negative for biofilm formation. Seven *E.coli* strains were serotyped (Table,4) and four serovars were identified O₁,O₆,O₁₂₅ and O₁₄₆ (28.57% – 14.29% – 28.57% – 28.57% respectively).

PCR results of pathotyping and virulence genes detection to *E.coli* serovares (table,7,8) showed that *tspE4c2* and *yjaA* pathotyping genes were detected in all ,but only 4 strains have *chuA* gene. While *hlyA*, *eaeA* and *stx1*genes was detected in 1 strain of O₁₂₅, *iss* gene was detected in another isolate of O₁₂₅and *stx2* was absent in all studied strains.

Four samples showed positive amplification of *chuA* gene at 279 bp (fig.1).

All samples showed positive amplification of *yjaA* gene at 211 bp (fig.2). All samples showed positive amplification of *tspE4c2* gene at 152 bp (fig.3). All samples showed negative amplification of *stx2* gene at 779 bp and one sample showed positive amplification of *stx1* gene at 614 bp (fig. 4). One sample showed positive amplification of *eaeA* gene at 248 bp (fig. 5). One sample showed positive amplification of *hly* gene at 450 bp and one sample showed positive amplification of *iss* gene at 323 bp(fig. 6). PCR results of virulence genes detection to *K.oxytoca* (table,9) showed that 3 of tested isolates had no *iroN* gene and other 2 samples were not done while all of them had *pehX* gene. All samples showed positive amplification of *pehX* gene at 343 bp (fig. 7) and all showed negative amplification of *iroN* gene at 847 bp (fig. 8).

Table (1) The isolated bacteria from diseased chickens (Pure infection) n=104:

Isolates	No.	%*	%**
<i>Acinetobacter lowoffii</i>	2	1.9	2.1
<i>Citrobacter freundii</i>	10	9.6	10.53
<i>Enterobacter cloacae</i>	2	1.9	2.1
<i>E. coli</i>	42	40.38	44.2
<i>Klebsiella oxytoca</i>	2	1.9	2.1
<i>Salmonella spp.</i>	8	7.6	8.42
Total	66	63.5	69.45

*= % according to total number of samples. n = 104.

**= % according to positive samples. n = 95.

Table (2) The isolated bacteria from diseased chickens (mixed infection) n=104 :

Isolates	No.	% *	% **
<i>Acinetobacter lowoffii</i> + <i>Salmonella spp.</i>	2	1.9	2.1
<i>Citrobacter freundii</i> + <i>Proteus vulgaris</i>	2	1.9	2.1
<i>Enterobacter cloacae</i> + <i>Salmonella spp.</i>	4	3.8	4.21
<i>Enterobacter pantoe</i> + <i>Salmonella spp.</i>	2	1.9	2.1
<i>E. coli</i> + <i>Enterobacter gergoviae</i>	2	1.9	2.1
<i>E. coli</i> + <i>Klebsiella oxytoca</i>	2	1.9	2.1
<i>E. coli</i> + <i>Salmonella spp.</i>	6	5.7	6.32
<i>E. coli</i> + <i>Salmonella spp.</i> + <i>Klebsiella oxytoca</i>	1	0.96	1.1
<i>Proteus vulgaris</i> + <i>Salmonella spp.</i>	2	1.9	2.1
<i>Salmonella spp.</i> + <i>Citrobacter freundii</i>	2	1.9	2.1
<i>Salmonella spp.</i> + <i>Edwardsiella tarda</i>	4	3.8	4.21
Total	29	27.9	30.55

*= % according to total number of samples. n = 104.

**= % according to positive samples. n = 95.

Table (3) Interpretation of zones inhibition for antimicrobial susceptibility (Modified from CLSI (2017))

Antimicrobial agents	Abbreviation	Disc content in ug	Diameter of inhibition to nearest mm.		
			Resistant	intermediate	Susceptible
Amikacin	Ak.	30 ug	14	15–16	17
Apramycin	Apr.	15 ug	12	12-14	>14
Gentamicin	CN.	120 ug	12	13–14	15
Kanamycin	K.	30 ug	13	14–17	18
Lincomycin	MY.	10 ug	11	12-14	>14
Neomycin	N.	30 ug	17	18-20	21
Oxytetracycline	OT.	30 ug	14	15-18	19
Spectinomycin	SH.	100 ug	10	14–17	14
Spiramycin	SP.	100 ug	14	15–18	19
Trimethoprim + sulfamethoxazole	SXT.	25 ug	10	11–15	16

Table (4) Biofilm results of *E. coli* isolated from diseased chickens (n = 43)

Total	Strong		Weak		Negative	
	No.	%	No.	%	No.	%
43	20	46.5	10	23.3	13	30.2

Table (5) Distribution of *E. coli* serovars isolated from diseased chickens

Total	O ₁		O ₆		O ₁₂₅		O ₁₄₆	
	No.	%	No.	%	No.	%	No.	%
7	2	28.57	1	14.29	2	28.57	2	28.57

Table (6): Oligonucleotide primers sequences for detection of 3 phylogenetic genes of *E. coli* and 7 virulence associated genes in *E. coli*, *Klebsiella oxytoca* isolates with PCR:

Gene	Primer Sequence 5'-3'	Amplified product	Reference	Pos. control	Neg. control		
<i>E. coli</i>	<i>chuA</i>	F GAC GAA CCA ACG GTC AGG AT R TGC CGC CAG TAC CAA AGA CA	279 bp				
	<i>yjaA</i>	F TGA AGT GTC AGG AGA YGC TG R ATG RAG AAT GCG TTC CTC AAC	211 bp	Jeong <i>et al.</i> (2012)	AJ413986		
	<i>tspE4C2</i>	F GAG TAA TGT CGG GGC ATT CA R CGC GYC AAC AAA GTA TTR CG	152 bp				
	<i>stx1</i>	F ACACTGGATGATCTCAGTGG R CTGAATCCCCCTCCATTATG	614 bp	Dipineto <i>et al.</i> (2006)	AJ413986		
	<i>stx2</i>	F CCATGACAACGGACAGCAGTT R CCTGTCAACTGAGCAGCACTTTG	779 bp		FN252457		
	<i>eaeA</i>	F ATGCTTAGTGCTGGTTTAGG R GCCTTCATCATTTTCGCTTTC	248 bp	Bisi-Johnson <i>et al.</i> (2011)	M58154		
	<i>hly</i>	F GGCCACAGTCGTTTAGGGTGCTTACC R GCGGTTTTAGGCATTCCGATACTCAG	450 bp.	Morales, (2004)	NG036728.1		
	<i>iss</i>	F CAGCAACCCGAACCACTTGATG R AGCATTGCCAGAGCGGCAGAA	323 bp.	Johnson , (2008 a)	DQ309287		
	<i>K. oxytoca</i>	<i>pehX</i>	F GATACGGAGTATGCCTTTACGGTG R TAGCCTTTATCAAGCGGATACTGG	343 bp	Chander <i>et al.</i> (2011)	***	***
		<i>iroN</i>	F ATC CTC TGG TCG CTA ACT G R CTG CAC TGG AAG AAC TGT TCT	847 bp	Ewers <i>et al.</i> (2007)	ATCCr13182	***

Staphylococcus aureus ATCC25923

Table (7) : Phylogenetic group determination to *E.coli* strains isolated

Sample no.	<i>E. coli</i> strains	Results			Grouping	Sub-grouping
		<i>chuA</i>	<i>yjaA</i>	<i>tspE4c2</i>		
1	O ₆	-	+	+	Un-typeable	**
2	O ₁₂₅	+	+	+	B ₂	B _{2 3}
3	O ₁₂₅	+	+	+	B ₂	B _{2 3}
4	O ₁	-	+	+	Un-typeable	**
5	O ₁	-	+	+	Un-typeable	**
6	O ₁₄₆	+	+	+	B ₂	B _{2 3}
7	O ₁₄₆	+	+	+	B ₂	B _{2 3}

(Table 8): Results of virulence genes of *E.coli* strains isolated

Sample no.	<i>E. coli</i> strains	Results				
		<i>stx1</i>	<i>stx2</i>	<i>eaeA</i>	<i>iss</i>	<i>hly</i>
1	O ₆	-	-	-	-	+
2	O ₁₂₅	-	-	-	+	-
3	O ₁₂₅	+	-	+	-	-
4	O ₁	-	-	-	-	-
5	O ₁	-	-	-	-	-
6	O ₁₄₆	-	-	-	-	-
7	O ₁₄₆	-	-	-	-	-

Table (9) PCR results of *K. oxytoca* isolated from diseased chickens :

<i>Klebsiella</i> sample	Results	
	<i>pehX</i>	<i>iroN</i>
1	+	-
2	+	-
3	+	-
4	+	ND
5	+	ND

ND = none done

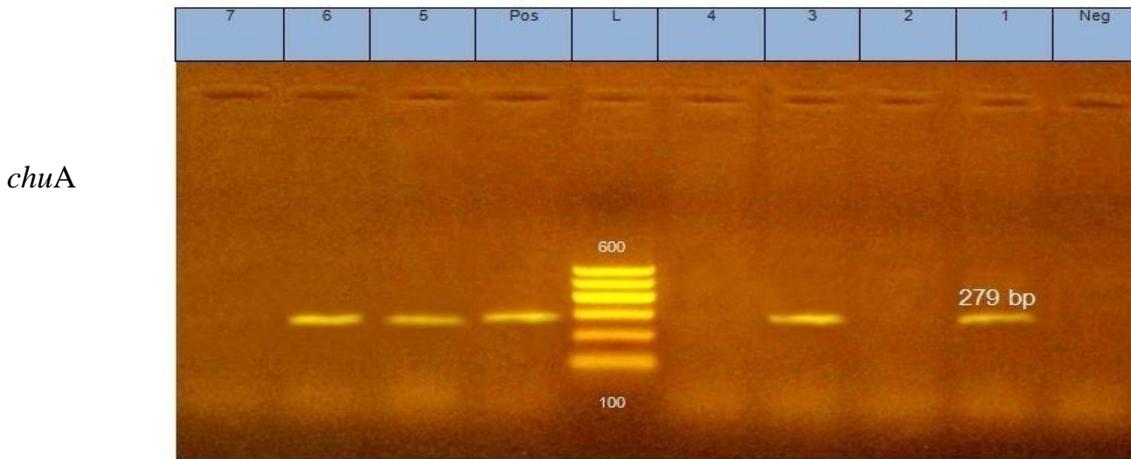


Fig .(1): Agarose gel electrophoresis of PCR for amplification products of hemeutilization protein A (*chuA*) gene of *7E.coli* isolates. Lanes 1,3,5,6 shows positive amplification of *chuA* gene at 279 bp. Lane L: DNA ladder at 100-600bp. Neg.: Negative control (*Staphylococcus aureus* ATCC25923). Pos.: Positive control (AJ413986).

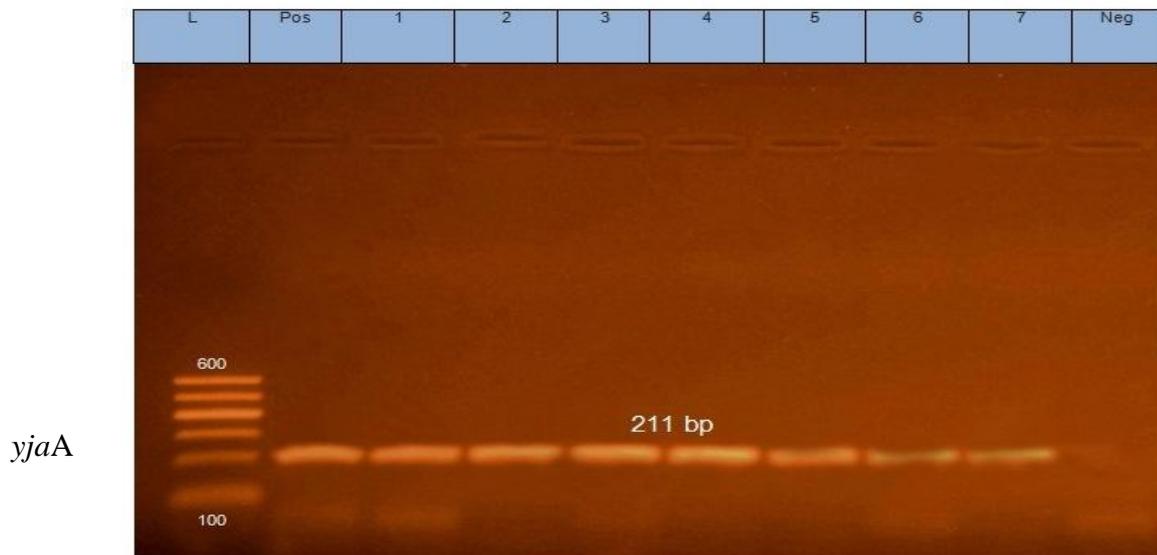


Fig. (2): Agarose gel electrophoresis of PCR for amplification products of inner membrane protien (*yjaA*) gene of *7E.coli* isolates. Lanes1,2,3,4,5,6,7 shows positive amplification of *yjaA* gene at 211 bp. Lane L: DNA Ladder at 100-600bp. Neg.: Negative control (*Staphylococcus aureus* ATCC25923). Pos.: Positive control (AJ413986).

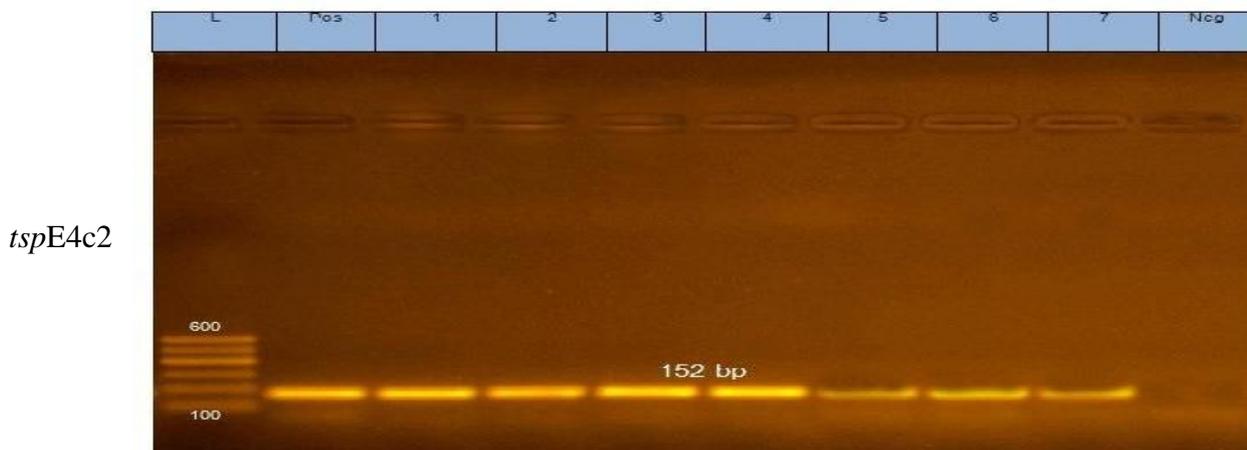


Fig. (3): Agarose gel electrophoresis of PCR for amplification products of specific DNA fragment (*tspE4c2*) gene of *E.coli* isolates. Lanes 1,2,3,4,5,6,7 shows positive amplification of *tspE4c2* gene at 152 bp. Lane L: DNA Ladder at 100-600bp. Neg.: Negative control. (*Staphylococcus aureus* ATCC25923). Pos.: Positive control (AJ413986).

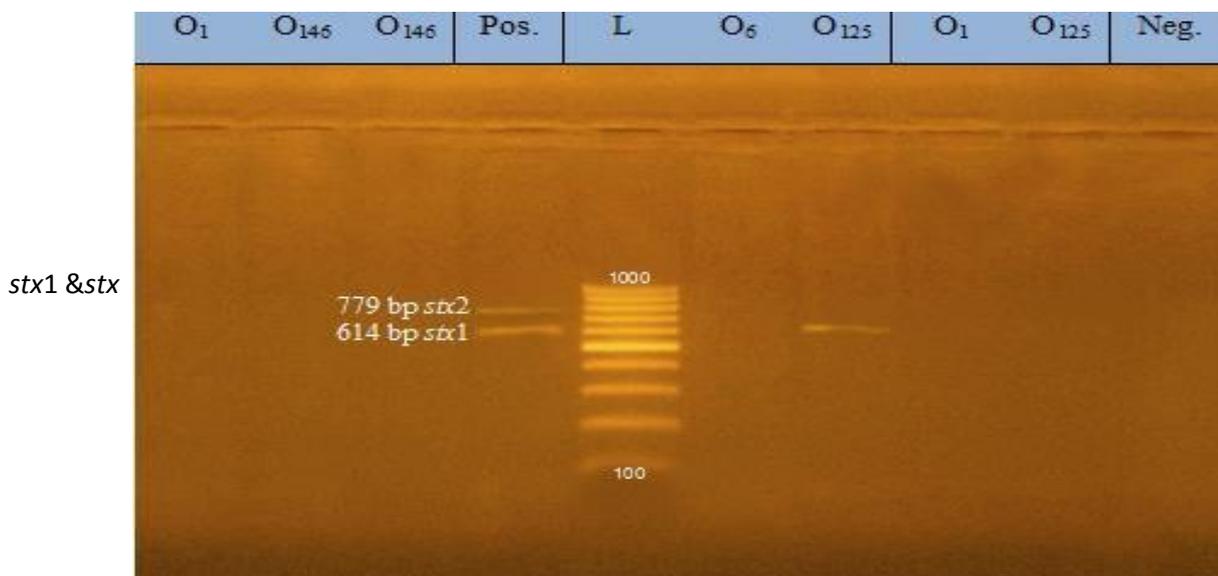


Fig.(4): Agarose gel electrophoresis of PCR for amplification products of shiga toxin 1 & 2 (*stx1 & stx2*) genes of *E. coli*. Lane 3 shows positive amplification of *stx1* gene at 614 bp ,but lanes 1,2,3,4,5,6,7shows negative amplification of *stx2* gene at 779 bp. Lane L: DNA Ladder at 100-1000bp. Neg.: Negative control (*Staphylococcus aureus* ATCC25923). Pos.: Positive control (*stx1 & stx2*) (AJ413986 & FN252457) .

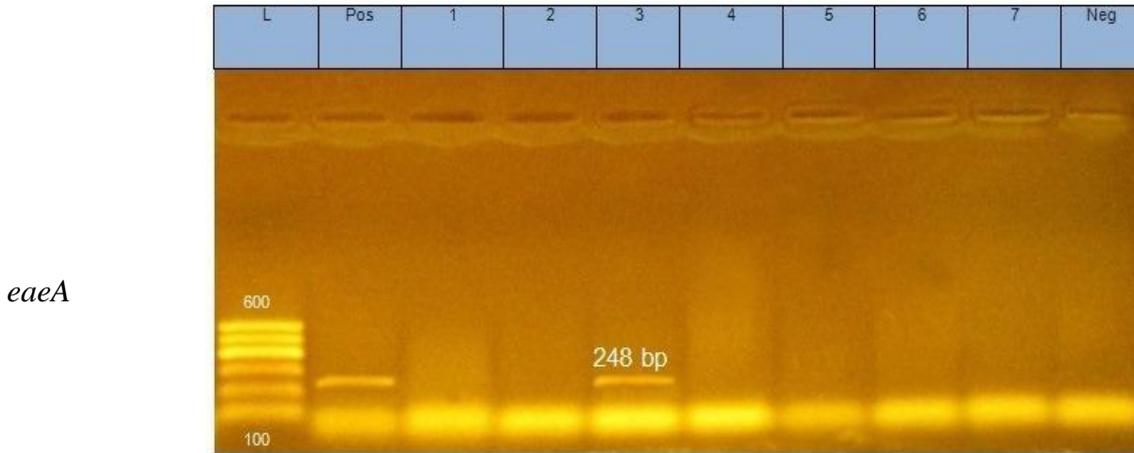


Fig.(5): Agarose gel electrophoresis of PCR for amplification products of intimin (*eaeA*) gene of *E. coli*. Lane 3 shows positive amplification of *eaeA* gene at 248 bp. Lane L: DNA Ladder at 100-600 bp. Neg.: Negative control (*Staphylococcus aureus* ATCC25923). Pos.: Positive control (M58154) .

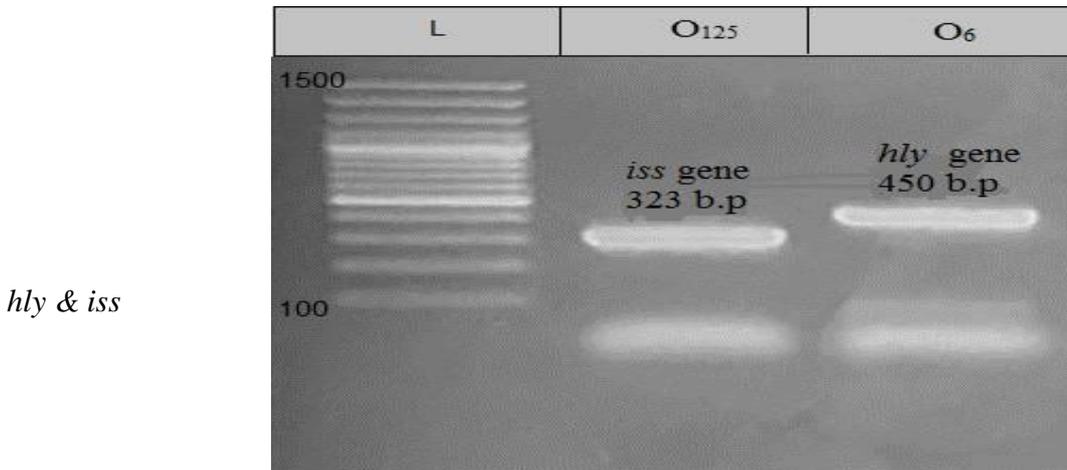


Figure (6): Agarose gel electrophoresis of of PCR for amplification products of hemolysine A (*hly*) and increased serum survival (*iss*) genes of *E.coli*. Lane 3 shows positive amplification of *hly* gene at 450 bp and lane 2 shows positive amplification of *iss* gene at 323 bp.Lane L: DNA Ladder at 100-1500bp. Neg.: Negative control (*Staphylococcus aureus* ATCC25923). Pos.: Positive control (*hly & iss*) (NG036728.1 & DQ309287) .

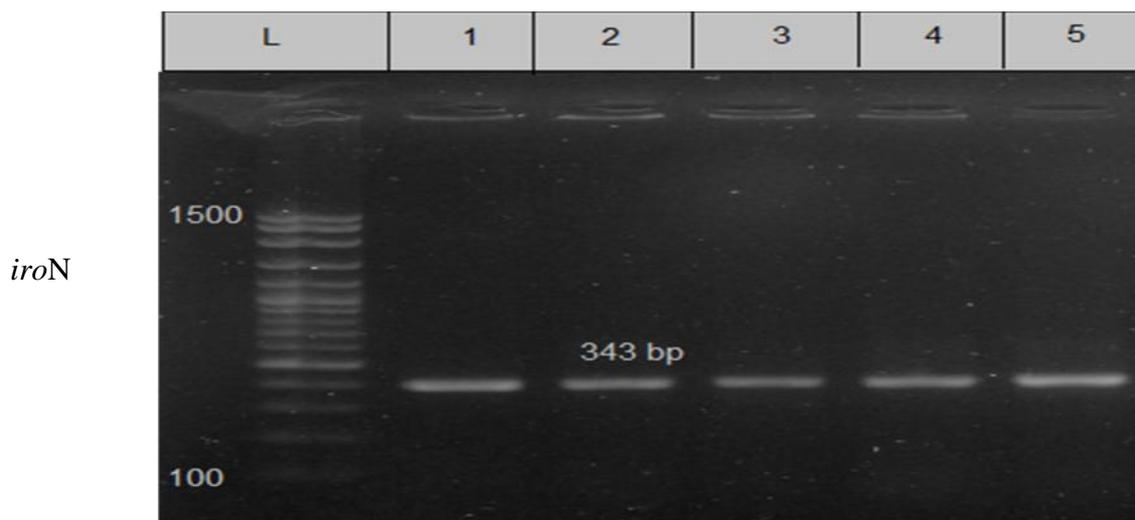


Fig. (7): Agarose gel electrophoresis of PCR for amplification products of *iroN* gene of *K. oxytoca*. Lanes 1,2,3 shows negative amplification of *iroN* gene at 847 bp .Lane L: DNA Ladder at 100-1000 bp. Pos.: Positive control (ATCCr13182) .

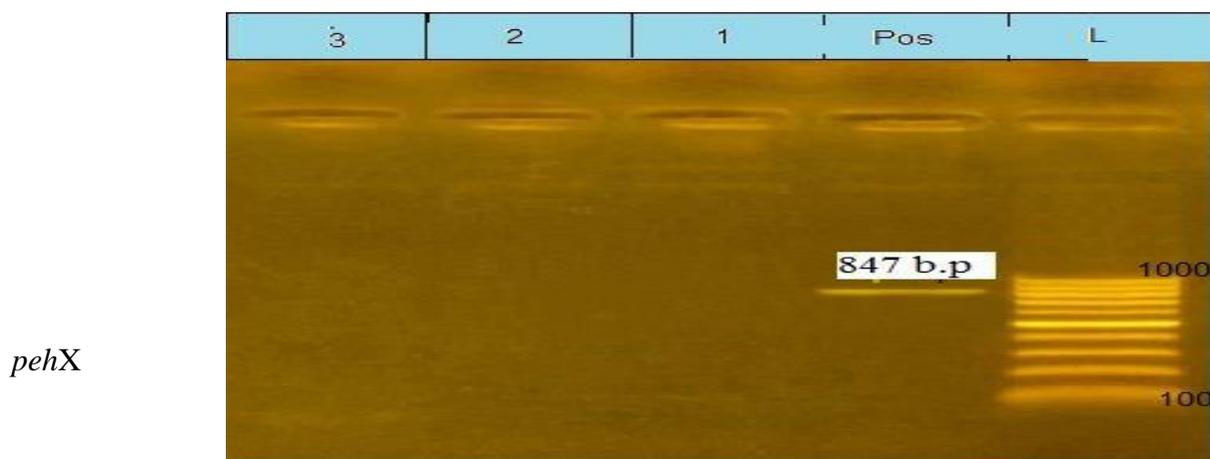


Fig.(8): Agarose gel electrophoresis of PCR for amplification products of Poly galacturonase gene (*pehX*) of *K. oxytoca*. Lanes 1,2,3,4,5 shows positive amplification of *pehX* gene at 343bp.Lane L: DNA Ladder at 100- 1500 bp.

4. DISCUSSION

Poultry represent an important source of meat, eggs and edible offals. Many bacterial agents were found to affect the raising of chickens. The present results revealed many bacterial agents isolated from

visceral organs of diseased chickens representing different ages and sexes. Ten bacterial species isolated were *E. coli* (53), *K. oxytoca* (5), *S. spp* (31), *Citrobacter freundii* (14), *Enterobacter cloacae* (6), *Enterobacter*

gergoviae (2), *Enterobacter pantoe* (2), *Acinetobacter lowoffii* (4), *Proteus vulgaris* (4), *Edwardsiella tarda* (4).

All samples collected indicated the unhygienic environment of the farm included feed, water, poor ventilation, wild animals and insects. Also contaminated hatchery and workers have a main role in infection or cross infection even its spread. The present infection resulted from horizontal infection through direct or indirect contact with other infected bird - cannibalism - or ingestion of infected feed (especially *E. coli*) or water with excreta or misusing of live vaccines (especially *Salmonella* species). Vertical transmission also occur through egg infection by fecal contamination of the egg surface with subsequent penetration of the shell and membranes. Moreover excessive and indiscriminate use of antimicrobials led to presence of strains highly resistant to most of them. *Escherichia coli* isolates were 53 isolates as in studies of Makhol et al.,(2010), Abd El Tawab et al.,(2016) and Halfaoui et al.,(2017). In spite of Abbott et al.,(2003), Hyma et al.,(2005) and Oaks et al.,(2010) isolated *E.alberti*, and Poulou et al.,(2008), Yamanaka et al.,(2010) isolated *E. hermannii*, and *E. vulneris* was found by Mohanty et al.,(2005) and Kilani et al.,(2008) and they were absent the isolates.

Salmonella species isolates were 31 isolates and agreed with Ammar et al.,(2016), Zhao et al., (2016) and Abd El Tawab et al.,(2017).

Five isolates of *K.oxytoca* were found as reported in Brisse et al.,(2006) and Jiang et al.,(2014). Despite of isolation of *K. pneumonia* from Keynan and Rubinstein (2007), Zadoks et al.,(2011) and Siu et al.,(2012) and *K. granulomatis* from Lagergard et al.,(2011) and this results were free of them. Fourteen *Citrobacter freundii* isolates were recovered although Borenshtein

and Schauer (2006) isolated *Citrobacter* spp. and VazMarecos et al.,(2012) isolated *C. koseri (diversus)*.

Enterobacter pantoe was 2 isolates as well as *Enterobacter gergoviae*, but *Enterobacter cloacae* was 6 isolates which agreed with Mezzatesta et al.,(2012). Al-Hasan et al.,(2011) isolated *Enterobacter* spp. Four isolates were recovered of *Acinetobacter lowoffii*, *Proteus vulgaris* and *Edwardsiella tarda*. These isolates do not reflect the picture of the disease as reflect the health and immune state of the bird. Also all isolated microorganisms are commensal bacteria which co-evolved with their hosts in gastrointestinal track especially the lower parts however, under specific conditions, they overcome protective host responses and exert pathologic effects.

E. coli represented the most common isolated bacteria from all samples that recovered from all tested organs. It was resistant to almost antimicrobial agents used except amikacin, gentamicin and apramycin as Makhol et al.,(2010) and Zhang et al.,(2012). When examined some *E. coli* isolates, found that there are extra-intestinal pathogenic *E. coli* as mentioned in Köhler and Dobrindt (2011) and Comery et al.,(2013). The strain of *E. coli* serovared O₁ agreed with Younis et al.,(2017) and Han et al.,(2018), that serovared O₂ agreed with Salama et al., (2007) and Halfaoui et al.,(2017), that serovared O₁₂₅ agreed with Abd El Tawab et al.,(2016) and Ozaki et al.,(2017) and that serovared O₁₄₆ agreed with Sánchez et al.,(2015) and Mughini-Gras et al., (2018). All results indicated that *E.coli* strains isolated were extra-intestinal pathogenic strains (Morales et al., 2004, Smith, et al., 2007, Mellata 2013 and Delannoy et al., 2017).

Genotypic characterization of tested *E.coli* revealed absence of *stx1* in all isolates, but Fierz et al.,(2017) found it , presence of *stx2*, *eaeA* and *hly* genes in one sample as mentioned in Momtaz and Jamshidi (2013), Alonso et al.,(2017) and Friesema et al.,(2014) and *iss* gene in another one as detected in Abd El Tawab et al.,(2014) and Johnson et al.,(2008).

K. oxytoca represented 5 isolates that recovered from all tested organs . It was resistant to almost antimicrobial agents used except amikacin , gentamicin and oxytetracycline as recorded in Wu et al.,(2016). All tested isolates had *pehX* gene that misidentified in other *Klebsiella* species which was agreed with Kovtunovych et al.,(2003) and Zedan et al., (2017). Antimicrobial sensitivity test to the other isolates showed marked fluctuation to many antimicrobial agents used due to the misuse of these chemotherapeutics in the routine raising of chickens which affect the bird immunity and body gain.

It is a wrong concept of using antimicrobial agents for growth promotion in animals. The overuse and misuse of these chemotherapeutics to chickens not only affect on them , but also affect on all human beings and animals. We should limit its use only to sick bird to eliminate chemotherapeutics from human food chain.

O₁₂₅ and O₁₄₆ are human specific *E. coli* and this indicate human ceacal infection by workers through bad hygiene and habits.

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