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UPDATE ON BROILERS CHICKEN AND HUMAN ESBL VIRULENT *SALMONELLA* SEROVARS

By

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ABSTRACT

The aim of this present study to survey the antimicrobial resistance, ESβL and virulence genes among *Salmonella* serovars isolated from broilers chickens and Human .300 broilers chickens and 60 stool human samples were investigated for *Salmonella* by cultural, biochemical and serotyping, 44 isolates were positive for *Salmonella* with the most predominant serotypes are *S,* Enteritidis and *S,* Typhimurium in all isolates from broilers chickens and Human. Broilers *Salmonella* isolates showed high resistance to Impiniem (83.3%) followed by Ceftriaxone (73.3%),On contrast showed high sensitive to Cephalexin (73.3%).But in human isolates showed high resistance to Ampicillin-Sulbectam (21.4%) ,On contrast showed highsensitivetoCeftazedime,AmikacinandTrimethoprime-Sulphamethoxazole (100 %) for each. By using PCR test for detection of four virulence genes (*invA,adrA,ompA* and *csgD)* and nine resistance genes (*int1, int2, int3, Bla_{TEM}, Bla_{CTX}, Bla_{OXA}, Mox,gyrA* and *gyrS*), detected the presence of *invA* virulence and Bla_{TEM} resistance gene in all serovars isolated from broilers chickens and human. Finally, in our study the results of genotypic and phenotypic analysis, found close relation between human and broilers chickens *Salmonella* strains.

Keyword

Salmonella serovars- virulence gene- ESβL - broilers - human.

INTRODUCTION

Salmonella bacterium is very important organism in poultry industry and for human health .*Salmonella* are gram negative, short bacilli, non-spore forming,non-capsulatedaerobic or facultative anaerobic organisms and classified under the family *Enterobacteriaceae* [21]. Their principal habitat is the intestinal tract of humans and animals that each year, this

bacterium is causes a significant number of diseases and deaths worldwide [47]. In 2006 in the United States *Salmonella* was estimated to represent the leading cause of food borne illnesses due to bacterial pathogens [44]. According to the Centers for Disease Control and Prevention (CDC) the genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori* [6]. The species *S. enterica* is further subdivided into six subspecies that are designated by taxonomic names such as *S.enterica* subsp. *enterica, S.enterica* subsp. *salamae, S. enterica* subsp. *arizonae,S. enterica* subsp. *diarizonae, S.enterica* subsp. *houtenae* and *S. enterica* subsp. *Indica* [6]. *Salmonella* is associated with approximately 2500 serovars. These serovars are separated based on differences in their lipopolysaccharide layer with regard to their somatic (O) and flagellar (H) antigens [1]. Identification of *Salmonella* by using biochemical and serological methods consuming time [17] ,so using molecular methods which based on DNA sequences .ability of *Salmonella* to infect host depend on the presence of virulence factors located on *Salmonella* pathogenicity isalnds(SPI) [23] .*Inv*A virulence gene is most important gene specific for cell invasion [22] .Misusing of antimicrobial agents in therapy,prophylaxis and as growth promotors [42] ,leading to great health problem of resistance to antibiotics and limitation of therapeutic options available to doctors for the human treatment from salmonellosis [20] . Nowadays Antimicrobial agents – penicillins caphalosporins, and B-Lactamsaredrugsofchoice fortreatment of *Salmonella* [7, 28]. Studies on *Salmonella* seovars.

For detecting resistance to antimicrobials and production of large spectrum bate-lactames [45, 33]. In Enterobacteriaceae, resistance to Cephalosporins in connected with the production of large spectrum beta-lactamase as ESβL s and AmpC beta-lactamase [16].

B-Lactams antibiotics used for treatment broad spectrum of gram-positive and gram-negative bacteria.ESBLs were firstly detected in 1979 [40]. ESβL s were beta-lactamase enzymes that hydrolyze extended - spectrum cephalosporins with an oxyimino side chain. ESβL s are plasmid endoded, these plasmids carry genes encoding resistance to other drugs as aminoglycosides [19] .In many studies on the world on *Salmonella* serovars. It is important to have knowledge of behavior of *Salmonella* strains against antibiotics agents. Therefore, the aim of this study to determine virulent and ESβL s *Salmonella* serovars isolated from broilers and human.

MATERIAL AND METHODS

Samples:

300 broiler chickens fecal samples and 60 human fecal samples were collected aseptically in plastic screw-top tubes containing 10 ml Buffered Peptone Water (BPW) and stored on ice until transported to laboratory ,where enrichment of samples was done immediately on arrival at 37^{o} C for 18 hours (OXOID) [3].

Enrichment:

0.1ml of BPW broth was transferred into tubes containing 10 ml Rappaport-Vassiliadis broth (RVB) medium and incubated at 41.5^{o} C for 24 hours (OXOID) [3].

Microbiological analysis:

A loopful from RVB was streaked onto xylose-lysine-deoxycholate (XLD) agar plates and incubated at 37 °C for 24 h. Black colonies with typical phenotypic characteristics were suspected as *Salmonella* which serotyped according to the Kauffman-White Scheme using slide agglutination with standered antisera [12] .

Antimicrobial susceptibility testing:

All the 44 *Salmonella* serovars were tested against 13 antimicrobial agents using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar following the guidelines of CLSI [8] .The antimicrobials selected were those commonly used in the poultry industry, namely Cephalosporin group (Cefotaxime CTX,Ceftazedime CAZ,Ceftriaxone CRO, CephalexinCL), Monobactams (Aztreonam), B-lactams (Ampicillin AM, Ampicillin-Sulbectam SAM, Amoxacillin and ClavulenicAMC,ImpenemIPM).Sulphonamides(Trimethoprimand Sulphamethoxazole SXT), Fluroquinolones (Ciprofloxacin CIP) as in (Table 1).

DNA extraction:

Genomic DNA of all the *Salmonella* isolates was extracted from the culture using the QIAamp DNA mini kit instructions (Qiagen, Germany, Gmbh). [14].

PCR for virulence and ESβL genes:

PCR was performed on the DNA extracted from all serovars. The *invA* gene used to confirm the presence of *Salmonella***.** The primers used to detect virulence and resistance genes are depicted in (Table 2). Gel electrophoresis of amplified products was then carried out in 1.5% agarose in a 1X TBE buffer containing Gel Red. After the gels were run, gel was photographed by a gel documentation system and the data was analyzed through computer software [41].

RESULTS

Occurrence of *Salmonella* serovars in broilers chickens and human stool samples.

From 300 broilers chickens fecal samples the result of *Salmonella* isolation and identification revealed that its occurrence was 10 % (30/300) and serotyped as Six strains *S.* Enteritidis (20%), three strains *S.* Kentucky, *S.*Typhimurium , *S.* Heidelberg , *S.* Hader and *S.* Newport for each in a percentage 10 % for each . five strains *S.*Infantis (16.6%) , two strains *S.* Blegdam (6.6%) and one strain *S.* Maloe and *S.* Geueletepee for each in a percentage 3.3 % as in (Table 3).

While for human stool samples;the *Salmonella* occurrence was 23.4 % (14/60) and serotyped as five strains *S.* Enteritidies (35.7%), five strains *S.* Typhimurium (35.7%), three strains *S.* Anatum (21.4%) and one strain *S.* Derby (7.1%) as in (Table 4).

Antimicrobial susceptibility test.

The results in (Table 5) revealed that *Salmonella* serovars recovered from avian fecal samples were exhibited resistance to Impenim (83.3%), Cefotaxime (80%), Ceftriaxone (73.3%), Trimethoprime-Sulphamethoxazole and Ciprofloxacin (66.6%) for each. Also there were showed sensitivity to Cephalexin (73.3%) and Gentamicin (70%), while *Salmonella* isolated fromhumanexhibitedsensitivity to Ceftazedime, Amikacin andTrimethoprime-Sulphamethoxazole in a percentage 100 %.

Detection of virulence genes in *Salmonella* spp.

PCR was used to screen for all four virulence genes which are *invA, adrA, csgD* and *ompA* genes and all genes screened were depicted in Fig. (1, 2, 3, 4) , (Table 6) showed the presence of *invA, csgD* and *ompA* genes in a percentage 100 % and *adrA* gene in a percentage 83.3%.in avian isolates while human isolates harbored all four virulence genes.

Detection of resistance genes in *Salmonella* serovars.

Observed PCR results indicated that detected *Salmonella* contained antimicrobial resistance genes known to confer resistance. The genes were illustrated in Fig. (5,6,7,8,9,10,11,12,13). The prevalence rates of genes detected were presented in (Table 6) that showed the presence of Bla_{TEM} genes in all *Salmonella* serovars isolated from broiler chickens and human in a percentage 100%,and presence of *Int1*and *Int3* in all human samples in a percentage 100%.

Correlation between virulence genes and antimicrobial resistance among *Salmonella* serovars isolated from broilers and human.

The presence of four virulence genes (*invA, adrA, CsgD* and *ompA*) in all *Salmonella* serovars isolated from broilers and human except one isolate *S.* Typhimurium and two isolates *S.* Infantis isolated from broilers as showed in (Table 7). A detailed analysis displayed associations of resistance and susceptibility phenotypes with potential virulence genes .this study confirmed relation between antibiotic resistance and virulence genes in *Salmonella* serovars isolated from avian and human origin.

DISCUSSION

Salmonella species are the most important agent causing great mortalities, morbidity and great economic loses in poultry industries [30]. The present study includes bacteriological examination of 300 Broilers samples and 60 stool Human samples. The obtained results showed that 44 *Salmonella* strains were recovered from 300 broiler cases and 60 humans' cases with percentage 12.2%. This percentage rates were differ from other studies as [34] (9.17%), [17] (10%), [37] (2%). Pre-enrichment of isolates on Buffered Peptone water (PBW) then enrichment on Rappapoert- Vassilisdis Broth (RVB),this agree with [46] who use this medium in enrichment of isolates which effective in detection of *Salmonella* isolates and inhibit other competing organisms. Cultural identification of *Salmonella* isolates by using XLD, BGA and MacCkoncy agar. Serotyping of 30 *Salmonella* isolates from broilers and Human by using Kauffman-White Scheme, showing *Salmonella* serovers isolated from broilersserotyped as Six strains [*S.*Enteritidis (20%),three strains *S.*Kentucky,*S.* Typhimurium, *S.* Heidelberg, *S,* Hader and *S,* Newport for each in a percentage 10 % for each. five strains *S.* Infantis (16.6%), two strains S. Blegdam(6.6%) and one strain *S.* Maloe and *S.* Geueletepee for each in a percentage 3.3 %. The results of serotyping of *Salmonella* isolates recovered from Human were 14 isolates were serotyped as five strains *S.* Enteritidies (35.7%); *five* strains *S.* Typhimurium (35.7%), three strains *S.* Anatum (21.4%) and one strain *S.* Derby (7.1%). Antimicrobial agents are effective tool to treat clinical diseases and to maintain healthy animals and maintain human health. So, In this present study made testing of *Salmonella* isolates from broiler against 13 antibiotic discs which are Cephalosporine groupe (Cefotaxime CTX, Ceftazedime CAZ , Ceftriaxone CRO , Cephalexine CL), Monobactams (Aztreonam ATM), B-Lactams groupe (Ampicillin AM, Ampicillin and Sulbectam SAM,

Amoxacillin and Clavulanic AMC,Impenem IPM), Amoniglycosides groupe (Amikacin AK, GentamicinCN),Sulphonamides (TrimethoprimandSulphamethoxazole SXT),Fluroquinolones (Ciprofloxacin CIP). In this present study 22 out from 30 *Salmonella* strains from broiler were sensitive to Cephalexin CL with a percentage 73, 3 % and 21 strains from 30 strains were sensitive to Gentamicin CN with a percentage 70 %, the highest resistance was detected to Impenim IPM with a percentage 83.3% indicating limited therapeutic value of this antibiotic to poultry, that differ from another authors who reported that *Salmonella* was sensitive to enrofloxacin and resistant to Ampicillin [25]. In our study tested 14 *Salmonella* strains from human against 13 antibiotic discs, reported that 100 % of *Salmonella* serovars from Human were senseitive to Ceftazidim CAZ, Amikacin AK ,and Trimethoprim and Sulphamethoxazole SXT followed by 92.8 % of *Salmonella* serovars from Human were sensitive to Cefotaxim CTX ,Ampicillin AM,Cephalexin CL,Amoxacillin-Clavulanic AMC and Ciprofloxacin CIP).Virulence of *Salmonella* related to its ability to invade host cell, replicate and resist both digestion by macrophages and destruction by complement components of plasma. All virulence genes were detected to *Salmonella* Pathogenicity Islands (SPI). In the current study ,Molecular detection of virulence genes (*invA* , *adrA* ,*OmpA* and *csgD*), found that, the presence of *invA* gene in all serovars isolated from broilers and Human,*invA* gene demonstrated by 284 bp PCR amplified fragment which agree with another studies (14,15,36]. *InvA* gene encoded in inner membrane of bacteria which responsible for epithelial cell invasion [12]. Also, detection of *ompA* and *csgD* virulence genes in all *Salmonella* serovars isolated from broilers and Human, and detection of *adrA* gene in all serovars except one isolate *S.* Typhimurium and two isolates *S.* Infantis. Another study [26] showed the presence of *csgD* gene in all *Salmonella* isolates. *OmpA* virulence gene play important role in bacterial adaptation to external environment stresses, which causing adhesion, invasion and host tissue damage .So, it considered from virulence factors [9]. In this current study, detection of ESβL s *[int1 ,int2 ,int3,* Bla_{TEM} , Bla_{CTX} , Bla_{OXA} *,MOX,gyrA*and *gyrS*] by using PCR test on *Salmonella* serovars isolated from boilers and Human. Detected the presence of Bla_{TEM} gene in all serovers isolated from broilers and Human .Another study, [48] detected Bla_{TEM} gene encoded for b-lactamase in 51.6% of *Salmonella* isolates. The widespread using of antibiotics leading to bacterial resistance, this antibiotic resistance not only in human medicine but also in veterinary and agricultural medicine.ESBLs are

plasmid encoded; these plasmids carry genes encoding resistance to drugs. ESβL s are classified according to two schemes: Amble Molecular Classification Scheme and Bush-Jacoby-Medieros System [2,4,38] .There are many types of ESβL s which are SHV-type, TEM-type,CTX-type,OXA-Type. ESβL-Enterobacteriaceae responsible for a lot of outbreaks of infection throughout world Bacteria which able to produce ESBLs enzyme are more resistant to many antibiotics which prescribed by doctors for treatment, thus making the infection which caused by an ESBL germ more difficult to treat. ESβL s are B-Lactamase which has resistance to Penicillins ,First-Second-and Third generation Cephalosporins and Aztreonam by hydrolysis of antibiotics [4].TEM-1 is the most common plasmid-mediatedB-Lactamaseofampicillin resistant gran-negative bacilli,TEM-2lesscommon with the same biochemical properities toTEM-1.[32] .OXA-type enzymes able to hydrolyze Penicillins,narrow-spectrum andThird-generationsCephalosporins and Monobactams [4] .TEM-Type ESβL s are first reported in 1965 form *E. Coli* isolates from patient in Ahen s, Greece ,named Temoneira [13] .CLSI recommended methods for ESβL s detection by 1. Disk diffusion methods by noting specific zone diameters [37], 2.Screening by dilution antimicrobial susceptibility tests using Ceftazidime,Aztreonam,Cefotaxime at screening concentration of 1 microgram/ml [37].In our study, found a correlation between virulence genes and resistance to commonly used antibiotics,most of molecular pathogenicity were located on chromosome or virulence-associated plasmid [25], but antibiotic resistance genes are located on extra chromosomal elements [5].The relation between virulence and antibiotic resistance among *Salmonella* serovars happened because of genetic determinants of antibiotic resistance and virulence genes [43,15]. Antibiotic resistance and virulence genes may be linked in same replicon [35]. Finally, in our study detection of chickens-to-human *Salmonella* strains transmission during farming widely demonstrated, this may happen through food chain or direct contact with live animals in broilers chickens industry [11, 29].

CONCLUSION

Salmonella bacterium is from the most important organisms that causing more serious economic losses in poultry industry and made great human health problem. The misuse of antibiotics in human medicine and in veterinary leading to resistance to antibiotics. In this present study found that *Salmonella* isolated from broilers have great resistance to Impineim in a percentage 83.3% and *Salmonella* isolated from Human have a great sensitivity to

Ceftazedime, Amikacin and Trimethoptime-Sulphamethoxazole in a percentage 100 % for each. By using PCR test for screening on four virulence genes (*invA* , *adrA* , *OmpA* and *csgD*) and screening on nine resistance genes *(int1* ,*int2* ,*int3* ,Bla_{TEM} ,Bla_{CTX},Bla_{OXA},*MOX* ,*gyrA* and *gyrS*) found that *invA* virulence gene which in marker for *Salmonella* are present in all serovars isolated from broilers and human and also the presence of Bla_{TEM} resistance gene in all serovars isolated from broilers and Human. ESBLs producing *Salmonella* is hazard to food safety, to public health, create sever therapeutic problems in the future. The relation between human and broilers chickens strains indicated that poultry industry act as most important reservoir for ESβL -producing *Salmonella* which transmitted to human by direct contact or by food Chain.

Competing interests:

All authors declare no competing interests.

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Table (1): List and classification of antimicrobial agents used in Human and Veterinary medicine.

Antibiotic	Disc concentration	Antimicrobial class	Medical importance
Aztreonam	**30 Mg**	**Monobactams**	**Critically important antimicrobials**
Imipenem	**10 Mg**	**Carbapenems**	**Critically important antimicrobials**
Cefotaxim	**30 Mg**	**Cephalosporins (third,fourth anf fifth generations)**	**Critically important antimicrobials**
Ceftazidime	**30 Mg**	**Cephalosporins (third,fourth anf fifth generations)**	**Critically important antimicrobials**
Ceftriaxone	**30 Mg**	**Cephalosporins(third,fourth anf fifth generations)**	**Critically important antimicrobials**
Cephalexine	**30 Mg**	**Cephalosporins(third,fourth anf fifth generations)**	**Critically important antimicrobials**
Gentamycin	**10 Mg**	**Aminoglycosides**	**Critically important antimicrobials**
Amikacin	**30 Mg**	**Aminoglycosides**	**Critically important antimicrobials**
Ampicillin	**10 Mg**	**Penicillins**	**Critically important antimicrobials**
Ampicillin & Sulbectam	**20 Mg**	**Penicillins**	**Critically important antimicrobials**
Amoxacillin & Clavulanic	**30 Mg**	**Penicillins**	**Critically important antimicrobials**
Ciprofloxacin	**5 Mg**	**Quinolones**	**Critically important antimicrobials**
Trimethoprime & Suphamethoxazole	**25 Mg**	**Suphonamides**	**Highly important antimicrobials**

Table (2): Oligonucleotide primers used to screen virulence and resistance genes in *Salmonella* serovars isolated from broilers and human.

Primer	Sequence	Amplified product	Reference
BlaOXA-1	**ATATCTCTACTGTTGCATCTCC**	**619 bp**	**Colom *et al*., 2003**
	AAACCCTTCAAACCATCC		
invA	**GTGAAATTATCGCCACGTTCGGGCAA**	**284 bp**	**Oliveira*et al*., 2003**
	TCATCGCACCGTCAAAGGAACC		
bla$_{TEM}$	**ATCAGCAATAAACCAGC**	**516 bp**	**Colome*tal*., 2003**
	CCCCGAAGAACGTTTTC		
Bla$_{CTX}$	**ATGTGCAGYACCAGTAARGTKATGGC**	**593 bp**	**Archambault *et al*., 2006**
	TGGGTRAARTARGTSACCAGAAYCAGCG		
	G		
qnrA	**ATTTCTCACGCCAGGATTTG**	**516 bp**	**Robicsek *et al*., 2006**
	GATCGGCAAAGGTTAGGTCA		
qnrS	**ACGACATTCGTCAACTGCAA**	**417 bp**	
	TAAATTGGCACCCTGTAGGC		
ompA	**AGT CGA GCT CAT GAA AAAGAC AGC TAT CGC**	**1052 bp**	**Kataria *et al*., 2013**
	AGT CAA GCT TTT AAG CCT GCG GCT GAG TTA		
Int1	**CCTCCCGCACGATGATC**	**280 bp**	**Kashif *et al*., 2013**
	TCCACGCATCGTCAGGC		
Int2	**TTATTGCTGGGATTAGGC**	**250 bp**	
	ACGGCTACCCTCTGTTATC		
Int3	**AGTGGGTGGCGAATGAGTG**	**484 bp**	
	TGTTCTTGTATCGGCAGGTG		
adrA	**ATGTTCCCAAAAATAATGAA**	**1113 bp**	**Bhowmick *et al*., 2011**
	TCATGCCGCCACTTCGGTGC		
csgD	**TTACCGCCTGAGATTATCGT**	**651 bp**	
	ATGTTTAATGAAGTCCATAG		
MOX	**GCT GCT CAA GGA GCA CAG GAT**	**520 bp**	**Pérez-Pérezand Hanson, 2002**
	CAC ATT GAC ATA GGT GTG GTG C		

Table (3): Serotypes of Broilers *Salmonella* isolates.

Serotype	n =	%
***S*. Kentucky**	**3**	**10**
***S*. Enteritidis**	**6**	**20**
***S*. Typhimurium**	**3**	**10**
***S*. Heidelberg**	**3**	**10**
S. Hader	**3**	**10**
***S*. Gueuletapee**	**1**	**3.3**
***S*. Newport**	**3**	**10**
***S*. Blegdam**	**2**	**6.6**
***S*. Infantis**	**5**	**16.6**
***S*. Maloe**	**1**	**3.3**
Total	**30**	**100**

Table (4): Serotypes of Human *Salmonella* isolates.

Serotype	n =	%
***S*. Enteritidis**	**5**	**35.7**
***S*. Typhimurium**	**5**	**35.7**
***S*. Anatum**	**3**	**21.4**
***S*. Derby**	**1**	**7.1**
Total	**14**	**100 %**

Table (5): The percentage of sensitivity and resistance of *Salmonella* serovars isolated from broilers and Human against 13 antimicrobial agents.

	Broliers				Human			
	Sensitive		Resistance		Sensitive		Resistance	
	n =	%	n =	%	n =	%	n =	%
ATM	9	30	21	70	12	85.7	2	14.3
IPM	5	16.6	25	83.3	12	85.7	2	14.3
CTX	6	20	24	80	13	92.8	1	7.2
AM	16	53.3	14	46.6	12	85.7	2	14.3
CAZ	14	46.6	16	53.3	14	100	--	--
CN	21	70	9	30	12	85.7	2	14.3
CRO	8	26.6	22	73.3	12	85.7	2	14.3
CL	22	73.3	8	26.6	13	92.8	1	7.2
AK	12	40	18	60	14	100	--	--
SAM	17	56.6	13	43.3	11	78.5	3	21.4
AMC	14	46.6	16	53.4	13	92.8	1	7.2
SXT	10	33.3	20	66.6	14	100	--	--
CIP	10	33.3	20	66.6	13	92.8	1	7.2

ATM :Aztreonam,IMP: Imipenem,CTX: Cefotaxim,AM:Ampicillin, CAZ : Ceftazedime , CN: Gentamicin,CRO: Ceftriaxone,CL: Cephalexin, AK : Amikacin ,SAM : Ampicillin-Sulbectam , AMC : Amoxacillin-Clavulanic , SXT : Trimethoprime-Sulphamethoxazole , CIP : Ciprofloxcin.

Table (6): Collection table for virulence and resistance genes for *Salmonella* serovars isolated from broilers and human.

Salmonella serovars from Brolier	RESULTS												
	invA	*adrA*	*csgD*	*ompA*	*Int1*	*Int2*	*Int3*	*blaTEM*	*blaOXA*	*blaCTX*	*MOX*	*qnrA*	*qnrS*
S. Kentucky	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Kentucky	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Enteritidis	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Enteritidis	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Typhimurium	+	-	+	+	-	-	-	+	-	-	-	-	-
S. Typhimurium	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Hedeilberg	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Hedeilberg	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Hader	+	+	+	+	+	-	-	+	-	-	-	-	+
S. Hader	+	+	+	+	+	-	-	+	-	-	-	-	+
S. Infantis	+	-	+	+	-	-	-	+	-	-	-	-	-
S. Infantis	+	-	+	+	-	-	-	+	-	-	-	-	-
S. Blegdam	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Blegdam	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Maloe	+	+	+	+	+	-	-	+	-	+	-	-	-
S. Gueuletapee	+	+	+	+	-	-	-	+	-	-	-	-	-
S. Newport	+	+	+	+	+	-	-	+	-	-	-	-	-
S. Newport	+	+	+	+	+	-	-	+	-	+	-	-	-
Salmonella serovars from Human													
S. Enteritidis	+	+	+	+	+	-	+	+	-	-	-	-	-
S. Anatum	+	+	+	+	+	-	+	+	-	-	-	-	-
S. Typhimurium	+	+	+	+	+	-	+	+	-	-	-	-	-
S. Derby	+	+	+	+	+	-	+	+	-	-	-	-	-

Table (7): Correlation between virulence genes and antimicrobial resistance between *Salmonella* serovars isolated from broilers and Human.

Salmonella serotypes from Brolier	Virulance genes	Antibiotic resistance	Resistance genes
S. Kentucky	invA,adrA.csgD and ompA	IMP,CTX,CN,CRO,AK,CIP	Bla_{TEM}
S. Kentucky	invA,adrA.csgD and ompA	IPM,CTX,CRO,SXT,CIP	Bla_{TEM}
S. Enteritidis	invA,adrA.csgD and ompA	ATM,IPM,CTX,CAZ,CRO,AK,SXT.CIP	Bla_{TEM}
S. Enteritidis	invA,adrA.csgD and ompA	ATM,IPM,CTX,AK,SAM,AMC,SXT,CIP	Bla_{TEM}
S. Typhimurium	invA,csgD,ompA	CTX,CAZ,CRO,AK,SAM,AMC,SXT,CIP	Bla_{TEM}
S. Typhimurium	invA,adrA.csgD and ompA	ATM,IPM,CTX,AM,CAZ,CN,CRO,SAM,SXT,CIP	Bla_{TEM}
S. Hedeilberg	invA,adrA.csgD and ompA	ATM,IPM,CTX,CN,CRO,CL,CIP	Bla_{TEM}
S. Hedeilberg	invA,adrA.csgD and ompA	ATM,IPM,CTX,AM,CAZ,CN,CRO,AK,AMC,SXT,CIP	Bla_{TEM}
S. Hader	invA,adrA.csgD and ompA	IPM , AM,CIP	Bla_{TEM}, *gyr S, Int 1*
S. Hader	invA,adrA.csgD and ompA	ATM,IPM,CTX,AM,CRO,CL,AMC,SXT , CIP	Bla_{TEM}, *gyr S, Int 1*
S. Infantis	invA , csgD , ompA	CAZ	Bla_{TEM}
S. Infantis	invA , csgD , ompA	IMP , AM , SXT	Bla_{TEM}
S. Blegdam	invA,adrA.csgD and ompA	ATM,IMP,CTX,CAZ,CRO,CL,AK,SAM,AMC	Bla_{TEM}
S. Blegdam	invA,adrA.csgD and ompA	ATM,IMP,CTX,CAZ,CN,CRO,AK,SAM,AMC,SXT,CIP	Bla_{TEM}
S. Maloe	invA,adrA.csgD and ompA	AM,AK	*Int 1*, Bla_{TEM}, Bla_{CTX}
S. Gueuletapee	invA,adrA.csgD and ompA	ATM,IPM,CTX,CN,CL,AK,AMC	Bla_{TEM}
S. Newport	invA,adrA.csgD and ompA	ATM,IPM,CTX,AM,CRO	*Int 1*, Bla_{TEM}
S. Newport	invA,adrA.csgD and ompA	ATM , AM , CRO	*Int 1*, Bla_{TEM}, Bla_{CTX}
Salmonella serotypes from Human			
S. Enteritidis	invA,adrA.csgD and ompA	ATM,IPM,CN,CRO,CIP	*Int 1*, *Int 3* Bla_{TEM}
S. Anatum	invA,adrA.csgD and ompA	AM,SAM	*Int 1*, *Int 3* Bla_{TEM}
S. Typhimurium	invA,adrA.csgD and ompA	ATM,IPM,CTX,AM,CN,CRO,CL	*Int 1*, *Int 3* Bla_{TEM}
S. Derby	invA,adrA.csgD and ompA	SAM,AMC	*Int 1*, *Int 3* Bla_{TEM}

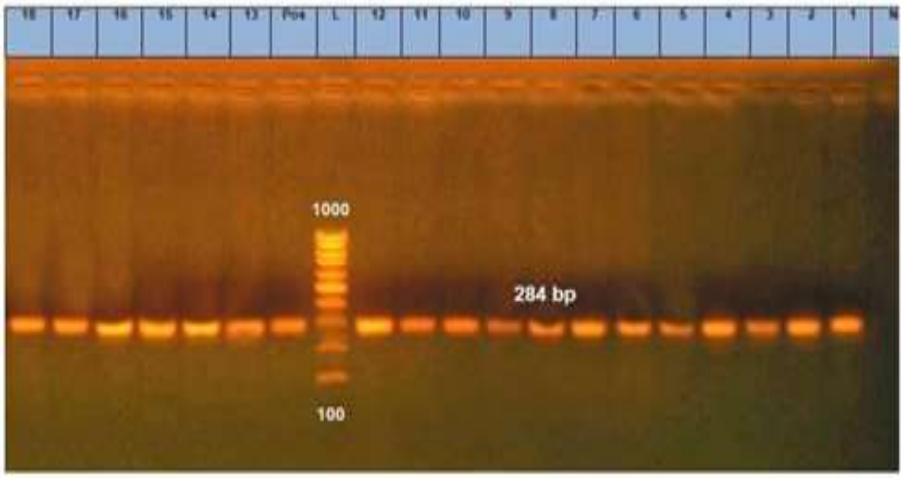

Fig. (1): Agarose gel electrophoresis showing positive amplification for *invA* gene at 284 bp. for *Salmonella* serovars.

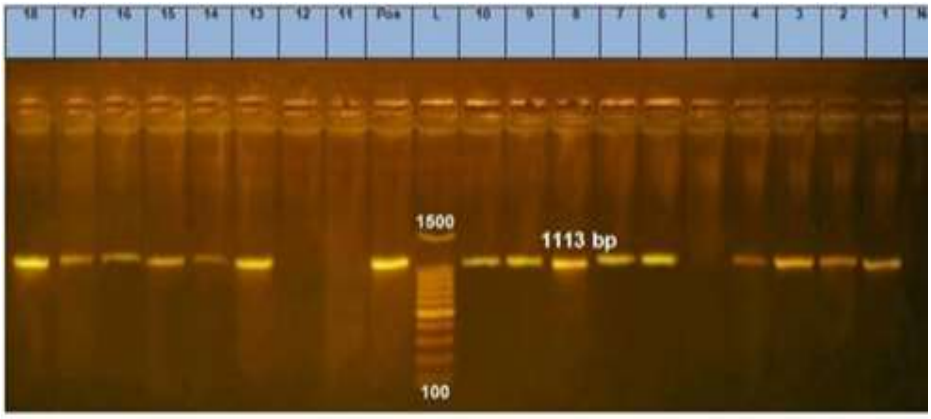

Fig. (2): Agarose gel electrophoresis showing positive amplification for *adrA* gene at 1113 bp. for *Salmonella* serovars.

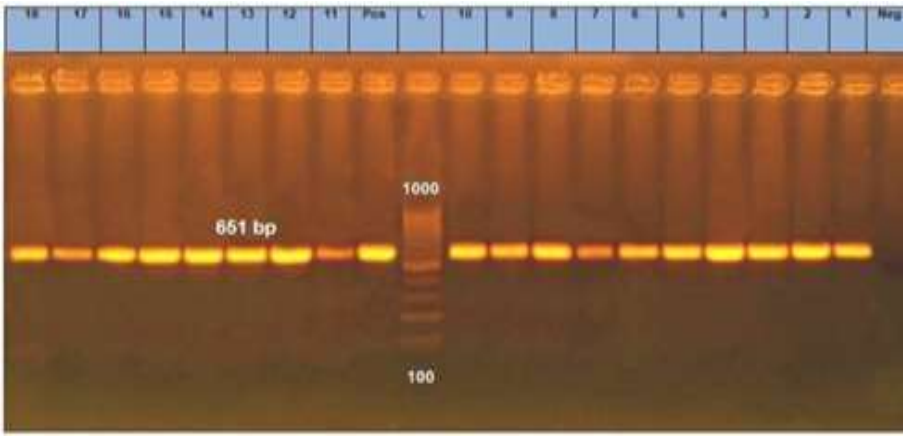

Fig. (3): Agarose gel electrophoresis showing positive amplification for *csgD* gene at 651 bp. for *Salmonella* serovars.

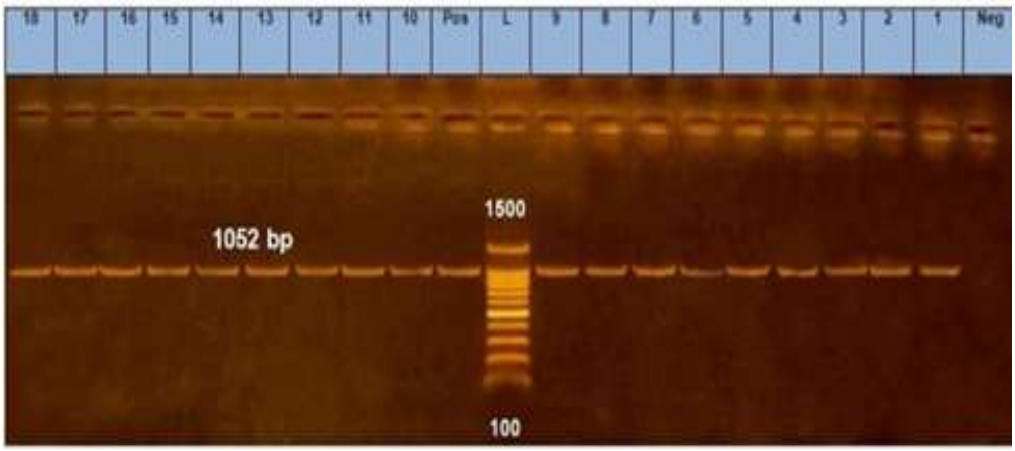

Fig. (4): Agarose gel electrophoresis showing positive amplification for *ompA* gene at 1052 bp. for *Salmonella* serovars.

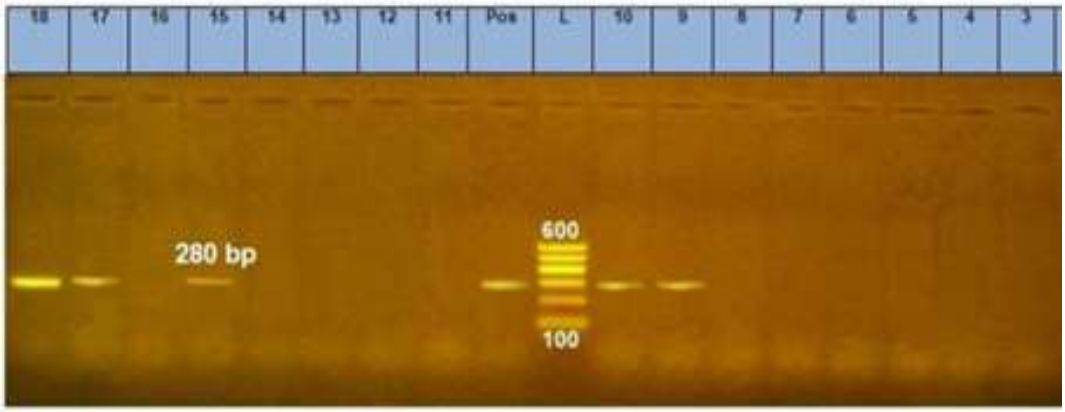

Fig. (5): Agarose gel electrophoresis showing positive amplification for *Int 1* gene at 280 bp for *Salomnella* serovars.

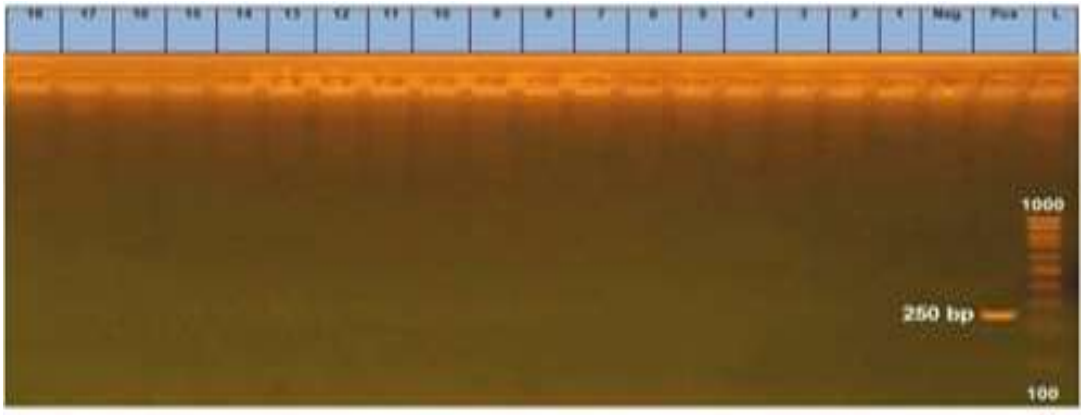

Fig. (6): Agarose gel electrophoresis showing positive amplification for *Int 2* gene at 250 bp. for *Salmonella* isolates.

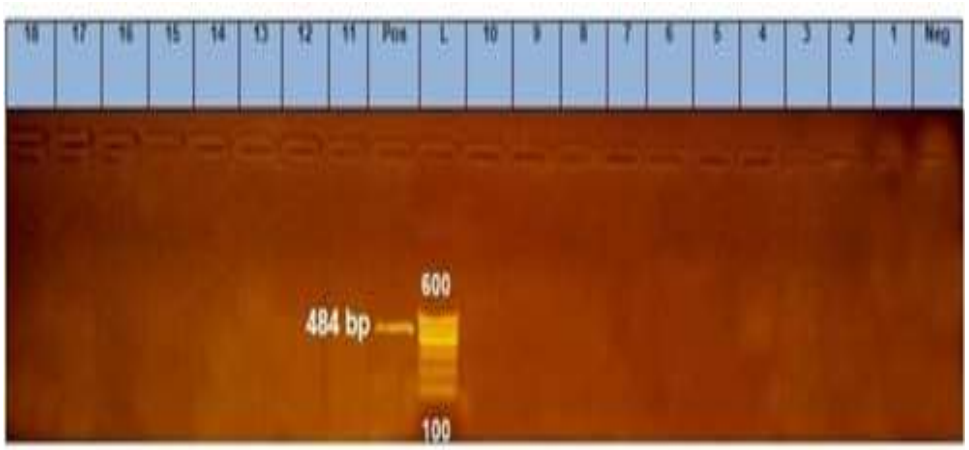

Fig. (7): Agarose gel electrophoresis showing positive amplification for *Int* 3 gene at 484 bp. for *Salmonella* isolates.

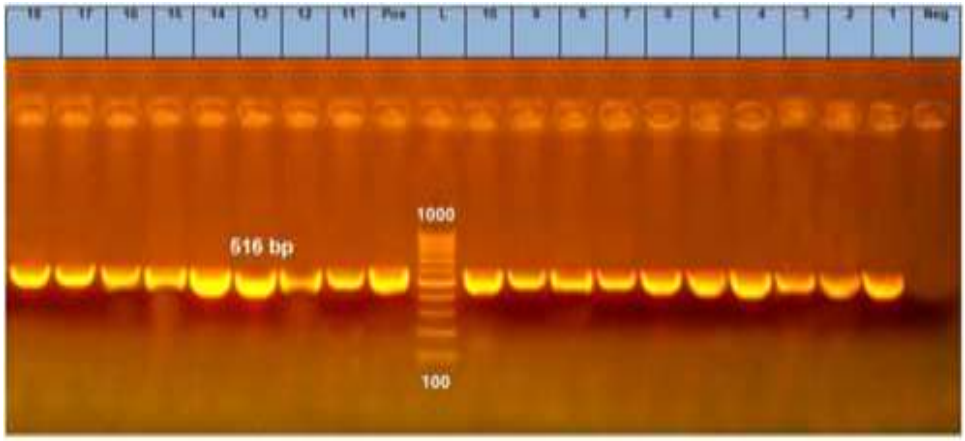

Fig. (8): Agarose gel electrophoresis showing positive amplification for Bla_{TEM} gene at 516 bp.in all *Salmonella* isolates.

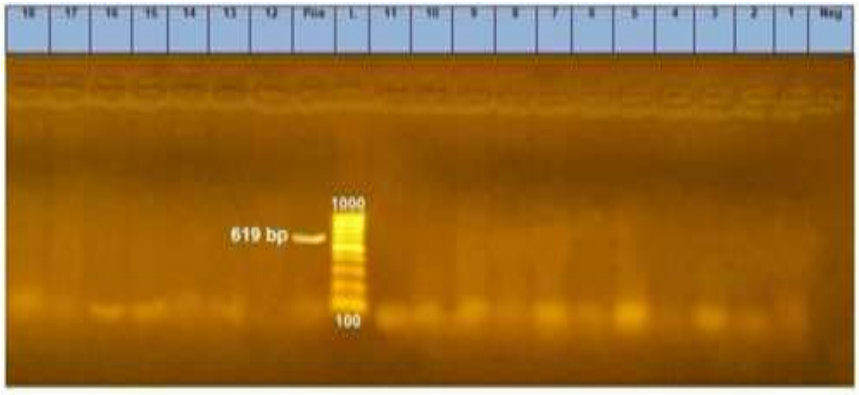

Fig. (9): Agarose gel electrophoresis showing positive amplification for Bla_{OXA} gene at 619 bp in all examined *Salmonella* isolates.

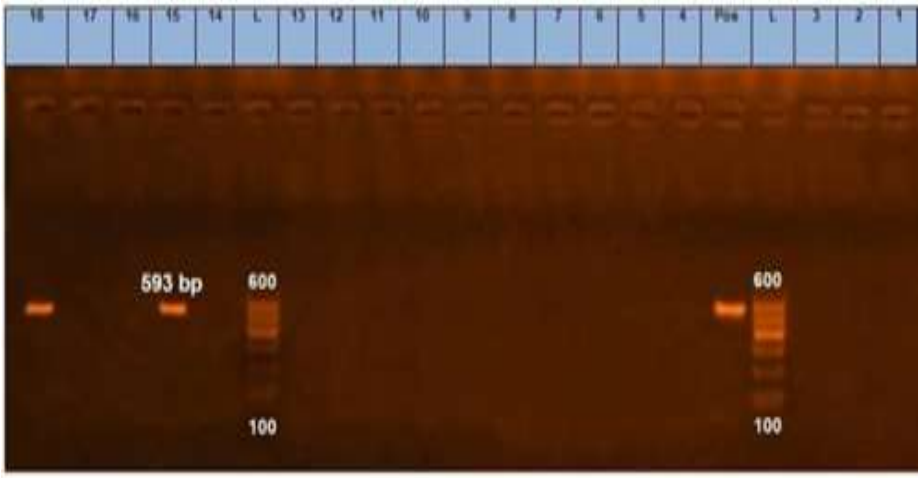

Fig. (10): Agarose gel electrophoresis showing positive amplification for *Bla$_{CTX}$* gene at 593 bp in *salmonella* isolates**.**

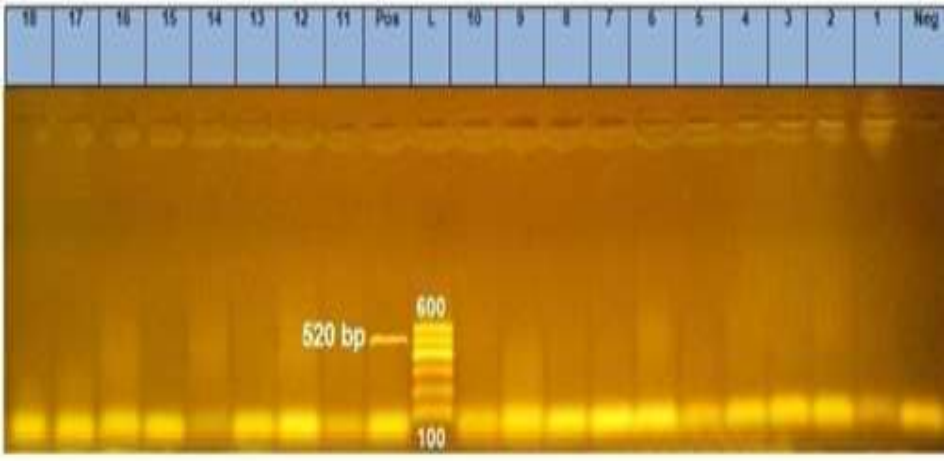

Fig. (11): Agarose gel electrophoresis showing positive amplification for *MOX* gene at 520 bp for *Salmonella* isolates**.**

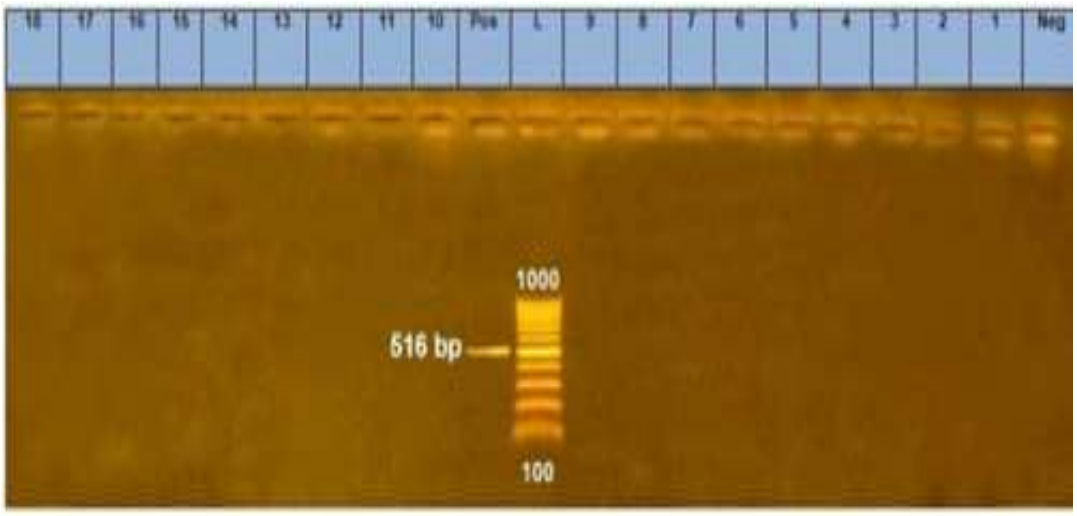

Fig. (12): Agarose gel electrophoresis showing positive amplification for *gyr A* gene at 516 bp for *Salmonella* isolates**.**

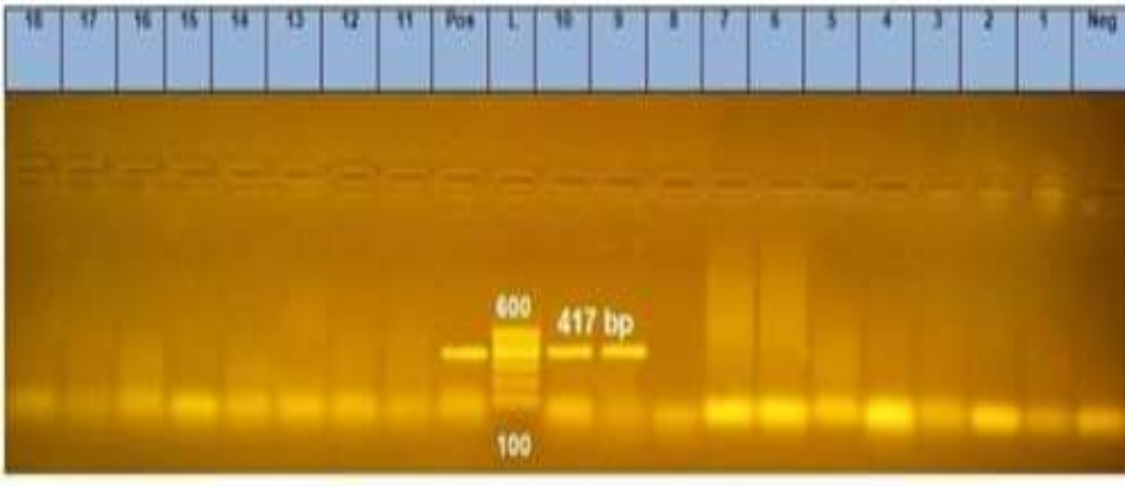

Fig. (13): Agarose gel electrophoresis showing positive amplification for *gyr S* gene at 417 bp for *Salmonella* isolates**.**