**Molecular Studies on Antimicrobial Resistance Genes in Multiple drug resistant *Salmonella Servoars* and *Staphylococcus aureus* Isolated from Chickens .**

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**ABSTRACT**

A total of 400 samples were collected from 100 broiler chickens represented by 100 for each of muscle ,liver, heart and 50 for each of spleen and joint from chicken flocks in Gharbia, Egypt during the period from February to June 2016. All samples were subjected for isolation of Salmonellaand *S.aureus,* antibiograms and application of PCR screen on resistant genes. The incidence of Salmonella and  *S.aureus* were 11 (2.75%) and 41(10.25%) respectively. For Salmonella the predominant servoars isolated were *S.Infantis* (36.36%) , *S.Typhimurium* (18.18%) , *S.Stanleyville* (18.18%) *, S.Magherafelt* (9%) , *S.Sinchew* (9%) , *S.Ratchaburi* (9%). All Salmonallae and *S.aureus* isolates (100%) showed multidrug resistance. All Salmonella isolates were sensitive to amikacin and gentamycin., Salmonalla isolates showed several antibiotic resistance by variable difference from 100% for pencillin, oxacillin , spiramycin and vancomycin to 27.2% for norfloxacin and cefoxitin. *S.aureus* isolates showed several antibiotic resistance by variable difference from 100% for oxacillin , ampicillin ,pencillin ,spiramycin to 12% for ceftriaxone and amikacin, By using PCR, For Salmonella showed that 9 isolates harbored *bla*TEM gene ,*Sul*1 and *flo*R (9/9)(100%) , 7 isolates harbored *ere*A *and tet*A (7/9)(77.7%), 6 isolates harbored *aad*A1 , class 1 integrons (6/9) (66.6%) and only one isolate harbored *qnr*S gene (1/9) (11.1%). But for *S.aureus* showed that 3 isolates harbored *mec*A gene (3/6) (50%) and 4 isolates harbored  *fem*A gene (4/6)

( 66.6%).

**Keywords :** Antimicrobial Resistance ,Chicken, Class I Integron, PCR.

**1. INTRODUCTION**

Antimicrobial resistance is now recognised as one of the most evidence of political traction, with endorsements of statements by the WHO and US Centers for Disease Control and Prevention describing a global crisis and an impending catastrophe of a return to the pre-antibiotic era. ([Organization, 2014](#_ENREF_29)). Amongst the foodborne pathogens, Salmonella and  *S.aureus* are the most common and frequent pathogens responsible for food poisoning and food related infections. Salmonellais a leading cause of enteric diseases in human and animal with millions of illness worldwide, whereas the non-typhoidal Salmonella species as a zoonotic agent are also predominantly associated with food borne infections ([Akbar and Anal, 2013](#_ENREF_7)). The increased level of antimicrobial resistance observed in Salmonellae has become a public health issue. The development of resistance in Salmonellae to antimicrobial agents is attributable to one of several mechanisms such as production of enzymes that inactivate antimicrobial agents through degradation or structural modification, reduction of bacterial cell permeability to antibiotics, activation of antimicrobial efflux pumps, and modification of cellular drug targets ([Crim *et al.*, 2015](#_ENREF_12)) Penicillin was the first choice of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase. More than 90% *S.aureus* strains were resistant to penicillin. However, methicillin, semi synthetic penicillin, was used to treat Penicillin Resistant *S. aureus* but resistance finally emerged ([Hendriksen *et al.*, 2011](#_ENREF_20)). Methicillin resistance arises due to the acquisition of the  *mec*A gene, which encodes an alternative penicillin-binding protein, PBP2a which has a low affinity for β-lactam antibiotics ([Weese, 2010](#_ENREF_42)).Integrons are mobile DNA elements with the ability to capture genes and can integrate gene cassettes, usually antibiotic resistance genes, by site-specifc recombination . To date, ten integron classes have been identified and, among them, class 1, 2, and 3 integrons have been implicated in antibiotic resistance.

This study aimed to investigate the incidience of some Antimicrobial resistance genes in multi-drug resistant isolates of Salmonella and *S.aureus* recovered from broilers in El-Gharbia ,Egypt.

2. MATERIALS AND METHODS

*2.1. Sample collection:*

Hundered broilers : ( 100 sample from muscle , 100 sample from liver , 100 sample from heart , 50 sample from spleen and 50 sample from joint ) were collected from different farms in El-Gharbia Governorate , Egypt between February to June 2016 then transferred to Tanta animal health laboratory under aseptic condition in ice box .

*2.2. Bacteriological examination : Isolation and identification of Salmonella servoars and S.aureus.*

*2.2.1.Salmonella servoars :*

2.2.1.Salmonella isolation and identification:

The procedure of isolation of Salmonella was carried out according to the standard methods recommended by ISO 6579 ([ISO, 2002](#_ENREF_21)). Typical colonies of Salmonella on XLD agar were pale pink with black center while on Salmonella-Shigella (S-S) agar salmonella colonies appear pale color with or without black centers .Smears from the suspected Salmonella colonies were stained with Gram’s stain and microscopically examined. Suspected colonies were identified as Salmonella spp. based on their colony morphology on selective media, and the biochemical testing using TSI agar, Urea agar, L-lysine decarboxylase, Voges Proskauer, Methyl red tests, Simmon's Citrate and Indole tests ([Cruickshank *et al.*, 1975](#_ENREF_13)). Finally ,Salmonella isolates were serotyped based on slide agglutination for O and H antigens according to Kauffmann-White (1972).

2.2.2. The procedure of isolation and identification of *S.aureus* was carried out according to ([Quinn *et al.*, 2011](#_ENREF_33)).

2..3.Antimicrobial Susceptibility testing of Salmonella and *S.aureus* ([Finegold and Martin, 1982](#_ENREF_17)) : By using the agar disc diffusion method on Mueller Hinton agar (Oxoid) plates. Twenty-one discs used were ampicillin (AMP) (10µg),penicillin (P)(10µg), cefoxitin (FOX) (30µg), ceftriaxone(CRO)(30µg), cefotaxime (CTX) (30µg) ,cephalothin (KF)(30µg), aztreonam(ATM)(30 µg), amoxicillin-Clavulanic acid (AMC)(30µg), oxacillin(OX)(10µg), nalidixicacid (NA)(30µg), norfloxacin(NOR)(10µg), enrofloxacin (ENR)(5µg), tetracycline (TE)(30µg), amikacin(AK)(30µg), gentamycin (CN)(10µg) ,streptomycin (S) (10µg) , chloromphenicol© (30 µg),erythromycin E (15 µg ) , spiramycin SP ( 100 µg ) ,vancomycin (30 µg ), sulphamethoxate-trimethoprim (SXT) (25µg) (Oxid,UK). The interpretation of inhibition zone of tested culture was according (CLSI.2014).

2.4- Detection of resistance genes and Class I integron using Polymerase chain reaction (PCR) for Salmonella and *S.aureus*

Oligoneucleotide primers were designated according to Integrated DNA Technology. The primers sequences were illustrated as in Table (1).

*2.2.4.1.DNA extraction and purification :*

The extraction was done by QIAamp® DNA MiniKit (Cat. No. 51304, Qiagen) used according to manufacturer’s instructions.

*2.2.4.2.Amplification and cycling protocol :*

for conventional PCR Using of PCR 1.1x ReddyMix TM Master Mix (Thermo SCIENTIFIC) with Cat. No. AB0575/LD-A.

*2.2.4.3.Detection of PCR products:* ([Sambrook *et al.*, 1989](#_ENREF_38))

Aliquots of amplified PCR products were mixed with gel loading buffer and electrophoresed in 1.5% agarose gel

**3.RESULTS.**

*3.1. Incidence of* Salmonellae *and S.aureus among the examined samples:*

*3.1.1.Salmonella:*

The Culturing and Serotyping identified 11 Salmonella isolates of 400 samples (2.75%) that belong to 6 serotypes distrubed as following 5% in liver, 4% in muscle , 2 % in heart and 0% in spleen and joints (Table 2). The predominant servoars isolated in this study were *S.Infantis* (36.36%) , *S.Typhimurium* (18.18%) , *S.Stanleyville* (18.18%) , *S.Magherafelt* (9%) , *S.Sinchew* (9%) and *S.Ratchaburi* (9%) .

*3.1.2. S.aureus:*

Out of 400 examined chicken samples coagulase positive *S.aureus* incidence was 10.25% (41 isolate). distrubed as following 18% in muscle, 11% in liver . 7% in heart . 6% in spleen and 4% in joints.(Table 3).

*3.2.Antimicrobial Susceptability testing for Salmonella and S.aureus:*

By Antimicrobial sensitivity test all Salmonalla and *S.aureus* isolates (100%) showed multidrug resistance (MDR;resistance to 3 or more antibiotics). Resistance percentage for Salmonella and *S.aureus* as shown in (Table 4 and 5).

*3.3.PCR results for detection of resistance genes in Salmonella and S.aureus:*

For Salmonella : 9 isolates out of 11 examined salmonella isolates harbored *bla*TEM gene ,*Sul*1 and *flo*R (100%) , 7 isolates out of 11 examined salmonella isolates harbored *ere*Aand *tet*A (77.7%) , 6 isolates out of 11 examined salmonella isolates harbored *aad*A1 and class I integron (66.6%) and only 1 isolate out of 11 examined salmonella isolates harbored *qnr*S gene (11.1%). (Table 6)

For *S.aureus* : 3 isolates harbored *mec*A gene (50%) and 4 isolates harbored *fem*A gene ( 66.6%) .(Table 7)

**4.DISCUSSION**

The present study tries to illustrate the problem of Antimicrobial resistance in Salmonella servoars and *S.aureus* in chicken in El-Gharbia Governorate,Egypt.

*4.1.Salmonella:*

Out of 400 examined chicken samples, 11 isolates (2.75%) of Salmonella spp were detected (Table 2) .This is aggreable with [Chiu *et al*., ( 2010](#_ENREF_10)) and [Akbar and Anal., (2013](#_ENREF_7)) who isolated Salmonella from chicken by (3.4%) and (5.2%) respectively . The incidence of *Salmonella* in chicken liver samples was 5 % higher than chicken meat samples (Muscle) (4%) and chicken heart samples (2%) while joint and spleen samples were free from *Salmonella*. (Table 2). Nearly similar results of incidence of *Salmonella* in different organs detected by [Abd-Elghany *et al*., (2015](#_ENREF_1)).Serological identification of Salmonella (Table 2)confirmed the isolation of 6 *Salmonella* servoars as the following*: S.Infantis* (36.36%) , *S.Typhimurium* (18.18%) *, S.Stanleyville* (18.18%) , *S.Magherafelt* (9%), *S.Sinchew* (9%) and *S.Ratchaburi* (9%) .This reflects the role of chicken as a potential source of zoonotic non-typhoidal salmonellosis in human as *S.Typhimurium* was mostly associated with human illness.([Hendriksen *et al.*, 2011](#_ENREF_20)).It was noticed that, *Salmonella Infantis* (36.3%) was the most predominant isolated *Salmonella* serotypes from broiler carcasses and organs. This Agree with [Akbar and Anal., ( 2013](#_ENREF_7)) and [Kaushik *et al*., (2014](#_ENREF_33)) who found that the most predominant serotype in chicken was *S.infantis* (56.36%) and (75%), respectively. Emergence of multidrug-resistant Salmonellaspecies has a great impact on human health ([Abd-Elghany *et al.*, 2015](#_ENREF_1)). All Salmonella serotypes showed MDR to ≥ 3 (100%) of the 21 Antimicrobial tested as shown in Table (4) that was similar to the previously recorded studies by [Abdel-Maksoud *et al*., (2015](#_ENREF_5)) who recorded MDR Salmonella isolates by 100% in Cairo. In the current study, it was found that Salmonella isolates were resistant to NA ,ENR and NOR by (72.7% ), (27.2%) and (27.2%), respectively as shown in Table (4). These result were agreed with [Ahmed and Shimamoto .,(2012](#_ENREF_6)) .These results counter [El-Sharkawy *et al*., (2017](#_ENREF_15)) who found that *S.infantis* shows good sensitivity to fluorinated quinolones. Among the β-lactams, Salmonella servoars resistance to to Pencillin ,Oxacillin (100%) , was found the highest, followed by Ampicillin , Azteronam (63.6%) , Cefotaxime (90.9%), Cephalothin(81.8%), Ceftriaxone(72.7%), AMC (18.1%), Cefoxitin (9%).(Table 5) These results similar to [Ahmed and Shimamoto., (2012](#_ENREF_6)) and [Gharieb *et al* ., (2015](#_ENREF_18)) . These results were disagreed with [Sodagari *et al*.,(2015](#_ENREF_39)) who found lower resistance to B-lactams and ceftriaxone.

In the current study, Strepomycin resistance was (54.5%) (Table 4) similar to [Sodagari *et al*., (2015](#_ENREF_53)) .Salmonellaisolates showed resistance to Erthromycin (81.8%) ,tetracycline (54.5%), chlorompenicol (45.4%) and Sulfamethoxate-trimethoprim (36.3%)( (Table 4) similar to [Halawa *et al*., (2016](#_ENREF_19)).

These results disagree with [Gharieb *et al.*,( 2015](#_ENREF_18)) and [Ahmed and Shimamoto.,(2012](#_ENREF_6)) who found higher resistance to tetracycline ,Chloromphenicol and trimethoprim-sulfamethoxazole. All Salmonella isolates were sensitive to Amikacin and Gentamycin similar to [El-Sharkawy *et al*., (2017](#_ENREF_15)) and [Abd El Tawab et al., (2015](#_ENREF_4)) . PCR was a perfect tool for accurate detection of Salmonella resistant genes , Phenotypical resistance to quinolonesconfirmed by presence of *qnrS* gene at 417bp (coding for plasmid-mediated quinolone resistance PMQR) , *qnr*S gene detected in only one Salmonella isolates (*S.Magherafelt)* (11.1%)(1/9)( Fig1)(Table 6) ,while other 8Salmonella isolates showed phenotypical resistance to quinolones without harbouring the *qnrS* gene, indicating that these strains possess another quinolone resistance mechanism *as qnrS* mediates resistance to NA ,NOR and ENR which is clearly reflected in the resistance phenotypes of these isolates (Table 4 ) .

Phenotypical resistance ofβ- lactam confirmedby presence of *bla TEM* gene at 516 bp , *bla TEM* gene detected in 100% (9/9) (Fig 2) (Table 6).Also It was found that all Salmonella isolates showed no specific amplicons with *bla*CMY-2 gene. These results agree with [Halawa *et al.*, (2016](#_ENREF_19)). In most of the isolates, which contained a gene cassette, the comparable antibiotic resistance phenotypes were detected except for some of the Salmonellaisolates were phenotypically sensitive to the streptomycin despite the presence of streptomycin modifying enzyme gene cassettes (*aad*A1) amplicons (484bp) at (66.6%) (6/9)(Fig 2)(Table 7) . As this suggests that some of the antimicrobial resistance genes are silent in bacteria in vitro; however, these silent genes can spread to other bacteria or turn on in  vivo, especially under antimicrobial pressure which in agreement with [El-Sharkawy *et al.*, (2017](#_ENREF_15)).

PCR screening showed that Class 1 integrons, which are the vehicles of spread for antibiotic resistant genes were detected in in (6/9) (66.6%) of tested Salmonella (Fig 6 )(Table 6). These Nearly similar to [Abd-Elghany *et al.*, (2015](#_ENREF_1)) and [Ahmed and Shimamoto, (2012](#_ENREF_6)) . Integrons were present in 6 out of the 9 Salmonella serovars. This shows that the integrons are not limited to specific Salmonella serovars and might occur in any serovars. The mechanism of multidrug resistance in integron carrying bacteria comes from their decreased susceptibility not only to antimicrobials where there respective gene included in the integron but also to other antimicrobials even if their resistance gene cassette was not included in integron as apparently a considerable number of antibiotic resistance genes are located outside the integrons either on chromosomes or plasmids.([Malek *et al.*, 2015](#_ENREF_24)).

*4.2.S.aureus*

The results of *Staphylococcus aureus* isolation from examined chicken samples ( Table 3) cleared that, a total of 41(10.25%) isolates of *S. aureus* were recovered from 400 samples. These results are higher than [Osman *et al.*, (2016](#_ENREF_30)) who isolated *S.aureus* by 3% in Cairo. These results lower than [Enany *et al.*,( 2013](#_ENREF_16)), [Momtaz *et al.*, (2013](#_ENREF_27)) and [Akbar and Anal, (2013](#_ENREF_7)) . The widespread use of antibiotics has undoubtedly accelerated the virulence of *S.aureus*, by acquiring multiple resistance genes, has become able to survive almost all antibiotic families ([Stefani and Goglio, 2010](#_ENREF_40)). All 41 *S.aureus* isolates (100%) exhibited multidrug resistance phenotype with resistance to ≥3 classes of antimicrobial agents (Table 5) similar to [Suleiman *et al.*, (2013](#_ENREF_41)) who detected MDR (100%) . *S.aureus* showing maximum resistance aganist Oxacillin , Ampicillin ,Pencillin ,Spiramycin (100% for each) followed by Vancomycin , Cephalothin, Tetracycline, Nalidixic acid (97.5% for each), Aztronam, Cefoxitin and Enrofloxacin (85.3% for each), Amoxicillin-Clavulanic (83%), Erythromycin (75.6%), Norfloxacin (48.7%), Chloromphenicol (34%) , Cefotaxime and Gentamycin (19.5% for each) , Ceftriaxone, Amikacin (12% for each) (Table 5). Theses results are similar to [Momtaz *et al.*, (2013](#_ENREF_65)), [Abd El-Tawab  *et al.*, (2017](#_ENREF_3)) and [Sallam *et al.*, ( 2015](#_ENREF_37)). Methicillin resistance arises due to the acquisition of the *mec*A gene, which encodes an alternative penicillin-binding protein, PBP2a ,which has a low affinity for β-lactam antibiotics.([Weese, 2010](#_ENREF_42)). Moreover, *mec*A alone does not solely confer the methicillin resistance as studies have shown that fem (factors essential for methicillin-resistance) or the auxiliary genes like *fem*A/B/X in addition to *mec*A are important in expression of methicillin resistance, the *fem*ABX operon encodes factors which are responsible for the formation of pentaglycine bridges in the cell wall of Staphylococci([Chikkala *et al.*, 2012](#_ENREF_9)) and there was correlation between genotypic content of the f*em*A and *mec*A genes and the phenotypic expression of them when tested by antibiotic disc diffusion method. The high resistance of *S.aureus* isolates from chicken meat and giblets to oxacillin (methicillin) , ampicillin , pencillin , spiramycin (100% resistance for each) confirmed by (50%) of *S.aureus* isolates showed the presence of *mecA* gene by PCR. (Table 7) . chicken samples tested. The results of PCR for amplification of *mec*A gene in *S. aureus* isolates (Fig 7 ) showed that, the *mec*A gene was amplified in 3 out of 6 examined strains (50%) giving product of 310 bp. The results came in harmony with those of [Momtaz *et al.*, (2013](#_ENREF_27)), [Osman *et al.*, (2016](#_ENREF_46)), [Abd El-Tawab  *et al.*, (2017](#_ENREF_3)) and [Sallam *et al.*, (2015](#_ENREF_37)). Phenotypic MRSA isolates not harboring the *mec*A gene were previously reported by [Pereira *et al.*, (2009](#_ENREF_75)) who found Also, 38% of the *S.aureus* isolates were resistant to oxacillin , but only 0.68% showed the presence of *mec*A gene, [Enany *et al.*, (2013](#_ENREF_16)) who detected 73.3% of isolates were methicillin resistant but only (20%) isolates were MRSA by PCR and [Abdalrahman *et al.*, ( 2015](#_ENREF_3)) who failed to detect *mec*A gene in poultry meat and its products and said that, this might be due to over production of β-lactamase enzymes or the presence of a variant *mec*A gene that does not amplify with the available PCR primers. Whole genome sequencing might help identify new *mec*A homologues. The results of PCR for amplification of *fem*A gene in *S. aureus* isolates (Fig 7) (Table 7 ) showed that, the *fem*A gene was amplified in 4 out of 6 examined strains (66.6%) giving product of 132 bp. The results came in harmony with those of [Abd El-Tawab  *et al.*, ( 2017](#_ENREF_3)) who detected *fem*A in 100% of examined isolates.

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Table (1) : Oligonucleotide primers sequences used.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target M.O. | Primer | Sequence  5'­­­­­- 3' | Amplified product | Reference |
| *Salmonella* | *Quinolones*  *qnr*S | ACGACATTCGTCAACTGCAA | 417 bp | Robicsek *et al*.,( 2006 ) |
| TAAATTGGCACCCTGTAGGC 5' |
| *Sulfonamides*  *Sul1* | CGGCGTGGGCTACCTGAACG | 443 bp | Sabarinath*et al*.,( 2011) |
| GCCGATCGCGTGAAGTTCCG |
| *B-lactams*  *bla*TEM | ATCAGCAATAAACCAGC | 516 bp | Colom *et al*., ( 2003 ) |
| CCCCGAAGAACGTTTTC |
| *Macrolides ere*(A*)* | GCCGGTGCTCATGAACTTGAG | 420 bp | Nguyen *et al*., ( 2009) |
| CGACTCTATTCGATCAGAGGC |
| *Phenicol*  *flo*R | TTTGGWCCGCTMTCRGAC | 494 bp | Doublet *et al*.,( 2003) |
| SGAGAARAAGACGAAGAAG |
| *ESBL*  *CMY-2* | TGG CCA GAA CTG ACA GGC AAA | 462 bp | Pérez-Pérez and Hanson, (2002) |
| TTT CTC CTG AAC GTG GCT GGC |
| *Aminoglycosides*  *aad*A1 | TATCAGAGGTAGTTGGCGTCAT | 484 bp | Randall *et al.* (2004)  Randall *et al.*  (2004) |
| GTTCCATAGCGTTAAGGTTTCATT |
| *Tetracycline*  *tet(*A*)* | GGTTCACTCGAACGACGTCA | 576 bp |
| CTGTCCGACAAGTTGCATGA |
| *Integrons*  *Int1* | CCTCCCGCACGATGATC | 280 bp | Kashik *et al*., (2013) |
| TCCACGCATCGTCAGGC |
| *S.aureus* | *B-lactams*  *fem*A | AAAAAAGCACATAACAAGCG | 132 bp | Mehrotra et al., (2000) |
| GATAAAGAAGAAACCAGCAG |
| *mec*A | GTA GAA ATG ACT GAA CGT CCG ATA A | 310 bp | McClure et al.,( 2006) |
| CCA ATT CCA CAT TGT TTC GGT CTA A |

Table (2) : Incidence of Salmonella Servoars in different chicken samples.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **%**  **\*\*** | **Total No of positive isolates** | **Joint** | | **Spleen** | | **Heart** | | **Muscle** | | **Liver** | | **Organs** |
|  |  | **50** | |  | **50** | **100** | | **100** | | **100** | | **Total number of examined samples** |
|  |  | **%\*** | **No of positive samples** | **%\*** | **No of positive samples** | **%\*** | **No of positive samples** | **%\*** | **No of positive samples** | **%\*** | **No of positive samples** |  |
| **36.36%** | **4** | **0** | **0** | **0** | **0** | **0** | **0** | **2** | **2** | **2** | **2** | ***S.Infantis* O 6,7,14:r ;1,5** |
| **18.18%** | **2** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **2** | **2** | ***S.Typhimurium* O 1 , 4 , [5] , 12 : i :1 , 2** |
| **18.18%** | **2** | **0** | **0** | **0** | **0** | **0** | **0** | **2** | **2** | **0** | **0** | ***S.Stanleyville* O 1, 4, [5] , 12 , [27] : z4 , z23 : [1,2]** |
| **9%** | **1** | **0** | **0** | **0** | **0** | **1** | **1** | **0** | **0** | **0** | **0** | ***S.Sinchew* O 3 ,10 : 1,v : z35** |
| **9%** | **1** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **1** | **1** | ***S.Ratchaburi* O 3 , 10 : z 35 : 1 ,6** |
| **9%** | **1** | **0** | **0** | **0** | **0** | **1** | **1** | **0** | **0** | **0** | **0** | ***S.Magherafelt* O 8 , 20 : i : 1 ,w** |
| **2.75%** | **11** | **0%** | **0** | **0%** | **0** | **2%** | **2** | **4%** | **4** | **5%** | **5** | ***Total*** |

**\*** percentage according to number of examined samples

**\*\*** percentage according to number of Salmonella isolates

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Coagulase Positive Samples | | | Number of examined samples | | Organs | |
| % | | Number |  | |  | |
| 18 | | 18 | 100 | | Muscle | |
| 11 | | 11 | 100 | | Liver | |
| 7 | | 7 | 100 | | Heart | |
| 6 | | 3 | 50 | | Spleen | |
| 4 | | 2 | 50 | | Joint | |
| 10.25% | | 41 | 400 | | Total | |

Table (3) : Incidence of *S.aureus* in different chicken organs.

Table (4) : .Results of Antimicrobial Sensitivity Test of Salmonella

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Antimicrobial Family | Antimicrobial disc | Resistant | | Intermediate | | Sensitive | |
|  |  | Number of isolates | % | Number of isolates | % | Number of isolates | % |
| Quinolones | Nalidixic acid(NA) | 8 | 72.7 | 1 | 9 | 2 | 18.1 |
| Norfloxacin (NOR) | 3 | 27.2 | 6 | 54.5 | 2 | 18.1 |
| Enrofloxacin (ENR) | 3 | 27.2 | 7 | 63.6 | 1 | 9 |
| *β-lactam* | Ampicillin (AMP) | 7 | 63.6 | 1 | 9 | 3 | 27.2 |
| Pencillin G (P) | 11 | 100 | 0 | 0 | 0 | 0 |
| Amoxicillin-Clavulanic (AMC) | 2 | 18.1 | 5 | 45.4 | 4 | 36.3 |
| Oxacillin (OX) | 11 | 100 | 0 | 0 | 0 | 0 |
| Cefoxitin (FOX) | 1 | 9 | 2 | 18.1 | 8 | 72.7 |
| Cefotaxime (CTX) | 10 | 90.9 | 1 | 9 | 0 | 0 |
| Ceftriaxone (CRO) | 8 | 72.7 | 3 | 27.2 | 0 | 0 |
| Cephalothin (KF) | 9 | 81.8 | 0 | 0 | 2 | 18.1 |
| Aztreonam (ATM) | 7 | 63.6 | 2 | 18.1 | 2 | 18.1 |
| Aminoglycosides | Amikacin (AK) | 0 | 0 | 1 | 9 | 10 | 90.9 |
| Gentamycin (CN) | 0 | 0 | 0 | 0 | 11 | 100 |
| Streptomycin (S) | 6 | 54.5 | 1 | 9 | 4 | 36.3 |
| Tetracycline | Tetracycline (TE) | 6 | 54.5 | 0 | 0 | 5 | 45.4 |
| Phenicols | Chloromphenicol (C) | 5 | 45.4 | 2 | 18.1 | 4 | 36.3 |
| Sulphonamides and  trimethoprim | Sulfamethoxate-Trimethoprim (SXT) | 4 | 36.3 | 1 | 9 | 6 | 54.5 |
| Macrolides | Erythromycin (E) | 9 | 81.8 | 0 | 0 | 2 | 18.1 |
| Spiramycin (SP) | 11 | 100 | 0 | 0 | 0 | 0 |
| Glycopeptide | Vancomycin (VA) | 11 | 100 | 0 | 0 | 0 | 0 |

Table (5) : .Results of Antimicrobial Sensitivity Test of  *S.aureus*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Antimicrobial Family | Antimicrobial disc | Resistant | | Intermediate | | Sensitive | |
|  |  | Number of isolates | % | Number of isolates | % | Number of isolates | % |
| Quinolones | Nalidixic acid (NA) | 40 | 97.5 | 0 | 0 | 1 | 2.4 |
| Norfloxacin (NOR) | 20 | 48.7 | 14 | 34 | 7 | 17 |
| Enrofloxacin (ENR) | 32 | 78 | 9 | 21.9 | 0 | 0 |
| *β-lactam* | Ampicillin (AMP) | 41 | 100 | 0 | 0 | 0 | 0 |
| Pencillin G ( P) | 41 | 100 | 0 | 0 | 0 | 0 |
| Amoxicillin-Clavulanic (AMC) | 34 | 83 | 0 | 0 | 7 | 17 |
| Oxacillin (OX) | 41 | 100 | 0 | 0 | 0 | 0 |
| Cefoxitin (FOX) | 32 | 78 | 0 | 0 | 9 | 22 |
| Cefotaxime (CTX) | 8 | 19.5 | 31 | 75.6 | 2 | 4.8 |
| Ceftriaxone (CRO) | 5 | 12 | 34 | 83 | 2 | 4.8 |
| Cephalothin (KF) | 40 | 97.5 | 1 | 2.4 | 0 | 0 |
| Aztreonam (ATM) | 32 | 78 | 0 | 0 | 9 | 21.9 |
| Aminoglycosides | Amikacin (AK) | 5 | 12 | 14 | 34 | 22 | 53.6 |
| Gentamycin (CN) | 8 | 19.5 | 2 | 4.8 | 31 | 75.6 |
| Streptomycin (S) | 34 | 83 | 0 | 0 | 7 | 17 |
| Tetracycline | Tetracycline (TE) | 40 | 97.5 | 1 | 2.4 | 0 | 0 |
| Phenicols | Chloromphenicol © | 14 | 34 | 7 | 17 | 20 | 48.7 |
| Sulphonamides  and trimethoprim | Sulfamethoxate-Trimethoprim (SXT) | 35 | 85.3 | 3 | 7.3 | 3 | 7.3 |
| Macrolides | Erythromycin (E) | 31 | 75.6 | 10 | 24.3 | 0 | 0 |
| Spiramycin (SP) | 41 | 100 | 0 | 0 | 0 | 0 |
| Glycopeptide | Vancomycin (VA) | 40 | 97.5 | 0 | 0 | 1 | 2.4 |

Table (6): Result of PCR of different resistance gene of *Salmonella Servoars.*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Salmonella  Serotype | Results | | | | | | | | |
| *B-Lactams* | | *Macrolide* | *Quinolones* | *Sulfonamides* | *Chloromphenicol* | *Aminoglocosides* | *TE* | *Int 1* |
| *blaTEM* | *Cmy-2* | *ereA* | *qnrS* | *Sul1* | *floR* | *aadA1* | *TetA* |
| *S.Infantis No (1)* | + | - | + | - | + | + | + | + | + |
| *S.Infantis No (2)* | + | - | - | - | + | + | + | + | + |
| *S.Infantis No (8)* | + | - | + | - | + | + | + | + | + |
| *S.Ratchaburi No (3)* | + | - | + | - | + | + | + | + | - |
| *S.Sinchew No (4)* | + | - | + | - | + | + | - | + | - |
| *S.Magherafelt No (5)* | + | - | + | + | + | + | - | + | + |
| *S.Stanyleville No (6)* | + | - | + | - | + | + | + | - | + |
| *S.Typhimurium No (7)* | + | - | + | - | + | + | - | - | - |
| *S.Typhimuruim No (9)* | + | - | - | - | + | + | + | + | + |
| Total Percentage%\* | 100% | 0% | 77.7% | 11.1% | 100% | 100% | 66.6% | 77.7% | 66.6% |

\* Percentage according to total number of examined Salmonella isolates by PCR ( 9 isolates )

Table (7): Result of PCR of different resistance gene of *S.aureus*

|  |  |  |
| --- | --- | --- |
| **No of *S.aureus* isolate** | **Results** | |
| **Methicillin (B-Lactams)** | |
| ***mec*A** | ***fem*A** |
| 1 | + | - |
| 2 | - | + |
| 3 | - | - |
| 4 | + | + |
| 5 | + | + |
| 6 | - | + |
| %\* | 50% | 66.6% |
|  |  |  |

\*Percentage according to total number of examined *S.aureus* isolates by PCR (6 isolates )

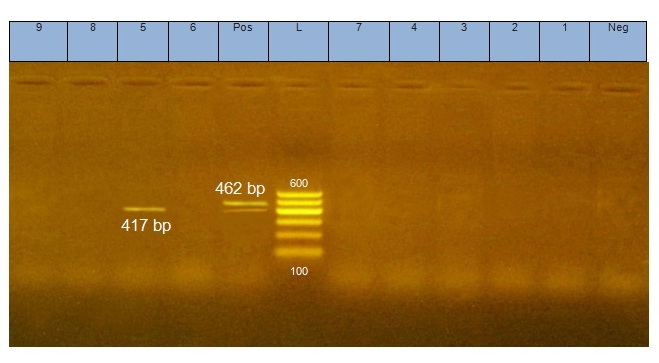


Figure (1): Agarose gel electrophoresis for amplified PCR product of quinolone resistance genes *(qnrS*) and β-Lactamase (*bla*CMY-2) in different Salmonella isolates

L ( DNA ladder 100 - 600 bp)

Neg (Negative control ), Pos ( positive control ) Positive and negative controls were recovered from previously tested and confirmed field isolates (Tested in the Reference laboratory for veterinary quality control on poultry production), Lane (1- 9) Salmonella isolates from chicken :

Lane 1,2,8 ( *S.Infantis*) ,Lane 3 (*S.Ratchaburi* ) , Lane 4 (*S.Sinchew*) ,

Lane 5 (*S.Magerhafelt*), Lane 6, (*S.Stanleyville*) , Lane 7,9 (*S.Typhimurum* )

(Amplicons of *qnr*S gene detected in lane 5 (*S.Magerhafelt*) at 417 bp )

( No amplicons of *bla*CMY-2 detected in any of Salmonella isolates tested at 462 bp )

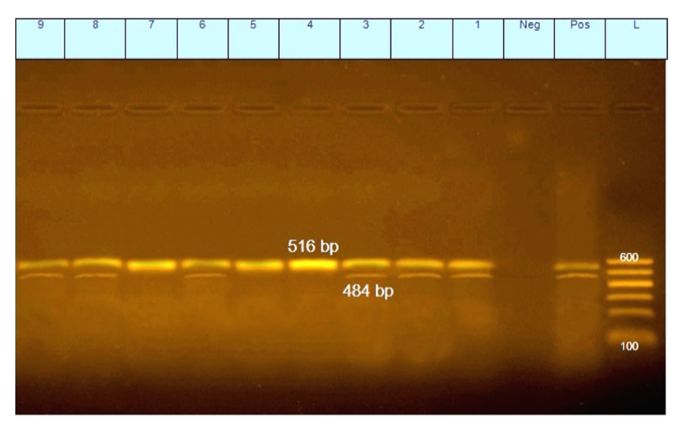
****

Figure (2): Agarose gel electrophoresis for amplified PCR product of the β-Lactamase gene (*bla*TEM) and of the Aminoglycoside resistance gene (*aad*A1) in different Salmonella isolates

L ( DNA ladder 100 - 600 bp) ,

Neg (Negative control ) ,Pos ( positive control) , Positive and negative controls were recovered from previously tested and confirmed field isolates (Tested in the Reference laboratory for veterinary quality control on poultry production), Lane (1- 9 ) Salmonella isolates from chicken : Lane 1,2, 8 ( *S.Infantis*) ,Lane 3 (*S.Ratchaburi* ) , Lane 4 (*S.Sinchew*) , Lane 5 (*S.Magerhafelt*), Lane 6, (*S.Stanleyville*) , Lane 7,9 (*S.Typhimurum* )

(Amplicons of *bla*TEM gene detected in lane 1, 2, 3, 4, 5, 6, 7,8 and 9 at 516 bp.) (Amplicons of *aad*A1 gene detected in lane 1, 2, 3, 6, 8 and 9 at 484 bp)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| I:\ \Research for people\Salm aada1, others, staph femA, antibiotic Maryem Tanta\tetA(A), floR.jpg9 | 8 | 7 | 6 | Pos | L | 5 | 4 | 3 | 2 | 1 | Neg |

600

100

300

100

576 bp

1500

300

100

494 bp

1500

300

100

Figure (3): Agarose gel electrophoresis for amplified PCR product of tetracycline resistance gene (*tet*A) and chlorompenicol resistance gene ( *flo*R*)* in different Salmonella isolates.

L ( DNA ladder 100 - 600 bp)

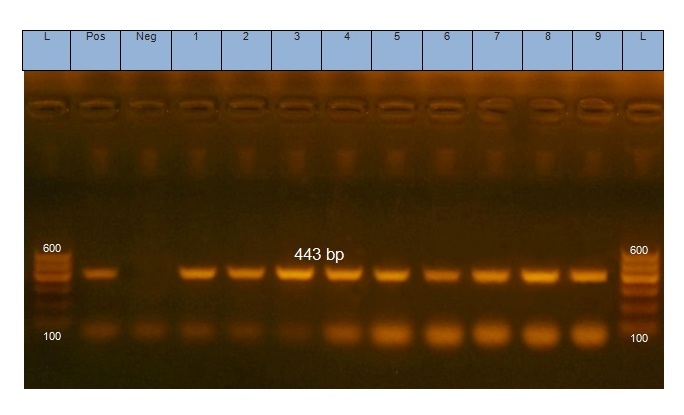
Pos ( positive control) , Neg (Negative control ) Positive and negative controls were recovered from previously tested and confirmed field isolates (Tested in the Reference laboratory for veterinary quality control on poultry production)

Lane (1- 9) Salmonella isolates from chicken : Lane 1,2, 8 ( *S.Infantis*) ,

Lane 3 (*S.Ratchaburi*) , Lane 4 (*S.Sinchew*) , Lane 5 (*S.Magerhafelt*), Lane 6 (*S.Stanleyville*) ,Lane 7,9 (*S.Typhimurum* )

( Amplicons of *tet*A gene detected in lane 1 , 2 , 3 , 4 , 5 , 8 , 9 at 576 bp )

( Amplicons of *floR* gene detected in lane 1,2 , 3,4,5,6,7,8, and 9 at 494 bp)

****

Figure(4) : Agarose gel electrophoresis for amplified PCR product of sulphonamide resistance gene (*sul* 1) in different salmonella isolates

L ( DNA ladder 100 - 600 bp)

Neg (Negative control), Pos( positive control) Positive and negative controls were recovered from previously tested and confirmed field isolates (Tested in the Reference laboratory for veterinary quality control on poultry production), Lane (1- 9 ) Salmonella isolates from chicken : Lane 1, 2, 8 ( *S.Infantis*) ,

Lane 3 (*S.Ratchaburi* ) , Lane 4 (*S.Sinchew*) , Lane 5 (*S.Magerhafelt*),

Lane 6 (*S.Stanleyville*) ,Lane 7,9 (*S.Typhimurum* )

(Amplicons of *sul*1 gene detected in lane 1, 2, 3, 4, 5, 6,7,8 and 9 at 443 bp).

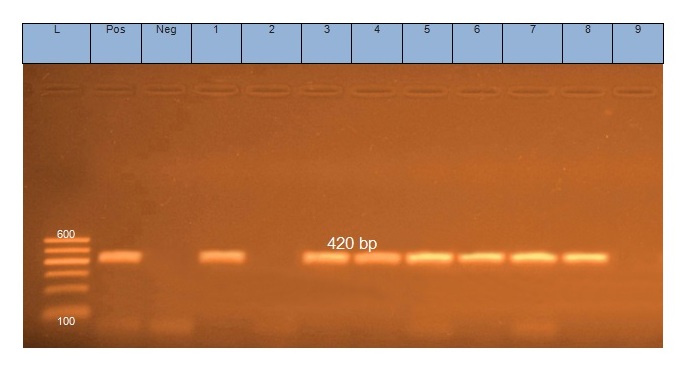
**494 b **

Figure (5) : Agarose gel electrophoresis for amplified PCR product of Macrolide resistace gene (*ere*A) in different Salmonella isolates.

L ( DNA ladder 100 - 600 bp)

Neg (Negative control) , Pos ( positive control) Positive and negative controls were recovered from previously tested and confirmed field isolates (Tested in the Reference laboratory for veterinary quality control on poultry production), Lane (1- 9 ) Salmonella isolates from chicken :Lane 1,2, 8 ( *S.Infantis*) ,

Lane 3(*S.Ratchaburi* ) ,Lane 4 (*S.Sinchew*) , Lane 5 (*S.Magerhafelt*),

Lane 6, (*S.Stanleyville*) , Lane 7,9 (*S.Typhimurum* )

( Amplicons of *ere*A gene detected in lane 1, 3, 4, 5, 6, 7 and 8 at 420 bp).

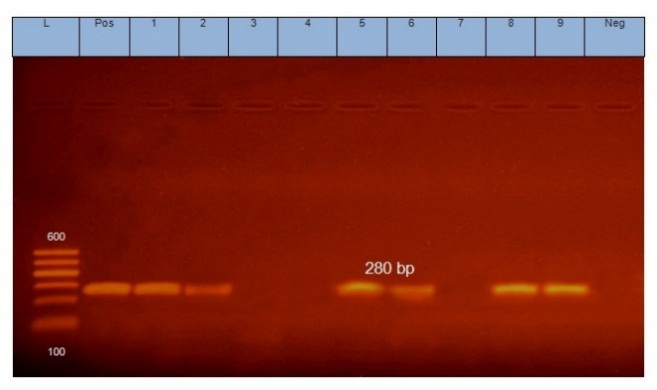


Figure (6) : Agarose gel electrophoresis for amplified PCR product of Integron gene cassette (int1) in different Salmonella isolates.

L( DNA ladder 100 - 600 bp)

Neg (Negative control ) , Pos ( positive control), Positive and negative controls were recovered from previously tested and confirmed field isolates (Tested in the Reference laboratory for veterinary quality control on poultry production), Lane (1- 9) Salmonella isolates from chicken : Lane 1,2,8 ( *S.Infantis*) ,

Lane3 (*S.Ratchaburi* ) , Lane 4 (*S.Sinchew*) , Lane 5 (*S.Magerhafelt*),

Lane 6 (*S.Stanleyville*) ,Lane 7,9 (*S.Typhimurum* )

(Amplicons of int1 gene detected in lane 1, 2, 5, 6,8 and 9 at 280 bp).

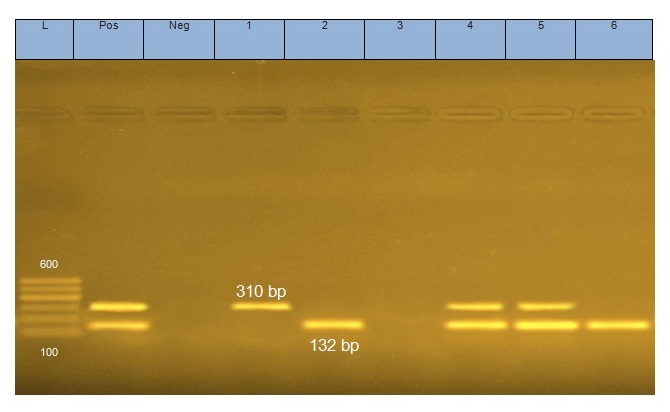


Figure ( 7 ) : Agarose gel electrophoresis for amplified PCR product of Methicillin resistance gene (*mec*A) and (*fem*A) in different *S.aureus* isolates

L ( DNA ladder 100 - 600 bp)

Pos ( positive control : reference strain deposited to gene bank with MRSA ATCC 43300 , Neg (Negative control: negative controls were recovered from previously tested and confirmed field isolates -Tested in the Reference laboratory for veterinary quality control on poultry production)

Lane (1- 6 ) *S.aureus* isolate.

( Amplicons of *mec*A gene detected in lane 1, 4 and 5 at 310bp) ,

( Amplicons of *fem*A gene detected in lane 2, 4, 5 and 6 at 132bp)