Research Article

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# Seroprevelance and Antibiotic Resistance of *Salmonella spp*. Isolated From Pigeons and Their Environment in Nineveh Governorate

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**Abstract**

This study was conducted to explore the antibody titers and antibiotic sensitivity of salmonella organisms isolated from pigeons and their surrounding in Nineveh governorate during the period from November 2021 to March 2022. Thirty out of 83 positive *Salmonella spp.* isolated from 400 pigeons and 150 samples of their environment in a previous study (unpublished data) were subjected to an antibiogram sensitivity test. Nearly half of the isolates (n=16) were tested for ß- lactamase production. Seroprevalence of 92 blood samples collected from the same pigeons (69/adults and 23/squabs) were used for the estimation of S. enteritides antibody titers. The antibiogram profile of tested Salmonella spp. revealed high resistance (73.33%) to Ciprofloxacin followed by Colistin (70%), Florphenicol (66.6%), Sulphamethoxazole-Trimethoprim (60%), Lincomycin and Amoxicillin (46.6), Spiramycin and Doxycycline (40%), Tetracycline (36.66%), Levofloxacin and Enrofloxacin (33.33%), Phosphomycin (26.6%), Norfloxacin (23.3%), Cephalexin, Neomycin and Gentamycin 16.66%. Beta-lactmase producing Salmonella spp. revealed that 3 isolates out of 13(18.75%) were positive by acidimetric, 7/16(43.75%) using extended spectrum ß -lactmase and 6/16(37.5%) in extended spectrum ß -lactmase type ampC methods. Antibody titers to S. enteritides naturally exposed pigeons obtained by Indirect ELISA revealed a significant (P≤0.ooo1) difference between adults and squabs mean titers. In conclusion, pigeons are considered one of the Salmonella spp. sources that may aid in the cross-contamination of Salmonella spp. from pigeons to the surroundings, humans or animals.

**Keywards:** Pigeons, Salmonella spp., Antimicrobial resistance, ß – Lactamase.

## مستوى الاضداد وحساسية جراثيم السالمونيلا للمضادات الحيوية للحمام في محافظة نينوى

**الخلاصة**

اجريت هذه الدراسة لمعرفة الحساسية لمختلف المضادات الحيوية ومستوى الاضداد لجراثيم السالمونيلا المعزولة من الحمام ومحيطه في محافظة نينوى خلال الفترة من تشرين الثاني 2021 إلى آذار 2022. ثلاثون من مجموع 83 عينة موجبة لجراثيم السالمونيلاتم تم عزلها من 186طائر (400)عينة و150 عينة من بيئة الطيورتم إخضاعها لاختبار حساسية المضادات الحيوية. كما وتم اختبار 16عزلة في قابليتها لإنتاج إنزيم بيتا لاكتاماز لتقدير مستوى الاضداد لجراثيم السالمونيلا. تم فحص 92 عينة لمصل الطيورذاتها التي جُمعت منها العينات ( 69 / حمام بالغًا و 23 / صغار الحمام ) لتقدير معيار الاضداد باستخدام تقنية الاليزا وعدة *S. enteritides*. أظهرت جراثيم السالمونيلا مقاومة عالية للسيبروفلوكساسين (73.33٪) يليها كوليستين (70٪)، فلورفينيكول (66.6٪)، سلفاميثوكسازول-تريميثوبريم (60٪) ، لينكومايسين وأموكسيسيلين (46.6 ٪) ، سبيرامايسين ودوكسيسايكلين (40٪) ، تتراسيكلين (36.66٪)، ليفوفلوكساسين وإنروفلوكساسين (33.33٪)، فوسفوميسين (26.6٪)، نورفلوكساسين (23.3٪)، سيفاليكسين، نيومايسين وجنتاميسين 16.66٪. اما نتائج عزلات جراثيم السالمونيلا المنتجة للبيتا لاكتاماز فقد اتضح ان 3 عزلات من مجموع 16 (18.75٪) كانت موجبة بواسطة القياس الحمضي ، 16/7 (43.75٪) باستخدام الطيف الممتد ß -lactmase و 16/6 (37.5٪) في الطيف الممتد ß -lactmase من النوع *ampC* , كشف معيار الاضداد لـ *S. enteritides* في الحمام باستخدام ELISA غير المباشر عن وجود فرق معنوي كبير (P≤0.ooo1) بين صغار الحمام و البالغين. في الختام ، يعتبر الحمام أحد مصادر *Salmonella spp.* التي قد تساهم في انتقال هذه الجراثيم من الحمام إلى البشر وبقية الحيوانات الأخرى.

## Introduction

Iraq country is one of the pioneers in the region in rearing fancy pigeons with multipurpose raising both domestic and feral pigeons in different areas of Nineveh/Iraq. They provide a source of meat, and financial source for families. Since pigeons may harbor different microorganisms, including *enterobactericeae,* so may be hazardous to their breeders, in the markets of birds or even in the farms of breeding different types of birds (1**).** The infection of pigeons with *Salmonella spp*. has been reported in Nineveh governorate (2**).** Salmonella infection may be noticed in diseased or even apparently healthy pigeons in houses or markets (3,4). since diverse sources related to infection by *Salmonella spp*. in pigeons including their parents, during incubation, rearing, in addition to the cross-contamination from feed, water, feces, cracked eggs or dead embryos and handlers (5).

Many authors referred to *Salmonella enteritidis* and *Salmonella typhimurium* as the frequently isolates from cases of Salmonellosis in pigeons (6, 7, 8, 9, and 10). The indiscriminate use of antibiotics in the treatments of human and animal diseases could emerge new antibiotic resistant strains of Salmonella in pigeons (11). There is an increase reports about the multi- drug resistant of salmonella isolates in poultry and their meat or egg products, making zoonosis and public health issue of great importance locally and globally (12). Nowadays, veterinarians or even human clinical physicians faces some difficulties in the treatment or in the control of some infectious diseases due to the increase of antibiotic resistance, posing a global public health problem (13, 14). Through different ways zoonotic agents could enter their hosts, of most important the food chain (12, 15). Making Pigeons as one of the important source of salmonellosis especially in younger peoples (16, 17, 18, and 19). No report about antibody titers against natural exposure to salmonella in pigeons was published in Mosul governorate, This study was, therefore, designed to different antibiotic resistance of *Salmonella spp.* isolated from pigeons raised in households and their surroundings in and around Nineveh governorate, as well as the estimation of antibody titers in the serum of these pigeons that were naturally exposed to Salmonella organisms.

## Materials and methods:

### Antibiotic sensitivity assay:

A total of 30 salmonella isolates from adult pigeons (n=10), squabs (n=10) and their environment (n=10), were subjected to16 antibiotics the Kirby–Bauer disk diffusion procedures (20). The already incubated salmonella inoculums were optimized to the 0.5 McFarland standard, streaked on Mueller-Hinton agar plates left for 15 min at room temperature to dry. Afterward, standard antibiotic disks, were placed with sterile forceps on MHA plates and incubated aerobically at 37°C for 24 h(3;21). Following the incubation, the organisms were categorized as “resistant”, “intermediate” and “susceptible” according to the CLSI guidelines (22**).** The antibiotics chosen for this study include the following compounds: Amoxicillin (AX 25

µg); Cephalexin (CL.30 µg); Colistin (CT 10 µg); Norfloxacin (NOR 10 µg); Ciprofloxacin (CIP, 10 µg); Levofloxacin(LEV,5 µg); Enrofloxacin (ENR 5 µg); Doxycycline (DO 10 µg); Tetracycline (TE, 30 µg); Gentamycin (CN, 30 µg); Neomycin(N,30 µg); Florphenicol (FFC,30 µg); Phosphomycin (FF10 µg); Lincomycin (L 10 µg); Spiramycin (SP, 30 µg); and sulphamethaxazole-Trimethoprim (SXT,25 µg).
The prevalence of resistance against specific antimicrobials in this trail was classified as either high, medium or low (>50%, 21-50% and 0-20%) respectively.

**Beta- Lactamase producing salmonella isolates:**

Sixteen salmonella isolates (five from each of adult pigeon, squabs, and environment including one sample from attenders hands), were tested for ß -Lactamase producing salmonella isolates using the following methods (23,24).:

**Acidimetric method:**

The reagents for this method were prepared by adding 1.2 gm penicillin G to tubes containing 2 ml (0.5%) of phenol red solution adjusted to pH (8.5). These tubes were inoculated with 0.1 ml of Salmonella spp. broth to give dense bacterial suspensions and left to stabilize at room temperature. Color change from violet to yellow is considered positive result (25).

**Disc spread method :**

Testing was performed on Muller-Hinton Agar, a general environment of the recommendation made by CLSI. Zone diameters were measured by an electronic calibrator after 24 hours of incubation at 37 °C(22).

**Extended Spectrum Beta-lactamase (ESBL):**

Five beta-lactam antibiotics, ceftiaxone (KF30µg), Aztreonam (ATM30µg), and Ceftazidim (CAZ30µg), and cefotaxime (ctx25µg) and cefoxopdime( 30 µg) were used on Muller Hinton agar already swabbed with *salmonella spp*., left for 15 minutes and then incubated for 24 hours at 37 ° (26,22).

**Extended Spectrum Beta-lactamase type *ampC* (ESBL *ampC*):**

Test phenotype of Cefoxitin sensitivity by Kirby-Bauer disc diffusion method:
Cefoxitin (30 µg) was used as a screening test for β-lactamase AmpC producing salmonella isolates using CLSI criteria. Positive phenotype isolates to cefoxitin (<18 mm diameter), was considered as potential *ampC* producers.

**Antibody titers against naturally exposed pigeons to *salmonella spp*.:**

Blood samples from 69 adult pigeons and 23 squabs were collected for estimation of antibodies against naturally exposed pigeons to *salmonella spp*. using *S.enteritides* kit (IDEXX), during the period from November 2021 to March 2022. Samples were gathered from three different districts in Nineveh governorate including 34 locations. Blood samples were left for 30-45 minutes at room temperature for serum collection in gel containing tubes. Separated serum was then collected in Eppendorf tubes and stored at -20°C until analyzing. Indirect enzyme-linked immunosorbent assay (iELISA) IDEXX (USA) was used to estimate the antibody titers according to the manufacturer's instructions using *S.enteritides* coated plates, pre-diluted, and ready-to-use reagents. In the case of iELISA, the antibody criterion was calculated from the optical density (OD) value of the serum dilution 1:500 using software program supported with the kit (27).

**Statistical analysis:**

In this study, chi-square was used to examine the difference between ELISA positive and negative antibody titers in adult pigeons and squabs, (28). The difference was considered significant when P-value was ≤ 0.05. The analysis was performed using the MedCalc.org (29).

## Results and Discussion

**Antibiotic sensitivity:**

Regarding the results of antibiotic sensitivity to *Salmonella spp*. isolated from adult pigeons (n=10) are represented in ( Table 1) and were as follows: highly resistant antibiotics (≥50%) were recorded with Florphenicol and Sulphamethoxazole-Trimethoprim (80%), Ciprofloxacin and Colistin (70%), Amoxicillin (50%); medium resistance (21-50%)were recrded to Enrofloxacin, Doxycycline, Tetracycline, Lincomycin and Spiramycin (40%), Levofloxacin (30%), Cephalexin, Norfloxacin, Gentamycin and Phosphomycin; (20%); while low resistance (0-20%) was noticed only with Neomycin (10%).

*Salmonella spp*. isolated from squabs (n=10) were highly resistant (≥50%) to Ciprofloxacin and Sulphamethoxazole-Trimethoprim (70%), Colistin (60%); medium resistance (21-50%), was shown to Amoxicillin, Levofloxacin, Doxycycline Florphenicol, and lincomycin (40%), Enrofloxacin and Phosphomycin (30%); while low resistance (0-20%) was recorded with Norfloxacin, Tetracycline, Spiramycin, Neomycin and Gentamycin (20%) and Cephalexin (10%) (Table 2).

Environment isolates (n=10), express high resistance (≥50%) to Colistin, Ciprofloxacin and Florphenicol (80%), lincomycin and spiramycin (60%); medium resistance (21-50%) was reported with Amoxicillin and tetracycline (50%); medium resistance (21-50%) was noticed with doxycycline, Norfloxacin, Levofloxacin, Enrofloxacin, Sulphamethoxazole-Trimethoprim and phosphomycin (30%); while low resistance (0-20%) was shown with Cephalexin, neomycin (20%); and Gentamycin (10%).(Table 3).

The overall Antibiogram profile of 30 salmonella isolates to 16 antibiotics is illustrated in (Figure 1). From table it is revealed that high resistance (73.33%) was reported to Ciprofloxacin followed by Colistin (70%), Flophenicol (66.6%), Sulphamethoxazole-Trimethoprim (60%), Lincomycin and Amoxicillin (46.6), Spiramycin and Doxycycline (40%), Tetracycline (36.66%), Levofloxacin and Enrofloxacin (33.33%), Phosphomycin (26.6%), Norfloxacin (23.3%), Cephalexin, Neomycin and Gentamycin 16.66%....

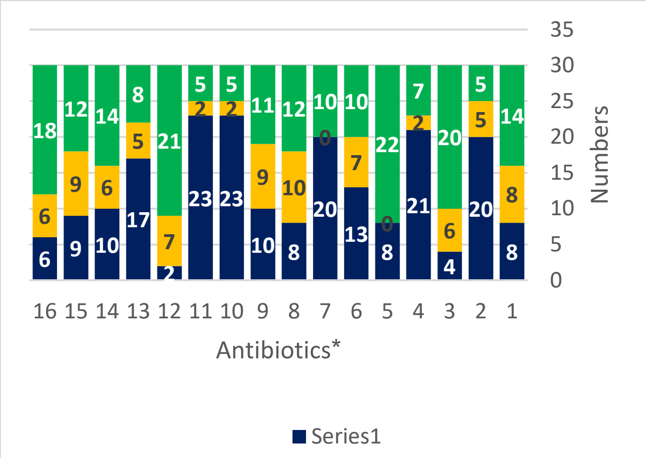


Figure 1: Number of sensitive, intermediate and resistant of 30 tested *salmonella spp* to different antibiotics. *1=Amoxicillin, 2= Cephalexin, 3= Colistin, 4= Norfloxacin, 5= Ciprofloxacin, 6= Levofloxacin, 7= Enrofloxacin, 8= Doxycycline, 9= Tetracycline, 10= Gentamycin, 11= Neomycin, 12= Florphenicol, 13= Phosphomycin, 14= Lincomycin, 15= Spiramycin, 16= Sulphamethoxazole - Trimethoprim

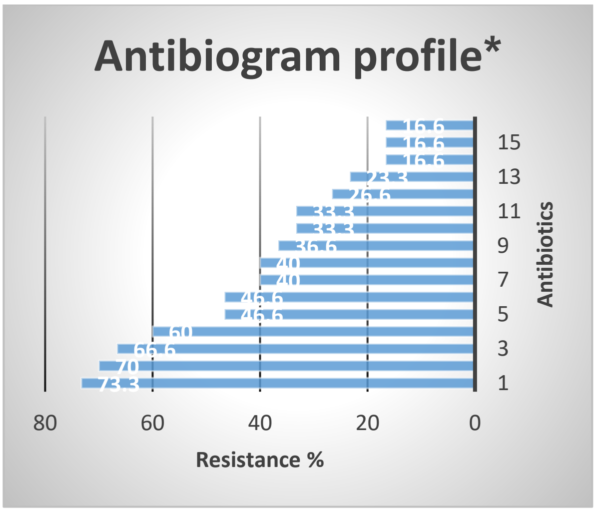


Figure 2: Summary of 30 tested *Salmonella spp.* resistance to antibiotics.

*1= Ciprofloxacin; 2= Colistin; 3= Florphenicol; 4= Sulphamethoxazole -Trimethoprim; 5= Lincomycin; 6= Amoxicillin; 7= Spiramycin; 8= Doxycycline; 9= Tetracycline; 10= Levofloxacin; 11= Enrofloxacin; 12= Phosphomycin; 13= Norfloxacin; 14= Cephalexin; 15= Neomycin; 16= Gentamycin

**Beta-lactamase producing Salmonella isolates:** The prevalence of positive ß -lactamase producing salmonella isolates using acidimetric method were only 3 out of 16 (18.75%) as shown in (table 4)

The result of positive Extended spectrum ß -lactamases salmonella producers using disk diffusion method revealed that more samples were positive, 7/16 (43.75%) as shown (Table 5 and Figures 2).

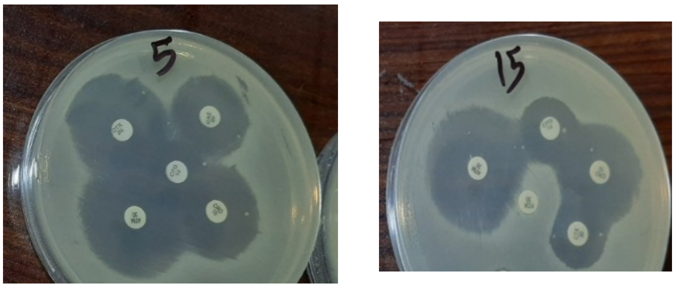


Figure (3): Positive result (plate labeled 15) of ß -lactamases salmonella isolate producer and negative producer isolate (plate labeled 5).

Extended spectrum ß -lactamases type AmpC (ESBL/AmpC) result as expressed in (Table 7), and (Figures 3), revealed that 6/10 (37.50%) Salmonella samples were positive for extended spectrum ß -lactamases type AmpC (ESBL/*ampC*) (table 7, Figure 4).

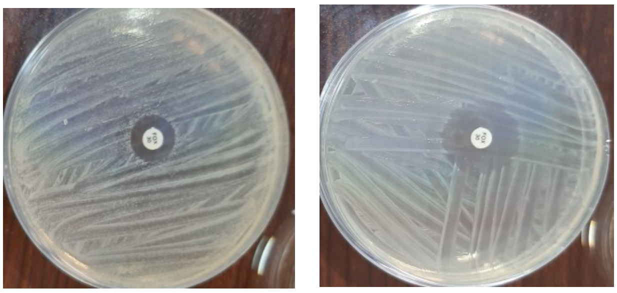

Figure (4): Positive result of ß -lactamases salmonella isolate type *apmC* (A) (<18 mm diameter), and negative result (B) (>18 mm diameter),

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**Antibody titers against naturally exposed pigeons to *salmonella spp.***

Five out of 23 (21.73%) squabs' serum samples were positive to *S. enteritides* using iELISA (IDEXX, USA). The mean Positive squabs titer to *S. enteritides* ELISA (681) was significantly ($p \leq 0.0001$) higher than the negatives (251.5) (Table 8 and Figure 5). In the mean while 8 out of 69 adult pigeons (11.59%) had positive titers in comparison to the negatives 61/69 (88.40%). The mean Positive titer in adult pigeons to S. *enteritides* (793.62) was significantly ($p \leq 0.0001$) higher than the negatives (202.65) (Table 7 and Figure5, 6).

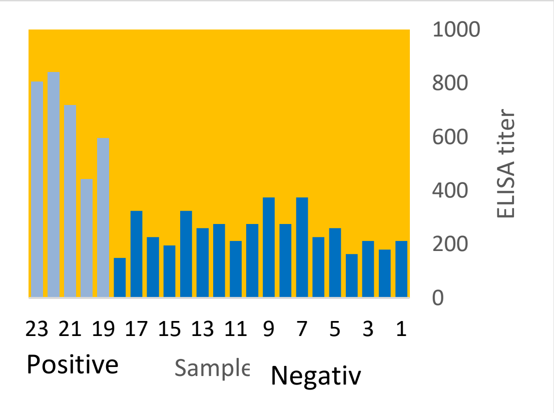


Figure (5) : Mean *S. enteritides* titers of squabs (< 6 months) using iELISA technique.

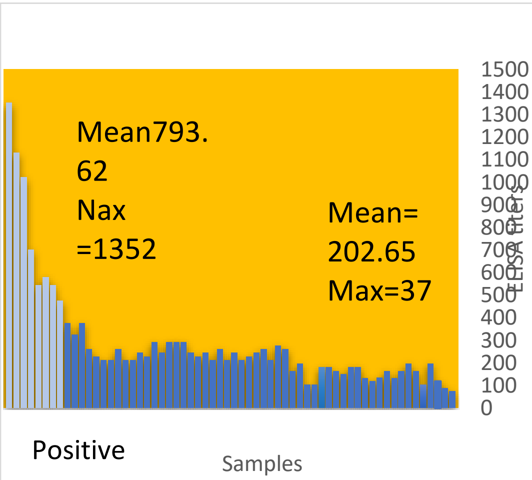


Figure (6): Mean S. *enteritides* titers of Adult pigeons (> 6 months) using iELISA technique.

In Nineveh governorate domestic pigeons are usually raised for many purposes like racing (among hobby peoples), exhibition (profit can be high), and nutritional.

Pigeons may transmit many different disease agents especially salmonella organisms to other birds and their handlers, as they are mostly reared in the same house at their owners, or through trading in the live-bird markets. A study on pigeon infection with Salmonella in Nineveh governorate was previously conducted by (2). In our previous study (unpublished data), the prevalence of *Salmonella spp.* in Nineveh governorate was found to be 13% in pigeons and 20% in their surroundings and handlers. A similar study of Salmonella in pigeon was conducted in Egypt with 5%, 3.5%, and 4.6% prevalence in squabs, pigeon, and environmental samples respectively (3). A higher prevalence was reported by ( ) who found a rate of 22.22%, 58.33%, and 27.50% of Salmonella isolates from cloacal swabs, footpads, and the feces respectively. In our study, the higher percentage of salmonella isolated from pigeons and their surrounding could be attributed to the density of pigeons reared in the boxes with their feeders and waters and to feed and water contamination from the bird's droppings.

Antibiogram profiling of *Salmonella spp.* isolated from pigeons and their surroundings to sixteen different antibiotics using the disk diffusion method were with different percentages of resistant (21). (Tables 1, 2 &3) to isolates from squabs, adult pigeons and their environment. The development of *Salmonella spp.* resistance to different antibiotics could be traced to cross-resistance and co-selection in a complex manner (30).

The indiscriminate and excessive use in clinical treatments of antibiotics helps to large extent the possible emergence of resistance problem as a new serious public health issue (31). It could be said that the circle of salmonella transmission between human-pigeon-environment may play a role in the spread of *Salmonella spp*., increasing by this the cost of health care, and making Salmonella of primary importance and so guide to promote research for the development of new antibiotics for Salmonella treatment (32, 33, and 34).

Moreover, antibiotic resistance by *Salmonella spp.* isolates can be achieved by mutations of exogenous resistance genes that can be disseminated horizontally between bacteria (35, 36). Several mechanisms are considered in antimicrobial resistance of *Salmonella spp*., including drug inactivation (37). Salmonella has also developed a mobile elements system of efflux pumps to streptomycin, spectinomycin, sulfonamides, chloramphenicol, florfenicol, tetracyclines, and ß - lactam antibiotics (37; 38), in addition to enzymes able to acetylate the ß-lactam ring of penicillin and some cephalosporins and chloramphenicol (33,39,40,41).

Although there is a negligible reports about the estimation of humeral antibody titers against *Salmonella spp.* in pigeons, so we try to compare our results of antibody titers of unvaccinated squabs and adult pigeons with *S. Enteritidis* vaccine 663.26 and 739.41 respectively(Figure 5&6), with the mean titers of unvaccinated control layer group of 776.1 1065.1 and 897.2 through their $1^{st}$, 2nd and $3^{rd}$ weeks respectively in experiment aimed to estimate antibody titers of vaccinated layers with *S. Enteritidis* vaccine (42). The humeral antibody titers found in the unvaccinated and unchallenged squabs and adult pigeons, reflect the natural exposure of these birds to *Salmonella spp* (43).

In conclusion it could be said that pigeons breeding in farmers houses could harbor resistant *Salmonella spp*. that may be of great threat to human and other animal's health. (44).

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Table 1: Percentage of "susceptible", "intermediate" and "resistant" *Salmonella spp.* isolated from adult pigeons.

| Class of antibiotics | Antibiotic | Abbreviation | Potency | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|---|
| B-Lactam antibiotics<br>Penicillins | Amoxicillin | AX | 25 µg | 2/10(20%) | 3/10(30) | 5/10(50%) |
| Cephalosporins | Cephalexin | CL | 30 µg | 7/10(70%) | 1/10 (10%) | 2/10(20%) |
| Peptide Antibiotic (Polymyxin) | Colistin | CT | 10 µg | 1/10(10%) | 2/10(205) | 6/10(70%) |
| Fluoroquinolones | | | | | | |
| 2nd generation | Norfloxacin | NOR | 10 µg | 8/10(80%) | 0/10 (0%) | 2/10(20%) |
| 3rd generation | Ciprofloxacin | CIP | 10 µg | 3/10(30%) | 0/10(0%) | 7/10(70%) |
| 4th generation | Levofloxacin | LEV | 5 µg | 4/10(40%) | 3/10(30%) | 3/10(30%) |
| | Enrofloxacin | ENR | 10 µg | 6/10(60%) | 0/10(0%) | 4/10(40%) |
| Tetracycline | | | | | | |
| Long acting | Doxycycline | DO | 10 µg | 2/10(20%) | 4/10(40%) | 4/10(40%) |
| Short acting | Tetracycline | TET | 30 µg | 3/10(30%) | 3/10(30%) | 4/10(40%) |
| Aminoglycosides | Gentamycin | CN | 30 µg | 7/10(70%) | 1/10(1%) | 2/10(20%) |
| | Neomycin | N | 30 µg | 7/10(70%) | 2/10(20%) | 1/10(10%) |
| Amphiplenicols | Florphenicol | FFC | 30 µg | 0/10(0%) | 2/10(20%) | 8/10(80%) |
| Phosphonic acid | Phosphomycin | FF | 10 µg | 6/10(60%) | 2/10(20%) | 2/10(20%) |
| Lincosamides | Lincomycin | L | 10 µg | 4/10(40%) | 2/10(20%) | 4/10(40%) |
| Macrolides | Spiramycin | SP | 30 µg | 4/10(40%) | 2/10(20%) | 4/10(40%) |
| Sulphonamides Intermediate acting and Trimethoprim | Sulphamethoxazole-Trimethoprim | SXT | 25 µg | 1/10(10%) | 1/10(1%) | 8/10(80%) |

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Table 2: Percentage of "susceptible", "intermediate" and "resistant" *Salmonella spp.* isolated from squabs.

| Class of antibiotics | Antibiotic | Abbreviation | Potency | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|---|
| B-Lactam antibiotics<br>Penicillins | Amoxicillin | AX | 25 µg | 3/10(30%) | 3/10(30%) | 4/10(40%) |
| Cephalosporins | Cephalexin | CL | 30 µg | 7/10(70%) | 2/10(20%) | 1/10(10%) |
| Peptide Antibiotic<br>(Polymyxin) | Colistin | CT | 10 µg | 2/10(20%) | 2/10(20%) | 6/10(60%) |
| Fluoroquinolones<br>2nd generation<br>3rd generation<br>4th generation | Norfloxacin<br>Ciprofloxacin<br>Levofloxacin<br>Enrofloxacin | NOR<br>CIP<br>LEV<br>ENR | 10 µg<br>10 µg<br>5 µg<br>10 µg | 7/10(70%)<br>3/10(30%)<br>5/10(50%)<br>7/10(70%) | 1/10(1%)<br>0/10(0%)<br>1/10(1%)<br>0/10(0%) | 2/10(20%)<br>7/10(70%)<br>4/10(40%)<br>3/10(30%) |
| Tetracycline<br>Long acting<br>Short acting | Doxycycline<br>Tetracycline | DO<br>TET | 10 µg<br>30 µg | 3/10(30%)<br>4/10(40%) | 3/10(30%)<br>4/10(40%) | 4/10(40%)<br>2/10(20%) |
| Aminoglycosi-des | Gentamycin<br>Neomycin | CN<br>N | 30 µg<br>30 µg | 7/10(70%)<br>8/10(80%) | 1/10(10%)<br>0/10(0%) | 2/10(20%)<br>2/10(20%) |
| Amphiplenic-ols | Florphenicol | FFC | 30 µg | 2/10(20%) | 3/10(30%) | 5/10(50%) |
| Phosphonic acid | Phosphomycin | FF | 10 µg | 5/10(50%) | 2/10(20%) | 3/10(30%) |
| Lincosamides | Lincomycin | L | 10 µg | 4/10(40%) | 2/10(20%) | 4/10(40%) |
| Macrolides | Spiramycin | SP | 30 µg | 3/10(30%) | 5/10(50%) | 2/10(20%) |
| Sulphonamid-es<br>Intermediate acting and<br>Trimethopri-m | Sulphamethoxazole-Trimethoprim | SXT | 25 µg | 1/10(10%) | 2/10(20%) | 7/10(70%) |

Table 3: Percentage of “susceptible”, “intermediate” and “resistant” *Salmonella spp.* isolated from environment.

| Class of antibiotics | Antibiotic | Abbreviation | Potency | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|---|
| B-Lactam antibiotics Penicillins | Amoxicillin | AX | 25 µg | 3/10(30%) | 2/10(30%) | 5/10(50%) |
| Cephalosporins | Cephalexin | CL | 30 µg | 6/10(60%) | 2/10(30%) | 2/10(20%) |
| Peptide Antibiotic (Polymyxin) | Colistin | CT | 10 µg | 1/10(10%) | 2/10(10%) | 8/10(80%) |
| Fluoroquinolones | | | | | | |
| 2nd generation | Norfloxacin | NOR | 10 µg | 6/10(60%) | 1/10(10%) | 3/10(30%) |
| 3rd generation | Ciprofloxacin | CIP | 10 µg | 2/10(30%) | 0/10(0%) | 8/10(80%) |
| 4th generation | Levofloxacin | LEV | 5 µg | 4/10(40%) | 3/10(30%) | 3/10(30%) |
| | Enrofloxacin | ENR | 10 µg | 7/10(70%) | 0/10(0%) | 3/10(30%) |
| Tetracycline | | | | | | |
| Long acting | Doxycycline | DO | 10 µg | 3/10(30%) | 3/10(30%) | 4/10(40%) |
| Short acting | Tetracycline | TET | 30 µg | 3/10(30%) | 2/10(30%) | 5/10(50%) |
| Aminoglycosides | Gentamycin | CN | 30 µg | 9/10(90%) | 0/10(0%) | 1/10(10%) |
| | Neomycin | N | 30 µg | 8/10(80%) | 0/10(0%) | 2/10(20%) |
| Amphiplenicols | Florphenicol | FFC | 30 µg | 0/1 0(0%) | 2/10(30%) | 8/10(80%) |
| Phosphonic acid | Phosphomycin | FF | 10 µg | 6/10(60%) | 1/10(10%) | 3/10(30%) |
| Lincosamides | Lincomycin | L | 10 µg | 2/10(30%) | 2/10(30%) | 6/10(60%) |
| Macrolides | Spiramycin | SP | 30 µg | 2/10(30%) | 2/10(30%) | 6/10(60%) |
| Sulphonamides Intermediate acting and Trimethoprim | Sulphamethoxazole-Trimethoprim | SXT | 25 µg | 4/10(40%) | 3 /10(30%) | 3/10(30%) |

Table (4) percentages of ß -lactamases salmonella isolates producers by acidimetric method.

| Total Number of isolates | Number of negative producers of ß -lactamases | Number of positive producers of ß -lactamases |
|---|---|---|
| 16 | 13(81.25% ) | 3(18.75%) |

Table (5) percentages of ß -lactamases salmonella isolates producers by using disk diffusion method

| Total Number of isolates | Number of negative producers of the extended spectrum ß -lactamases | Number of positive producers of the extended spectrum ß -lactamases |
|---|---|---|
| 16 | 9(56.25% ) | 7(43.75) |

Table (6) Percentages of salmonella isolates producing the extended spectrum ß -lactamases type ApmC by disk diffusion method

| Total Number of isolates | Number of positive producers of the extended spectrum ß -lactamases type AmpC | Number of negative producers of the extended spectrum ß -lactamases type AmpC |
|---|---|---|
| 16 | 6( 37.5% ) | 10(62.5%) |

Table (7): means of antibody titer to *S. enteritides* of squabs and adult pigeons.

| Total samples | Positive | | ELISA titers | Negative | | ELISA titers |
|---|---|---|---|---|---|---|
| | **No.** | **%** | | **No.** | **%** | |
| Squabs < 6 months | | | | | | |
| **23** | **5** | **21.73** | ***681** | **18** | **78.26** | 251.5 |
| Adults > 6 months | | | | | | |
| **69** | **8** | **11.59** | ***793.62** | **61** | **88.40** | 202.65 |

## Conclusion

Pigeons, squbs and their environment are considered a resovoir for multi antibiotic resistant Salmonella microorganisims and so regarded as a serious threat of public health importance.

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## Conflict of interest

Authors declared that there is no conflict of interests.

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