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ANTI-BACTERIAL RESISTANCE OF COMMENSAL ESCHERICHIA COLI STRAINS OF MECONIUM ORIGIN IN APPARENTLY HEALTHY CHICKS

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ABSTRACT

Antibiotics are a vital part of modern life through disease prevention and treatment to enhance our quality of life. However, some poultry farmers employ one or more antibiotics, at various birds' ages, as a regular practice for rearing poultry, leading to antimicrobial resistance (AMR). The purpose of the current investigation is to detect the result of unnecessary usage of antibiotics on commensal Escherichia coli strains of meconium origin and integrons Class 1 existence in the isolated strains of *E. coli* obtained from one-day-old chicks from different sources. The results recorded that the isolated strains were multidrug resistant. Antimicrobial resistance profile of the strains that were reported multidrug resistance against Penicillin, Ampicillin, Amoxicillin Clavulanic acid, Cephradine, Cephalexin, and Cephalothin with 100% percentage of resistance, 96.6% for Cefotaxime, 86.6% against Oxillinc acid, Tobramycin, Erythromycin, Ceftriaxone, Cephradine, 83.3% against Doxycycline, and Oxytetracycline, 76.6% against Amoxicillin and Streptomycin, 56.6% against Colistin sulphate, 50% against Trimethoprime-sulfamethoxazole and Norfloxacin showed the lowest resistance percentage 36.6%. In commensal *E. coli* isolated from native chick farms, 100% resistance was reported against Penicillin, Ampicillin, Amoxicillin Clavulinic acid, Cephalexin, Cephalothin, Streptomycin and Streptomycin. In commensal *E. coli* isolated from imported chicks 100% drug resistance was reported against Penicillin, Ampicillin, Amoxicillin clavulinic acid, Cephradine, Cephalexin, and Cephalothin. In commensal *E. coli* isolated from hatcheries, 100% drug resistance was reported against Oxillinc acid, Tobramycin, Penicillin, Ampicillin, Cephradine, Cephalexin and Cephalothin. In nine out of the ten MDR isolates, the integrone gene was found with a percentage of (90%). In order to identify the main risk variables that raise the prevalence of AMR in broilers' cycle of production, more research is required. To sum up, the aim of the study was to reduce the use of antibiotics unless it is necessary to use them in order to minimize multidrug resistance.

Key Words: Commensal Escherichia coli, multidrug resistance, chicks, integron.

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INTRODUCTION

Although Escherichia coli is a typical component of poultry intestinal microbial flora, it may also be a pathogen related to severe illnesses, such as colibacillosis. Furthermore, Escherichia coli may be the origin of resistance genes that can be transferred to humans, creating a hazard to the public's health (Shang *et al*., 2018).

Following the acquisition of virulence genes and pathogenicity enhancement, commensal strains of *E. coli* may be the ancestors of pathogenic strains via random point mutations or acquisition of chromosomal virulence operons (Duriez *et al.*, 2001).

Antimicrobial resistance among bacteria has become a difficulty due to antimicrobials increasingly being used as growth stimulants, preventing agents and medicinal substances in the chicken business over the past few decades (Nhung *et al.,* 2017). The administration of multiple antimicrobials during chicken rearing may cause the spread of antibiotic resistance, and the widespread use of antibiotics in chickens has raised the risk of bacterial resistance, particularly in gram-negative microbes like *E. coli.* (Simoneit *et al.,* 2015). Multiple-drug resistance (MDR) has been linked to selective pressure brought on by the use of antimicrobials in the poultry sector to treat both pathogenic and commensal *E. coli.* (Gyles, 2008). This occurrence is caused not just by bacterial innate ability to survive and reproduce in large numbers, but by horizontal gene transfer via plasmid as well (Apata 2009). Numerous studies have discovered a connection between the use of antibiotics in livestock agriculture and the development of antibiotic resistance in human illnesses (Silbergeld *et al*., 2018).

Integrons have been linked to multidrug resistance in enteric organisms, such as *E. coli*. (Deng *et al.,* 2015).

Integrons are a unique type of recombination system that can capture and express resistance to disinfectants and antimicrobials in gene cassettes (Hall, 2012).

Numerous researches have been conducted in recent years to identify the existence and the type of resistance cassettes, the integrons' structure, and the relationship between the presence of integrons and MDR in pathogenic and commensal *E. coli* isolated from animal and human samples (Kaushik *et al.,* 2018). Both in people and animals, gram-negative bacteria typically carry Class 1 integrons. The majority of integrons in clinical isolates belong to this class also known as clinical integrons (Gillings 2017). In order to ascertain the prevalence of MDR in commensal Escherichia coli bacteria of meconium origin in seemingly healthy chicks, as well as the distribution of class 1 integron-integrase gene (*intI1*) in these isolates, this study was set out to identify these two factors, in addition to examine AMR in commensal *E. coli* in chicks and decrease the antibiotics use in farming by identifying efficient substitute therapies, utilizing more resistant breeds, and enhancing animal welfare.

MATERIALS AND METHODS

Samples

Samples were taken from 30 batches (10 batches from farms, 10 from imported chicks and 10 from hatcheries), each batch has 30 chicks. Chicks were apparently healthy. The samples were taken from meconium and internal organs (liver, heart, and lung). So, the examined samples are 900 samples (300 from each group).

E. coli isolation and identification

According to (Nolan *et al.,* 2020), Escherichia coli was isolated and identified. All collected samples underwent a pre-enrichment step in buffered peptone water (Lab M, UK) and were then incubated aerobically for 24 hours at 37°C. On

MacConkey agar (Neogen, US) and eosin methylene blue agar (Lab M) plates, a loopful of the broth culture was used as an inoculum. The plates were then incubated at 37°C for 24 hours. Utilizing urea, Simmons' citrate agar, peptone water, and oxidase strips from Oxoid in the UK, Kovacs reagent from HiMedia in India, and triple sugar iron agar from Lab M, the isolated colonies were identified morphologically and biochemically.

Testing for antimicrobial sensitivity (AST)

A disk diffusion test was used to investigate all positive isolates. against 22 antibiotics (HiMedia®), which were Oxolinic acid (OA,2 µg), sulfamethoxazole-trimethoprim (SXT, 25µg), tobramycin (TOB, 10 µg), erythromycin (E, 15µg), doxycycline (DOX, 30µg), oxytetracyclin e(OT, 30 µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), Norfloxacin (NX, 10µg), Penicillin (P, 10µg), amoxicillin (AMX,10µg), ampicillin (AMP, 10µg), Amoxicillin- clavulanate (AMC, 30µg), cefotaxime (CTX, 30 µg), ceftriaxone (CTR, 30µg), cephradine (CE, 30µg), cephalexin (CL, 30 µg), cephalothin (KF, 30 µg), streptomycin (S,10 µg), aztreonam (ATM, 30µg), colistin sulfate (CT, 10µg) and fosfomycin (FOS, 200µg). Clinical and Laboratory Standard Institute recommendations were followed after aerobic incubation at 37°C for 18–24 h to determine and interpret the sensitivity of E. coli isolates to various antibiotic drugs. (CLSI 2020).

Molecular assessment

Polymerase chain reaction (PCR) was used to perform additional testing on the 10 chosen *E. coli* isolates to determine whether the class 1 integron was present.

DNA extraction. Adapting the manufacturer's instructions, the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to extract DNA from samples. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K, and then 200 µl of 100% ethanol was added to the lysate after incubation. After that, the sample was cleaned and centrifuged, following the manufacturer's instructions. The kit's 100 µl of elution buffer was used to elute the nucleic acid.

Priming oligonucleotide.

The primers used are indicated in Table (1) and were provided by Metabion (Germany).

PCR amplification.

A 25 µl reaction containing 12.5 µl of DreamTaq Green PCR Master Mix (2X) (Thermo Scientific), 1 µl of each primer at a concentration of 20 pmol, 5.5 µl of DNAas free water, and 5 µl of DNA template was used to test the primers. Thermal cycler 2720 from Applied Biosystems was used to carry out the process.

Examining the PCR products.

The PCR products were separated by electrophoresis on 1% agarose gel in 1x TBE buffer using gradients of 5V/cm (Applichem, Germany, GmbH). For gel analysis, 20 µl of the PCR products were loaded in each gel slot. Generuler 100 bp DNA ladder (Fermentas, Sigma) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1: Target genes, sequences of primers, amplicon sizes and cycling conditions.

Target gene	Sequences of primers	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Int1	CCTCCCGCA CGATGATC TCCACGCAT CGTCAGGC	280	94˚C 5 min	94˚C 30 sec	50˚C 30 sec	72˚C 30 sec	72˚C 7 min	**(Kashif *et al.*, 2013)**

RESULTS

Isolation and identification of *Escherichia coli*

A commensal strain of *E. coli* was examined in 30 batches (each batch contains 30 chicks), so the number of examined chicks was 900. Pooled internal organs (heart, lungs, liver) and meconium were obtained from each batch. Commensal *E. coli* strains were found in all examined meconium samples with a percentage of (100%). The number of final examined samples was 30 samples (10 samples from each group). Each sample represented one batch.

E. coli colonies on Macconkey agar were pink. and violet on violet red bile agar, Eosin methylene blue agar were used for differentiation between pathogenic *E.coli* and commensal *E.coli*. Pathogenic *E.coli* were shin metallic colonies On EMB. Biochemical tests were applied for confirmation.

Antimicrobial Susceptibility of Isolated Strains:

Table 2: Interpretative criteria of antimicrobial drugs of commensal *E.coli* isolated from one-day-old native chicks (farms).

Antimicrobial agent	Interpretative criteria		
	Resistant	intermediate	Sensitive
Oxillinc acid	9/10(90%)	-	1/10(10%)
Trimethoprim-sulfamethoxazole	7/10(70%)	-	3/10(30%)
Tobramycin	8/10(80%)	-	2/10(20%)
Erythromycin	9/10(90%)	1/10(10%)	-
Doxycycline	9/10(90%)	-	1/10(10%)
Oxytetracycline	9/10(90%)	-	1/10(10%)
Ciprofloxacin	6/10(60%)	2/10(20%)	2/10(20%)
Levofloxacin	6/10(60%)	2/10(20%)	2/10(20%)
Norfloxacin	5/10(50%)	2/10(20%)	3/10(30%)
Penicillin	10/10(100%)	-	-
Amoxicillin	9/10(90%)	1/10(10%)	-
Ampicillin	10/10(100%)	-	-
Amoxicillin clavulinic acid	10/10(100%)		
Ceftriaxone	7/10 (70%)	1/10(10%)	2/10(20%)
Cefotaxime	9/10(90%)	-	1/10(10%)
Cephalexin	10/10(100%)	-	-
Cephalothin	10/10(100%)	-	-
Streptomycin	10/10(100%)	-	-
Aztreonam	6/10(60%)	-	4/10(40%)
Colistin sulphate	10/10(100%)	-	-

The isolated 10 Commensal *E.coli* isolates demonstrated multidrug resistance in the native one-day-old chicks. 100% resistance was reported against Penicillin, Ampicillin, Amoxicillin clavulinic acid, Cephalexin, Cephalothin, and Streptomycin. High resistance (90%) was recorded against Oxillinc acid, Erythromycin, Doxycycline, Oxytetracycline, Amoxicillin, and Cefotaxime. More than 50% resistance was shown against used Fluoroquinolones (Ciprofloxacin, Levofloxacin, Ceftriaxone and Aztreonam and 50% against Norfloxacin as displayed in Table (2).

Table 3: Interpretative criteria of antimicrobial drugs of commensal *E.coli* in one-day-old Imported chicks.

Antimicrobial agent	Interpretative criteria		
	Resistant	**intermediate**	**Sensitive**
Oxillinc acid	7/10(70%)	-	3/10(30%)
Trimethoprim-sulfamethoxazole	3/10(30%)	-	7/10(70%)
Tobramycin	7/10(70%)	3/10(30%)	-
Erythromycin	10/10(100%)	-	-
Doxycycline	9/10(90%)	-	1/10(10%)
Oxytetracycline	8/10(80%)	-	2/10(20%)
Ciprofloxacin	2/10(20%)	2/10(20%)	6/10(60%)
Levofloxacin	3/10(30%)	2/10(20%)	5/10(50%)
Norfloxacin	4/10(40%)	2/10(20%)	4/10(40%)
Penicillin	10/10(100%)	-	-
Amoxicillin	6/10(60%)	3/10(30%)	1/10(10%)
Ampicillin	10/10(100%)	-	-
Amoxicillin clavulinic acid	10/10(100%)	-	-
Ceftriaxone	2/10(20%)	2/10(20%)	6/10(60%)
Cefotaxime	6/10(60%)	2/10(20%)	2/10(20%)
Cephradine	10/10(100%)	-	-
Cephalexin	10/10(100%)	-	-
Cephalothin	10/10(100%)	-	-
Streptomycin	5/10(50%)	5/10(50%)	-
Aztreonam	1/10(10%)	3/10(30%)	6/10(60%)
Colistin sulphate	2/10(20%)	1/10(10%)	7/10(70%)

High drug resistance was reported against Penicillin 100%, Ampicillin 100%, Amoxicillin clavulinic acid 100%, Cephradine100%, Cephalexin 100%, and Cephalothin100%. Resistance of more than 50 % was reported against Doxycycline 90%, Oxytetracycline 80%, Amoxicillin 60%, and Cefotaxime 60%. as displayed in Table (3).

Table 4: Interpretative criteria of antimicrobial drugs of commensal *E.coli* in new Hatched chicks.

Antimicrobial agent	Interpretative criteria		
	Resistant	**intermediate**	**Sensitive**
Oxillinc acid	10/10(100%)	-	-
Trimethoprim-sulfamethoxazole	5/10(50%)	-	5/10(50%)
Tobramycin	10/10(100%)	-	-
Erythromycin	7/10(70%)	2/10(20%)	1/10(10%)
Doxycycline	7/10(70%)	2/10(20%)	1/10(10%)
Oxytetracycline	8/10(80%)	-	2/10(20%)
Ciprofloxacin	9/10(90%)	1/10(10%)	-
Levofloxacin	9/10(90%)	1/10(10%)	-
Norfloxacin	1/10(10%)	1/10(10%)	8/10(80%)
Penicillin	10/10(100%)	-	-
Amoxicillin	8/10(80%)	2/10(20%)	-
Ampicillin	10/10(100%)	-	-
Amoxicillin clavulinic acid	10/10(100%)	-	-
Ceftriaxone	5/10(50%)	1/10(10%)	4/10(40%)
Cefotaxime	8/10(80%)	-	2/10(20%)
Cephradine	10/10(100%)	-	-
Cephalexin	10/10(100%)	-	-
Cephalothin	10/10(100%)	-	-
Streptomycin	8/10(80%)	2/10(20%)	-
Aztreonam	6/10(60%)	1/10(10%)	3/10(30%)
Colistin sulphate	5/10(50%)	-	5/10(50%)

100% drug resistance was against Oxillinc acid, Tobramycin, Penicillin, Ampicillin, Cephradine, Cephalexin and Cephalothin resistance of more than 50 % was reported against Erythromycin, Doxycycline, Oxytetracycline, Ciprofloxacin, Levofloxacin, Amoxicillin, Cefotaxime, Streptomycin and Aztreonam as displayed in table (4).

Table 5: Commensal *E.coli* antimicrobial profile of resistance isolates collected from native chicks, imported chicks and newly hatched chicks:

Antimicrobial agent	No. of isolates that showed resistance	Percentage of resistance
1- Oxillinc acid	26/30	86.6%
2- Trimethoprim-sulfamethoxazole	15/30	50%
3- Tobramycin	26/30	86.6%
4- Erythromycin	26/30	86.6%
5- Doxycycline	25/30	83.3%
6- Oxytetracycline	25/30	83.3%
7- Ciprofloxacin	17/30	56.6%
8- Levofloxacin	18/30	60%
9- Norfloxacin	11/30	36.6%
10- Penicillin	30/30	100%
11- Amoxicillin	23/30	76.6%
12- Ampicillin	30/30	100%
13- Amoxicillin clavulinic acid	30/30	100%
14- Ceftriaxone	26/30	86.6%
15- Cefotaxime	29/30	96.6%
16- Cephradine	26/30	86.6%
17- Cephalexin	30/30	100%
18- Cephalothin	30/30	100%
19- Streptomycin	23/30	76.6%
20- Aztreonam	16/30	53.3%
21- Colistin sulphate	17/30	56.6%

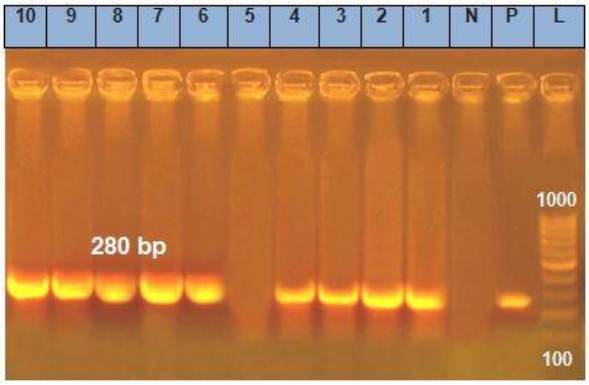
From Table (5) we explain that all the isolated strains showed multidrug resistance.

High drug resistance was reported against Penicillin, Ampicillin, Amoxicillin clavulinic acid, Cephradine, Cephalexin, and Cephalothin the percentage was 100%, 96.6% against Cefotaxime, 86.6% against Oxillinc acid, Tobramycin, Erythromycin, Ceftriaxone, Cephradine for Each, 83.3% against Doxycycline, and Oxytetracycline, 76.6% against Amoxicillin and Streptomycin, Colistin sulphate 56.6%, Trimethoprime-sulfamethoxazole 50% and Norfloxacin showed the lowest resistance percentage which was 36.6%.

Class 1 integron detection among isolates with multiple drug resistance

Conventional PCR was applied on ten MDR *E. coli* isolates for the detection of the integrase gene.

Nine out of the ten MDR isolates had the integrase gene, with a percentage of (90%) giving characteristic bands at 280 bp **(**Fig. 1).



Agarose gel electrophoresis of DNA from ten isolates of multidrug-resistant E. coli reveals amplification of the integrase gene by PCR at a 280 bp fragment. (L):1000-100 bp DNA ladder, Neg.: Negative Control,

Pos.: Positive control, Lan (1-4 & 6-10): positive samples. Lane 5: negative sample.

DISCUSSION

Despite all the examined chicks were not administered antibiotics before the examination of the samples, all the isolated *E. coli* strains were found to be MDR. The results are in line with the most current report from the European Food Safety Authority (EFSA). (EFSA and ECDC2020). This might be determined by a vertical or horizontal acquisition of resistance from breeders. (Osman *et al*., 2018 and Marin *et al*., 2020) or the environment (Montoro *et al*., 2020 and Oikarainen *et al*., 2019), respectively. These results show the importance of MDR acquired from the environment of breeding, hatching, or transportation (Poulsen *et al*., 2017; Dame *et al*., 2019). It has been observed that breeders' microbiota can directly vertically infiltrate one-day-old chicks (Nilsson *et al*., 2014) or by the bacteria that are resistant and persist in the hatchery or on delivery surfaces (Oikarainen *et al*., 2019; Projahn *et al*., 2017; Projahn *et al*., 2018). To reduce the selective AMR/MDR impact on breeders, hatcheries, and farm environments, they must be managed strictly in the early phases (Aarestrup 2015, Dierikx *et al*., 2013). In our study, high drug resistance was reported against Penicillin, Ampicillin, Amoxicillin clavulinic acid, Cephradine, Cephalexin, and Cephalothin, and the percentage was 100%, 96.6% against Cefotaxime, 86.6% against Oxillinc acid, Tobramycin, Erythromycin, Ceftriaxone, Cephradine for each, 83.3% against Doxycycline, and Oxytetracycline, and 76.6% against Amoxicillin and Streptomycin. A low percentage of drug resistance was reported against Colistin sulphate 56.6% Trimethoprime-sulfamethoxazole 50% and Norfloxacin 36.6%.

According to the outcomes listed by the (EFSA 2020) and (Martins da Costa *et al*., 2009), who identified resistant bacteria from one-day-old chicks to ampicillin, cephalothin, tetracycline, streptomycin, gentamicin, and enrofloxacin but discovered no *E. coli* resistant to chloramphenicol. Since no antimicrobial drugs had previously been administered to the chicks used in this investigation, vertical transmission of resistant strains from parent flocks is possible. (Giovanardi *et al*., 2005) or contamination in the environment of the hatchery (Dierikx *et al*., 2013) possibly be the primary factors. (Bortolaia *et al*., 2010). They concluded that *E. coli* resistance to -lactams and fluoroquinolones was caused by vertical transmission through parent hens in the same framework. Baron *et al*., (2014) suggested that *E. coli* resistance may be introduced to the hatchery facilities, either through true vertical transmission when parent poultry stocks are contaminated or through very early contamination in the hatchery itself, or during transport when the immature digestive flora is probably very receptive to early colonization. Even yet, it is impossible to exclude further later-occurring contamination incidents on the production farm.

In this investigation, 90% of the *E. coli* isolates had integrons, which is similar to (Abdel-Rahman *et al*., 2023) who identified integrons in *E. coli* isolates from Egyptian broiler farms, but differs from previous research by (Moawad *et al*., 2017), who did not record integrons

in *E. coli* isolates from samples of raw chicken in Egypt.

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مقاومة عترات الايشيريشيا كولاي ذات الأصل من الميكونيوم للمضادات الحيوية في الكتاكيت السليمة ظاهريا

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المضادات الحيوية لها دور مهم في الحياة من خلال منع وعلاج المرض ولكن يتم اعطاء واحد او اكثر من المضادات الحيوية بصفة روتينية في بعض مزارع الدجاج في أعمار متعددة مما يؤدي إلي حدوث مشكلة المقاومة المتعددة للمضادات الحيوية.

وهدفت هذه الدراسة إلى تحليل مدى مقاومة الايشيريشيا كولاي, المعزولة من الكتاكيت الفاقسة, للمضادات الحيوية المختلفة تحديد نتيجة الإستخدام الغير ضروري للمضادات الحيوية وتأثيرها علي الميكروب القولوني ذات الأصل الميكونيوم وتحديد أحد الجينات المقاومة (الانتجرون الفئة ١) في ٩٠٠ معزولة من الايشيريشيا كولاي التي تم عزلها من الكتاكيت ذات عمر يوم واحد.

وأوضحت النتائج أن كل المعزولات متعددة المقاومة للمضادات الحيوية والمقاومة سجلت مقاومة ضد البنسيلين والأمبيسلين والأموكسيسيلين وحمض الكلافيولينك والسيفادرين والسيفالوكثين والسيفالوثين بنسبه ١٠٠%. وكانت نسبه المقاومة ٩٦,٦% ضد حمض الاوكرالينك والتوبراميسين والايريثروميسين والسيفترايكسون والسيفادرين. وكانت نسبه المقاومة ٧٦,٦% ضد الستريبتوميسين وكانت ضد الكولستين سلفات ٥٦,٦% وضد الترايميسوبريم سلفاميسوكازول ٥٠%. أما النورفلوكساسين سجل أقل نسبة مقاومة ٣٦,٦%.
بالمقارنة بين مصادر الكتاكيت المختلفة, أوضحت الدراسة أن الكتاكيت ذات الأصل المحلي سجل الميكروب القولوني مقاومة بنسبة ١٠٠% ضد البنسيلين والأمبيسلين والأموكسيسيلين وحمض الكلافيولينك والسيفالوكثين والسيفالوثين والستريبتوميسين. ولكن المقاومة كانت ١٠٠% في الكتاكيت ذات الأصل المستوردة ضد البنسيلين والأمبيسلين والأموكسيسيلين وحمض الكلافيولينك والسيفالوكثين والسيفالوثين والسيفادرين. وفي كتاكيت الحضانات كانت ١٠٠% ضد البنسيلين والأمبيسلين حمض الاوكرالينك والتوبراميسين والسيفالوكثين والسيفالوثين والسيفادرين. ووجد الجين الانتجرون في تسع من المعزولات بنسبه ٩٠% .وفي النهاية توصي هذه الدراسة بتقليل استخدام المضادات الحيوية التى لا ضرورة من استخدامها للتقليل من حدوث ظاهرة المقاومة المتعددة للمضادات الحيوية.