How to Cite:
Radi, N. K. ., & Al-Marzoqi, A. H. (2022). Detection of virulent genetic markers like bla tem, bla imp1 of pathogenic e. coli strain isolated from Iraqi frozen chicken meat. *International Journal of Health Sciences*, *6*(S1), 6864–6874. https://doi.org/10.53730/ijhs.v6nS1.6679

Detection of virulent genetic markers like *bla tem, bla imp1* of pathogenic e. coli strain isolated from Iraqi frozen chicken meat

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***Abstract*---**This research was conducted at the Department of Biology, College of Science faculty for Women, University of Kufa, Iraq. From August 2020 to April 2021, one hundred twenty samples (frozen chicken) were collected from various brands (15) and origins (5) from various locations in AL-Najaf Province. The goal of this study was to look at the incidence of *Escherichia coli* with extended-spectrum -lactamase genes (*blaTEM, bla IMP1*) in chicken meats in Iraq. The samples were obtained aseptically in a sterile plastic container then transferred to the laboratory inside an icebox for bacteriological investigation. The results revealed that *Escherichia coli* had the highest percentage(33, 27%)out of 120 of bacterial species isolated from frozen chicken meat then *Enterobacter clocace* ssp dissolvens(30, 25%) then different species as *Klebsiella pneumonia* ssp pneumonia(21,18%), *Klebsiella oxytoca* (10,8%), *Staphylococcus lantus* (5,4%), *Enterococcus faecalis* (5,4%), *Enterococcus gallinarum* (3,3%), *Enterobacter kobi* (4,3%), *Leclercia adecarboxylata*(2,2%), *Kluyvera cryocrescens* (3,2.5%), *Vagococcus fluvialis*(2 ,2%), *Enterobacter hormaechei* (1,1%), *Enterobacter ludwigii*(1,1%). Recognition of resistance genes by PCR test yielded a negative outcome to *bla IMP1* identification in all *Escherichia coli* isolates, while 8 (24.2 percent) of isolates were positive for *blaTEM* gene of *Escherichia coli* out of 33 examined isolates.

***Keywords*---**Resistance gene, E. coli, blaTEM, PCR, ARS.

Introduction

The Enterobacteriaceae, such as Salmonella spp., *Escherichia coli*, *Klebsiella* and *proteus,* might be considered a significant food safety issue in polluted cow and poultry meat (Paterson,.2006). Foodborne microorganisms can be spread through the handling of chicken flesh. The fact that *Salmonella* spp. as well as *Campylobacter enteritis* seem undetectable in live birds and that considerable numbers of bird corpses were identified throughout process and slathering procedures suggest that poultry may be a major food-borne disease reservoir (Cavitte,.2003) As a result of the increasing use of antibiotics as therapeutic prophylactics or growth promoters in livestock, antibiotic resistant bacteria, including *Salmonella* poisoning (Schroeder,.2004), have emerged in chickens. There has been an upsurge in the prevalence and distribution of ESBLs and AmpC in bacteria due to the widespread usage of beta-lactam and third-generation cephalosporins for human and veterinary use (Colom etal,.2003). In chickens, 88.2 percent of E. coli was resistant to a variety of medicines. Veterinarians and animal health experts are deeply concerned about it (Yassin etal,.2017). Antibiotic-resistant *Escherichia coli* bacteria can spread from hens to humans via food or other animals. The resistance capabilities of resistant bacteria can be transferred to regular flora by colonization. Genes for resistance can be passed from one genus or family to another, or even from one bacterial species to another, Because of this, horizontal gene transfer (HGT) was already considered to be a key factor in spreading antibiotic resistance. (Talebiyan,.2014)

Since *E. coli* is a prevalent microorganism in the human digestive tract, the high incidence of ESBLs and AmpC—lactamases in the bacteria raises public health concerns worldwide. Human activities, such as cattle as chicken meat, hospital as well as municipal wastewater, and so on, are the primary sources of antibiotic-resistant *Escherichia coli* entering aquatic ecosystems. One of the earliest known bacterial resistance transposons, the blaTEM gene encodes an enzyme known as the TEM -lactamase that responsible for resistance for penicillin family drugs like ampicillin. A plasmid RP1 (or RP4, R18, R68, or RK2) initially had Tn801, Tn22, and Tn33 from IncP1- plasmids RSF1030 and Tn4 from plasmid R1. [Yassin etal,.2017, Colom etal,.2003]. Gram-negative bacteria's TEM enzymes play a critical role in determining resistance Gram-negative bacteria, as well as more than 180 variations of the TEM-1 or TEM-2 -lactamase have been identified. Extensive spectrum -lactamases (ESBLs) can hydrolyze third-generation cephalosporins, for example, whereas others, due to the inclusion of TEM and SHV enzymes, can resist inhibitors created to counteract -lactam resistance. (Singh etal,.2018).

Material and Methods

Chicken and meat Samples

Following correct aseptic technique, 25 g of every meat random selection were tested with a weighing scale and transferred to a completely seperate clean a septic mixer jar; 225 ml from 0.1 percent sterilized peptone water then added to the contents of the jar to generate a 10-1 dilution. To obtain a food homogenate, each sample was homogenized in a mixer at 2000 rpm approximately 1-2 minutes (Bouchrif etal,.2009). Then, 1 mL of the meat preparation was moved to 9 mL of

0.1 percent sterile peptone water to obtain the 10-2 dilution, and the dilution was repeated in the this series until the bacterial count reached 10-5 (AL-Tamimi and AL-Khafaji,.2021).

Plating and Media Culture

Homogenized meat specimens in 0.1 percent sterile peptone water; as well as Loop full from cultured swab samples in Nutrient broth, that were prepared throughout the last 2 phases from animal flesh as well as swabs specimens, have been spread on to the MacConkey agar (MAC), Nutrient Agar, and chrome agar plates then put them in an incubator at 37^{o}C for 24 hours. (AL-Tamimi and AL-Khafaji,.2021).

Susceptibility testing and antibiotic prescribing with the VITEK2 Compact System Susceptibility

Sensitivity analysis and antibiotic prescribing with the VITEK2 Compact Kit Susceptibility.

Identification of resistance genes with the use of (PCR)

Integrated DNA Technology was used to name the oligoneucleotide primers. The primer sequences are depicted in Table (1). Extraction and purification of DNA The extraction was carried out by promega.company in accordance with the manufacturer's instructions (Protocol involving conventional PCR amplification and cycling, PCR 1.1x ReddyMix TM Master Mix (Kit (thermo) was used.PCR product detection: Aliquots of amplified PCR outcome being combined with gel loading buffer before being electrophoresed in a 1.5 percent agarose gel. (Bauer etal,.2007).

Table 1
Sequences of primers of genes of this study

Gene for *Escherichia coli*	Primers		
	Forward	Reverse	bp
blaTEM	TTAGACGTCAGGTGGCACTT	GGACCGGAGTTACCAATGCT	972
blaIMP-1	GAAGGCGTTTATGTTCATAC	GTAAGTTTCAAGAGTGATGC	587

Detection of *blaTEM blaIMP-1.genes* of *Escherichia coli*

The gradient conditions for *Escherichia coli* 's blaTEM and blaIMP-1 genes are listed in the table below (3-11). In a reaction mixture volume of 20 µl, the gradients PCR reaction mixture contained 4 µl template DNA, 10 µl master mix, and 3 µl of each forward and reverse primer. The blaTEM and blaIMP-1.genes got amplified using traditional PCR. As illustrated in the table (3-11) After determining the optimal annealing temperature for the blaTEM and blaIMP-1 genes through choosing the best and most accurate band, which is (55 C°), the PCR mixture was 4 µl DNA, 10l master mix, 3 µl forward and 3 µl reverse primer, and PCR products were dissected on a 2 percent agarose gel and recolored with 5 g/ml Red safe nucleotide bases acid staining solution (Cabral etal,.2017) .

The Study Results

The result in Figure.1, illustrated that the bacterial species isolated from frozen chicken meat reveled that : *Eschericha coli* had the highest percentage(33, 27%)out of 120 of bacterial species isolated from frozen chicken meat then *Enterobacter clocace ssp dissolvens*(30, 25%) then different species as

Table (2)
Gradient condition for blaTEM blaIMP-1gene.

steps	Temperature C°		Time/min		cycles	
	blaTEM	*blaIMP-1*	*blaTEM*	*blaIMP-1*	*blaTEM*	*blaIMP-1*
Step denaturation	95°C	94°C	2min	2min	1cycle	1
Denaturation	95°C	94°C	1min	1min	30cycles	30
Annealing Zones	55°C	55°C	1min	1min		
Extension	72°C	72°C	1min	1.5min		
final extension	72°C	72°C	5 min	5min	1	1
Storage	4°C			Hold		

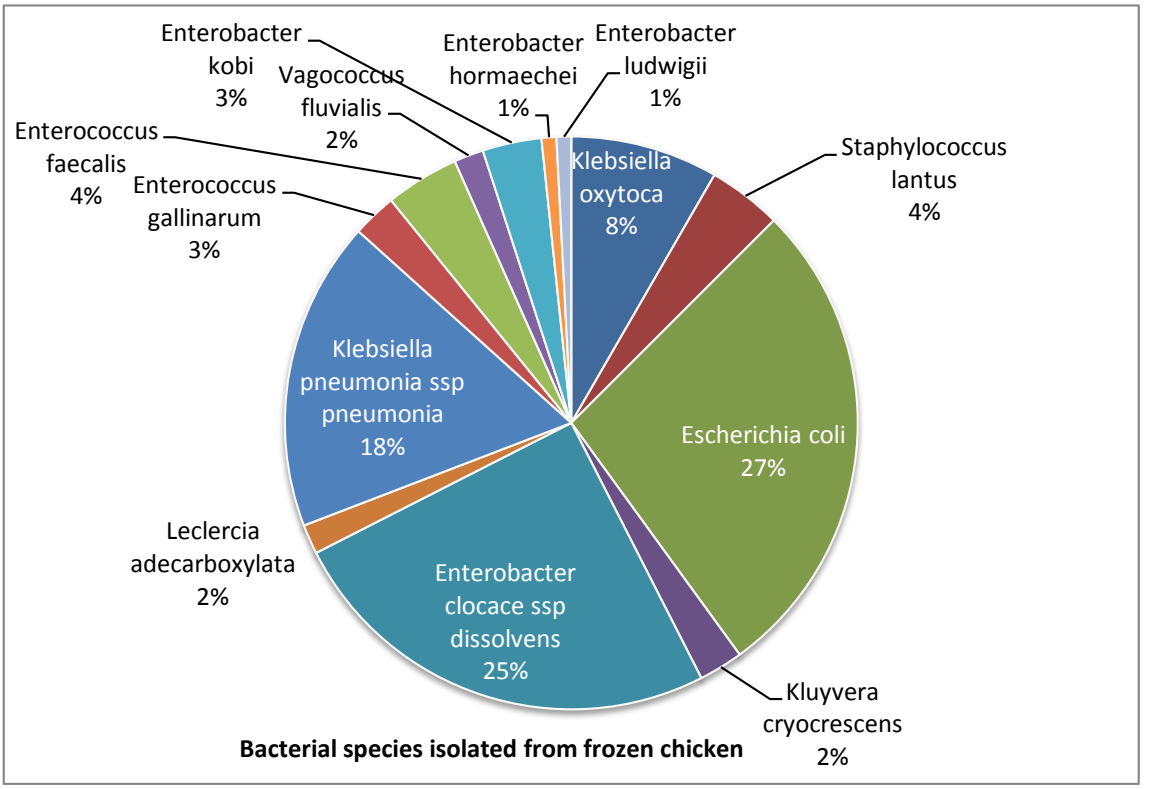


Fig. 1:-bacterial species isolated from frozen chicken meat

Klebsiella pneumonia ssp pneumonia(21,18%) ,*Klebsiella oxytoca* (10,8%), *Staphylococcus lantus* (5,4%), *Enterococcus faecalis* (5,4%), *Enterococcus gallinarum* (3,3%), *Enterobacter kobi* (4,3%), *Leclercia adecarboxylata*(2,2%), *Kluyvera cryocrescens* (3,2.5%), *Vagococcus fluvialis*(2 ,2%), *Enterobacter hormaechei* (1,1%), *Enterobacter ludwigii*(1,1%).

Electrophoresis Pattern of *bla team* gene isolated from Ecoli of frozen chicken

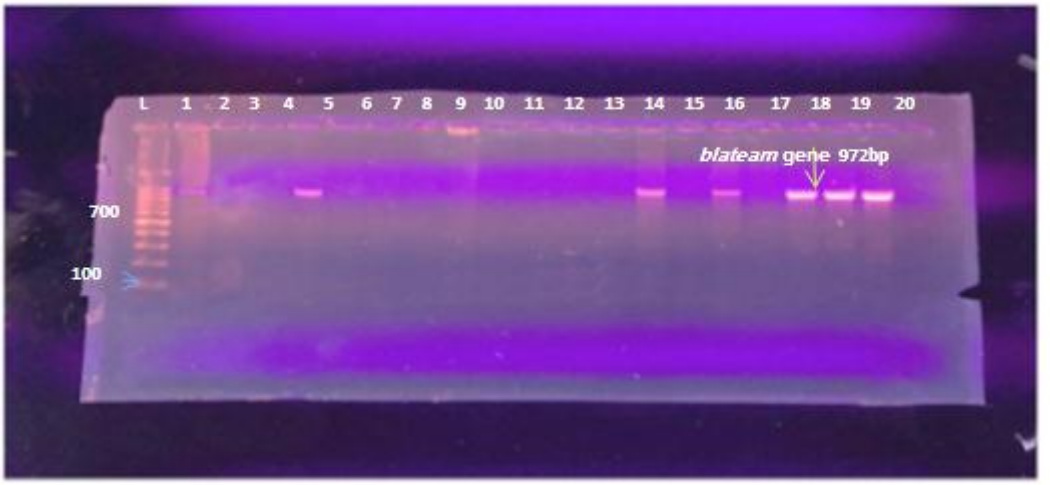
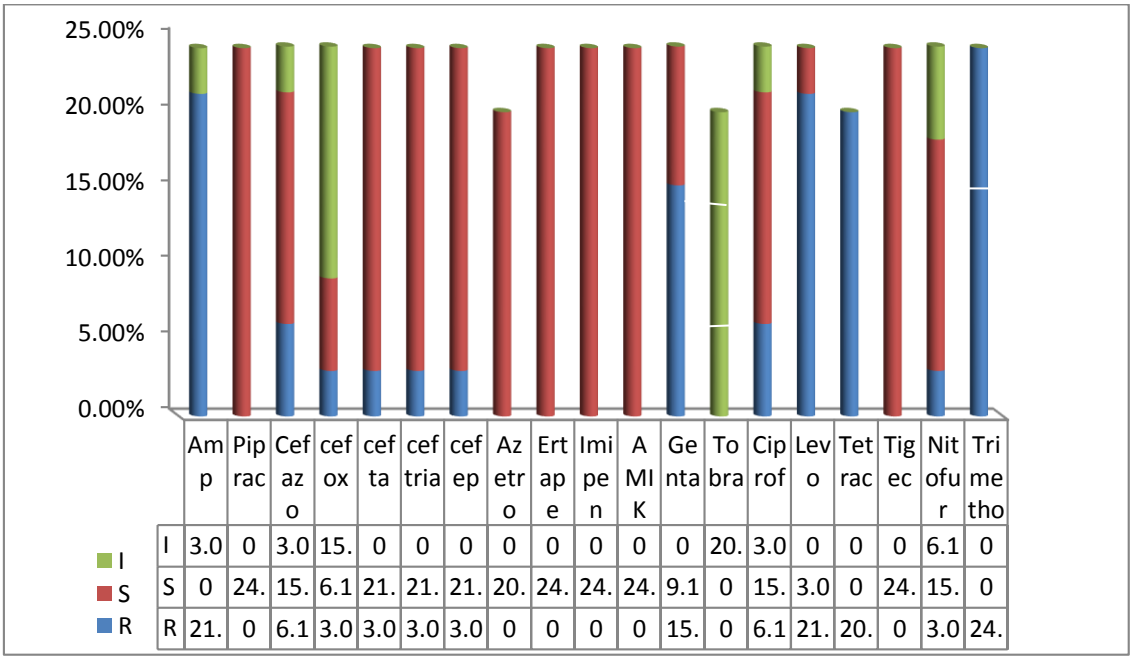


Fig. 2:-The Electrophoresis Pattern of *bla team* gene isolated from *Ecoli* of frozen chicken.L lane contain the 100 bp DNA Ladder.



	Amp	Piprac	Cefazo	cefox	ceftа	ceftria	cefep	Azetro	Ertape	Imipen	AMIK	Genta	Tobra	Ciprof	Levo	Tetrac	Tigec	Nitofur	Trimetho
I	3.0	0	3.0	15.	0	0	0	0	0	0	0	0	20.	3.0	0	0	0	6.1	0
S	0	24.	15.	6.1	21.	21.	21.	20.	24.	24.	24.	9.1	0	15.	3.0	0	24.	15.	0
R	21.	0	6.1	3.0	3.0	3.0	3.0	0	0	0	0	15.	0	6.1	21.	20.	0	3.0	24.

Fig. 3: distribution of antibiotics resistance against *bla TEM* gene of *Escherichia coli.*

Figure 3 demonstrate that (tetracycline Levofloxacin, Trimethoprim, Ampicilin) had a larger percentage of *Ecoli* resistant to antibiotics, whereas (tetracycline Levofloxacin, Trimethoprim, Ampicilin) had a higher percentage of *Ecoli* sensitive to antibiotics (Amikacin,. Impenem,. Ertapenem, Azetreonam). When it came to intermediate, cefoxitin had the highest percentage (15%).

Table 3
Distribution of genes in Relation to sources and brand market

Source				blaTEM		bla IMP1	
				-ve	+ve	-ve	+ve
Iraq	Brand market	Alfaihaa	F	9	2	11	0
			%	45	10	55	0
		Jouharat Baghdad	F	0	1	1	0
			%	0	5	5	0
		tazage	F	3	1	4	0
			%	15	5	20	0
		Abdullah	F	1	0	1	0
			%	5	0	5	0
		suran	F	1	0	1	0
			%	5	0	5	0
	Total:20 local inducitry		F	14	4	18	0
			%	46	12.12%	60	0
Turki	Brand market	Bakpi	F	3	2	3	0
			%	23.1	6.6	23.1	0
		alezeta	F	1	1	2	0
			%	7.7	7.7	15.4	0
		Banvet	F	4	1	7	0
			%	30.8	7.7	53.8	0
		damas	F	1	0	3	0
			%	7.7	0	10	0
	Total:13		F	9	4	15	0
			%	69.2	12.12%	50	0

Total 33	F	23	8	33	0
	%	76%	24.24%	100	0
***p-value**		0.438 N.S		- N.S	

N.S=no significant, -ve=negative result, +ve =positive result.

From Table .3. There is still a non-significant correlation among brand market and thus the two genes blaTEM, bla IMP1 at p-value 0.05, and revealed that Distribution of *Escherichia coli* genes in relationship towards the sources as well as brand market that display the same percent (4,12 percent)of bla team gene sequestered from local and imported refrigerated chicken whereas (–ve)negative result of bla IMP1 gene of *Ecoli* bacteria were detected from frozen chicken chicken meat.

Discussion

The *blaTEM* gene was previously identified as the predominant ampicillin resistance gene in human commensal *E. coli*. One of most frequent resistance mechanism with this family of antibacterial drugs in clinically significant Gram-negative bacteria is Beta -lactamase hydrolysis of beta-lactam antibiotics. Form Figure (1) ,The result demonstrated that 13 different bacterial serotypes were isolated from broiler meat including , 10 *Klebsiella oxytoca*, 5 *Staphylococcus lantus , 33 Escherichia coli, 3 Kluyvera cryocrescens, 30 Enterobacter clocace ssp dissolvens , 2 Leclercia adecarboxylata, 21 Klebsiella pneumonia ssp pneumonia, 3 Enterococcus gallinarum, 5 Enterococcus faecalis, 2 Vagococcus fluvialis, 4 Enterobacter kobi, 1 Enterobacter hormaechei, 1 Enterobacter ludwigii* out of 120 and These findings are nearly accepted with (Noori and Alwan ,.2016). A team of researchers determined that broiler meat had a total of 39 Salmonella species, six Pseudomonas species, six *Citrobacter* species, 29 E. coli species, and five *Proteus* species. Broiler meat is a major source to food-borne infections can cause human sickness or death if correct treatment is not performed. Chicken flesh contaminated with fecal organisms, such as *Salmonella* spp., *E. coli, Proteus and Klebsiella* spp., might be regarded an important food hygiene hazard, as established by (Paterson,.2006). We looked on the prevalence and genetic connection of Extended - spectrum beta *E. coli* as *blaTEM, bla IMP1 gene* in chickens in Nagaf province. The identification of resistance genes by PCR test resulted in a negative result for bla IMP1 identification in all *Escherichia coli* isolates, but 8 (24.2 percent) of isolates were positive for *Escherichia coli* blaTEM gene out of 33 investigated isolates and this disagreed with(Parvin etal,.2020) who found that The blaTEM gene was found in all of the isolates while our finding agreed with the findings of (Jiang etal,2011) they found an impressive 88.9% (10) of the chicken *E. coli* strains tested positive for the blaTEM gene in china. blaTEM gene was also found by (Brinas etal,.2003b) Of the five *E.coli* isolates tested, roughly 48. percent had positive three-sample blaTEM PCR results, and another isolate also had positive three-sample PCR results. These percentages significantly lower than the ones found in this study.

As figure(3) reveled the distribution of antibiotics resistance against *bla TEM* gene of *Escherichia coli* . The results demonstrate that (tetracycline Levofloxacin, Trimethoprim, Ampicilin) had a larger percentage of *Ecoli* resistant to antibiotics, whereas (tetracycline Levofloxacin, Trimethoprim, Ampicilin) had a higher percentage of *Ecoli* sensitive to antibiotics (Amikacin,. Impenem,. Ertapenem, Azetreonam). When it came to intermediate, cefoxitin had the highest percentage (15%).and these finding wre in line with(Jacoby *etal*,.2004) Many Enterobacteriaceae and a few other species express cephalosporinases, which seem to be molecular class C enzymes. This class of antibiotics is more powerful than benzylpenicillin and appears to be resistant to inhibition by clavulanic acid. Cephamycins, such as cefoxitin, are also of interest to them. These enzymes exhibit a significant affinity for the antimicrobial agent aztreonam, unlike class A cephalosporinases. Other hallmarks include the absence of cefoxitin, inhibition by clavulanate or Tazobactam, and resistance to cefotaxime and not ceftazidime (AL-Tamimi and AL-Khafaji,.2021) Many bacteria, including *Citrobacter* known research, *Enterobacter cloacae* plus *Serratia marcescens* and *Pseudomonas aeruginosa*, have low levels of ampC expression. However,beta -lactams such amoxicillin, ampicillin, imipenem and clavulanic acid can stimulate this gene's expression (Jacoby etal,.2004). Other organisms, such as *Acinetobacter baumannii* and *Escherichia coli*, lack one or more components of the induction system. When synthesized in significant quantities, particularly in hosts with low -lactam aggregation, group 1 enzymes can offer resistance to carbapenems, particularly ertapenem (Yu etal,.2008).

Table (3)shows the distribution of genes in connection to origin and manufacturer market that show higher percent (6,20 percent) of blateam gene separated from export frozen poultry mean while the lower percent's (4,13 percent) of same gene separated from local poultry meat origin turkey that was a focus of our study. These finding disagree with (Yu etal,.2008). who were found that 53 and 30 ESBL-Ec from Brazilian hens out of 111 total. For domestic chickens, blaTEM (36 percent) and blaSHV (15 percent) were found, while blaCTX-M (100 percent) and blaTEM (20 percent) were found for imported birds. Imported chicken isolates had the highest prevalence of blaCTX-M-2 (53 percent) and blaCTX-M-8 (43 percent) whereas domestic chicken isolates had the lowest prevalence of blaCTX-M-2 (45 percent) and blaCTX-M-1 (34 percent). Tetracycline (83 percent), next by streptomycin (70 percent) and nalidixic acid (40 percent) were the most often resistant antibiotics in domestic chicken isolates (62 percent). Nalidixic acid (63 percent) and tetracycline resistance were the most common among imported chicken isolates (77%). (57 percent). Specifically, 60 percent (32/53) and 70 percent (21/30) ESBL-Ec of imported and domestic chickens, respectively, were shown to have considerable multidrug resistance. ESBL-Ec from indigenous and imported hens contained virulent genes involved with diarrheagenic and extraintestinal pathogenic *E. coli*. ESBL-Ec in retail poultry foods may serve as a reservoir for antibiotic resistant determinants, according to these findings, and some of these resistance determinants may be detrimental to people, According to Abd El Tawab and his group nonetheless, the link between antibiotic resistance and the blaTEM gene in E. coli isolated from frozen chicken flesh.(Abd El Tawab etal,.2016 ; Hardiati etal,.2021).

Conclusion

Tetracycline, Levofloxacin, Trimethoprim, and Ampicilin were all resistant to the *Escherichia coli* isolated from Iraqi frozen chicken meat, The antimicrobial resistance gene identified is *blaTEM*.

Acknowledgments

It is with great gratitude that we acknowledge the work of all the beta-lactamase researchers. We would want to express our gratitude to DR. Nawfal, the facility's management, and AL-Amen center staff. As a result of their assistance.

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