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Research Article

Molecular identification of antibiotics resistance genes of pathogenic bacteria isolated from different clinical sources

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Abstract

Most of the acquired antibacterial resistance can be obtained by mutations occurring in genes due to certain physical and chemical factors. These mutations are present in chromosomal gene and plasmids , among other types of mobile genetic elements. Aim of the current study is to determine the resistance genes in isolated pathogenic bacteria and their effectiveness in resisting pathogenic bacteria to antibiotics. In the present study, Samples from patients suffering from burns, wounds and urinary tract infection were collected . These samples were cultured on the proper media and colonies were identified by different biochemical tests. Identified pathogenic bacteria were applied to investigate their resistance against wide range of antibiotics using VITEK system. PCR technique was used to detect the antibiotics resistant genes among bacterial isolates. Results showed that *K. pneumoniae* carried all tested genes. However, *tem* and *shv* genes were absent in *St. aureus*, *E. coli* and *P. aeruginosa*. It can be concluded that all tested bacteria were dangerous due to the antibiotics resistance activity but *K. pneumoniae* were the most dangerous.

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Introduction

Antibiotics have been considered as the high significant classify of pharmaceuticals which have been employed for decades for fighting microbes [1], as well as, represented the most significant medical innovations of the 20th century. Employing antimicrobial in this field has been increased in the world over the years. A previous study conducted by Klein [2]showed that the consumption of antibiotics was increased by 65% during period of 2000 and 2015 in 76 countries. However, the extensive usage of antibiotics have widely caused bacteria to develop and spread antibiotic resistance , which has turned into a serious issue, lead to bacterial diseases harder or impossible to treat [3]. Resistance of bacteria is defined as the ability of bacterial cells to prohibit antibiotic bacteriostatic or bactericidal impacts [4]. The report of World Health Organization (WHO) in 2019, revealed that a round 700,000 people were died yearly due to the infections by antibiotic-resistant bacteria and estimated to be reach for over 20 million per year was death in long future)[5]. As result of not finishing a course of antibiotics by any person or took antibiotics too often for the wrong reasons, these routines will lead to occurring modifications within the bacteria resulted to lose antibiotics their activity against target bacteria [6,7]. On the other hand, such factors including bad society hygiene, bad controlling in hospitals infection and clinics infection control in hospitals and clinics, environmental accumulation of antibiotics may develop the bacterial resistance [8].

However, most of the acquired antibacterial resistance can be obtained by mutations occurring in genes due to certain physical and chemical factors [9]. These mutations are present in chromosomal gene and plasmids , among other types of mobile genetic elements. Antibiotics resistance bacteria passage a copy of their genes to another bacterial strain which they don't have resistant feature via transformation, conjugation, or transduction [10]. In several studies such as [11,12] appeared active removal of the antibiotic, reduction of intracellular antibiotic concentrations and modification of the antibiotic target enhanced gene expression encoding for efflux pumps can lead to arisen bacterial resistance.

Thus, the goal of the present study was to investigate the bacterial pathogen resistance activity for a wide range of antibiotics and searching for genes encoded for this antibiotics resistance.

Materials and methods

Samples collection:

One hundred and ten samples were collected from various sources, including burns, urinary tract infections, wound infections, and taken from both sexes at the Imam Hussein Teaching Hospital. Using sterile cotton swabs containing a carrier medium to maintain bacteria through transporting them to the laboratory after they were clinically diagnosed .

Bacterial isolation:

All specimen collected were inoculated on selective and differentiated media such as blood and MacConkey agar purchased from (Hymedia-India). The grown samples were incubated at 37°C for one day. Bacteria were identified by Gram stain, phenotypic characteristics of the colony, and depending on biochemical tests such the enzymetic reaction for Indol, urease, coagulase , oxidase, and catalase tests.Five isolates of *Staphylococcus aureus* were isolated from burns, seven isolates of *Escherichia coli* samples were cultured from wounds and urine, three isolates of *Pseudomonas aeruginosa* were identified from burns and wounds, and nine isolates of *Klebsiella pneumoniiae* were isolated from wounds and burns.

Antibiotic susceptibility:

Antibiotic susceptibility test was performed by VITEK system.

PCR technique for the molecular detection of resistance genes:

DNA extraction was obtained from aforementioned isolates by using a DNA extraction kit from Addbio, Korea for detecting the antibiotics resistance genes including (*ermA, qnr, shv, tem,aac(6')-Ib -cr*) by using specific designed primers shown in the Table 1.

Table 1: Primers sequences to detection of antibiotics resistance-genes in pathogenic bacteria				
primer type	nitrogen bases sequence table		the size(bp)	Reference
Tem	TCCGCTCATGAGACAATAACC	F	931bp	[13](Alikhani.,2013)
	TTGGTCTGACAGTTACCAATGC	R		
Shv	CTTTACTCGCCTTTATCG	F	827	[14] (PEYMANI *et al*.,2017)
	TCCCGCAGATAAATCACCA	R		
ErmA	TCTAAAAAGCATGTAAAAGAA	F	645	[15] Juda *et al*., (2016)
	CTTCGATAGTTTATTAATATTAGT	R		
QnrA	GATAAAGTTTTTCAGCAAGAGG	F	593	[16](Jacoby *et al*., 2003)
	ATCCAGATCCGCAAAGGTTA	R		
aac(6')-Ib -cr	TTGCGATGCTCTATGAGTGGCTA	F	482	[17]Kim *et al*., (2009)
	CTCGAATGCCTGGCGTGTTT	R		

PCR reactions were performed, and primer solutions were prepared according to their manufacturing company. The components of each PCR tube were as the following: Master Mix 12.5 µl , forwards primer 0.25-2.5 µl, reverses primer 0.25-2.5 µl, 1-5 µl of DNA template Nuclease-Free water were added into tubes to complete the final volume to 25 µl. Each tube was carefully mixed with a vortex mixer for 10 sec, then inserted into a Thermocycler PCR device for the purpose of performing the reaction, using the specific program for the amplification of target gene Table 2 .

Table 2: Thermocycler conditions for each tested gene.							
genes	**Initial denaturation**	**cycles**	**denaturation**	**annealing**	**extension**	**final extension**	**references**
tem	94 ˚C / 5 min	Cycles (30)	94 ˚C /30s	53 ˚C / 30s	72 ˚C /1 min	72 ˚C /7 min	[18](Yang *et al*., 2018)
shv	94 ˚C / 5 min	Cycles (35)	94 ˚C /30s	53 ˚C / 30s	72 ˚C /1 min	72 ˚C /7 min	(Yang *et al*., 2018)
erma	94 ˚C / 5 min	Cycles (35)	94 ˚C /30s	51 ˚C / 30s	72 ˚C /1 min	72 ˚C /7 min	[19](Sutcliffe *et al*., 1996)
qnrA	94 ˚C / 5 min	Cycles (35)	94 ˚C /30s	53 ˚C / 30s	72 ˚C /1 min	72 ˚C /7 min	[20](AbdelRahman *et al*.,2020)
aac(6')-Ib -cr	94 ˚C / 5 min	Cycles (35)	94 ˚C /30s	53 ˚C / 30s	72 ˚C /1 min	72 ˚C /7 min	[20](AbdelRahman *et al*.,2020)

PCR products was loaded onto agarose gel with (1%) prepared by dissolving one gram of agarose powder in 100 ml of X1 TBE buffer, and the solution was heated until completely dissolved using a water bath,. Solution then was left for cooling until reached a temperature of approximately 50 °C. Three µl of ethidium bromide was added to the solution for staining the DNA. The agarose gel was poured gently and continuously to avoid bubbles in the tray mold after the combs was fixed. The gel was left to solidify at room temperature , and the comb after that was removed with careful [21]. Ladder 100bp was loaded alongside with the PCR products for comparing the PCR product sizes. Products were run on voltage of (70) volts for (50) min.

Ethical consideration:

This study was accepted by Ethical Committee at College of Science/ University of Karbala. All subjects enrolled in this work were informed and agreement gained verbally from each one before the collection of sample.

Statistical analysis

All presented data are expressed as means ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's HSD multiple range *post hoc* testing were used to determine the significant differences between number of isolates which carry each gene.The accepted levels of significance were $P < 0.05$. results management and analysis were performed using MiniTab statistical software version 17, IBM (Pennsylvania, USA).

Result:

110 samples were collected from various clinical sources, including burns, urinary tract infections, wound infections,. Current study was undertaken at the period (August 3, 2022 - January 5, 2023) in the Imam Hussein (peace be upon him) Teaching Hospital, from the burns lobby, and from the Obstetrics and Gynecology Hospital, using sterile cotton swabs. It contains a transport medium to preserve the bacteria during its transport to the laboratory, as well as samples from the urine and discharge of children from the Children's Teaching Hospital in Karbala, with Staphylococcus aureus5 samples from burns and urine, *E.coli*, 7 samples from wounds and urine, Pseudomonas aeruginosa 3 from burns and wounds, and Klebsiella pneumoniae 9 samples from wounds. And burns after patients are subjected to examination and treatment. Samples are obtained using a swab with transport media to maintain the viability of bacteria until they are cultured on appropriate media. Different types of media used to culture the samples such as MacConkey and blood purchased from (Hemidia-India).

Biochemical identification of bacterial isolate

In the present study Five isolates of *Staphylococcus aureus* were isolated from burns and urine, seven isolates of *Escherichia coli* samples were cultured from wounds and urine, three isolates of *Pseudomonas aeruginosa* were identified from burns and wounds, and nine isolates of *Klebsiella pneumoniiae* were isolated from wounds and burns.

Table(3): Antibiotic sensitivity results for isolated bacteri

Antibiotic	resistance rate			Antibiotic	resistance rate for
	E.coli	*p. aeruginosa*	*k.pneumoniae*		*S. aureus*
Ticarcillin	86%	100%	89%	Ciprofloxacin	20%
Ticarcillin\ clavulanic Acid	86%	100%	89%	Oxacillin	40%
				Rifampicin	60%
Piperacillin	57%	66.6%	89%	Fusidic Acid	60.4%
Cefepime	42.8%	33%	78%	Moxifloxacin	60%
Piperacillin\Tazobactam	57%	33%	56%	Ampicillin	40%
Ceftazidime	28.5%	33%	77.7%	Teicoplanin	60.1%
Azithromycin	42.8%		78%	Imipenem	20%
Tobramycin	71%		66.6%	Linezolid	60%
Imipenem	28.5%	67%	77.7%	Tigecycline	80%
Meropenem	42.8%	33.4%	78%	Cefoxitin Screen	60%
Gentamicin	71%	67%	89%	Rifampicin	60,2%
Minocycline	71%		38%		

The present data showed that *E. coli* were showed the highest resistance against Ticarcillin, Ticarcillin\clavulanic Acid which represented 86% from the total number and the lowest resistance (28.5%) to Ceftazidime, Imipenem . This result is inagreement with study of [24] in Algeria and [25], respectively. Moreover, *Klebsiella* revealed the highest resistance to Ticarcillin , Ticarcillin\clavulanic Acid , Gentamicin,Piperacillin as 89% from the total number and lowest resistance minocycline (38%). These data were in agreement with Raheem and his colleagues whileconflicted with the Gentamicin[26] and (2022)**[27]**. Minocycline inhibits protein synthesis by binding to the 16S rRNA component of the 30S ribosomal subunit and inhibiting aminoacyl-tRNA delivery to the A site, thus preventing the elongation step, Minocycline has broad intrinsic Gram-negative activity, but resistance is common, which is primarily due to efflux pump activity[28]. *Furthermore,* all isolates of *P.aeruginos* were resisted to Ticarcillin Ticarcillin\clavulanic acid 100%, , and the lowest resistance to Cefepime, Meropenem, cipfloxacin **(**33%). This result is similar with study of [29] conducted in Pakistan , [30] in hospitals in Sokoto, northwest Nigeria and [31] in Iran.

Finally, the results showed that ***S. aureus*** were less resistant to antibiotics Benzylpencillin, Ciprofloxacin ,Imipenem (20%) and the highest resistance to Tigecycline (80%) . These results are consistence with Petrillo's study in Italy [32]. Antibiotic resistance is a significant development that arises from the prescription of excessive and inappropriate usage of antibiotics . The incidence of multiple drug resistance and the use of more than four antibiotics were the main indicators of bacterial resistance.

The highest resiste of *p.aeruginosa* was to Ticarcillin Ticarcillin\clavulanic acid 100%, , and the lowest was Cefepime, Meropenem, cipfloxacin 33% .
The genetic study of the bacteria isolated in the current study and comparison of its results with the phenotypic resistance to antibiotics shows that some genes play an active role in this resistance, which poses a threat to the community health and treatment system, this is in consistent with PCR technition was used in the current study to detect the found of the *acc* gene in the isolated pathogenic bacteria .

PCR technique was used in the current study to detect the presence of *aac(6')-Ib -cr* gene in the isolated pathogenic bacteria. Figure (1) shows the electrophoresis of the PCR products, which was successfully identified these genes by specific primers designed for A-*aac(6')-Ib -cr B- erm* A, C- *qnr,* D- *shv,* E- *tem genes with bands in size of* (482 bp), (645 bp), (645 bp), (593 bp), and (593 bp) , *respectively.*

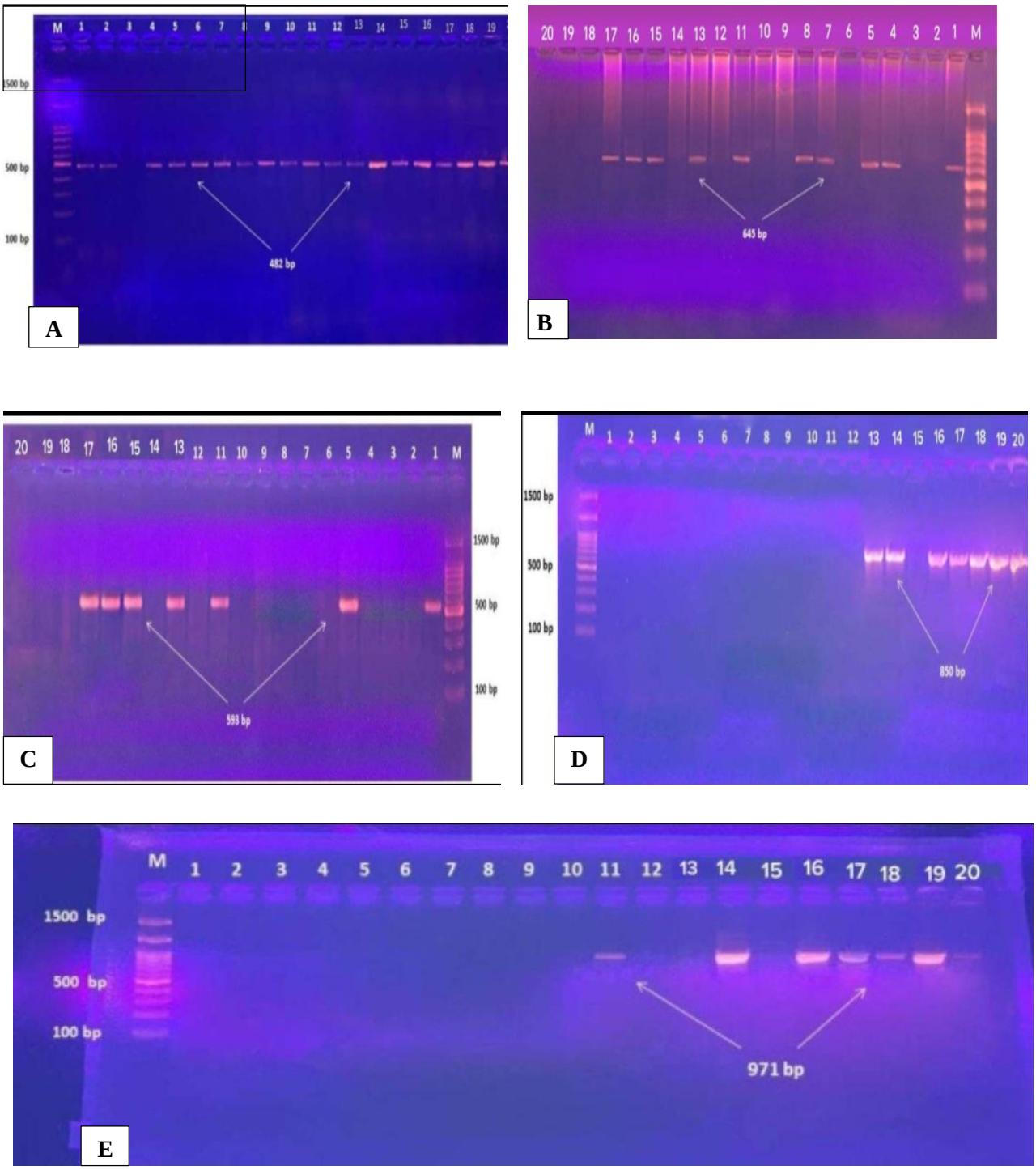

Figure (1) Electrophoresis for PCR products by specific primers for the tested genes. at a gel concentration of (1.5%) and a voltage of (70) volts for (50) min. *Sample code:A- aac(6')-Ib -cr* gene (482 bp),*B- erm* A (645 bp), C- *qnr* gene (645 bp), D- *shv* gene (593 bp), E- *tem* gene (593 bp).

Furthermore, Figure (2) showed that *8 isolates out of 10 isolates of K. pneumoniae* carried the *aac(6')-Ib -cr* gene which were significantly higher than the other genera, whereas *P. aeruginosa* were significant lower in having this gene, knowing that this gene encodes for antibiotic resistance of the aminoglycoside type ($P \leq 0.05$). On the other hand, the number of gene having bacteria isolates was significantly lower and higher in compare to *Klebsiella pneumoniae* and other bacteria, respectively. This result was similar to what was obtained by [33] where *the aac(6')-Ib-cr* gene was detected in both *E.coli* and *Klebsiella*, and this gene was the most common among the quinolones resistant genes (QNG).

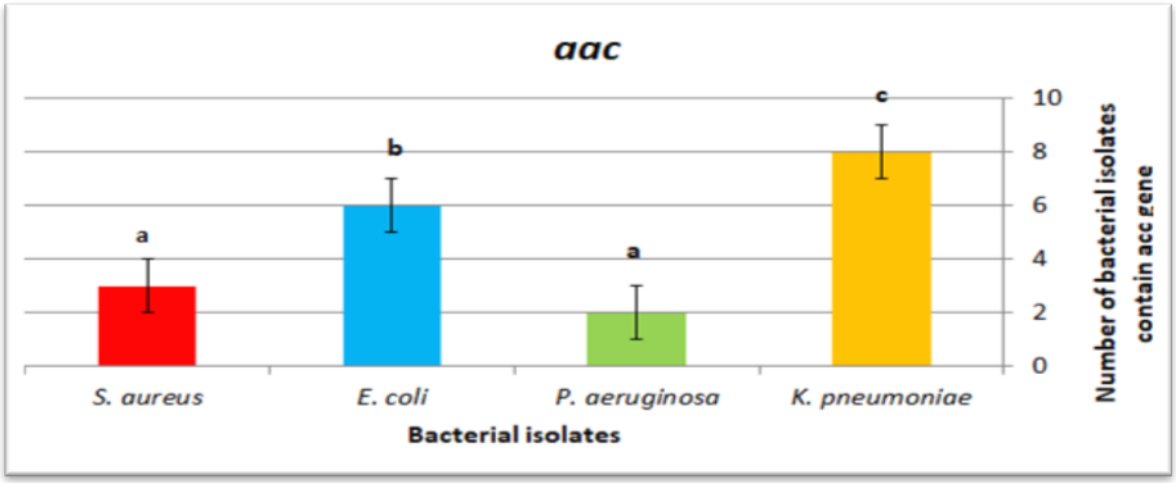

Figure (2) Number of bacterial isolates carry *aac(6')-Ib -cr* gene. Columns having different letters indicate for a significant difference in the number of isolates that carry this gene ($P < 0.05$) .

Existence of *aac(6')-Ib -cr* genes in *E. coli* depends on geographical location, excessive use of antibiotics, and the types of bacterial strains. Literature indicate variable detection rates, ranging from 20% to 80% depending on the *aac(6')-Ib -cr* variant and the specific region. The study of Ruiz and his colleagues in 2012 was similar to the diagnosis of this gene in *E. coli* bacteria [33]. The presence of these genes can significantly limit treatment options for *E. coli* infection, increasing the risk of treatment failure and potentially life-threatening complications [34]. *S. aureus*, still poses major challenges to public health in many regions due to antibiotic resistance problems associated with the *aac(6')-Ib-cr* gene presente [35]. Literature show varying prevalence rates of this gene depending on many factors such as patterns of antibiotic use and specific types of *S. aureus* strains. The presence of the *aac(6')-Ib-cr* gene greatly reduces the effectiveness of antibiotics belonging to the aminoglycoside family, which it limits options for infections caused by resistant *S. aureus* and poses a major challenge in healthcare settings, contributing to morbidity and mortality [36] .

Figure (3) shows that the *P.aeruginosa* were significantly lower in their possession of *erm*A gene, which encodes resistance against erythromycin-type antibiotics ($P = 0.031$). Although, other bacterial isolates having this gene in different numbers, there were no significant differences between them. . This result was similar to a study conducted in 2023, when three isolates of *pseudomonas* carrying *erm*A gene among 92 samples of bacteria [37].

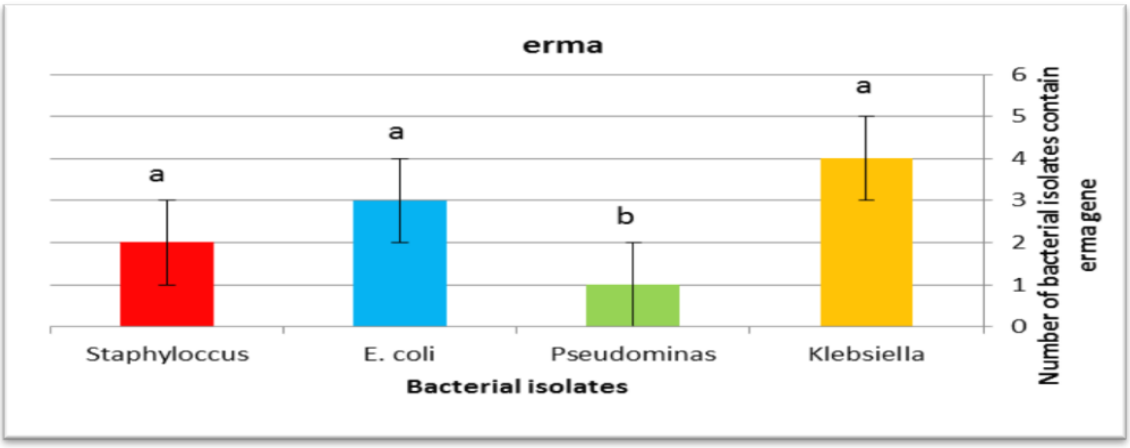

Figure (3)Number of bacterial isolates carry *erm*A gene. Columns having different letters indicate for a significant difference in the number of isolates that carry this gene ($P < 0.05$) .

The results showed that *K.pneumoniae* was significantly higher in numbering of their isolates having this gene 4 isolates out of 10(P =0.014). In contrast, only one isolate having this gene out of the total number which indicate for no significant differences. The existence of this gene could be encodes for resistance to ceftazidime antibiotics Figure (4)..

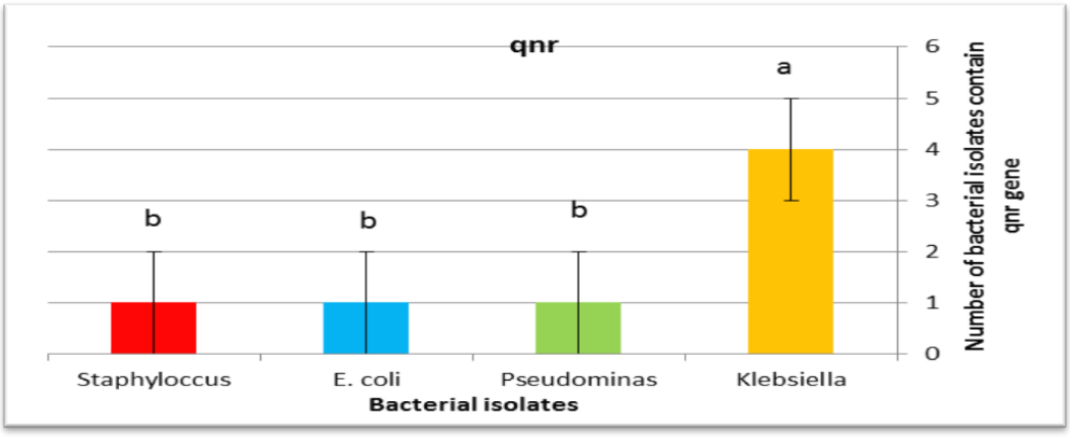

Figure (4) Number of bacterial isolates carry *qnr*.gene. Columns having different letters indicate for a significant difference in the number of isolates that carry this gene (P=0.014).($P < 0.05$) .

Frequency of *qnr* gene among the tested bacteria isolates in present study is higher than the frequency that was found in the study of Rezazadeh [38], and similar to (Wang *et al*., 2008). in spite of the fact that *qnr* genes are the farthest resistant agents contra (β-lactam and quinolone), the rate of resistance to other antibiotics has risen sharply recently and increased detection of *qnr* genes poses a challenge due to limited treatment options for quinolone-resistant bacteria.

It is possible to observe this tactic in quinolone resistance. Since the quinolones that were discovered were wholly artificial antimicrobials, it was thought that quinolone resistance (qnr) genes did not occur naturally. Previous research has verified that clinical isolates of *E. coli*, *S. aureus*, and *P. aeruginosa* had the lower frequencies of *qnr* gene. However, prior research on *S. aureus* demonstrated a strong association between resistance to aminoglycosides, chloramphenicol, clindamycin, erythromycin, trimethoprim, and tetracycline and the presence of ermA gene. For instance, it was

found that 83.4% of S. aureus strains resistant to erythromycin carry ermA gene [46].

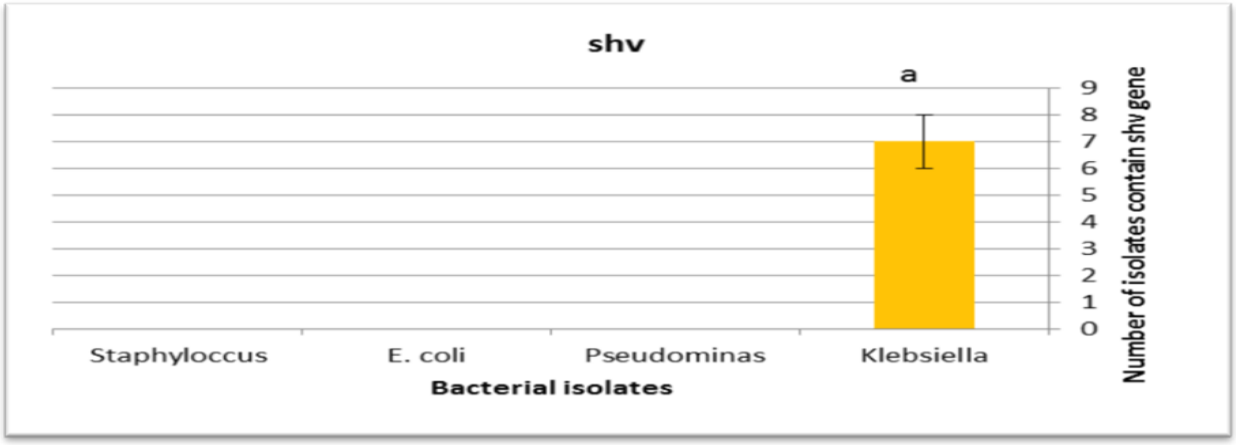

Figure (5) Number of bacterial isolates carry *shv gene*. Columns having different letters indicate for a significant difference in the number of isolates that carry this gene (P=0.000).

Seven isolates of K. pneumoniae out of 10 were found to carry shv gene which significantly in comparison to other genera which were have no ability to have this gene ($P \leq 0.05$), Figure (5).

This result was comparable to a local study carried by (Al- Tuhmazi & Al-Hisnawi, 2023)[39]. In contrast the present data is not in agreement with study of Ghenea *et al*., (2022)[40] who found that among 14 isolates of *E.coli* contained the *bla shv-1* gene

Determining the antibiotic susceptibility of bacterial isolates, especially as resistance increases, is often crucial (Banerjee and Patel., 2023)[41]. It is shown from Figure (6) isolates out of 10 isolates of *Klebsiella* were significantly carried *tem* gene higher than *P. aeruginosa* which was only one isolate was carried this gene. *N contrast this gene was absent in all isolates of S. aureus and E. coli.* This result is similar to the study [39] who found that all isolates of E.coli did not carry tem gene.

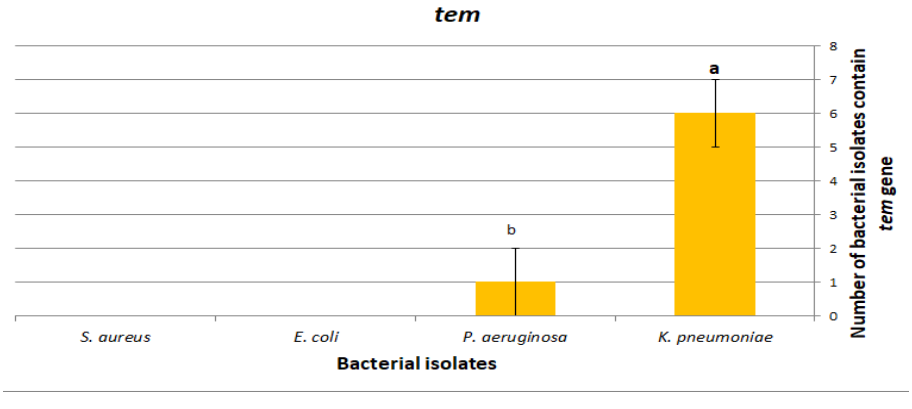

Figure (6) Number of bacterial isolates the *tem* resistance gene. Columns having different letters indicate for a significant difference in the number of isolates that carry this gene (P=0.000).

Antibiotic resistance can occur due to the different mechanisms such as enzyme production that enhance the antimicrobial agent, or alternative enzyme for the specific enzyme that is inhibited. Resistance might occur under mutation condition in the structure of the target on which the antibiotic acts, which decrease the antimicrobial agent binding or post-transcriptional yield or post-translational modification of the target of the antimicrobial agent occurs, which reduces binding of the antimicrobial agent In some cases of resistance there is a decrease in the rate of absorption of antibiotics by microorganisms, or it may be the result of an artificially active biological agent [42].

Table 4 : Number of bacterial isolates that carried resistance genes study

Bacterial isolate	*aac(6')-Ib -cr (6')-Ib -cr*	*ermA*	*qnr*	*shv*	*tem*	*P value*
Staph	3	2	1	0	0	0.125
E.coli	6	3	1	0	0	0.003 *
pseudomonas	2	1	1	0	0	0.191*
Klebsiella	8	4	4	7	6	*0.002
P value	0.00001	100.00	0.014	0.031	100.00	/

*Indicates for significant differences

Table 3 showed that *Klebsiella* spp. carried all tested genes include tem and *shv* genes that encode the resistance trait to the β-Lactam family by [43]. However, these two genes were completely missing in other gnera of bacteria.

The study of antibiotic resistance genes, which is the ability of bacteria to progresses defense tactic that make them difficult to treat, has become of great importance. The emergence of mutations in the genes that cause resistance may make treating bacterial infections more difficult, antimicrobial resistance develops spontaneously as a result of genetic mutations or as a result of the transfer of resistance from one species that possesses it to another that does not in genetic manner)[48].

Conclusions:

All tested bacteria were found to reveal antbiotics resistance aginst wide rang of antibiotics and *Klebsiella* spp. were the only bacteria to have all tested genes which indicate that these bacteria are difficult to kill by using chemical medication.

discussion:

E.coli has been shown that the highest resistance86%toTicarcillin, Ticarcillin\clavulanic Acid and this result is similar to stady in Algeria 82.5% *E.coli* obtained from clinical sources[24]. the low resist to Ceftazidime, Imipenem 28.5% This study Inconsistent with to a local study of *E.coli* samples, an exit sample collected from children with diarrhea appearance the resistance of Ceftazidime 89.3%and Imipenem 0%[25].

The incidence of multiple durg resistance and the use of more than four antibiotics were the main indicators of bacterial resistance. Antibiotic resistance is a significant development that arises from the prescription of excessive and inappropriate usage of antibiotics .

On the other hands, *Klebsiella* high resistance to Ticarcillin , Ticarcillin\clavulanic Acid , Piperacillin89 %that agreement with

Raheem and his colleagues whileconflicted with the
Gentamicin[26] and Ali and Kamil [27]and the lowest resist to minocycline 38%,Minocycline inhibits protein synthesis by binding to the 16S rRNA component of the 30S ribosomal subunit and inhibiting aminoacyl-tRNA delivery to the A site, thus preventing the elongation step, Minocycline has broad intrinsic Gram-negative activity, but resistance is common, which is primarily due to efflux pump activity[28].
The highest resiste of *p.aeruginosa* was to Ticarcillin Ticarcillin\clavulanic acid 100%, , and the lowest was Cefepime, Meropenem, cipfloxacin 33% .
This result Ticarcillin100% contradicted a study conducted in Pakistan of samples taken from various clinical sources, including swabs, pus, wounds, blood, sputum, urine, and burns samples[29]and Cefepime33% This percentage is similar to the study in hospitals in Sokoto, northwest Nigeria While the result contradicted with Ticarcillin\clavulanic acid[30].
cipfloxacin 33% This result was inconsistent with a study conducted in Iran of samples taken from a clinical source [31].
Staph showed the highest resistance to Tigecycline 80% while the lowest was resistance to Ciprofloxacin ,Imipenem 20% These results were compared with Petrillo's study in Italy on samples taken from clinical sources where a higher rate of resistance appeared .
while they agreed with him in the presence of a high rate of resistance to Tigecycline [32].
The genetic study of the bacteria isolated in the current study and comparison of its results with the phenotypic resistance to antibiotics shows that some genes play an active role in this resistance, which poses a threat to the community health and treatment system, this is in consistent with PCR technition was used in the current study to detect the found of the *acc* gene in the isolated pathogenic bacteria .
The Figure (1) shown the an electrophoresis of the PCR production, which successfully identified by specific primers designed for the *acc* gene with band in size of (482 base pair)
and Figure (2) show that the *K.pneumoniae* bacteria carried the *aac(6')-Ib -cr* gene significantly more than the rest of the other genera, while the *P. aeruginosa* were the least significant in their possession of this gene, which encodes antibiotic resistance of the aminoglycoside type. Figure (2) also shows clear significant differences under p=0.05in bacterial number that carrying that gene. This result was similar to what was obtained [33] where *the aac(6')-Ib-cr* gene was detected in both *E.coli* and *Klebsiella*, and this gene was the most common among the quilonones resistant genes (QNG).
The found of *aac(6')-Ib -cr* genes in *E. coli* depends on geographical location, excessive use of antibiotics, and types of bacterial strains. Studies indicate variable detection rates, ranging from 20% to 80% depending on the *aac(6')-Ib -cr* variant and the specific region. The study of Ruiz and his colleagues in 2012 was similar to the diagnosis of this gene in *E. coli* bacteria[33], The presence of these genes can significantly limit treatment options for *E. coli* infection, increasing the risk of treatment failure and potentially life-threatening complications [34], As for *Staphylococcus aureus*, it still poses major challenges to public health in many regions due to antibiotic resistance problems associated with the *aac(6')-Ib-cr* gene presente [35], Studies show varying prevalence rates of this gene depending on many factors such as patterns of antibiotic use and specific types of *S. aureus* strains. The presence of the *aac(6')-Ib-cr* gene greatly reduces the effectiveness of antibiotics belonging to the aminoglycoside family, which It limits options for infections caused by resistant *S. aureus* and poses a major challenge in healthcare settings, contributing to morbidity and mortality [36] .
Polymerase Chain Reaction technique was used in current study to detect the presence of the *erm*A gene in isolated pathogenic bacteria. Figure (2) appears electrophoresis of the PCR products, through which it was shown that the primer for the *erm*A gene was successfully amplifying this gene through the appearance of a band in size of (645 basepai).

Figure(4) shows that the *P.aeruginosa* were significantly low in their possession of the *erm*A gene, which encodes resistance against erythromycin-type antibiotics, while the rest of the bacterial genera were insignificant in their possession of the gene, despite the fact that *E.coli* bacteria were higher in number in It possesses the gene, and this result was similar to a study conducted in 2023, when three isolates carrying the *erm*A gene appeared among 92 samples of bacteria by researcher Duman [37].

while Figure (5) indicates the success of the primers for the *qnr* gene in the isolated pathogenic bacteria through the appearance of a 645 bp band during the electrophoresis process of the PCR products. The results showed that the *K.pneumoniae* bacteria was significantly higher It possesses this gene that encodes resistance to ceftazidime antibiotics (p=0.014), while no significant differences appeared between the rest of the bacterial genera in the number of genes they carry.

qnr gene frequency amongst clinical isolates of pathogenic bacteria described in present study is higher than the frequency that was confirmed in the study of researcher Rezazadeh in 2016 [38], and the result was also similar to a study conducted by researcher Wang and his group in 2008 . Although *qnr* genes are the farthest resistant agents contra (β-lactam and quinolone), the rate of resistance to other antibiotics has risen sharply recently and increased detection of *qnr* genes poses a challenge due to limited treatment options for quinolone-resistant bacteria. These genes give bacteria the capability to change or shield the antimicrobial drug's target region, obstructing the efficacious course of treatment.

It is possible to observe this tactic in quinolone resistance. Since the quinolones that were discovered were wholly artificial antimicrobials, it was thought that quinolone resistance (qnr) genes did not occur naturally.

The *shv* gene was also detected in pathogenic bacteria isolated from various clinical sources in this study by using the polymerase chain reaction (PCR) technique. Figure 7 explain the electrophoresis of PCR productive, during it was shown that the primers) of the *shv* gene was successfully in amplified this gene by the appearance of 593 bpBand.

It appears from Figure (8)that Klebsiella bacteria are the only bacteria studied, as seven isolates out of eight carried the *shv* gene, while the rest of the bacterial genera were missing these genes. This result was comparable to a local study carried in 2023 [39], There is also a study conducted in 2022 by researcher Ghenea and others that among 14 isolates of *E.coli* contained the *bla shv-1* gene [40]. Figure (9) deal with the electrophoresis of PCR production, that showed the primers for the *tem* gene clearly amplified the gene through the appearance of a band of 850 bp.

Determining the antibiotic susceptibility of bacterial isolates, especially as resistance increases, is often crucial [41]. It is shown from Figure 10 that the only *Klebsiella* bacteria that carried the *tem* gene were significantly different from the rest of the genera, followed by *Pseudomonas aeruginosa* with the presence of one isolate, while all the isolates of *Staphylococcus* and coliform bacteria did not carry the gene. This result is similar to the study of researchers [39] in The missing of this gene in *E .coli* bacteria.

Resistance can occur due to the different mechanisms such as enzyme presence that enhance the antimicrobial agent, or alternative enzyme for the specific enzyme that is inhibited. Resistance might occur under mutation condition in the structure of the target on which the antibiotic acts, which decrease the antimicrobial agent binding or post-transcriptional yeild or post-translational modification of the target of the antimicrobial agent occurs.

which reduces binding of the antimicrobial agent, In some cases of resistance there is a decrease in the rate of absorption of antibiotics by microorganisms, or it may be the result of an artificially active biological agent [42]. Table (4) shows the number and types of antibiotic resistance genes that were isolated from bacteria used under study.

It is noted in Table (3) that the number of bacterial isolates of *Klebsiella* carrying genes that encode resistance to the studied antibiotics was significantly higher compared

to the rest of the bacterial genera under study ($p \leq 0.05$), as these are observed in the results of the current investigation regarding the presence of isolates of bacteria. *Klebsiella* carries the Tem and *shv* genes that encode the resistance trait to the β-Lactam family by [43] also the presence of isolates carrying the aac gene with the study of Doi and his group[44]. As for the presence of bacterial isolates carrying the *ermA* gene, it agrees with What was found [45].

On the other hand, the results displayed in this table indicate that the level of probability ($p < 0.05$) of *qnr* gene presence varies significantly between the clinical isolates. Previous research has verified that clinical isolates of *E. Coli*, *S. aureus*, and *P. aeruginosa* had the lower frequencies of *qnr* genes. However, prior research on *S. aureus* demonstrated a strong association between resistance to aminoglycosides, chloramphenicol, clindamycin, erythromycin, trimethoprim, and tetracycline and the presence of resistance genes encoding these substances. For example, it was found that 83.4% of S. aureus strains resistant to erythromycin carry the ermA gene [46].

The dominance of the ermA gene dominant the other erythromycin resistance genes in *S. aureus* has previously been confirmed [47], but in the current study the average number of isolates that showed the *erm*A resistance gene was less than the number of isolates that showed the *aac6'-Ib-cr* resistance gene .

The study of antibiotic resistance genes, which is the ability of bacteria to progresses defense tactic that make them difficult to treat, has become of great importance. The emergence of mutations in the genes that cause resistance may make treating bacterial infections more difficult, antimicrobial resistance develops spontaneously as a result of genetic mutations or as a result of the transfer of resistance from one species that possesses it to another that does not in genetic manner [48].

These bacterial isolates were successfully isolated using PCR technology, which is characterized as a simple, fast technology with high sensitivity and specificity. PCR technology was depended in the current study to detect the presence of five types of genes (*ermA, qnr, shiv aac(6')-Ib -cr, tem*) in the pathogenic bacterial species under study.

Reference:

1. Bin Zaman S, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A review on antibiotic resistance: alarm bells are ringing. Cureus 2017, http://dx.doi.org/10.7759/cureus.1403
2. Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc. Natl. Acad. Sci. Unit. States Am. 115, E3463–E3470. https://doi.org/10.1073/pnas.1717295115
3. Huemer, M., Mairpady Shambat, S., Brugger, S. D., & Zinkernagel, A. S. (2020). Antibiotic resistance and persistence—Implications for human health and treatment perspectives. *EMBO reports*, *21*(12), e51034.
4. Munita, J. M., and Arias, C. A. 2016. Mechanisms of Antibiotic Resistance. Microbiology spectrum, 4(2), 10.1128/microbiolspec.VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016- 2015
5. **Bacterial Antibiotic Resistance: The Most Critical PathogensGiuseppe Mancuso, Angelina Midiri ,Elisabetta Gerace Carmelo Biondo,**Department of Human Pathology, University of Messina, 98125 Messina, ItalyIRCCS Centro Neurolesi "Bonino-Pulejo", 98100 Messina, Italy Author to whom correspondence should be addressed. *Pathogens* **2021**, *10*(10), 1310; **https://doi.org/10.3390/pathogens10101310**

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6. Sreeja MK, Gowrishankar NL, Adisha S, Divya KC. Antibiotic resistance-reasons and the most common resistant pathogens – a review. ResJ Pharm Technol 2017;10:1886–90, http://dx.doi.org/10.5958/0974-360X.2017.00331.6.
7. Sulis G, Daniels B, Kwan A, Gandra S, Daftary A, Das J, et al. Antibiotic overusein the primary health care setting: a secondary data analysis of standardisedpatient studies from India, China and Kenya. BMJ Glob Heal 2020;5:3393,http://dx.doi.org/10.1136/bmjgh-2020-003393.
8. Collignon, P.; Beggs, J.J. Socioeconomic Enablers for Contagion: Factors Impelling the Antimicrobial Resistance Epidemic.Antibiotics **2019**, 8, 86. [CrossRef] [PubMed]
9. Majeed, H. T., and Aljanaby, A. A. J. 2019. Antibiotic Susceptibility Patterns and Prevalence of Some Extended Spectrum Beta-Lactamases Genes in Gram-Negative Bacteria Isolated from Patients Infected with Urinary Tract Infections in Al-Najaf City, Iraq. Avicenna journal of medical biotechnology, 11(2),192.
10. Partridge SR, Kwong SM, Firth N et al. Mobile genetic elements associated with antimicrobial resistance. ClinMicrobiol Rev 2018; 31: e00088–17
11. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13: 42 – 51
12. Sun, D.; Jeannot, K.; Xiao, Y.; Knapp, C.W. Editorial: Horizontal Gene Transfer Mediated Bacterial Antibiotic Resistance. Front. Microbiol. 2019, 10, 1933.
13. Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic E. coli isolated from adolescents and adults in Hamedan, Western Iran. Iranian journal of microbiology 2013; 5(1): 42-7.
14. PEYMANI,A :NASERPOUR-FARIVAR, T : and AZARHOOSH E. ZARE, KH.(2017). Distribution of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing P. aeruginosa isolated from Qazvin and Tehran hospitals, Iran. J PREV MED HYG ; 58: E155-E160.
15. Juda, M., Chudzik-Rzad, B., & Malm, A. (2016). The prevalence of genotypes that determine resistance to macrolides, lincosamides, and streptogramins B compared with spiramycin susceptibility among erythromycin-resistant *Staphylococcus epidermidis*. *Memórias do Instituto Oswaldo Cruz*, *111*(3), 155-160.
16. Jacoby, G. A., Chow, N., Waites, K. B. 2003. Prevalence of Plasmid-Mediated Quinolone Resistance .Antimicrobial Agents and Chemotherapy, 47(2): 559-562.
17. Kim E.S.; Jeong J.; Jun J.; Choi S.; Lee S.; Kim M.; Woo J. and Kim Y. (2009). Prevalence of aac(6')-Ib-cr Encoding a Ciprofloxacin-Modifying Enzyme among Enterobacteriaceae Blood Isolates in Korea. Antimicrob Agents Chemother; 53(6):2643-2645
18. Yang, S. C., Lin, C. H., Sung, C. T., & Fang, J. Y. (2014). Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. Frontiers in Microbiology, 5, 241. https://www.frontiersin.org/journals/microbiology
19. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L.(1996) Detection of erythromycin-resistant determinants by PCR. Antimicrobial agents and chemotherapy,; 40(11):2562–2566. https://doi.org/10.1128/ AAC.40.11.2562 PMID: 8913465
20. H., Abdelrahman, Hassan, D. I. N. A., El-Moaafy, M. A., Saleh, H., A. A., & Abdallah, S. E. (2022). Characterization of Quinolone Resistance Genes in Uropathogenic Escherichia coli Isolates, in Egypt. *Records of Pharmaceutical and Biomedical Sciences*, *6*(2), 163-172.
21. Tay, W. T., Mahon, R. J., Heckel, D. G., Walsh, T. K., Downes, S., James, W. J., ... & Gordon, K. H. (2015). Insect resistance to Bacillus thuringiensis toxin Cry2Ab is conferred by mutations in an ABC

transporter subfamily A protein. *PLoS genetics*, *11*(11), e1005534.
22. Katz, D. S. (2010). Coagulase test protocol. *American Society for Microbiology Laboratory Protocols. Available online: https://www. asmscience. org/content/education/protocol/protocol, 3220.*
23. Bo L, Dandan Z, Siyu Z, Lihong Ch, Jian Y. VFDB 2022: a general classification scheme for bacterial virulence factors, Nucleic Acids Research,2022 and 50(7): 912–D917, https://doi.org/10.1093/nar/gkab1107)
24. Ait-Mimoune, N., Hassaine, H., & Boulanoir, M. (2022). Bacteriological profile of urinary tract infections and antibiotic susceptibility of Escherichia coli in Algeria. *Iranian journal of microbiology*, *14*(2), 156.
25. Shamki, J. A., Al-Charrakh, A. H., & Al-Khafaji, J. K. (2012). Detection of ESBLs in Enteropathogenic E. coli (EPEC) isolates associated with infantile diarrhea in Kut City. *Medical Journal of Babylon*, *9*(2), 403-412.
26. Raheem, H. Q., Hussein, E. F., Ghosh, S., & Alkafaas, S. S. (2021). Resistance of Klebsiella pneumoniae from Different Clinical Samples to Penicillin, Cephalosporin, Carbapenem and Fluoroquinolone (Vol. 44, Issue 06). http://atlas.ecdc.europa.eu/public/index.aspx?Instance
27. Ali, S. Q., & Kamil, Y. M. (2022). Identifying the Resistant Bacterial Pattern in Patients with Diabetic Foot Ulcer. Journal for Research in Applied Sciences and Biotechnology, 1(4), 151–158. https://doi.org/10.55544/jrasb.1.4.20
28. Brennan-Krohn, T., Grote, A., Rodriguez, S., Kirby, J. E., & Earl, A. M. (2022). Transcriptomics reveals how minocycline-colistin synergy overcomes antibiotic resistance in multidrug-resistant Klebsiella pneumoniae. *Antimicrobial Agents and Chemotherapy*, *66*(3), e01969-21.
29. Khan, T., Ullah, H., Nasar, A., & Ullah, M. (2023). Antibiotic Resistance and sensitivity pattern of Pseudomonas aeruginosa obtained from clinical samples. *Lett Appl NanoBioScience*, *12*(4), 112.
30. Olowo-okere, A., Ibrahim, Y. K. E., Ehinmidu, J. O., Mohammed, Y., Nabti, L. Z., & Olayinka, B. O. (2020). Emergence of VIM metallo-β-lactamase among carbapenem-resistant Pseudomonas species in northwest Nigeria. *Gene Reports*, *21*, 100877.
31. Nazari-Alam, A., Badie, F., Shaeri, M., Moniri, R., Akbari, H., & Mansoori, M. (2021). The Bacterial Profile and Microbial Susceptibility of Acute and Chronic Dacryocystitis in Matini Hospital, Kashan, Iran. Jundishapur Journal of Microbiology, 14(5).
32. Petrillo, F., Folliero, V., Santella, B., Franci, G., Foglia, F., Trotta, M. C., ... & Galdiero, M. (2020). Prevalence and antibiotic resistance patterns of ocular bacterial strains isolated from pediatric patients in University Hospital Of Campania "Luigi Vanvitelli," Naples, Italy. International Journal of Microbiology, 2020, 1-6.
33. Ruiz, E. , Sáenz,Y. , Zarazaga,M., Rocha-Gracia,R., Martínez-Martínez,L., Arlet, G. and Torres,C. (2012) . *qnr*, *aac(6')-Ib-cr* and *qepA* genes in *Escherichia coli* and *Klebsiella* spp.: genetic environments and plasmid and chromosomal location, *Journal of Antimicrobial Chemotherapy*, Volume 67, Issue 4, April 2012, Pages 886–897, https://doi.org/10.1093/jac/dkr548
34. Urban-Chmiel R, Marek A, Stępień-Pyśniak D, Wieczorek K, Dec M, Nowaczek A, Osek J. (2022). Antibiotic Resistance in Bacteria-A Review. Antibiotics (Basel). Aug 9; 11(8):1079.
35. Patrick E. Akpaka, Rashida Roberts, Stefan Monecke, (2017). Molecular characterization of antimicrobial resistance genes against Staphylococcus aureus isolates from Trinidad and Tobago, Journal of Infection and Public Health, Volume 10, Issue 3, 2017, Pages

36. Safarpoor Dehkordi F, Gandomi H, Basti AA, Misaghi A, Rahimi E.(2017). Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrob Resist Infect Control. 2017 Oct 4;6:104. doi: 10.1186/s13756-017-0257-1. PMID: 29034091; PMCID: PMC5628482.
37. Duman, M., Woo, S. J., Altun, S., & Satıcıoğlu, I. B. (2023). Tentative Epidemiological Cut-Off Values and Distribution of Resistance Genes in Aquatic Pseudomonas Species Isolated from Rainbow Trout. *Current Microbiology*, *80*(5), 157.
38. Rezazadeh M, Baghchesaraei H, Peymani A.(2016). Plasmid-Mediated Quinolone-Resistance (qnr) Genes in Clinical Isolates of Escherichia coli Collected from Several Hospitals of Qazvin and Zanjan Provinces, Iran. Osong Public Health Res Perspect. 2016 Oct and 7(5):307-.
39. Al-Tuhmazi, H., A., (2023) . Determine the Activity of Antibiotics Resistance against Bacterial Causative Agents of Diarrhea among Children Under the Age of 5 Years . Department of Biology, College of Sciences, Kerbala University, Kerbala, Iraq
40. Ghenea, A. E., Zlatian, O. M., Cristea, O. M., Ungureanu, A., Mititelu, R. R., Balasoiu, A. T., ... & Balasoiu, M. (2022). TEM, CTX-M, SHV genes in ESBL-producing Escherichia coli and Klebsiella pneumoniae isolated from clinical samples in a county clinical emergency hospital Romania-predominance of CTX-M-15. *Antibiotics*, *11*(4), 503.
41. Banerjee R and Patel R.(2023). Molecular diagnostics for genotypic detection of antibiotic resistance: current landscape and future directions. JAC Antimicrob Resist. 2023 Feb 17 and 5(1):dlad018
42. Murugaiyan J, Kumar PA, Rao GS, Iskandar K, Hawser S, Hays JP, Mohsen Y, Adukkadukkam S, Awuah WA, Jose RAM, Sylvia N, Nansubuga EP, Tilocca B, Roncada P, Roson-Calero N, Moreno-Morales J, Amin R, Kumar BK, Kumar A, Toufik AR, Zaw TN, Akinwotu OO, Satyaseela MP, van Dongen MBM.(2022). Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics. Antibiotics (Basel). 2022 Feb 4;11(2):200. doi: 10.3390/antibiotics11020200. PMID: 35203804; PMCID: PMC8868457.
43. Li CF, Tang HL, Chiou CS, Tung KC, Lu MC, Lai YC.(2018). Draft genome sequence of CTX-M-type beta-lactamase-producing Klebsiella quasipneumoniae subsp. similipneumoniae isolated from a Box turtle. J Glob Antimicrob Resist. 2018 and 12:235–6.
44. Doi Y, Wachino JI, Arakawa Y. (2016). Aminoglycoside resistance: The emergence of acquired 16S ribosomal RNA methyltransferases. Infect Dis Clin North Am. 2016 and 30(2):523–37. DOI: 10.5897/AJMR12.1517 ISSN 1996-0808 ©2013 Academic Journals
45. Safika, S., Nilasari, Z. and Pasaribu, F.H., (2022). Detection of antibiotic resistance coding gene in Klebsiella pneumoniae bacteria isolated from broiler chickens in West Java, Indonesia. Journal of Applied Pharmaceutical Science, 12(7), pp.190-198.
46. Aggarwal S, Jena S, Panda S, Sharma S, Dhawan B, Nath G, Singh NP, Nayak KC, Singh DV.(2019). Antibiotic Susceptibility, Virulence Pattern, and Typing of *Staphylococcus aureus* Strains Isolated From Variety of Infections in India. Front Microbiol. 2019 Dec 4;10:2763. doi: 10.3389/fmicb.2019.02763. PMID: 31866962; PMCID: PMC6904308.

47. Kaur, D. C., and Chate, S. S. (2015). Study of antibiotic resistance pattern in methicillin resistant Staphylococcus aureus with special reference to newer antibiotic. J. Glob. Infect. Dis. 7, 78–84.

48. Reygaert WC.(2018). An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. Jun 26;4(3):482-501. doi: 10.3934/microbiol.2018.3.482. PMID: 31294229; PMCID: PMC6604941.