The effect of mutation in 23SrRNA regions on *cfr, fexA, cmlA*, and *floR*, genes amplification of bacteria resistant to chloramphenicol and florfenicol

Majid A. Kadhim and Munaff J. Abd Al-Abbas

Biology Department, College of Science, University of Basrah, Basrah, Iraq

Abstract

Bacterial resistance to chloramphenicol and florfenicol become one of the modern problems that threatens humans and animals alike, 107 bacterial isolates from animal (42) and human (75) were subjected to the four reactance genes *cfr, floR, floR* and *cmlA*. Although the resistance was appeared for florfenicol and chloramphenicol (55% and 72% versus 48% and 50% respectively) on the plate, but not any gene was detected by PCR.

The nucleotides sequenced of region 1 of the *23SrRNA* gene for the 9 isolates No. 40- *S. epidermidis*, 73- *S. hominis*, 105- *S. aureus*, 7- *A. baumannii*, 11-*E. indicum*, 128-*S. equinus*, 129- *S. bovis* and 141- *P. aeruginosa* showed many mutations except to isolate No.14-*B.safensis* with no mutation. Therefore, there were many different alleles at this region in comparison with 101 different bacterial species from Genbank. Furthermore, all the nucleotide sequences among these 9 isolates were varied greatly. While, the 23SRNA region 2 sequencing of 128-*S. equinus, 129- S. bovis,73-S. hominis, 70- S. epidermidis* and *93- S. epidermidis* showed identical or very little mutations, but a wide range of different alleles were found in this region when compared to 101 different bacterial species of GenBank. Many differences in nucleotide sequencing were observed among these 5 bacterial species. On the other hand, the eight isolates 73 -*S. hominis*, 105- *S. aureus*, 7 *A. baumannii*, 11 *E. indicum*, 128 *S. equinus* and 141 *P. aeruginosa* showed numerous nucleotide mutations with the exception of 70 *S. epidermidis* and 14 *B. safensis* which had very few mutations. However, the region 3 sequencing of each of those isolates

compared to 101 distinct bacterial species from GenBank showed several different alleles. But the nucleotide sequences among these eight bacterial species revealed a high percentage of allelic similarity.

Comparing to *16SrRNA* gene identification, region 1 of the *23SrRNA* gene was the most effective for accurately identifying bacteria (82%) with a significant difference from regions 3 (72%) and 2 (45%). Since, the three regions for *S. hominis* and *S. equinus* were amplified 100% at the time, followed *S. epidermidis, S. aureus, A. baumannii, E. indicum, B. safensis, S. bovis and P. aeruginosa* (66% for each), and 40 *S. epidermidis* and *S. epidermidis* produced only 33% for each.

Keyword : Florfenicol , chloramphenicol and cfr, fexA, cmlA gene resistance

Introduction

Antimicrobial resistance mutations change the way antibiotics action through one of the following mechanisms: (1) changes to the antimicrobial target reducing the drug's affinity especially 23SrRNA region (2) a reduction in drug uptake (3) activation of efflux mechanisms to extrude the toxic molecule (4) global changes in key metabolic pathways through regulatory network modulation, therefore, changes in drug targets, active efflux, decreased membrane permeability and enzymatic inactivation of antimicrobial drugs are some of the main mechanisms by which antibiotic resistance develops in bacteria [1],[2]. Compared to chloramphenicol, the derivative florfenicol exhibits lower toxicity and it is effective against bacteria that have developed a gene making them resistant to chloramphenicol. However, florfenicol is currently used only in veterinary medicine, where it is used to treat illness in animals, numerous bacterial species have been found to acquire resistances to florfenicol [3]. However, inhibitors of bacterial protein production include chloramphenicol and florfenicol, bacterial pathogens have developed and/or acquired chloramphenicol resistance as a result of the use of chloramphenicol in human and veterinary medicine [4].

Some researchers found few frequency of *fexA, fexB* and *cfr* [5]. Also, [6] investigated *cfr* negative MRSA strains isolated from animals and humans in different geographical areas

of China, even these isolates were resistant to chloramphenicol and florfenicol. As these antimicrobial agents may potentially induce cross-resistance between animal and human bacterial pathogens, their prudent use in veterinary medicine is highly recommended [7]

This study is deal with the different bacterial species from animal and human sources resistant to chloramphenicol and florfenicol and their relation to the mutation in 23SrRNA region.

Material and methods

Bacterial isolates

All the 107 bacterial species were received from the previous study of [8], [9] ″under publishing″

Antibiotic Susceptibility discs

The bacterial isolates were collected at 37ºC for 24 h on blood agar base (HIMEDIA, India) supplemented with 5% shape blood and 30µg /ml chloramphenicol (HIMEDIA, India). All the chloramphenicol resistant bacterial isolates were growth on Muller –Hinton agar (HIMEDIA, India) for 30µg florfenicol disc (HIMEDIA, India) investigation as modified of [10].

Antibiotic susceptibility (PCR)

***Cfr* , *fexA*, *cmlA*, *floR* and *23SrRNA* gene primer**

The *cfr* gene primer, reagents and program according to [10] are listed in Table (1- 3).

Table (1): Primer sequence

Primer name	DNA sequences (5′-3′)	Product size (bp)	Reference
cfr	F-TGA AGT ATA AAG CAG GTT GGG AGT CA R- ACC ATA TAA TTG ACC ACA AGC AGC	746	Kehrenbergand Schwarz (2006)
fexA	F-AGAGTTTGATCCTGGCTCAG R-GGTTACCTTGTTACGACTT	1.272	Kehrenbergand Schwarz (2006)
cmlA	F- CCGCCACGGTGTTGTTGTTATC R-CACCTTGCCTGCCCATCATTAG	698	Kuo *et al*., (2009).
floR	F-AGAGTTTGATCCTGGCTCAG R-GGTTACCTTGTTACGACTT		Kuo *et al*., (2009).
23S1	F- GGTTAAGTTAATAAGGGCGC R- TTTCGACTACGGATCTTAGC	1011	Miyoshi *et al*. (2005)
23 center	F- CTGTTTGGGTGAGGGGTCC R- ACCTGCATCTTCACAGGTAC	1060	Miyoshi *et al*. (2005)
23S 3	F- CGGCGGCCGTAACTATAACG R- TTGGATAAGTCCTCGAGCTATTAG	103	Miyoshi *et al*. (2005)

Table (2): Reagents (25µl) of PCR amplification

Reagent	Volume (µl)
Go Taq green master mix	12
DNA template	2
Primer forward (10 picomol\ml)	1
Primer reverse (10 picomol\ml)	1
Nuclease Free Water	9
Total volume	25

Table (3): PCR program amplification

Stage	Temperature	Time	Cycles
Initial denaturation	95° C	5 min	1
Denaturation	94° C	1 min	35
Annealing	55° C*	2min	
Extension	72° C	3 min	
Final extension	72° C	7 min	1

**cfr* =55ºC, *fexA*=58ºC, *cmlA*= 55ºC, *floR*=55ºC, and= *23SrRNA*= 55ºC

The bands were detected on agarose gel (1.5%) electrophoresis and photographed under UV transilluminator (Wisl, Korea).

Sequencing of *23SrRNA* gene

Only 11 bacterial species (3 regions for each =33) that showed antibiotic resistance to chloramphenicol and florfenicol on the plate but negative to their genes (PCR) were nucleotides sequenced for the 3 regions of *23S rRNA* gene for each bacterial species according to the Macrogen Biotechnology Company protocol and the *23S r RNA* gene of each of the 11 samples were sent for sequencing.

Bacterial species identification and the phylogenetic tree of the 3 regions of 23SrRNA

Each region of the 23SrRNA of the 11 bacterial species were identified by the 23SrRNA sequence using BLAST program and aligned together with their reference strains of GenBank using multiple sequence alignment. The phylogenetic tree was constructed by MAFFT program of Molecular Evolutionary GeneticsAnalysishttp://www.megasoftware.net [13].

Results

The antibiotic discs for bacterial species (n=75) isolated from human were resistant to Chloramphenicol (72%) and Florfenicol (53%). While, 50% and 48% of the bacteria isolated from animals (n= 42) as Table (4 and 5) respectively, with no significant differences (P≤ 0.05). However, all bacterial species showed negative results for *cfr, fexA, floR* and *cmlA* genes.

Table (4): Chloramphenicol and florfenicol resistant discs and genes (*cfr, fexA, cmlA* and *floR)* of bacterial species from human

No	No isolate	no	Resistance					
			Discs n (%)		PCR			
			C	F	*cfr*	*fexA*	*floR*	*cmlA*
1	*S.epidermidis*	14	11 (79)	9 (62)	-	-	-	-
2	*E.coli*	11	6 (54)	4 (36)	-	-	-	-
4	*S. homins*	4	2 (50)	2 (50)	-	-	-	-
3	*S.aeruus*	7	5 (71)	3 (49)	-	-	-	-
5	*E. feacalis*	6	4 (67)	2 (33)	-	-	-	-
6	*S. hemolytic*	4	2 (50)	3 (75)	-	-	-	-
7	*K. pneumonia*	4	4 (100)	2 (50)	-	-	-	-
8	*P. aeruginosa*	10	9 (90)	7 (70)	-		-	-
9	*Acinetobacter Baumannii*	2	2 (50)	2 (100)	-	-	-	-
10	*E.mori*	1	1 (100)	1 (100)	-		-	-
11	*E.fergusonii*	1	1 (100)	1 (100)	-	-	-	-
12	*E buggndesis*	1	-	-	-	-	-	-
13	*E .horamechie*	7	5 (71)	3(42)	-	-	-	-
14	*E . ludwigii*	1	-	-	-	-	-	-
15	*P.msselii*	1	1 (100)	-	-	-	-	-
16	*B.safensis*	1	-	1(100)	-	-	-	-
	Total no(%)	75	54 (72)	40 (53)	-	-	-	-

*P≤ 0.05

*Chloramphenicol (C), Florfenicol (F). -: Negative PCR result

Table (5): Chloramphenicol and florfenicol resistant discs and genes (*cfr, fexA, floR*, and *cmlA*) for bacterial species from animals

N0	Bacterial species	Antibiotic resistance						
		Discs n (%)			PCR			
			C	F	*cfr*	*fexA*	*floR*	*cmlA*
1	*Staphylococcus aureus*	2	1(50)	-	-	-	-	-
2	*Lysinibacillus fusiformis*	1	-	-	-	-	-	-
3	*Lysinibacillus xylanilyticus*	1	-	-	-	-	-	-
4	*Mammaliicoccus sciuri*	1	1(100)	-	-	-	-	-
5	*Enterococcus cloacae*	4	1(25)	-	-	-	-	-
6	*Bacillus mycoides*	1	-	-	-	-	-	-
7	*Bacillus cereus*	4	4(100)	2(50)	-	-	-	-
8	*Bacillus thuringiensis*	1	-	1(100)	-	-	-	-
9	*Bacillus paramycoides*	1	-	1(100)	-	-	-	-
10	*Bacillus safensis*	1	1(100)	1(100)	-	-	-	-
11	*Acinetobacter venetianus*	1	1(100)	1(100)	-	-	-	-
12	*Acinetobacter baumannii*	2	2(100)	2(100)	-	-	-	-
13	*Escherichia coli*	2	-	-	-	-	-	-
14	*Exiguobacterium indicum*	1	1(100)	-	-	-	-	-
14	*Mammaliicoccus caseolyticus*	1	1(100)	-	-	-	-	-
15	*Staphylococcus warneri*	2	-	1(100)	-	-	-	-
16	*Staphylococcus sciuri*	1	1(100)	1(100)	-	-	-	-
17	*Staphylococcus pasteuri*	1	-	1(100)	-	-	-	-
18	*Staphylococcus felis*	1	-	1(100)	-	-	-	-
19	*Streptococcus equinus*	1	-	-	-	-	-	-
20	*Streptococcus bovis*	1	1(100)	1 (100)	-	-	-	-
20	*Pseudomonas aeruginosa*	2	2(100)	2 (100)	-	-	-	-
22	*Pseudomonas parafulva*	1	1(100)	1 (100)	-	-	-	-
23	*Comamonas kerstersii*	1	-	-	-	-	-	-
24	*Shewanella xiamensis*	1	-	-	-	-	-	-
24	*Klebsiella pneumonia*	1	1(100)	1(100)	-	-	-	-
38	*Alacligenes faecalis*	2	1(100)	1(50)	-	-	-	-
25	*Enterococcus gallinarum*	1	1(100)	-	-	-	-	-
26	*Bacillus rugosus*	1	-	-	-	-	-	-
27	*Pantoea agglomerans*	1	-	-	-	-	-	-
Total animal (%)		42	21 (50)	20 (48)	(0)	(0)	(0)	(0)

*P≤ 0.05

*Chloramphenicol (C), Florfenicol (F)

- : Negative PCR result

Region 1 of *23SrRNA*

Amplification of region 1 for 11 different bacterial species were detected by gel electrophoresis as bands at 1001 bp (Figure 1).

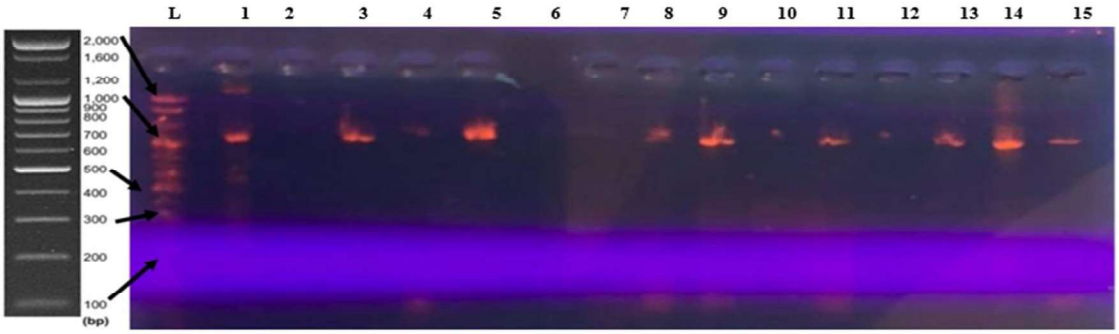
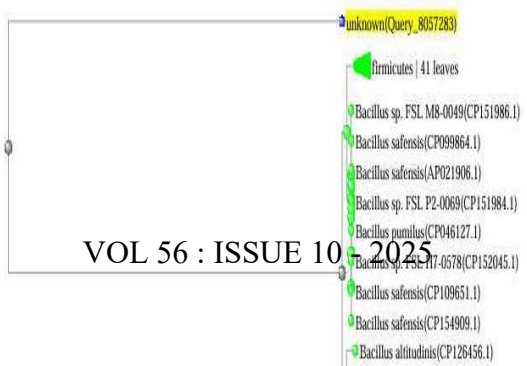


Figure (1): Agarose gel electrophoresis (1.5%) showed a model of region 1 band of *23SrRNA* gene (appromixlty1003bp).Lane L: 100bp marker. Lane 1, 3, 4, 5 ,8, 9, 10,11, 12, 13, 14 and 15 Region 1bands of *23Sr RNA*.

Alleles of *23SrRNA* region 1

The region 1 sequencing of *23S rRNA* gene for the 9 isolates No.40- *Staphylococcus epidermidis*, 73- *staphylococcus homins* ,105- *S .Staphylococus aureus*, 7- *Acinitibacter baumannii*, 11- *Exiguobactererium indicum,* 128 – *Sterptococus equinus*, 129 – *Sterptococcus bovis* and 141 – *Pseudomona aeruginosa* from the present study compared with their closely type strians from the GenBank showed many nucleotide mutations at this region except of isoloate No.14-*Bacillus safensis* with no any mutation, therefore, the comparison of the region 1 sequencing of each of those isolates with this region of 101 different bacterial species from GenBank showed many different allels at this region (Figur 2).



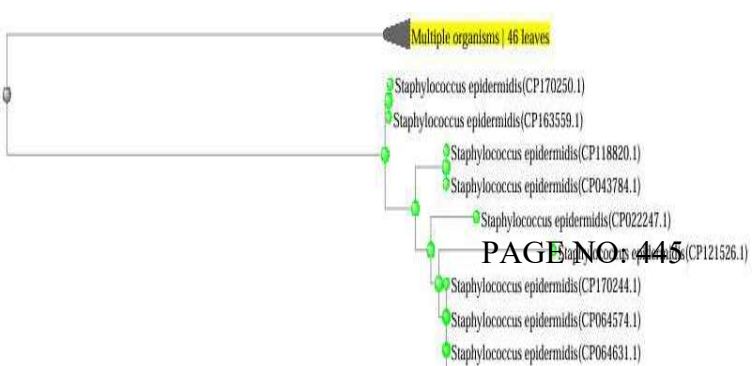


Figure (2): Comparison of regions 1 of *23S rRNA* gene in *Staphylococcus epidermidis* from the present study with the same region in 101 different bacterial species from Genbank showed 19 different alleles (19%).

Figure (10): Comparison of regions 1 of *23S rRNA* gene in *Pseudomonas aeruginosa* from the present study with the same region in 101 different bacterial species from Genbank showed 15 different alleles (15%).

Comparison among *23S rRNA* gene region 1 sequencing

The region 1sequencing of *23S RNA* gene of the 9 bacterial species bacterial species 40- *S. epidermidis,73- S. hominis,* 105- *S. aureus, 7 - A. baumannii,* 11-*E. indicum,* 14- *B. safensis,* 128-*S.*

equinus, 129- *S. bovis and* 141- *P. aeruginosa* showed very different nucleotides sequencing among these isolates except only 8 nucleotide at all the alignment (Figure 3).

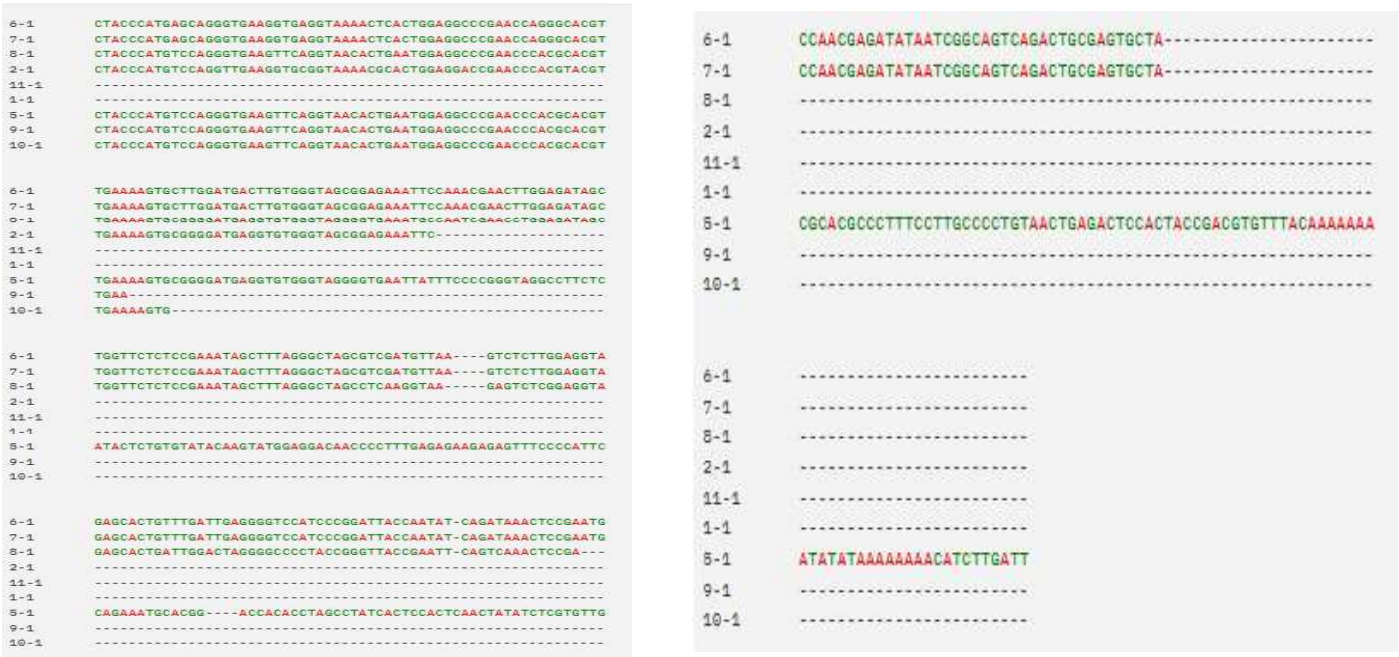


Figure (3): Multiple sequence alignments of *23Sr RNA* region 1of the 9 studied isolates showing different along the nucleotide sequence.

Region 2 of *23SrRNA*

Amplification of region 2 for 11 different bacterial species were detected by gel electrophoresis as bands at 1000bp (Figure 4).

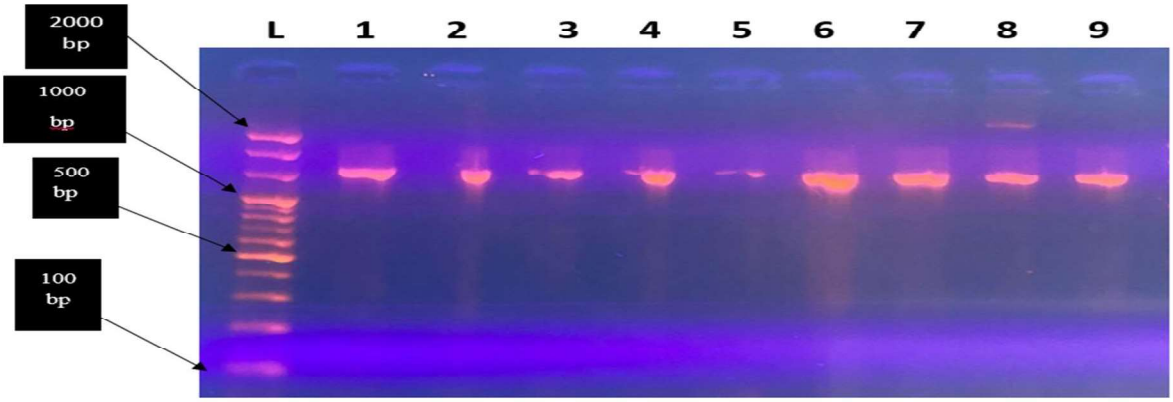


Figure (4): Agarose gel electrophoresis (1.5%) showed a model of amplified region 2 of *23SrRNA* gene at (1060bp).Lane L: 100bp marker. Lane 1, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14 and 15: Region 2 bands of *23Sr RNA.*

Alleles of *23SrRNA* region 2

The regions 2 sequencing of *23S RNA* gene for the 5 isolates No. 73-*Staphylococcus hominis,* 70-*Staphylococcuse epidermidis,* 93-*Staphylococcus epidermidis* 128-*Streptococcus equinus* and 129 - *Streptococcus bovis* from the present study compared with their closely type strain from GenBank showed more closely or identical nucleotide sequencing especially for *S. epidermidis*, respectively. Moreover, the comparison of this region of each of those isolates with this region in 101 different bacterial species of the GenBank showed many different alleles at this region (Figure5).

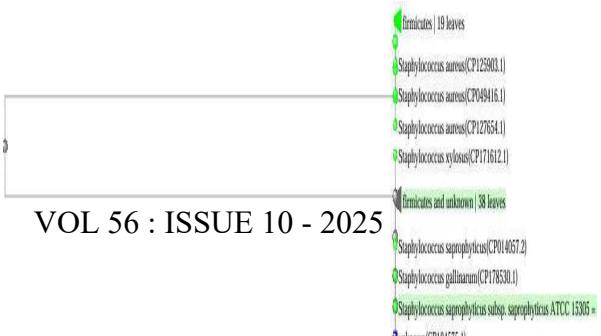


Figure (5): Comparison of regions 2 of *23SrRNA* gene in *Staphylococcus hominis* from the present study with the same region in 101 different bacterial species bacteria from Genbank showed 10 different alleles (10%).

Comparison among 23S *rRNA* gene region 2 sequencing

The region 2 sequencing of *23S rRNA* gene of the 5 bacterial species 73- *S. hominis*, 70- *S. epidermidis,* 93-*S.epidermidis,* 128- *S. equinus* and 129- *S. bovis* showed very different nucleotide sequencing among these isolates (Figure 6).

Figure (6): Multiple sequence alignments of *23S rRNA* region 2 of the 5 studied isolates showing differences along the nucleotide sequences.

Region 3 of *23SrRNA*

Amplification of region 3 for the 11 different bacterial species detected by gel electrophoresis as bands at 1000bp (Figure7).

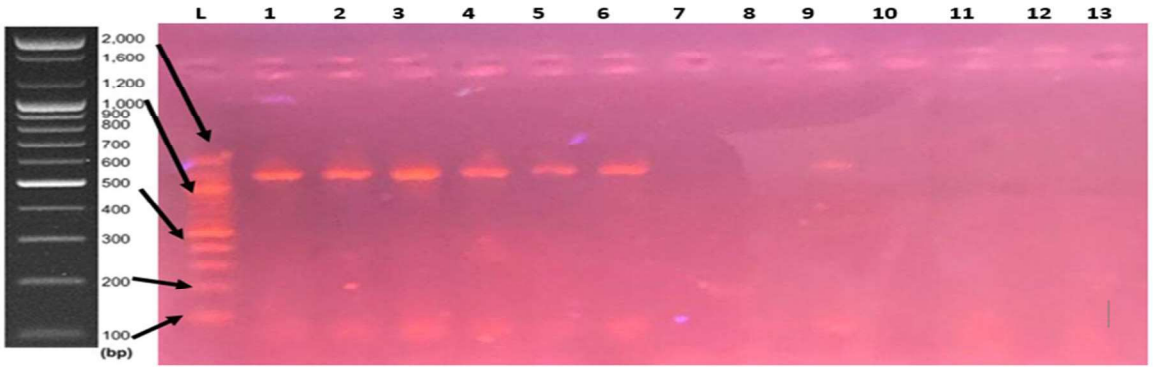


Figure (7): Agarose gel electrophoresis (1.5%) showed a model of region 3 band of *23SrRNA* gene (100bp). Lane L: 100bp marker. Lane 1, 2, 3, 4, 5, 6 and 9: Region 2 bands of *23Sr RNA.*

Alleles of *23SrRNA* region 3

The region 3 sequencing of *23S rRNA* gene for the 8 isolates, 73- *Staphylococcus hominis*, 105-,*Staphylococcus aureus*, 7-*Acinetobacter baumannii*, 11-*Exigubacterium indicum,128- Streptococcus equinus* and 141- *Pseudomonas aeruginosa* from the present study compared with their closely type strain from the GenBank **showed** many nucleotide mutations at this region except for 70 -*S . epidermidis* and 14 *B. safensis* were with very few mutations. On the other hand, the comparison of the region 3 sequencing of each of those isolates with this region of 101 different bacterial species in the GenBank showed many different alleles at this region (Figure 8).

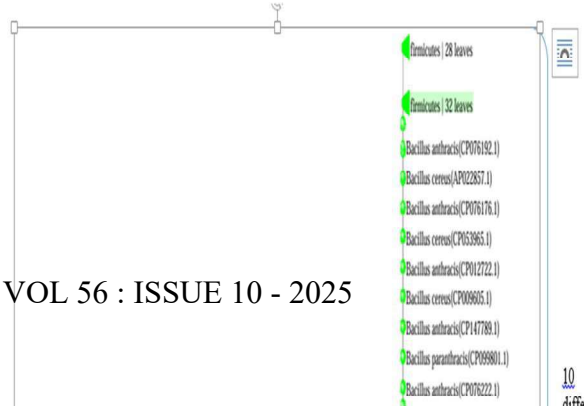
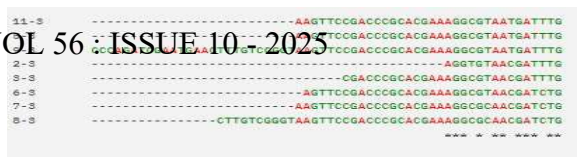


Figure (8): Comparison of regions 3 of *23S rRNA* gene in *Staphylococcus hominis* from the present study with the same region in 101 different bacterial species from Genbank showed 10 different alleles (10%).

Comparison among *23S rRNA* gene region 3 sequencing

The region 3 sequence of *23Sr RNA* gene of the 8 bacterial species *73- S .hominis, 70 –S. epidermidis, 105 – S .aureus, 7 A .baumannii, 11- E. indicum, 14- B. Safensis, 128 – S .equinus and 141- P. aeruginosa* showed a large percentage of similarly among the nucleotide sequencing of these isolate (Figure 9).



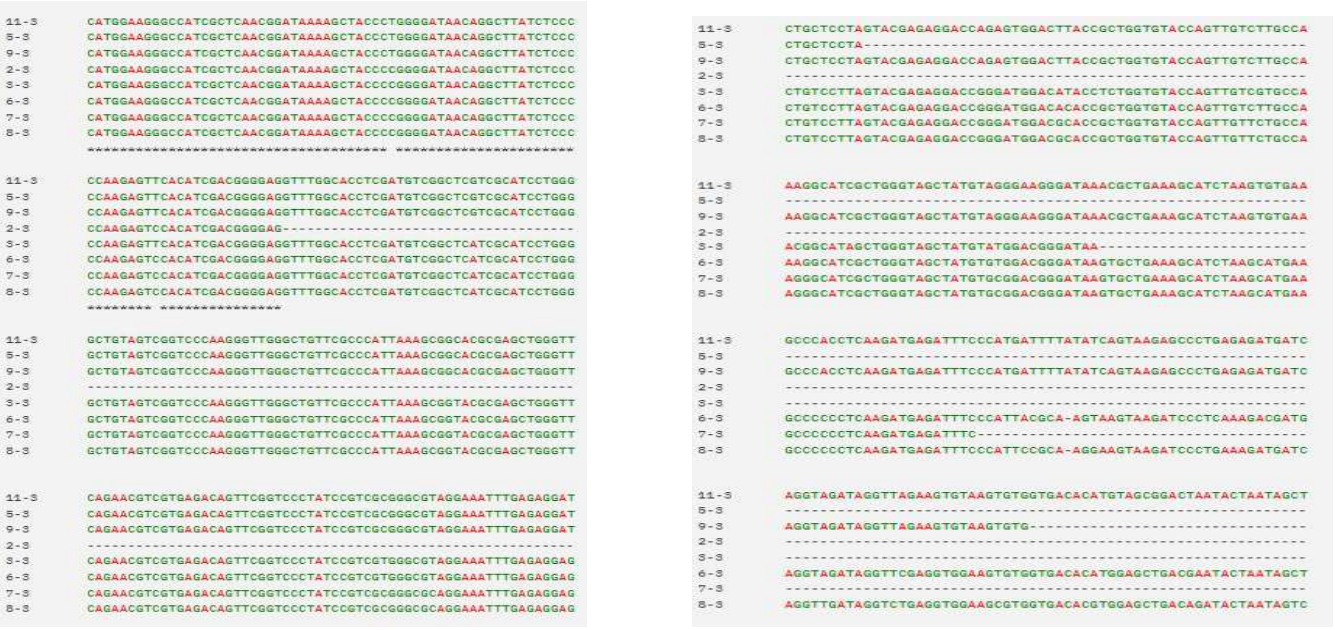
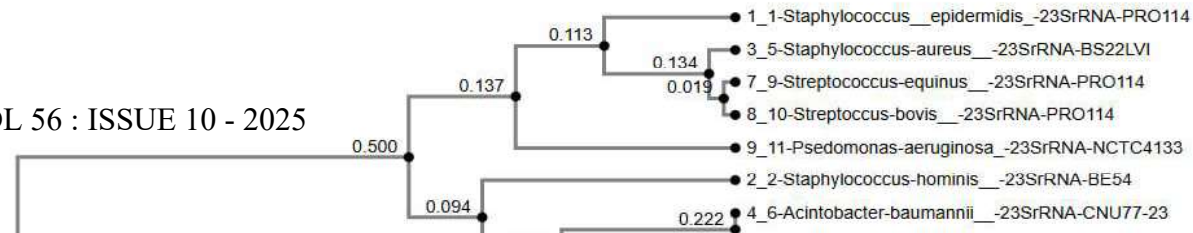


Figure (9): Multiple sequence alignments of *23SrRNA* regions 3 of the 8 studied isolates showing a high percentage of similarity along the nucleotide sequences.

Phylogenetic tree of the 3 regions of *23S rRNA* gene for Bacterial species

The rooted phylogenetic tree for *23SrRNA* sequencing of the 3 regions the studied bacterial species showed all the 11 bacterial species were separated to a single region 1, 2 or 3 according to the sequencing (Figure 10).



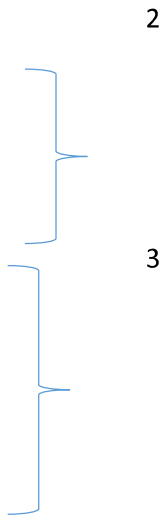


Figure (10): Phylogenetic tree based on *23S rRNA* gene sequences regions 1, 2 and 3 of the 23SrRNA region. The isolates are grouped into three main phylogenetic clusters according their region.

Bacterial identification by *23S RNA* gene versus *16Sr RNA* gene sequencing

Table (6) appeared that the region 1 of the *23SrRNA* gene was the best for precisely bacterial identification (82%) comparing with *16SrRNA* gene sequencing with the significant difference at $p \leq 0.05$ following by region 3(72%) and region 2 (45%). No one region gave a mistake in any bacterial identification, but only for that having no PCR result.

Table (6): Bacterial species by *16S rRNA* and the regions of *23S rRNA*.

No	no species	*16RrRNA*	23SrRNA regions			no (%)
			1	2	3	
1	40	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	Non sequence	Non sequence	*33*
2	73	*Staphylococcus hominis*	*Staphylococcus hominis*	*Staphylococcus. hominis*	*Staphylococcus hominis*	*100*
3	70	*Staphylococcus epidermidis*	Non sequence	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*66*
4	93	*Staphylococcus epidermidis*	Non sequence	*Staphylococcus epidermidis*	Non sequence	*33*
5	105	*Staphylococcus aureus*	*Staphylococcus aureus*	Non sequence	*Staphylococcus aureus*	*66*
6	7	*Acinetobacter baumannii*	*Acinetobacter. baumannii*	Non sequence	*Acinetobacter baumannii*	*66*
7	11	*Exiguobacterium indicum*	*Exiguobacterium indicum*	Non sequence	*Exiguobacterium indicum*	*66*
8	14	*Bacillus safensis*	*Bacillus safensis*	Non sequence	*Bacillus safensis*	*66*
9	128	*Streptococcus equinus*	*Streptococcus equinus*	*Streptococcus equinus*	*Streptococcus equinus*	*100*
10	129	*Streptococcus bovis*	*Streptococcus bovis*	*Streptococcus bovis*	Non sequence	*66*
11	141	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Non sequence*	*Pseudomonas aeruginosa*	*66*
		Total n (%)	9 (82)*	5(45)	8 (72)	

Dissection

Antibiotic susceptibility

Table (4) of human bacterial isolates showed chloramphenicol was 72% and florfenicol 53% for antibiotic resistance .While, Table (5) of bacteria isolated from animals showed chloramphenicol (50%) and florfenicol (48%), respectively. These results agreed with other studies, Since *Staphylococcus aureus* and *S. sciuri* are a relevant agent of skin and soft tissue infections in animals were low resistance to chloramphenicol and florfenicol [14],[15],[16].

The high resistance of chloramphenicol and florfenicol of the human bacteria may be due to the uncontrolled using of antimicrobes by human than in animals especially with

closed culturing information by with the veterinary medicine in Iraq. Since, bacteria have evolved a variety of defense mechanisms over the time to get chloramphenicol and florfenicol inhibitory effects [17]. A widely recognized and consistent nomenclature for genes implicated in resistance to chloramphenicol and florfenicol is also desperately needed, as was previously demonstrated for the nomenclature of tetracycline resistance and macrolide, lincosamide, streptogramin resistance genes [4]. However all the bacterial species from human or animals sources in the present study showed negative for the resistant genes *cfr, fexA, floR* and *cmlA*. Therefore, the resistance on the plates may be due to the plasma membrane permeability barriers or to the multiple nucleotides mutation in the bacterial *23SrRNA* gene. Since, chloramphenicol and florfenicol acts on 23SrRNA and any mutations lead to unidentified gene resistance or may be the genes responsible for chloramphenicol and florfenicol resistance are not found in Iraq bacterial strains, as a result to the biological environment distribution of strains and biological horizontal transfers element [18[,[19], [20],[21]. Furthermore, if the specific resistance genes have mutations in the specific primer site it may not be recognized by the primers used in the PCR (Mahdi *et al*., 2021[14], [22].

The existence of the florfenicol and chloramphenicol resistance genes *fexA* and *cfr* were examined in of *Staphylococcus* bacteria that were resistant to chloramphenicol, only one *Staphylococcus aureus* isolate out of the 114 human isolates had a higher MIC to florfenicol but it lacked the *cfr* and *fexA* resistance genes. However, out of the 188 *Staphylococci* derived from animal sources, 11 were shown to be resistant to florfenicol and possessed either fexA in five isolates, cfr in one isolate or both resistance genes in five isolates [10].The majority of bacteria belonged to the multidrug-resistant *Enterobacteriaceae* according to the antimicrobial sensitivity results, but only 4.72% of the strains had the *cfr* gene and 7.01% positive for *floR* [23]. The existence of particular florfenicol related resistance genes *floR* and *cfr* was assessed in the bacterial strains, while none of the them had *cfr* the *floR gene* [24]. *E. coli* isolates were obtained from bovine

mastitis in Ningxia Province, China. these isolates were then subjected to PCR analysis to check for the *floR* gene and the florfenicol resistance gene, approximately only 7.35% tested positive for the *floR gene* [25].

Based on the above researches there is a relative difference in the percentage of presence of the resistant genes in bacteria, which confirms that they are not widely available in all types of bacteria, and they may have undergone mutations in the strains, or perhaps these primers do not match the bacterial samples isolated from the environment in Basra.

Although resistance to chloramphenicol and florfenicol was existent in the plate but not detection by PCR method, that may support the opinion that resistance to antibiotic especially chloramphenicol and florfenicol is due to the change in plasma membrane canals. Since, fluoroquinolones, tetracycline, chloramphenicol and small hydrophilic antibiotics like β-lactams, their resistance may resulted from any reduction, deletion or functional alteration in these compounds' capacity or rote of entry [26].

Alleles of 23SrRNA region 1, 2 and 3

In general, region 1 ,2 or 3 , each has many nucleotides mutation changing the sequence to give an unique allele mostly in each bacterial isolate encouraging the hypothesis of the antibiotic resistance especially for that acting on the *23SrRNA* gene like chloramphenicol and florfenicol [27]. Since, mutations produced different degrees of clindamycin and macrolide resistance, resistance to valnemulin, chloramphenicol, florfenicol and linezolid was conferred. Furthermore, some mutants had decreased sensitivity to spiramycin and josamycin, whereas the other mutants demonstrated decreased susceptibility to the 16-membered macrolides tylosin, spiramycin and josamycin. Additionally, the tandem mutations affected 16-membered macrolide resistance simultaneously. Therefore, mutations in the 23SrRNA are important contributors to resistance to clinically effective antibiotics that target the large ribosomal subunit and resistance to 16-membered macrolides is impacted by the combined effects

of the double mutations [28]. Moreover, the use of *23SrRNA* gene for bacterial identification as in Table (6), only two bacteria *S. hominis* and *S. equinus* shared all the 3 regions of *23SrRNA* gene to give the same bacterial identification of *16SrRNA* gene. In comparison to 16S rDNA, the 23S rDNA region has the following benefits as a target for a pan-bacterial PCR: (1) more variable sequence stretches, (2) distinct insertions and deletions and (3) the potential for improved phylogenetic resolution [29]. All things considered, this technique might offer a more accurate way to identify chronic bacteria and might be more broadly applicable for identifying other bacterial infections [30]. Therefore, many resrarches used 23SrRNA for identifying the bacteria [31], [32], [28], [33[, [34]. Nevertheless, the absence of well-established broad-range bacterial PCR amplification, sequencing primers hinders, many mutations, the use of the *23SrRNA* gene for bacterial population research were the less than *16SrRNA* gene [32], [19],[8], [35b], [21].

Conclusion

The chloramphenicol and florfenicol resistance in the bacterial isolates is due to the membrane permeability of the bacteria confirming that with absent of *cfr, fexA, cmlA* and *floR* genes.

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