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ORIGINAL ARTICLE

Comparison of Antibiotic Resistances of Different Bacterial Species Isolated from Human and Animal Sources and Their Relation to *sea* Gene

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Key words:
Bacterial resistance, Molecular detection, Sea gene, IRQBAS-230

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ABSTRACT

Background: *Bacterial resistance to antibiotics has become one of the major modern challenges, posing threats to both humans and animals, particularly resistance to chloramphenicol and florfenicol.* ***Objective:*** *To study the distribution of different bacterial species in animal sources and their resistance to various antibiotics, as well as to detect the presence of the sea gene in bacteria isolated from animal sources.* ***Methodology:*** *Forty-two nasal and oral samples were collected from different animals between November 2021 and November 2022. Additionally, 75 human-identified bacterial isolates were obtained from a previous study for comparison.* ***Results:*** *A phylogenetic tree was constructed to show the relationships between 30 different bacterial species isolated from animals and their respective type strains from GenBank. Nine animal bacterial strains were identified as novel global strains and were designated as IRQBAS-230 to IRQBAS-238. Bacterial species isolated from humans exhibited higher resistance to chloramphenicol (72%) and florfenicol (55%) compared to those isolated from animals (50% and 48%, respectively). Furthermore, human isolates showed greater resistance to streptomycin, kanamycin, clindamycin, and other antibiotics than animal isolates. The sea gene was detected in 11 out of 35 (31%) bacterial isolates from animals.* ***Conclusion:*** *There is a direct relationship between resistance to chloramphenicol and florfenicol and resistance to other antibiotics studied. Moreover, the sea gene was detected in various animal bacterial species.*

INTRODUCTION

Because of their exceptional genetic flexibility, bacteria can adapt to a variety of environmental threats, such as exposure to antibiotic compounds that may endanger their survival[1]. Antimicrobial resistance arises through several mechanisms: alterations to the antibiotic's target site (reducing drug affinity), reduced drug uptake, activation of efflux pumps that expel the drug, and global changes in key metabolic pathways through regulatory network modulation. As a result, changes in drug targets, active efflux, decreased membrane permeability, and enzymatic inactivation of antimicrobial drugs are among the main mechanisms by which bacteria develop resistance[2].

Antibiotic Resistance in Animal Isolates

Overall, bacterial isolates from animals showed resistance to ampicillin (83%), enrofloxacin (83%), ciprofloxacin (81%), oxytetracycline (100%), and trimethoprim-sulfamethoxazole (100%). Resistance was approximately 29% for florfenicol and 79% for chloramphenicol among the phenicol antibiotics[3].

When compared to chloramphenicol, its derivative florfenicol exhibits lower toxicity and remains effective against bacteria that have acquired genes conferring resistance to chloramphenicol. However, florfenicol is currently authorized only for veterinary use, primarily in treating respiratory infections and illnesses in animals. Despite this, numerous bacterial species have been reported to acquire resistance to florfenicol[4].

Chloramphenicol and its fluorinated derivative, florfenicol, inhibit bacterial protein synthesis. Due to the extensive use of chloramphenicol in both human and veterinary medicine, various bacterial species and genera have developed or acquired resistance to this drug. Florfenicol, introduced in clinical veterinary settings in the mid-1990s, is used exclusively in veterinary medicine[5].

Staphylococcus aureus and Food Poisoning

Staphylococcus aureus is considered the most virulent pathogen in the genus Staphylococcus due to its ability to produce a wide range of staphylococcal enterotoxins (SEs) and exotoxins. It is a major cause of food poisoning and clinical syndromes such as skin infections, inflammation, pneumonia, and sepsis[6].

These SEs are small, globular proteins with emetic and superantigenic properties. They tolerate a broad pH range and are resistant to proteolytic enzymes, heat, and cold. SEs have been implicated in numerous staphylococcal food poisoning (SFP) outbreaks, clinical cases, and have been isolated from various animal strains. To date, over 24 SE genes (including *sea*) have been thoroughly documented[7].

Study Purpose

This study aims to examine the distribution of different bacterial species from animal sources and their resistance to several antibiotics, particularly **chloramphenicol** and **florfenicol,** and to compare them with bacterial isolates from human sources. Additionally, it investigates the **presence of the *sea* gene** in bacterial isolates obtained from animals.

METHODOLOGY

Bacterial isolates

Preparation of Culture Media

The culture media used in this study included Brain Heart Infusion Broth (BHIB), Blood Agar (BA) supplemented with 5% sheep blood, Muller-Hinton Agar (MHA), and Nutrient Agar (NA). All media were prepared according to the manufacturer's instructions (Himedia, India).

Sample Collection

A total of 42 swab samples were collected from various animals, including cats, dogs, sheep, buffaloes, and birds, between November 2021 and November 2022. Samples were taken from the oral and nasal cavities and cultured on Blood Agar. Additionally, 75 human bacterial isolates were obtained from clinical specimens, including blood, burns, nasal swabs, stool, urine, wounds, body fluid, sputum, ear, cerebrospinal fluid (CSF), eyes, and throat. These isolates had been previously collected and identified by Abd Al-Wahid and Abd Al-Abbas[8].

Bacterial Identification by 16S rDNA Gene Sequencing

For molecular identification, a single bacterial colony from each isolate was cultured in 5 ml of sterilized BHIB and incubated at 37 °C for 24 hours. DNA was then extracted using the Presto™ Mini gDNA Bacteria Kit, following the manufacturer's protocol. For 16S rRNA gene amplification, primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used (Pragma, USA)[9]. The PCR reaction mixture (50 µl total volume) consisted of 2 µl of each primer, 25 µl of GoTaq® Green Master Mix (Promega, USA), 19 µl of nuclease-free water (Bioneer, Korea), and 2 µl of DNA template. The amplification was performed using a thermocycler (Bioneer) with the following conditions: an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute. A final extension was carried out at 72 °C for 2 minutes. PCR products of approximately 1500 base pairs were visualized using 1.5% agarose gel electrophoresis.

Bacterial species were identified using the Basic Local Alignment Search Tool (BLAST), and the sequences of each isolate were concatenated and aligned with type strains obtained from GenBank. Phylogenetic analysis was carried out using Clustal Omega, and a phylogenetic tree was constructed using the Multiple Alignment Program for Nucleotide Sequences[10].

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI, 2020)[11] guidelines. Ten antibiotics from Himedia (India) were selected: ampicillin (15 µg), erythromycin (15 µg), kanamycin (30 µg), chloramphenicol (30 µg), clindamycin (15 µg), tetracycline (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25 µg), florfenicol (30 µg), and streptomycin (10 µg). Among these, ampicillin, chloramphenicol, tetracycline, streptomycin, erythromycin, kanamycin, and gentamicin were most commonly tested.

One colony from each of the 117 bacterial isolates (42 from animals and 75 from humans) was inoculated into a glass tube containing 3 ml of 3.5% sterile saline solution. The bacterial suspension was adjusted to match the turbidity of a 0.5 McFarland standard (approximately 1.5×10^{8} CFU/ml). A 100 µl aliquot of this suspension was spread onto MHA plates using a sterile L-shaped spreader. Antibiotic discs were placed on the inoculated agar surface, and plates were incubated at 37 °C for 24 hours. The diameters of inhibition zones were measured, and isolates were classified as sensitive or resistant based on CLSI interpretive criteria.

For florfenicol, 0.01 mg of the powdered antibiotic was dissolved in 1000 ml of sterilized distilled water, filtered through a 0.45 µm membrane filter, and added to Nutrient Agar plates for susceptibility testing.

Detection of the *sea* Gene

Polymerase chain reaction (PCR) was used to detect the *sea* gene in 35 bacterial isolates from animals[12]. The primer sequences were as follows: forward primer 5′-CCTTTGGAAACGGTTAAAACG-3′ and reverse primer 5′-TCTGAACCTTCCCATCAAAAAC-3′. The PCR reaction mixture (25 µl) consisted of 12 µl of GoTaq® Green Master Mix (Promega, USA), 2 µl of DNA template, 1 µl of each primer (Macrogen, Korea), and 9 µl of nuclease-free water (Bioneer, Korea). The thermocycling conditions included an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 35 seconds, annealing at 55 °C for 35 seconds, and extension at 72 °C for 1 minute. A final extension was performed at 72 °C for 10 minutes. PCR products were separated on a 1.5% agarose gel and visualized under a UV transilluminator (Wisd, Korea). A positive band of 127 base pairs indicated the presence of the *sea* gene.

RESULTS

The *16S rRNA* gene of 42 bacterial isolates was showed as a single band for each isolate on agarose gel electrophoresis at a position 1500 bp in comparison with a standard molecular DNA ladder (Figure 1).

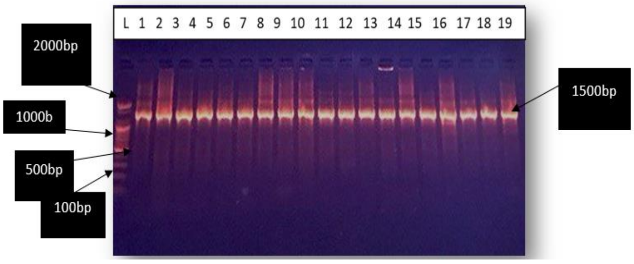

Fig. 1: A model of agarose gel (1.5%) electrophoresis. Lane L: 100 bp Marker, Lane 1-19 bands of 16*SrRNA* gene (1500bp).

The 16S rRNA gene of all 42 bacterial isolates was successfully sequenced, and the bacterial species were identified. Among them, *Enterobacter cloacae* and *Bacillus cereus* were each represented by 4 isolates (10%). Two isolates (5% each) were identified as *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, and *Staphylococcus warneri*. The remaining species were represented by a single isolate each (2%) and included: *Lysinibacillus fusiformis*, *Staphylococcus pasteuri*, *Enterococcus gallinarum*, *Staphylococcus felis*, *Pantoea agglomerans*, *Bacillus mycoides*, *Bacillus thuringiensis*, *Bacillus paramycoides*, *Acinetobacter venetianus*, *Bacillus rugosus*, *Klebsiella pneumoniae*, *Streptococcus equinus*, *Streptococcus bovis*, *Citrobacter kerstersii*, *Macrococcus caseolyticus*, *Lysinibacillus xylanilyticus*, *Staphylococcus sciuri*, *Mammaliicoccus sciuri*, *Pseudomonas parafulva*, *Bacillus safensis*, *Enterobacter indicium*, and *Stenotrophomonas xiamensis*. The differences in isolate distribution were not statistically significant ($p < 0.05$).

The phylogenetic tree revealed that each bacterial species was grouped with its closest or identical type strain obtained from GenBank (Figure 2). Novel bacterial strains were identified by comparing their nucleotide sequences to the corresponding type strains. These new strains were submitted to the DNA Data Bank of Japan (DDBJ) and also published in the National Center for Biotechnology Information (NCBI) and GenBank databases.

One of the new strains, 83-*Lysinibacillus fusiformis* (IRQBAS-230), was found to be 99% identical to *L. fusiformis* XKS50.1, with a transversion mutation (G →T) at position 856 bp.

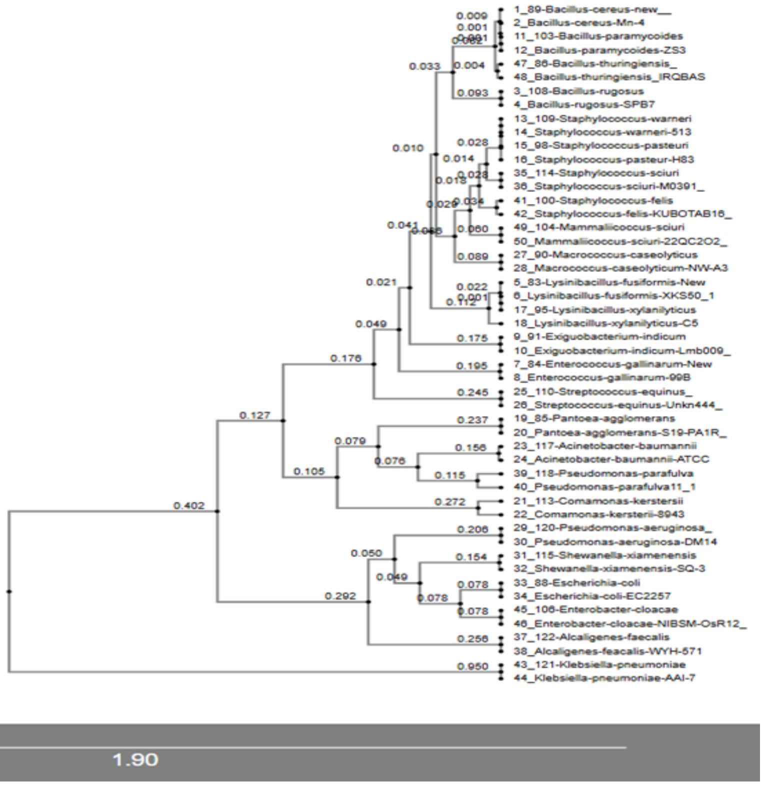

Fig. 2: Rooted Neighbor Joining phylogenetic tree constructed form alignment of 16*SrRNA* sequences gene by MAFFT program, each bacterial species identified by its type strain from the GenBank

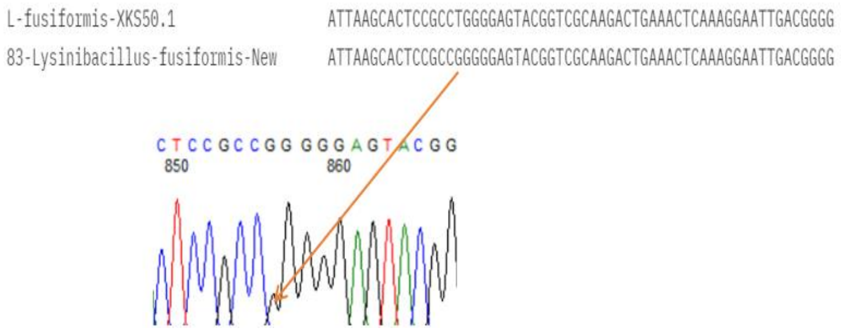

Fig. 3: *16SrRNA* nucleotides sequence of isolate *L. fusiformis* No.83 from present study (IRQBAS-230) with its type of strain, transversion mutation (G-T) at position 856 bp.

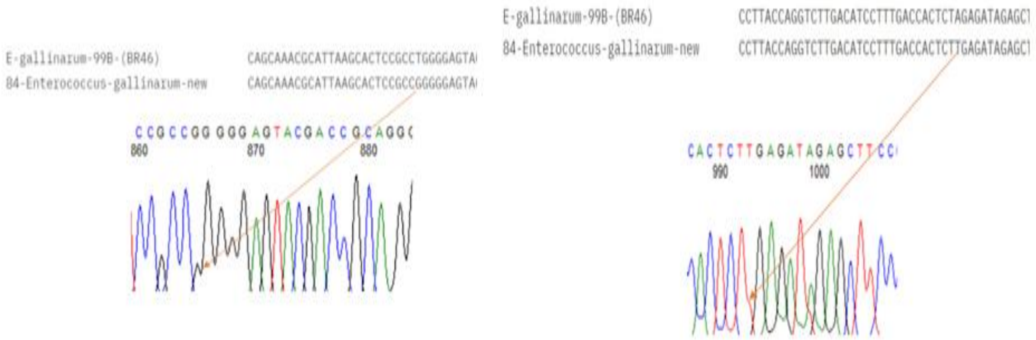

Fig. 4: *16SrRNA* nucleotides sequence of isolate *E. gallinarum* No.84 from present study (IRQBAS-231) with its type strain, transversion mutation (G-T) at position 865 bp and T-A at position 933 bp.

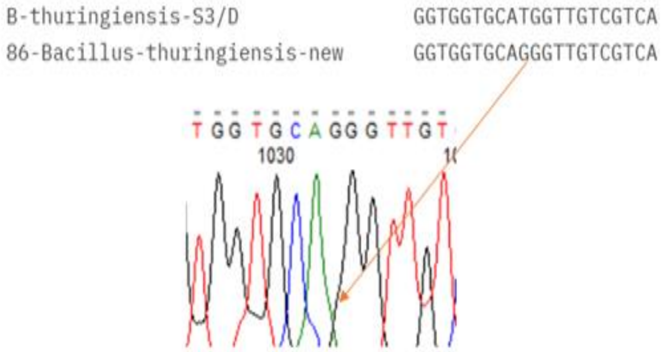

Fig. 5: *16SrRNA* nucleotides sequence of isolate *B. thuringiensis* No.86 from present study (IRQBAS-232) with its type of strain, transvers ion mutation (G-T) at position 1032 bp.

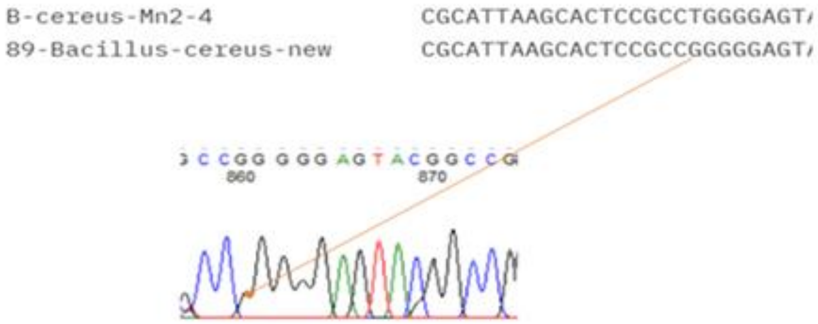

Fig. 6: *16SrRNA* nucleotides sequence of isolate *B. cereus* No.89 from present study (IRQBAS-233) with its type of strain, transversion mutation (T-G) at position 860 bp.

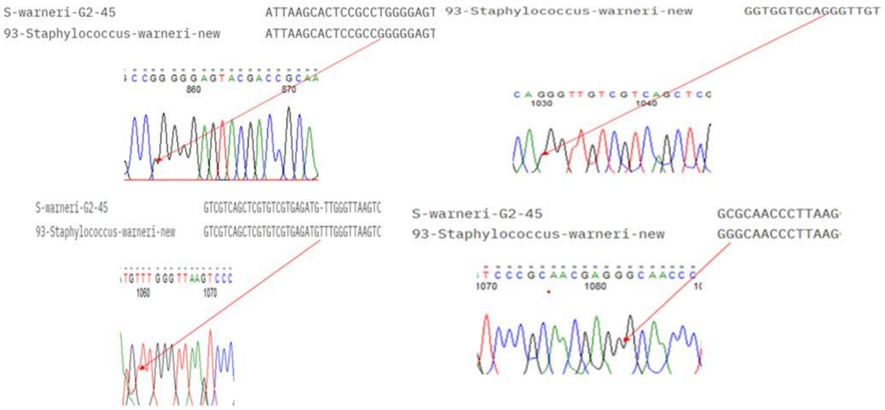

Fig. 7: *16SrRNA* nucleotides sequence of isolate *S. warneri* No.93 from present study (IRQBAS -234) with its type strain, transversion mutation (G-T) at position 856bp, (G-T) at position 1030 bp, deletion mutation (T) at position 1060 bp and transversion mutation (G-C) at position 1083 bp.

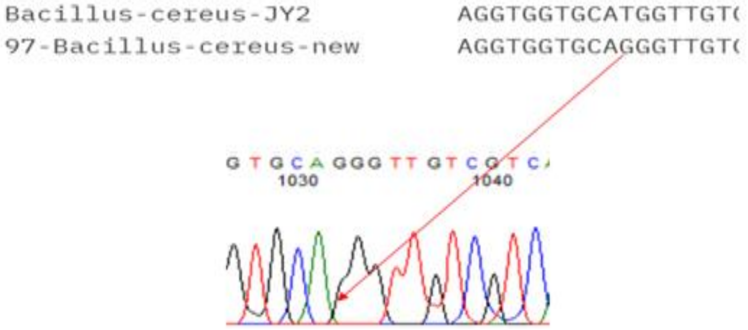

Fig. 8: *16SrRNA* nucleotides sequence of isolate *B. cereus* No.97 from present study (IRQBAS-235) with its type of strain, transversion mutation (G-T) at position 1030 bp.

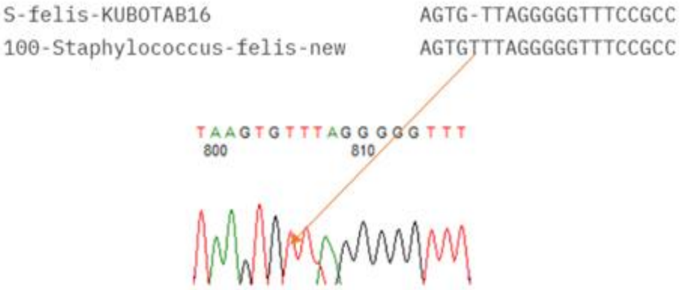

Fig. 9: *16SrRNA* nucleotides sequence of isolate *S. felis* No.100 from present study (IRQBAS-236) with its type of strain, frame shift mutation (insertion T) at position 805 bp.

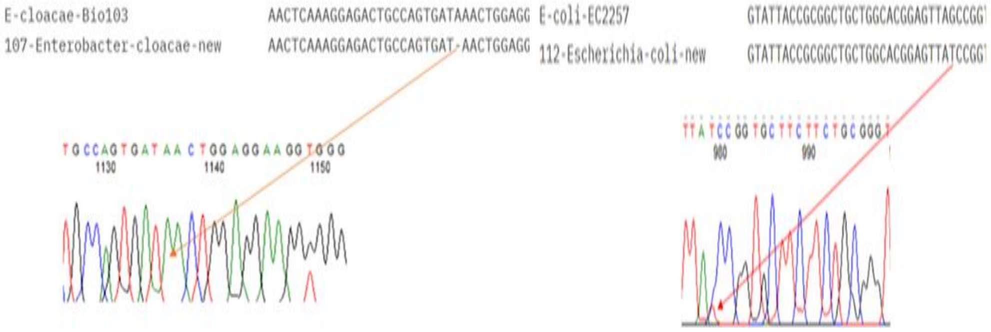

Fig. 10: *16SrRNA* nucleotides sequence of isolate *E. coli* No.112 from present study (IRQBAS-238) with its type of strain, transversion mutation (T-G) at position 979 bp.

The second strain, 84–*Enterococcus gallinarum* (IRQBAS-231), was closely related (99%) to *Enterococcus gallinarum* 99B (BR46), with transversion mutations (G →T) at position 865 bp and (T→A) at position 933 bp. The third strain, 86–*Bacillus thuringiensis* (IRQBAS-232), was 99% similar to *Bacillus thuringiensis* S3/D, with a transversion mutation (G →T) at position 1032 bp. The fourth strain, 89–*Bacillus cereus* (IRQBAS-233), showed 99% similarity to *Bacillus cereus*, with a transversion mutation (G →T) at position 860 bp.

The fifth strain, 93–*Staphylococcus warneri* (IRQBAS-234), was closely related (99%) to *Staphylococcus warneri* G2-4 and exhibited several mutations, including transversion mutations (G →T) at positions 856 bp and 1030 bp, a deletion mutation (T) at position 1060 bp, and a transversion mutation (G → C) at position 1083 bp. The sixth strain, 97–*Bacillus cereus* (IRQBAS-235), showed 99% similarity to *Bacillus cereus* JY2, with a transversion mutation (G →T) at position 1030 bp. The seventh strain, 100–*Staphylococcus felis* (IRQBAS-236), was closely related to *Staphylococcus felis* KUBOTA16 and exhibited a frameshift mutation caused by a T insertion at position 805 bp. The eighth strain, 107–*Enterobacter cloacae* (IRQBAS-237), was 99% similar to *E. cloacae* Bio103 and showed a frameshift mutation due to an A deletion at position 1136 bp. The ninth strain, 112–*Escherichia coli* (IRQBAS-238), was closely related (99%) to *E. coli* EC2257, with a transversion mutation (T→ G) at position 979 bp.

Regarding antibiotic susceptibility, the bacterial species isolated from human sources (n = 75) exhibited higher resistance to chloramphenicol and tetracycline, with 54 isolates (72%) resistant to each. This was followed by streptomycin and kanamycin (69% each), trimethoprim-sulfamethoxazole (68%), and erythromycin (66%). Resistance rates to ampicillin, florfenicol, gentamicin, and clindamycin were 61%, 55%, 52%, and 51%, respectively. As shown in Table 1, there were no statistically significant differences in resistance among these antibiotics ($p \leq 0.05$).

Table 1: Antibiotic resistance discs for bacterial species from human

Bacterial species			**Antibiotic resistance**									
			Discs (%)									
no	No isolate	no	C	S	K	TM	CD	G	E	T	AM	F
1	*S. epidermidis*	14	11(79)	12(85)	10(71)	12(85)	6(42)	6(42)	10(71)	11(79)	11(79)	9(62)
2	*E. coli*	11	6(54)	8(72)	10(90)	9(80)	11(100)	3(27)	10(90)	11(100)	5(45)	4(36)
4	*S. hominis*	4	2(50)	4(100)	4(100)	4(100)	2(50)	3(49)	2(50)	2(50)	1(25)	2(50)
3	*S. aureus*	7	5(71)	5(71)	5(71)	4(57)	4(57)	3(49)	4(57)	4(57)	4(57)	3(49)
5	*E. faecalis*	6	4(67)	4(67)	4(67)	5(83)	5(83)	5(83)	4(67)	5(83)	5(83)	2(33)
6	*S. haemolyticus*	4	2(50)	1(25)	4(100)	4(100)	4(100)	2(50)	2(50)	2(50)	4(100)	3(75)
7	*K. pneumoniae*	4	4(100)	4(100)	2(50)	3(75)	4(100)	2(50)	4(50)	3(75)	1(25)	2(50)
8	*P. aeruginosa*	10	9(9)	6(60)	5(50)	6(60)	7(70)	7(70)	6(60)	7(70)	7(70)	7(70)
9	*Acinetobacter baumannii*	2	2(50)	1(50)	1(50)	1(50)	1(50)	2(100)	1(50)	1(50)	1(50)	2(100)
10	*E. mori*	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
11	*E. fergusonii*	1	1(100)	1(100)	1(100)	1(100)	1(100)	-	1(100)	1(100)	-(0%)	1(100)
12	*E. bugandensis*	1	-	-	-	-	-	-	-	-	-	-
13	*E. hormaechei*	7	5(71)	3(42)	3(42)	3(42)	3(42)	3(42)	4(71)	4(71)	4(71)	3(42)
14	*E . ludwigii*	1	-	-	-	-	1(100)	-	1(100)	1(100)	1(100)	-
15	*P. mosselii*	1	1(100)	1(100)	1(100)	1(100)	-	1(100)	-	-	1(100)	-
16	*B. safensis*	1	-	1(100)	1(100)	1(100)	1(100)	1(100)	-	1	-	1(100)
	Total no (%)	75	54 (72)	52 (69)	52 (69)	55 (68)	51 (51)	39 (52)	50 (66)	54 (72)	46 (61)	40 (55)

Chloramphenicol (C), Streptomycin (S), kanamycin (K), Trimethoprim – sulfamethoxazole TMR, Clindamycin (CD), Gentamycin (GM). Erythromycin (E), Tetracycline (T), Ampicillin (AM), Florfenicol (F).

Table 2. Antibiotic resistance discs for bacterial species from animals

Bacterial species		**Antibiotic resistance**										
		Discs (%)										
No	**Bacterial species**		**C**	**S**	**K**	**TRM**	**CD**	**G**	**E**	**T**	**AM**	**F**
1	*Staphylococcus aureus*	2	1(50)	1(100)	-	-	-	-	-	-	-	-
2	*Lysinibacillus fusiformis*	1	-	-	-	-	-	-	-	-	-	-
3	*Lysinibacillus xylanilyticus*	1	-	-	-	-	-	-	-	-	-	-
4	*Mammaliicoccus sciuri*	1	1(100)	-	-	-	-	-	-	-	-	-
5	*Enterococcus cloacae*	4	1(25)	1 (100)	2(50)	2(50)	1(25)	2(50)	-	-	-	-
6	*Bacillus mycoides*	1	-	-	-	-	-	-	-	-	-	-
7	Bacillus cereus	4	4(100)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)
8	Bacillus thuringiensis	1	-	-	-	-	-	-	-	1(100)	1(100)	1(100)
9	Bacillus paramycoides	1	-	1(100)	1(100)	1(100)	1(100)	1(100)	-	-	-	1(100)
10	Bacillus safensis	1	1(100)	-	-	-	-	-	-	-	-	1(100)
11	Acinetobacter venetianus	1	1(100)	1(100)	-	-	-	1(100)	1 (100)	1(100)	1(100)	1(100)
12	Acinetobacter baumannii	2	2(100)	1(50)	1(50)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	2(100)
13	Escherichia coli	2	-	2(100)	-	2(100)	1(50)	1(50)	-	-	-	-
14	Exiguobacterium indicum	1	1(100)	-(0)	-(0)	1(100)	-(0)	1(100)	1(100)	1(100)	1(100)	-
14	Mammaliicoccus caseolyticus	1	1(100)	1(100)	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	-
15	Staphylococcus warneri	2	-	1(50)	1(50)	2	1(50)	1(50)	1(50)	1(50)	1(50)	1(100)
16	Staphylococcus sciuri	1	1(100)	-	1(100)	-	-	-	-	-	-	1(100)
17	Staphylococcus pasteuri	1	-	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
18	Staphylococcus felis	1	-	-	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
19	Streptococcus equinus	1	-	1(100)	1(100)	-	1(100)	-	-	-	-	-
20	Streptococcus bovis	1	1(100)	1(100)	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
22	Pseudomonas parafulva	1	1(100)	-	-	1(100)	1(100)	-	-	-	1(100)	1(100)
23	Comamonas kerstersii	1	-	1(100)	-	-	1(100)	1(100)	-	-	-	-
24	Shewanella xiamensis	1	-	-	-	1(100)	1(100)	-	-	-	-	-
24	Klebsiella pneumoniae	1	1(100)	1(100)	1(100)	-	-	-	-	-	1(100)	1(100)
38	Alacligenes faecalis	2	1(100)	-	-	-	1(50)	1(50)	-	-	-	1(50)
25	Enterococcus gallinarum	1	1(100)	-	-	-	-	-	-	-	-	-
26	Bacillus rugosus	1	-	-	-	-	-	-	-	-	-	-
27	Pantoea agglomerans	1	-	-	-	-	-	-	-	-	-	-
	Total animal (%)	42	21 (50)	16 (38)	15 (36)	15 (36)	17 (40)	18 (43)	12 (29)	18 (43)	15 (36)	20 (48)

Chloramphenicol (C), Streptomycin (S), kanamycin (K), Trimethoprim –sulfamethoxazole TMR, Clindamycin (CD), Gentamycin (GM). Erythromycin (E), Tetracycline (T), Ampicillin (AM), Florfenicol (F).

The bacterial species isolated from animal sources (n = 42) exhibited lower resistance to chloramphenicol (50%) and florfenicol (48%), followed by tetracycline (43%), gentamicin (43%), clindamycin (40%), streptomycin (38%), kanamycin (36%), trimethoprim-sulfamethoxazole (36%), ampicillin (36%), and erythromycin (29%), as shown in Table 2. No statistically significant differences in resistance patterns were observed ($p \leq 0.05$). Importantly, the antibiotic resistance profiles of bacterial isolates from both human and animal sources indicated a direct relationship between resistance to chloramphenicol and florfenicol and resistance to the other eight antibiotics tested. This suggests possible co-selection or shared resistance mechanisms.

The sea gene was detected in 11 of 35 (31%) bacterial isolates from animals, as shown in Figure 12 and Table 3. These included isolates of *Staphylococcus aureus* (2 isolates, 100%), *Enterobacter cloacae* (2 isolates, 50%), *Staphylococcus sciuri* (1 isolate, 100%), *Streptococcus bovis* (1 isolate, 100%), *Pseudomonas aeruginosa* (2 isolates, 100%), *Klebsiella pneumoniae* (1 isolate, 100%), *Staphylococcus pasteuri* (1 isolate, 100%), and *Citrobacter kerstersii* (1 isolate, 100%). These findings indicate that the sea gene, which encodes staphylococcal enterotoxin A, may be more widespread across diverse bacterial species from animal sources than previously thought.

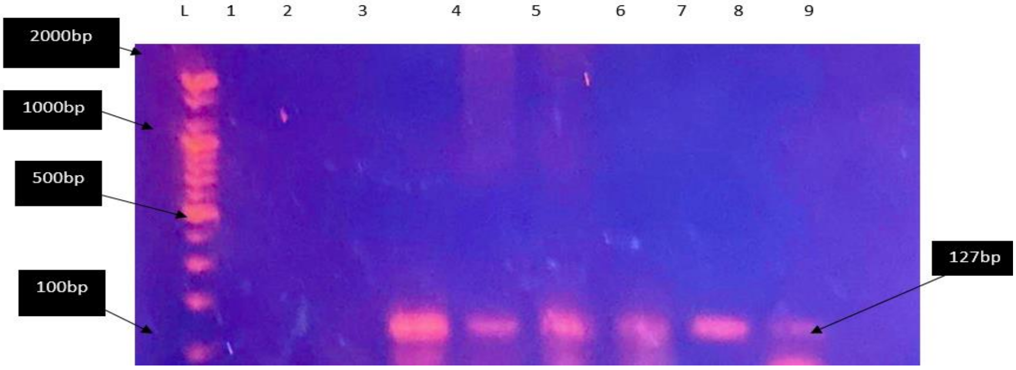

Fig. 11: Agarose gel electrophoresis (1.5) percentage showing amplified *Sea* gene (127 bp). Lane L: 100 bp Marker. Lane 3, 4, 5, 6, 7, 8 and 9: *Sea gene* bands.

Table 3: Distribution of the *Sea* gene among bacterial species isolated from animals

No	Bacterial species	No.	*Sea gene* n(%)
1	*S. aureus*	2	2(100%)
2	*E. cloacae*	4	2(50%)
3	*B. ceruse*	3	0
4	*E. coli*	2	0
5	*A. baumannii*	1	0
6	*A. venetianus*	1	0
7	*L. fusiformis*	1	0
8	*E gallinarum*	1	0
9	*P. agglomerans*	1	0
10	*M. caseolyticus*	1	0
11	*E. indicum*	1	0
12	*S. warneri*	1	0
13	*B. safensis*	1	0
14	*L. xylanilyticus*	1	0
14	*S. felis*	1	0
15	*B. mycoides*	1	0
16	*B. paramycoides*	1	0
16	*B. rugosus*	1	0
17	*S. bovis*	1	1(100%)
18	*P. aeruginosa*	2	2(100%)
19	*A. faecalis*	2	0
20	*K. pneumoniae*	1	1(100%)
21	*S. pasteuri*	1	1(100%)
22	*S. equinus*	1	0
23	*S. sciuri*	1	1(100%)
24	*C. kerstersii*	1	1(100%)
Total %		35	11(31%)

DISCUSSION

The 16S rRNA gene is the most commonly used housekeeping genetic marker for bacterial taxonomy and phylogeny research. Consequently, 16S rRNA gene sequencing can be utilized to identify and discover new bacterial species[13,14]. By enabling conclusive diagnosis and guiding patient management, 16S rRNA gene sequencing is particularly valuable for identifying a wide variety of bacterial species, including those that fail to grow in clinical cultures, often due to prior empirical antibiotic therapy. This is especially important in cases of severe infections[8,13,15]. However, the results of the present study showed no significant differences in the distribution of bacterial species isolated from animal samples, which can be attributed to the precise genetic identification provided by 16S rRNA sequencing.

In this study, nine new bacterial strains were recorded in GenBank. Mutations, including substitutions, transversions, and nucleotide deletions, led to the emergence of these new strains. According to numerous studies, a similarity range of approximately 99% is generally sufficient for classification as a new strain[13]. Various factors can cause mutations, such as errors during DNA replication, environmental influences, horizontal transfer of antibiotic resistance elements, and exposure to UV light, all of which can result in permanent genetic changes[16].

Furthermore, 16S rRNA sequencing has facilitated the identification and reclassification of several new bacteria from domestic animals. Examples include two Moraxella species from cows and goats, a new Capnocytophaga species from cats, an alpha-hemolytic Streptococcus species from guinea pigs, the reclassification of thermophilic Campylobacter species found in pigs to the newly identified genus *Exercitatus* within the Pasteurellaceae family, and the renaming of *Clostridium spiroforme*, a cause of diarrhea and anorexia in domestic rabbits, to *Thomasclavelia spiroformis* comb. nov. in 2023. Additionally, *Mannheimia ovis* was renamed *Mannheimia pernigra*[17]. Many other new bacterial strains have also been detected[8,10,18,19].

Notably, all the new bacterial strains identified in the present study were isolated from animal sources, underscoring the limited research focus on animal-derived bacteria compared to human sources. Animals represent a significant reservoir of many yet-undiscovered bacterial species and may contribute to the discovery of novel resistance genes, some of which could pose risks to both human and animal health. Moreover, bacterial research has traditionally focused primarily on pathogenic microorganisms. The use of 16S rRNA-based molecular diagnostics has greatly contributed to the discovery of these new strains.

Human bacterial isolates showed antibiotic resistance rates of 72% for chloramphenicol and 55% for florfenicol. In contrast, bacteria isolated from animals exhibited resistance rates of 50% to chloramphenicol and 48% to florfenicol, along with varying resistance to other antibiotics. These findings are consistent with previous studies. For example, *Staphylococcus aureus* is a significant agent of skin and soft tissue infections in animals, typically showing low resistance to erythromycin, clindamycin, kanamycin, tobramycin, gentamicin, tetracycline, and chloramphenicol[20]. *S. sciuri* isolated from animals displayed resistance to trimethoprim-sulfamethoxazole (51.3%), florfenicol (30.8%), and gentamicin (23.1%) in antimicrobial susceptibility testing[21]. Similarly, *Pseudomonas aeruginosa* strains isolated from dogs were found to be sensitive to gentamicin[22].

On the other hand, there is a scarcity of research on antimicrobial susceptibility for many bacterial species isolated from animals. There is particularly limited drug sensitivity information for *Enterobacter cloacae*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus paramycoides*, *Bacillus safensis*, *Acinetobacter venetianus*, *Exiguobacterium indicum*, *Mammaliicoccus caseolyticus*, *Staphylococcus pasteuri*, *Pseudomonas parafulva*, *Comamonas kerstersii*, *Alcaligenes faecalis*, *Enterococcus gallinarum*, *Bacillus rugosus*, *Pantoea agglomerans*, *Shewanella xiamensis*, *Lysinibacillus fusiformis*, and *Lysinibacillus xylanilyticus*.

The higher resistance observed in human bacterial isolates may be attributed to the uncontrolled use of antimicrobials in humans compared to animals, especially given the restricted access to veterinary antibiotics in Iraq. Bacteria have evolved various defense mechanisms over time to counteract the inhibitory effects of chloramphenicol and other antibiotics. One common mechanism is enzymatic inactivation through acetylation of the drug by several forms of chloramphenicol acetyltransferases. Additional mechanisms include permeability barriers, phosphotransferase-mediated inactivation, efflux systems, and target site alterations. Notably, inconsistencies in the nomenclature of antibiotic resistance genes have emerged as the number of genes associated with resistance and related medications has increased. Different genes conferring resistance have been given multiple designations, creating confusion. There is an urgent need for a widely accepted and consistent nomenclature for genes involved in resistance to chloramphenicol and florfenicol, similar to the standardized nomenclature already established for tetracycline and macrolide-lincosamide-streptogramin resistance genes[23].

The detection of the *sea* gene in bacterial isolates from animal sources aligns with previous findings by Banaszkie et al.[24] Although this gene is commonly found in Gram-positive bacteria[25], it has also been detected in Gram-negative species. Importantly, in this study, the sea gene was detected for the first time in *Pseudomonas aeruginosa*, *Streptococcus bovis*, *Staphylococcus sciuri*, *Enterococcus faecalis*, and *Comamonas kerstersii*. This suggests the possibility of horizontal gene transfer of the *sea* gene from humans to animals, as well as its transfer among different bacterial species[3,26]. The presence of the *sea* gene in animal bacteria may contribute to enterotoxin production, potentially causing illness in these animals, although this remains to be fully elucidated.

Previous studies have reported the isolation of *Bacillus cereus* strains carrying enterotoxin genes such as *hblA*, *hblC*, and *hblD* in all isolates following PCR amplification[27]. Similarly, enterotoxin production has been detected in bacteria isolated from animals, including *Staphylococcus aureus*[28], other *Staphylococcus* species[29], and *Bacillus cereus*[30]. In the present study, molecular identification of the *sea* gene was performed for the first time in bacterial species including *Lysinibacillus xylanilyticus*, *Mammaliicoccus sciuri*, *Shewanella xiamenensis*, and *Alcaligenes faecalis*.

CONCLUSION

There was no dominant bacterial species identified in the animal sources, and these isolates exhibited lower antibiotic resistance compared to those from humans, particularly to chloramphenicol and florfenicol. However, a direct relationship was observed between resistance to these two antibiotics and resistance to the other eight antibiotics studied. Additionally, the *sea* gene was detected across various bacterial species isolated from animals.

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