RECORD OF AEROBIC BACTERIAL SPECIES FROM THE CYPRINID FISH *CYPRINUS CARPIO* FROM FISH FARMS IN SULAIMANI PROVINCE, KURDISTAN REGION, IRAQ

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Abstract. Major bacterial pathogens responsible for infectious diseases in *Cyprinus carpio* Linnaeus 1758 have a negative economic impact on aquaculture and decrease the quantity of fish production. A total of 174 *C. carpio* were collected from fish farms in Sulaimani City. The fish samples were examined for clinical signs of infectious diseases prior to habitual bacteriology. Identification of bacterial isolates to species level by using VITEK®2 and sequencing of 16S rDNA was done. Antibiotic susceptibility testing was done by the Kirby-Bauer disk diffusion method. This research revealed the occurrence of 21 species of bacteria, belonging to eight families (Enterobacteriaceae, Pseudomonadaceae, Aeromonadaceae, Comamonadaceae, Bacillaceae, Staphylococcaceae, Streptococcaceae, and Moraxellaceae). The most common pathogen of gram-negative isolates was *Aeromonas sobria* (15.87%), while gram-positive was *Exiguobacterium profundum* ((9.52%). Results of antibiotic susceptibility testing showed that all isolates were multidrug resistant; *Staphylococcus aureus* and *S. warneri* were determined to be methicillin-resistant. Recording of 12 species of bacteria (*Bacillus cereus***,** *Priestia megaterium***,** *Comamonas aquatica*, *Citrobacter freundii*. *Enterobacter hormaechei* subsp. *xiangfangensis***,** *E. mori***,** *Escherichia fergusonii*, *Exiguobacterium aestuarii*, *E. profundum*, *Lactococcus taiwanensis*, *Pantoea agglomerans*, and *S. warneri*) from *C. carpio* are considered to be the first records in Iraq.
Keywords: *bacterial fish, molecular, antibiotic, multidrug resistant, mecA gene, first record*

Introduction

Cyprinus carpio Linnaeus 1758 considered the most prevalent variety of fish that is currently cultured in Iraq. It is tolerant of a variety of water quality characteristics including a wide pH range of 5 to 9, a large thermal range of 1 to 35°C, and modest halotolerance (Ahmed et al., 2020). Global aquaculture and fishing sectors are facing serious problems due to zoonotic diseases caused by fish and aquatic organisms (Ziarati et al., 2022). According to human health safety, fish must be free of contagious pathogens and have long-lasting nutritional value because bacterial infections were thought to be the primary cause of high mortalities and economic losses among fish and fish farms (Tsfaye et al., 2018).

A few data exist on bacterial illnesses affecting *C. carpio* in the Kurdistan Region, A study that identified the agents responsible for skin ulceration in ponds housing carp fish in the Sulaimani province was conducted by (Abid and Al-Hamdani, 2016). Another study by (Mala and Abdullah, 2022) found that the Taqtaq District in the Erbil Province had 13 kinds of bacteria from *C. carpi*o. However, a recent study revealed that one of the most consumed and demanded aquatic food in the Kurdistan Region of Iraq is the common carp *C. carpio* which is sensitive to bacterial infections, and

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veterinarians prefer to treat the condition with the antibiotic oxytetracycline (Othman et al., 2023). In addition to that, there have been some bacterial isolate examinations in Iraq. Jassim et al. (2019) used the VITEK 2 system test to identify four species of bacterial isolates in Basrah Province. While, Al-Gburi (2020) in the province of Baghdad, discovered a bacterial isolate from common carp *C. carpio* using traditional culture method, biochemical testing, and VITEK 2.

Lakes and ponds become contaminated with pathogenic bacteria from a variety of sources including residential regions, agricultural systems, livestock systems, and the industries involved in the slaughter and processing of animals, this decreases the water quality and makes it unsuitable for recreational activities such as fishing and aquaculture as well as irrigation (Parlapani et al., 2023). The main signs of different bacterial infections in farmed freshwater fish are skin ulceration and hemorrhagic septicemia (Irshath et al., 2023). Also, gill disease is another growing problem that causes significant losses in farmed fish. while complex gill disease is known to be brought on by many pathogens or causes (O'Halloran et al., 2022). The major disease-causing bacterial genera infecting tropical freshwater fish species are *Streptococcus, Pseudomonas, Edwardsiella, Flavobacterium, Vibrio,* and *Aeromonas* (Rahman et al., 2017).

Developments in molecular biology have sped up the evolution of novel techniques in order to identify fish illnesses. In comparison to more traditional techniques, molecular diagnostic procedures are quicker, more precise, and more sensitive when determining the causes of fish disease in an epidemic area (Ador et al., 2021). Microorganisms that are resistant to antibiotics could emerge as a result of prolonged antibiotic use. These antibiotics are capable of entering aquatic environments, where they can selectively stimulate microorganisms, enriching them and triggering the evolution of antibiotic-resistance genes (Su et al., 2019). Fish should only be treated with antibiotics when an antibiogram is complete since blind antibiotic treatments lead to resistance. The rapid spread of antibiotic resistance in bacteria is facilitated by horizontal gene transfer, and the prevalence of numerous antimicrobial resistance genes in Gram-negative bacteria has been detected (Gazal et al., 2020). MDR poses a severe threat to the entire world because of its rapid global expansion and the few antibiotic treatment choices available for diseases caused by MDR pathogens that are difficult to treat (Gaibani et al., 2022). Considering the obvious effects of AMR and the rise in intensive aquaculture which require the usage of antibiotics, there is a lack of knowledge on AMR for fish microorganisms in our environment. So reference data from the current study was provided to avoid fish infectious illnesses and evaluate the risk of antibiotics. Consequently, this investigation was carried out to ascertain the bacterial profile and antimicrobial resistance pattern of bacterial isolates from external parts of *C. carpio* from different aquacultures in Sulaimani City.

Materials and methods

Fish sampling

A total of 174 common carp were collected from Shakhaswr of Sharbazher in Sulaimani Province, Kurdistan Region, Iraq, during the period from June 2021 to May 2022. Three fish farms were involved (45, 68, and 61) samples were taken from each farm. Sulaimani Province is located in the northeast of Iraq; it is situated between latitudes 35° 05' and 36° 30' and between longitudes 44° 25' and 46° 20' (*Fig. 1*). The samples were moved to the advanced bacteriology lab of the biology department of the

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College of Science at the University of Sulaimani. The total length, standard, and body weight of each fish sample were recorded, among other morphometric measurements.

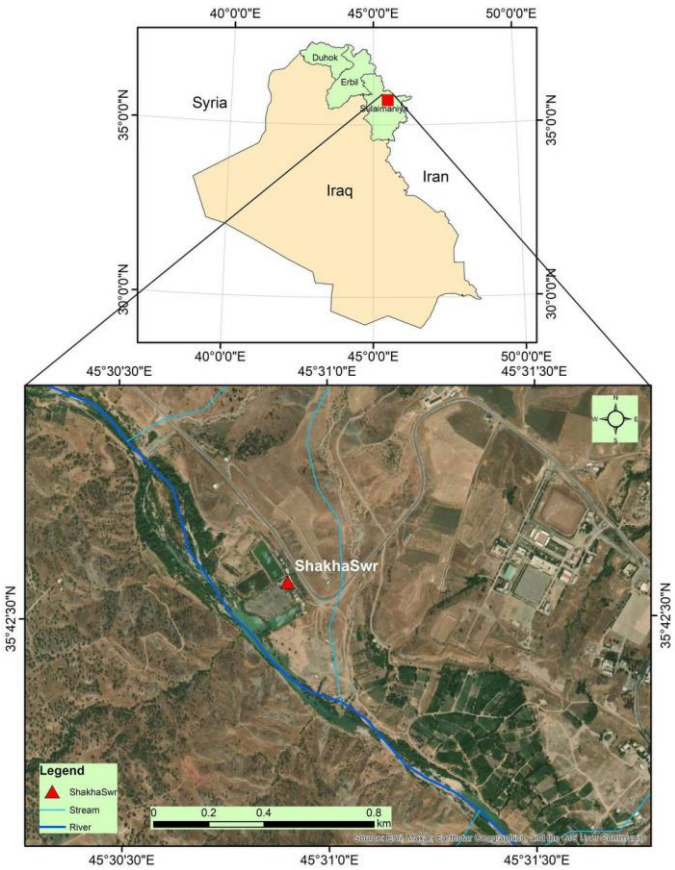

Figure 1. *(A) Map of Iraq, showing the Sulaimani province. (B) Map showing the study area (Google Maps)*

Bacterial isolation and identification:

The bacterial isolations specimens were taken by swabbing from the surface of skin ulcers, erosion, tail, fin, and gills, specifically from ulcers that were visible to the naked eye of *C. carpio*. Suspect fish were then examined for the existence of pathogenic bacterial species, and the inoculated tubes and palates were then incubated at 37°C for 24 h. The bacterial colonies were first identified by Biochemical assays and VITEK®2 Systems (bioMérieux, USA), and finally, the 16S rRNA of 21 bacteria was amplified by PCR and sequenced.

Molecular characterization of isolated bacteria

Genomic DNA was extracted using the AddPrep Bacterial Genomic DNA Extraction Kit, Korea in accordance with the manufacturer's recommended laboratory procedures. Universal 16SrRNA bacterial primers forward F16S (5-TGGCTCAGATTGAACGCTGGCGGC-3), and reverse R16S (5-TACCTTGTTACGACTTCACCCCA-3), Lee et al. (2008) were used to amplify 1500 bp of genomic DNA isolated from each strain. Polymerase chain reaction (PCR) (Techne,

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USA) was performed with the initial denaturation temperature for 5 min at 94°C, followed by 35 cycles of 94°C for 30 s,62°C for 40, 72°C for 40 s with a final extension of 5 min at 72°C and a 4°C hold. The PCR results were electrophoresed in 1% agarose gel with Tris-boric-EDTA buffer containing ethidium bromide (0.5 g/ml) (TransGen, China), the fragments were observed by gel documentation (BIO-RAD, USA), and fragment sizes were verified using bands of a 100 bp DNA ladder (Promega, USA). The amplicon was then purified using the QIAquick PCR Purification kit (QIAGEN, Germany).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing for the isolated bacteria was carried out using the Kirby-Bauer disc diffusion method on the Müller-Hinton agar medium (Oxoid, UK). To adjust 0.5 McFarland turbidity standards, the bacterial suspension was modified and then transferred to the Mueller-Hinton agar plate Using a sterile cotton swab, the entire agar surface was rubbed against to evenly seed the plate, which was then left incubating for 24 h. Using sterile forceps, antibiotic-impregnated disks were placed on the inoculated plates in double replicates after the inoculums had dried. The plates were then incubated aerobically at 37°C for 24 h. A total of 21 different genera and species from gram-positive and gram-negative bacterial isolates were examined. The standard antibiotic discs (Himedia, India) and their concentrations used against the isolates were, CD (clindamycin 2 μg) TOB (tobramycin 10 μg) AZM (Azithromycin 10 μg) MRP (Merpopenem 10 μg), AK (Amikacin 30 μg), LE (Levofloxacin 5 μg), CTR (Ceftriaxone 30 μg), CEC (Cefotaxime/clavulanic acid 30/10 μg), DO (Doxycycline 30 μg), AMC (amoxycilin clavulanic-acid 30 μg), CIP (Ciprofloxacin 5 μg), A/S (ampicillin sulbactam 10/10 μg), RIF (Rifampicin 5 μg), GEN (Gentamicin 10 μg), CAZ (Ceftazidime 30 μg), CFM (Cefixime 5 μg), NA (Nalidixic acid 30 μg), CX (Cefoxitin 30 μg), TE (Tetracycline 30 μg), E (Erythromycn 15 μg), PI (piperacillin 100 μg), NX (Norfloxacin 10 μg), VA (Vancomycin 30 μg), CTX (cefotaxime 30 μg), CPM (Cefepime 30 μg), CL (Colistin 25 μg), C (Chloramphenicol 30 μg), TR (trimethoprim 5 μg), CLR (Clarithromycin 15 μg), AT (Aztreonam 30 μg), IPM (Imipenem 10 μg), PIT (Piperacillin/tazobactam 100/10) and P (Penicillin-G 10U). Finally, The zone of inhibition formed around the discs was measured in (mm) by using a ruler. The zone of diameters was interpreted as (susceptible, intermediate, and resistant) to the critical points recommended by According to the recommendations from the Clinical and Laboratory Standards Institute (CLSI, 2021).

Detection of methicillin-resistant among Staphylococcus sp.

Methicillin resistance of *S. aureus* and *S. warneri* was detected by using 30 g discs of cefoxitin and the presence of the *mecA* gene in their genomes. PCR was used to amplify a 314 bp fragment of the *mecA* gene using the primer sets forward FMecA (5-CCTAGTAAAGCTCCGGAA-3), reverse RMecA (5-CTAGTCCATTCGGTCCA-3), PCR parameters were previously described by Duran et al. (2012).

Results

Identification and distribution of bacterial isolates

A total of 63 bacterial colonies were recovered from the 174 examined gill and skin of common carp fish in different aquacultures. The average values for the

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morphological characteristics of *C. carpio* fish were as follows: total weight 934.62 + 460 gm, total length 32.90 + 6.70 cm, and standard length 27.82 + 6.09 cm. The results of the bacteriological investigation showed that *A. sobria* was the most common isolate (15.87%), followed by *E. mori* (11.11%), *A. baumannii* (6.34%), *P. agglomerance* and *C. freundii* (7.93%), *A. veronii* and *E. profundum* (9.52%), and *C. muytjensii*, *E. fergusonii*, and *E. aestuarii* (4.76%). However, other remaining bacterial isolates showed the lowest 1.58% (*Fig. 2*). The majority of isolates are from Gram-negative bacteria rather than Gram-positive isolates.

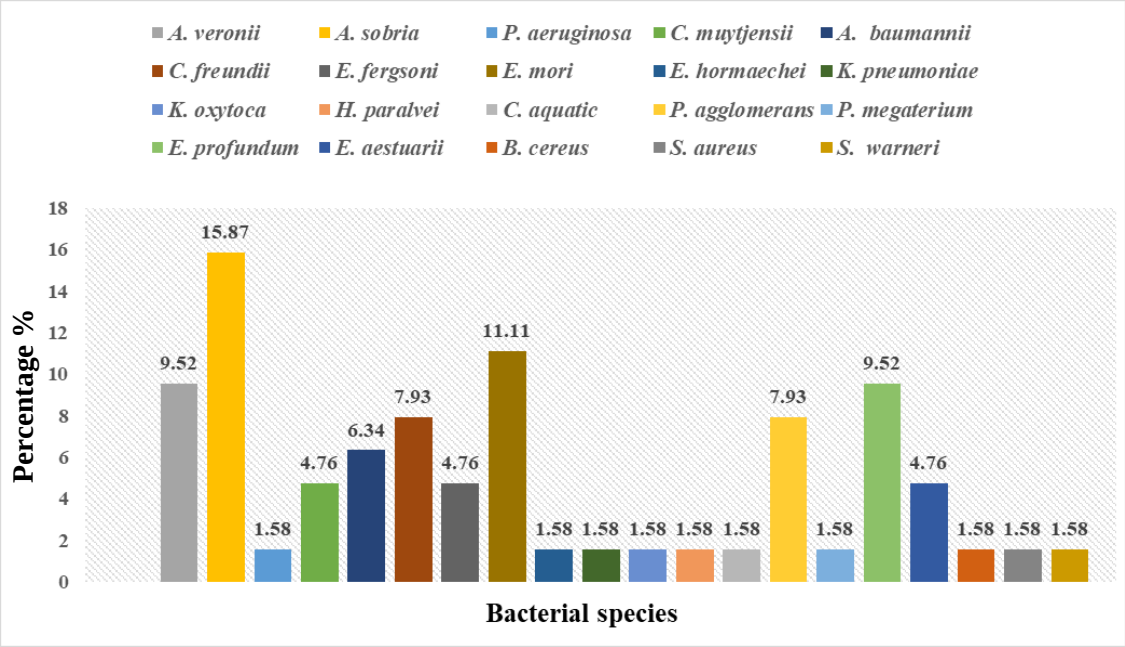

Figure 2. *The percentage of bacterial isolates from the external surface of C. carpio in Sulaimani aquacultures*

Analysis of 16S rRNA gene

Amplification of the 16S rRNA genes was performed for the 21 bacterial isolates (fourteen gram-negative and seven gram-positive bacteria) using universal primers that demonstrated ~ 1500 bp band size as shown in (*Figs. 3* and *4*). Bacterial strains were analyzed for the 16S rRNA gene using a Genetic Analyzer (Applied Biosystems/3500, Korea) and compared to those in the GenBank database based on nucleotide homology levels ranging from 99–100%. Isolates were categorized under specific accession numbers as presented in *Table 1*. The study determined that these bacterial isolates belong to the family of Enterobacteriaceae, Pseudomonadaceae, Aeromonadaceae, Comamonadaceae, Bacillaceae, Staphylococcaceae, Streptococcaceae, and Moraxellaceae.

Antibiotic susceptibility pattern of isolates

The results of *Table 2* analysis of the susceptibility profiles of 14 isolates of Gram-negative bacteria to 31 different antibiotics from several class groups showed that, with the exception of *A. veronii* to RIF, other isolates were completely resistant

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to CD, VA, and RIF, which belongs to the Lincosamides, Glycopeptides, and Ansamycins class categories. However, sensitivity to certain additional classes, including PIT-B-lactam, NX-second generation fluoroquinolones, PI-penicillin, carbapenems, and aminoglycosides, was found. Although *C. aquatica* represented the most common susceptible bacteria. Resistant to almost all generations of fluoroquinolones was found in *E. fergusonii*. Among other isolates, including *E. fergusonii* were resistant to the second generation-TOB of the class Aminoglycosides. Susceptibility to second-CEC and fourth-generation CPM cephalosporin was detected among entire isolates. The result found that most gram-negative were resistant to cephalosporins from the second-generation CX excluding *E. fergusonii* and *C. aquatic*, and *A. veronii*. Approximately most of the isolates were reported as third-generation cephalosporin (CFM, CTX, CAZ, and CTR) sensitive. However, *E. fergusonii* has shown resistance to fluoroquinolone antibiotics from three different generations. Only *K. oxytoca* was colistin-resistant. Excluding *A. baumannii*, susceptibility to AT of the class monobactam was found. The variability of isolates against antibiotics was detected.

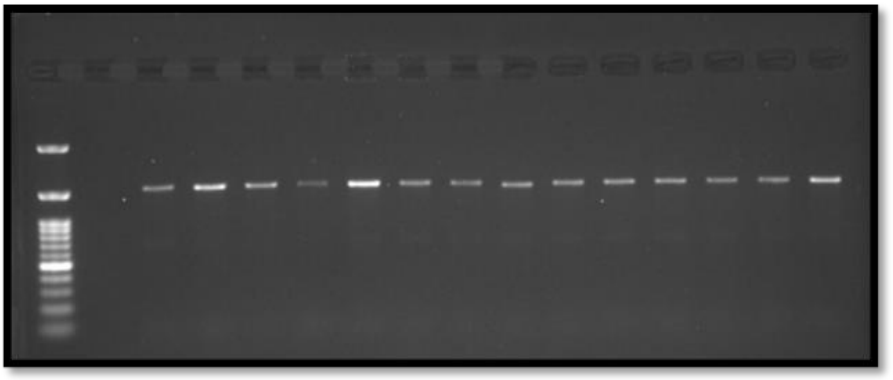

Figure 3. *Gel electrophoresis of PCR product of gram-negative isolates. Lane M: 100 bp DNA ladder, lane 1 represents Negative Control, lanes 2 -15 represent PCR amplification of 16S rRNA gene fragment of bacterial isolates*

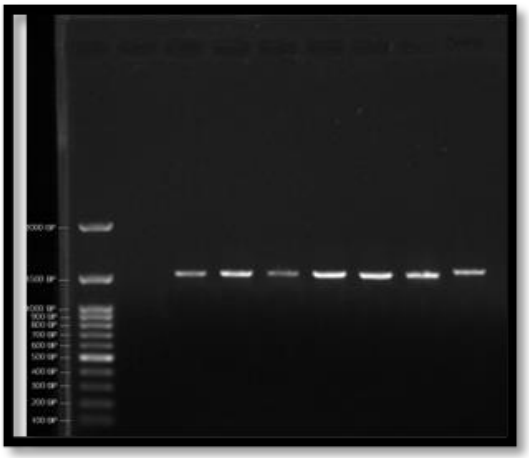

Figure 4. *Gel electrophoresis of PCR product of gram-positive isolates. Lane M: 100 bp DNA ladder, lane 1 represents Negative Control, lanes 2-8 represents 16S rRNA gene fragment of bacterial isolates*

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Table 1. *Accession numbers provided by NCBI for the bacterial isolates from C. carpio*

Isolate NO.	Bacterial isolates	Accession number	Percent identity
1	*Aeromonas veronii*	OP419528	100%
2	*Aeromonas sobria*	OP959873	99.90%
3	*Acinetobacter baumannii*	OP419527	100%
4	*Bacillus cereus*	OP959871	100%
5	*Citobacter freundii*	OP419529	99.90%
6	*Comamonas aquatica*	OP419530	100%
7	*Cronobacter muytjensii*	OP419531	100%
8	*Escherichia fergusonii*	OP419533	99.69%
9	*Enterobacter mori*	OP419532	99.71%
10	*Enterobacter hormaechei*	OP959869	100%
11	*Exiguobacterium profundum*	OP419540	99.80%
12	*Exiguobacterium aestuarii*	OP419534	99.90%
13	*Hafnia paralvei*	OP959870	99.54%
14	*Klebsiella pneumoniae*	OP959867	99.91%
15	*Klebsiella oxytoca*	OP959868	99.74%
16	*Lactococcus taiwanensis*	OP419539	100%
17	*Pantoea agglomerans*	OP419535	99.79%
18	*Pseudomonas aeruginosa*	OP419536	99.90%
19	*Priestia megaterium*	OP419538	99.90%
20	*Staphylococcus warneri*	OP419537	99.90%
21	*Staphylococcus aureus*	OP959872	100%

While the result of *Table 3*, demonstrated that gram-positive bacteria completely were susceptible to aminoglycosides, carbapenems, PIT-B-lactam, PI-penicillin, and glycopeptides classes but totally resistant to monobactams. The most prevalent sensitive isolate to antibiotics was *E. profoundum*. Resistance to first-generation E-macrolide was found among isolates with the exception of *B. cereus* and *E. profoundum*. The sensitivity of *L. taiwanensis* to ansamycin among isolates was detected. The result determined that *S. aureus, S. warneri, P. megaterium,* and *B. cereus* were found to be resistant to P-penicillin. *S. aureus, P. megaterium, L. taiwanesis*, and *E. austuarii* were shown to be resistant to all three generations of fluoroquinolones. *B. cereus* was the most well-known isolate that was resistant to generations class cephalosporin. In addition, entire isolates showed sensitivity to second- and fourth-generation cephalosporine antibiotics CPM and CX respectively.

Phenotypic multiple drug resistance (MDR) of bacterial isolates

The result determined that all isolates were MDR patterns. Thirty-two different antibiotics from fourteen different class categories were used. Most MDR isolates against the majority of the antibiotic used, which are divided into ten classes, were found in *L. taiwanensis*. While *C. aquatica* and *E. profundum* were shown to have the lowest MDR resistance to 3 and 4 classes, respectively. However, *A. baumannii, P. aeruginosa, K. oxytoca, P. megaterium,* and *S. aureus* were resistant to nine types of class antibiotics. In addition, various resistance among isolates were detected as seen in *Table 4*.

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Table 2. *Antimicrobial susceptibility patterns of Gram-negative bacterial isolates*

		Gram-negative bacterial isolates													
Class category	**Antimicrobial agent generations**	***K. penmonia***	***K. oxytoca***	***E. hormaechei***	***E. mori***	***E. fergusonii***	***C. muytjensii***	***C. freundii***	***P. agglomerans***	***H. paralvei***	***P. aeruginosa***	***A. baumannii***	***C. aquatica***	***A. veronii***	***A. sobria***
Lincosamides	CD	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Aminoglycosides	1st GEN	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	2nd TOB	S	S	S	S	R	S	I	I	S	S	S	S	S	S
	AK	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Macrolides	2nd AZM	R	R	S	R	R	S	R	R	R	R	S	S	S	S
	1st E	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Carbapenems	2nd MRP	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	1st IPM	S	S	S	S	S	S	S	S	S	S	S	S	I	S
Tetracyclines	2nd DO	R	R	R	S	R	S	I	S	S	R	S	S	S	S
	1st TE	R	R	R	S	R	S	S	S	S	R	S	S	R	S
Penicillin	AMC	R	R	R	R	S	S	R	S	R	R	R	S	R	S
	PI	S	S	S	S	S	S	S	S	S	S	S	S	S	S
B-lactam inhibitor	A/S	R	R	S	I	S	S	S	R	R	R	S	S	R	R
	PIT	S	S	S	S	S	S	S	I	S	S	S	S	S	S
Ansamycins	RIF	R	R	R	R	R	R	R	R	R	R	R	R	S	R
Cephalosporin	2nd CECCX	S	S	S	S	S	S	S	S	S	S	S	S	S	S
		R	R	R	R	S	R	R	R	R	R	R	S	I	R
	CFM	S	S	I	S	S	R	S	S	S	R	R	S	S	S
	3rd CTX	S	S	S	S	S	S	I	S	S	S	S	S	S	S
	CAZ	S	I	S	S	S	I	S	S	I	S	S	S	S	S
	CTR	S	S	S	S	S	I	S	S	S	S	I	S	S	S
	4th CPM	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Fluoroquinolones	1st NA	I	I	S	I	R	S	S	I	S	R	S	S	R	I
	2nd CIP	I	I	S	S	R	S	R	S	S	S	S	S	S	S
	NX	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	3rd LE	S	S	S	S	R	S	S	S	S	S	S	S	R	S
Glycopeptides	VA	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Lipopeptides	CL	S	R	S	S	S	S	S	S	S	S	S	S	S	S
Phenicols	C	S	S	S	S	R	R	R	S	S	S	R	S	R	R
Folate pathway inhibitor	TR	S	S	S	S	R	R	S	S	I	R	R	S	S	S
Monobactams	AT	S	S	S	S	S	S	S	S	S	S	R	S	S	S

Table 3. *Antimicrobial susceptibility patterns of Gram-positive bacterial isolates*

Class category	**Antimicrobial agents**	**Gram-positive bacterial isolates**						
		S. aureus	***S. warnerii***	***P. megaterium***	***B. cereus***	***L. taiwanesis***	***E. profoundom***	***E. austuarii***
Lincosamides	CD	R	S	R	S	R	S	R
Aminoglycosides	1st GEN	S	S	S	S	S	S	S
	2nd TOB	S	S	S	S	R	S	S
	2nd AK	S	S	S	S	S	S	S
Macrolides	1st AZM	R	R	R	S	R	R	R
	CLR	I	S	R	S	R	S	R
	E	R	R	R	S	R	S	R
Carbapenems	2nd MRP	S	S	S	S	S	S	S
	1st IPM	S	S	S	S	S	S	S

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Penicillin	AMC	S	S	S	R	S	S	S
	PI	S	S	S	S	S	S	S
	P	R	R	R	R	S	S	S
B-lactam inhibitor	A/S	S	S	S	R	S	S	S
	PIT	S	S	S	S	S	S	S
Ansamycins	RIF	S	S	S	S	R	S	S
Tetracyclines	2nd DO	S	S	R	R	R	S	R
	1st TE	R	S	R	R	R	S	R
Fluoroquinolones	1st NA	R	R	R	S	R	R	R
	2nd NX	R	S	R	S	R	S	R
	CIP	R	S	R	S	R	S	R
	3rd LE	R	S	R	S	R	S	R
Glycopeptides	VA	S	S	S	S	S	S	S
Cephalosporin	2nd CEC	S	S	S	S	S	S	S
	CX	R	R	R	R	R	S	S
	CAZ	I	S	S	R	S	S	S
	3rd CTR	S	S	S	R	S	S	S
	CFM	R	I	I	R	I	R	R
	CTX	I	S	S	R	S	S	S
	4td CPM	S	S	S	R	S	S	S
Phenicols	C	R	R	R	S	R	S	S
Folate pathway inhibitor	TR	R	S	R	R	R	S	R
Monobactams	AT	R	R	R	R	R	R	R

All antibiotic abbreviation was written in the material and method. S-Susceptible, I- Intermediate, R-Resistant

Table 4. *Antibiotic resistance pattern of multidrug resistant bacterial isolates*

Bacterial isolates	Resistant pattern	No. of resistance classes	MDR status
A. veronii	AMC, LE, CD, A/S, NA, TE, E, VA, C	7	MDR
A. sobria	CD, A/S, RIF, CX, E, VA, C	7	MDR
A. baumannii	CD, AMC, RIF, CFM, CX, E, VA, C, TR, AT	9	MDR
P. aeruginosa	CD, AZM, DO, AMC, A/S, RIF, CFM, CX, NA, TE, E, TR, VA	9	MDR
C. aquatica	VA, RIF, CD	3	MDR
P. agglomerans	CD, AZM, RIF, A/S, CX, E, VA	6	MDR
C. muytjensii	CD, RIF, CX, E, CFM, VA, TR, C	8	MDR
E. fergsonii	CD, TOB, AZM, LE, DO, CIP, RIF, NA, TE, E, VA, C, TR	8	MDR
C. freundii	CD, AZM, AMC, CIP, RIF, CX, E, VA, C	7	MDR
E. mori	CD, AMC, AZM, RIF, CX, E, VA	7	MDR
E. hormaechei	CD, AMC, DO, RIF, CX, TE, E, VA	7	MDR
K. pneumoniae	CD, AZM, DO, AMC, RIF, A/S, CX, TE, E, VA	8	MDR
K. oxytoca	CD, AZM, DO, AMC, RIF, A/S, CX, TE, E, VA, CL	9	MDR
H. paralvei	CD, AZM, AMC, RIF, A/S, CX, E, VA	6	MDR
S. warneri	AZM, NA, CX, E, C, AT, P	5	MDR
S. aureus	AZM, CD, CX, LE, CIP, CFM, NA, TE, E, NX, C, TR, AT, P	9	MDR
L. taiwanensis	CD, TOB, AZM, LE, DO, CIP, RIF, NA, CX, TE, E, NX, C, TR, CLR, AT	10	MDR
E. profundum	CFM, NX, AZM, AT	4	MDR
E. aestuarii	CD, AZM, LE, DO, CIP, CFM, NA, TE, E, NX, TR, CLR, AT	6	MDR
P. megaterium	CD, AZM, LE, DO, CIP, NA, CX, TE, E, NX, C, CLR, AT, TR, P	9	MDR
B. cereus	CTR, AMC, DO, CAZ, CFM, A/S, CX, TE, CTX, CPM, TR, AT, P	7	MDR

Resistance genes among Staphylococcus spp.

The results were interpreted in accordance with (CLSI, 2021), based on the cefoxitin inhibition zone diameter of ≤ 21 mm, The findings showed that 1.58% of both *S. aureus*

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and S. warneri are methicillin-resistant, due to cefoxitin resistance and the presence of the *mecA* gene, which was successfully detected 314 bp of the gene by PCR as shown in *Figure 5*.

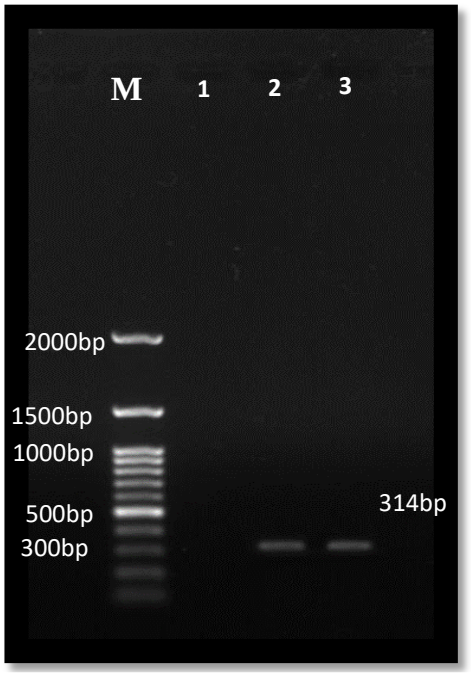

Figure 5. *Agarose gel electrophoresis of the PCR products confirmed the mecA gene and showed amplicons (~ 314 bp), Lane M: 100 bp DNA ladder, lane 1 represent negative control, lanes 2 and 3 show mecA gene in S. aureus and S. warneri respectively*

Discussion

Fish are an essential source of nutrition for humans and are vulnerable to infectious diseases brought on by the wide variety of bacterial infections found in aquaculture facilities where they are farmed (Al-Shammari et al., 2019). The present study detected several bacterial species depending on 16S rRNA gene analyses, which belong to eight different families (*Table 1*). This is, in accordance with other studies of isolated bacteria (Oh et al., 2019; Keiz et al., 2023). According to Mhaisen (2023), 12 species of bacteria (*B. cereus, B. megaterium, C. aquatica, C. freundii. E. hormaechei subsp. xiangfangensis, E. mori, E. fergusonii, E. aestuarii, E. profundum, L. taiwanensis, P. agglomerans* and *S. warneri*) from *C. carpio* are regarded as the first to be recorded in Iraq.

The majority of pathogens in the aquacultures of Sulaimani belong to the genus *Aeromonas*, with *A. sobria* accounting for 15.87% of those (*Fig. 2*). The result was in agreement with the findings of (Chen et al., 2019), which identified *Aeromonas spp*. as the primary pathogen in fish farms and *A. veroni* has been more frequently infecting fish. Whereas the result does not agree with Chakraborty et al. (2022). *Aeromonas sp.* incidence in aquaculture and other environments is correlated with stress factors, such as environmental changes, temperature swings, insufficient harvest, irregular transport, and factors related to insufficient forms of commercialization, in addition to high densities (Barcellos et al., 2008). *P. aeruginosa* is a significant opportunistic fish pathogen that affects stressed and immune-compromised fish and causes Pseudomonas

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septicemia (Ali et al., 2021). The results of *P. aeruginosa* infection were similar to those of Wamala et al. (2018) but varied with the results of Garabawi et al. (2022). Host susceptibility, environmental factors, and the sampling season all had an impact on prevalence variations (Algammal et al., 2020). Instead of being a human pathogen, *Comamonas spp.* are significant environmental bacteria that are connected to environmental bioremediation (Ryan et al., 2022). It has been isolated from a variety of habitats and is predicted to have a low pathogenicity (Pavone et al., 2021). No information was available to us about the incidence rate of the bacteria from Iraq, although there were a few records of *Comamonas spp*. in different species of fish in Uganda (Wamala et al., 2018). *Acinetobacter*, *Aeromonas*, and *Pseudomonas* are all positively linked with the presence of comamonas (Palanisamy et al., 2022). As fish farming becomes more intensive, the unique bacteria will cause fish disease to become more severe in the future, a practice characterized by high stocking density, poor water quality, and more human meddling. *A. baumannii* is among the many bacterial septicaemic illnesses, and fish mortality which is distributed in different environments (Malick et al., 2020). Considering the environmental conditions involved, it was believed that *Acinetobacter sp.* in common carp was a developing opportunistic bacterium for aquaculture (Dadar et al., 2016).

Microorganisms from the Enterobacteriaceae family are the main source of infection in humans, with 11.11% of *E. mori* having the greatest recorded prevalence. Salgueiro et al. (2020) made a similar discovery, in contrast to Garabawi et al. (2022), who found that *C. freundii* was the most common bacterial infection. According to El-Barbary and Hal (2016), *K. oxytoca* is an opportunistic disease that affects fish and is thought to be a symptom of sewage contamination. Despite the fact that Enterobacteriaceae are commonly found in the normal microbiota of fish, incorrect handling of professionals that work with fish farms, and the indiscriminate use of antibiotics can lead to the onset of diseases in fish transmittable to the human consumer (Oliveira et al., 2017). Additionally, the degree of environmental pollution has been identified as a further factor contributing to differences in the occurrence of Enterobacteriaceae in freshwater fish (Rawash et al., 2019).

The results also showed that *E. profundum* was the most common of Gram-positive bacteria, the findings concur with a study by Manan et al. (2022) that found *E. aestuarii* and *E. profundum* to be dominant, but they were not supported by Agbeko et al. (2022) who found *S. aureus* to be the dominating organism. *E. aestuarii* can be suggested as a probiotic for *L. vannamei* and improve the water quality in aquaculture (Kim et al., 2022). Several fish species, including silver carp and rainbow trout in Spain, have been documented to have illnesses caused by *Staphylococcus* species (Seçil et al., 2014). *B. megaterium*, formerly known as *P. megaterium*, was deliberately created for biotechnological uses (Biedendieck et al., 2021). *B. cereus* has the potential to cause disease in many fish species, including *C. carpio* (Algammal et al., 2020). About *L. taiwanensis* in fish, no information has been acquired. Other prior investigations have established *L. garvieae*, including one by Soltani et al. (2021). In this research, the predominant isolates were represented by Gram-negative bacteria rather than Gram-positive isolates, which was consistent with Ojasanya et al. (2022). Overall, the distribution of bacterial isolates becomes comparable with our findings depending on the species, sampling duration, geographic scope, fish species, the methods used to find the bacteria, and their sensitivity and specificity (Vivekanandhan et al., 2005).

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Globally, antimicrobial resistance (AMR) has become a serious hazard to public health (Gajic et al., 2022). Resistance against CD, VA, and RIF antibiotics among gram-negative was detected, perhaps gram-negative bacteria characterized by their intrinsic resistance, often chromosomal mediated and movable to consecutive progeny during cell division (Reygaert, 2018). Resistant against (A/S) β-lactams and fluoroquinolones (first and second generation) not consistent with Dhanapala et al. (2021) in Srilanka. Contrarily, the most vulnerable to aminoglycosides and resistant to β-lactams and lincosamide antibiotics concur with the present findings (Zdanowicz et al., 2020). The same conclusion about CX-cephalosporin and quinolone resistance was reported from the study of (Conte et al., 2021), but not the same regarding carbapenem and aminoglycoside susceptibility. The majority of antibiotics used in aquaculture are effective against gram-negative bacteria because aerobic, gram-negative rods represent the majority of the bacterial pathogens in aquatic animals (Petty et al., 2020).

The source of Aeromonas sp., as well as the frequency and kind of antimicrobial medications used to treat Aeromonas infections in cultured fish in different geographic locations, produced diverse antibiotic study outcomes (Son et al., 1997). The results showed that *K. oxytoca* was resistant to colistin, but WHO (2020) demonstrated that *Klebsiella spp.* were naturally susceptible to it. The resistance and sensitivity of *A. baumannii* toward several antibiotics in the present findings are not similar to the previous studies (Adewoyin et al., 2021; Al-Sheboul et al., 2022). *Acinetobacter* reports of carbapenem resistance are on the rise, making MDR *Acinetobacter* infections challenging to treat (Almasaudi, 2018). Previous reports of Carbapenem-resistant in *A. baumannii* were not consistent with our findings because human activities have contaminated fish farms from many aquatic sources.

P. aeruginosa is intrinsically resistant to several antibiotics, due to its bigger genome's distribution in aquatic settings and its outer membrane's reduced permeability, which has resulted in variations in the drug response (Vaisvila et al., 2001). However, antibiotics including CTX, are taken into consideration as a possible drug in the treatment (Ali et al., 2021). The data on *Comamonas spp.* antibiotics susceptibility was reviewed by (Rayan et al., 2022). Also, the bacteria exhibit resistance to the β-lactams of antibiotics as a result of the presence of multiple genes (Zhuang et al., 2017). Resistance of pathogens towards new generation cephalosporins probably raised major challenges. The presence of plasmids in 92% of the isolates studied of Enterobacteriaceae, including their plasmid-mediated AMR, demonstrated the severe spread of AMR in aquaculture ecosystems (Preena et al., 2021). Cephalosporin-resistant Enterobacteriaceae isolates have previously been discovered in Swiss fish and aquatic environments (Abgottspon et al., 2014). Since a heat-labile toxin is present on a plasmid of *E. fergusonii* potentially resists many drugs (Hamza et al., 2020). The sensitivity to NX-fluoroquinolones is inconsistent with (MAO et al., 2019), which discovered that many Gram-negative bacteria in the fish pond sediments were norfloxacin resistant. The result presented large numbers of antibiotic-resistant gram-negative bacteria from the external surface of *C. carpio,* which have negative environmental and public health impacts. Therefore, Antimicrobial agent usage in fish culture must be properly regulated.

There have been few studies on the antibiotic resistance pattern of *Exiguobacterium spp*. in aquacultures. *Exiguobacterium spp*. harbored some antimicrobial resistance genes, such as tetracycline and macrolide, as detected by Chen et al. (2017), which were observed in the current result, excluding aminoglycoside and phenicol. Resistance of *Exiguibacterium spp*. to Macrolides, Lincosamides, Tetracyclines, and Monobactams

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was not found in the current study, despite the presence of Fluoroquinolones (Jain and Kamble, 2018). *Bacillus spp*. can be used as probiotics in aquaculture to decrease the use of antibiotics and prevent antibiotic-resistance genes from spreading (Jinendiran et al., 2019). According to the study results of Algammal et al. (2020), *B. cereus* possessed tetracycline and macrolide resistance genes, which is compatible with our findings. While *L. taiwanensis* is the most common resistant isolate against ten different antibiotic classes. Previous studies, when compared to the current results, found *L. garvieae* instead of *L. taiwanensis*. Also, the study of Kim et al. (2020) demonstrated that *L. taiwanensis* has antimicrobial activity against pathogenic bacteria. According to Korun et al. (2021), erythromycin is used to treat sick fish in farms where lactococcus is a problem, so erythromycin resistance in our findings should be taken seriously. Tetracyclines, sulphonamides, and quinolones were the most important antibiotic classes used as antimicrobial compounds (Lulijwa et al., 2020). The current investigation finds that MDR bacteria are present in fish farms (*Table 4*), which is consistent with the results of the earlier study by Koudou et al. (2020), as a result of the indiscriminate and uncontrolled use of antibiotics for humans, veterinary, and food purposes.

S. aureus (MRSA) has been identified as a zoonotic pathogen in humans and a number of animal species (Aires-de-Sousa, 2017). Methicillin resistance requires the presence of the *mecA* gene, this was done using the PCR method, which is considered to be the gold standard. However, the Cefoxitin disk diffusion method can be utilized as an alternative to PCR for the identification of MRSA (Koupahi et al., 2016). The *mecA* gene has been found in *S. warneri* (*Fig. 5*) for the first time in the Iraq region, which was consistent with the finding of Grema et al. (2015). *S. aureus* gains more virulence factors and methicillin resistance resulting from horizontal gene transfer of the *mecA* gene from *S. epidermidis* and other coagulase-negative staphylococci. In addition, the occurrence of *S. aureus* regarding MRSA in Fish and Fishery products was documented by Vaiyapuri et al. (2019). The sensitivity of *S. aureus* to CTR was confirmed by Masood and Aslam (2010), which is used as a first-line treatment. It was determined that certain antibiotic-resistance genes provide resistance to aminoglycosides, tetracyclines, β-lactams, macrolides, and lincosamides (Hammad et al., 2012). *Staphylococcus spp.* has become resistant to antibiotics as a result of aquaculture antibiotic use, terrestrial contamination of water sources, or contamination from fish processing facilities (Smith et al., 2013). Additional focus should be placed on fish care and the cleanliness of fishing equipment to prevent bacterial colonization and fish infection. Therefore, it is necessary to continue studying and monitoring the resistance of these bacteria affects fish raised in aquaculture in the Kurdistan region.

Conclusion

The study concluded that Gram-negative bacteria were more frequently found than Gram-positive isolates which offers extra data for quantitative risk assessment that was not available in earlier studies. The finding of the study supported the use of several antibiotics in aquaculture fish, which is relevant to the economic expansion of fishing. While increasing multidrug resistance among bacterial isolates in the present study are become endangering human health. These findings were made for the first time in such a broad line of occurrence of a variety of bacterial species and their resistance to antibiotics in Sulaimani fish farms.

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