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Microbial Association with Gallstone Disease in Maysan Province, Iraq

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ABSTRACT

Good health and well-being are among the UN Sustainable Development Goals. Based on this, the present study aimed to investigate a prevalent disease in Iraq, namely gallstone disease, along with the microbial infections associated with cholelithiasis and their antibiotic sensitivity patterns. The study was conducted at Teaching al-Sadr Hospital and Al-Zahrawi Surgical Hospital in Maysan, Iraq, from November 2021 to December 2022. Sixty cases of cholelithiasis in men and women between the ages of 20 and 50, who underwent cholecystectomy, were selected.

The most common organism identified was *Escherichia coli*, found in 20 patients (33%). This was followed by *Pseudomonas fluorescens* in 7 patients (11.6%), *Klebsiella pneumoniae* in 6 patients (10%), *Serratia marcescens* in 6 patients (10%), *Pseudomonas aeruginosa* in 5 patients (8.3%), *Kluyvera cryocrescens* in 5 patients (8.3%), *Proteus vulgaris* in 5 patients (8.3%), *Raoultella orithinolytica* in 3 patients (5%), *Klebsiella oxytoca* in 2 patients (3.3%), and *Enterobacter cloacae* in 1 patient (1.6%). The biliary organisms showed various antibiotic sensitivity patterns. The highest sensitivity was observed with Cefotaxime (71%). The other antibiotics arranged as following; Amikacin (56%), Gentamicin (50%), Ciprofloxacin (46%), Meropenem (40%), Ceftazidime (35%), Imipenem (35%), Ertapenem (10%), Ampicillin (10%), Amoxicillin clavulanate (10%), and Cefepime (8%).

In addition, female gender and advanced age were noted as contributing factors to gallstones. Cholesterol stones were found to be more common than other kinds of gallstones.

KEYWORDS: Gallstone disease, Microbial infection, Cholelithiasis, Antibiotic sensitivity

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INTRODUCTION

Gallstone disease (GSD) has been a persistent and socially significant public health issue, entailing substantial costs. In recent years, extensive research has been conducted, encompassing human and animal studies to explore the involvement of the microbiota in different segments of the gastrointestinal tract as a novel connection to GSD etiopathogenesis. Notably, the microbiome of bile correlates with the bacterial composition of saliva, while the microbiome of the biliary tract exhibits a striking resemblance to the microbiota found in the duodenum (1).

In the study of Eslami et al. (2), 100 patients had cholecystectomy. Out of these individuals, 63 samples showed evidence of bacterial growth, with 11 samples showing the presence of two bacterial species. There were 63 gram-negative bacteria (including *E. coli*, *Klebsiella*, *Aerobacter*, *Pseudomonas*, *Proteus*, *Citrobacter*, *Providencia*, and *Acinetobacter*) and a total of 10 gram-positive bacteria (including enterococci and staphylococci).

Forty patients were enrolled in the study conducted by Al-Zuharri in 2011(3), which took place from April 2008 to October 2008 in Al-Najaf province. Among them, 16 had cholecystitis, while 24 had cholelithiasis. All patients underwent cholecystectomy. The results indicated that all patients had positive cultures. *Escherichia coli* was the most commonly isolated bacterium, accounting for 75% of cholecystitis cases and 79.17% of cholelithiasis cases. β-hemolytic streptococci were present in 12.5% of cholecystitis cases and 8.33% of cholelithiasis cases. *Klebsiella* spp. were found in 6.25% of cholecystitis cases and 12.5% of cholelithiasis cases. *Proteus* spp. were isolated from 6.25% of patients with cholecystitis.

Forty stones were collected in the study conducted by Abd-Alkareem in 2011(4). These stones exhibited bacterial growth, with the following bacteria being identified: *Escherichia coli* in 12 stones (48%), *Klebsiella pneumoniae* in 4 stones (16%), *Pseudomonas aeruginosa* in 3 stones

(12%), *Salmonella enterica* (S.Typhi) in 4 stones (16%), and *Staphylococcus aureus* in 2 stones (8%).

The findings of the study conducted by Arteta et al. (5) suggested that out of the 149 patients included in the study, 28 individuals (19%) tested positive for bacterial cultures. Among the patients with positive cultures, 21 individuals (75%) were from the Medellin metropolitan area. The most commonly isolated bacteria in this geographical location were *Pseudomonas* spp. (38%), *Klebsiella* spp. (23%), and *Proteus* spp. (9%), along with a few cases of other bacterial species. In the city of Apartado, the isolated bacteria were *Enterobacter cloacae* (50%) and *Raoultella terrigena* (32%). Interestingly, one male patient (18%) had both *Enterobacter cloacae* and *Raoultella terrigena* present in their culture. Furthermore, five out of the 149 patients (3.3%) tested positive for the hilA gene of Salmonella spp. using the polymerase chain reaction (PCR) method. All of these patients were female and residents of the Medellin metropolitan area.

According to a study by Thomas et al. in 2019 (6), 70 stones were cultured for bacteria. Out of these, 52 stones (74.3%) were found to be sterile, meaning no bacteria were detected. On the other hand, 18 stones (25.7%) tested positive for bacterial growth. Among the stones with bacterial growth, 16 showed the presence of a single type of bacteria (mono-microbial growth). In contrast, two showed the presence of multiple types of bacteria (poly-microbial growth).

Aim of the study: The aim of the study was to shed light on the incidence of gallstones in Maysan Province and to identify associated bacterial infections.

MATERIALS AND METHODS

Stone Sampling:

For the stone sampling, 60 patients participated in the study. These patients provided gallstone samples after cholecystectomy at Al-Sadr Teaching Hospital and Al-Zahrawi Surgical Hospital in Maysan Province, Iraq. The sampling period spanned from November 2021 to December 2023. The age range of the participants was between 20 and 50 years. In addition to collecting gallstone samples, relevant information such as age, gender, recorded using a questionnaire list.

Out of 60 patients, 40 patients with cholelithiasis (15 men and 25 women) were prepared for stone chemical analysis collected after cholecystectomy. The stones were washed with distilled water and dried. Then they were powdered in a pestle and mortar to detect the organic and inorganic compounds (7). The detection process involved different approaches depending on the type of compound. For cholesterol and bilirubin stone, manual isolation was performed based on visual color. Cholesterol stones were yellowish-white in appearance (8) bilirubin stones were identified by their black or brown color (9).

For the analysis of other types of compounds, a Stone Analysis Set from BIOLAB SAS in France was used. This kit provided the necessary tools and reagents to identify and analyze the composition of the gallstones.

MICROBIAL SAMPLING

A total of 60 patients participated in the study. To collect the samples, special swabs containing a gel were used. These swabs were designed to effectively capture the desired specimen. After collection, the samples were promptly delivered to the laboratory for further processing.

In the laboratory, the collected samples were plated on MacConkey agar, which is a type of culture medium manufactured by Guangdong Huankai Microbial Sci and Tech. CO, China. The plated samples were then incubated at a temperature of 37°C for a period of 18 to 24 hours. This incubation period allowed the growth and development of microorganisms present in the samples. By utilizing this method, the study aimed to isolate and identify bacterial species present in the collected samples.

MICROSCOPICAL EXAMINATION AND STAINING

After colonies appeared on the MacConkey agar plates, the colony color and type frequency were documented in table 5 of the study.

To further analyze the bacterial isolates, Gram staining was performed. The Gram staining procedure was conducted following the manufacturing guidelines of AFCO, a manufacturer based in Jordan. Gram staining is a common laboratory technique used to differentiate bacteria into Gram-positive and Gram-negative categories based on their cell wall composition.

Biochemical identification

To identify bacterial isolates and determine their drug sensitivity, samples were analyzed using the VITEK 2 System, manufactured by Biomerieux in France. The analysis involved testing the isolates against more than 18 antibiotics at various concentrations, including the determination of the minimum inhibitory concentration (MIC).

To prepare the samples for the system, the turbidity of the bacterial culture was measured using three plastic tubes to create a bacterial solution. Additionally, a small device called the VITEK 2 Densi-Chek, also manufactured by Biomerieux in France, was used to measure the turbidity of the bacterial culture accurately.

RESULTS AND DISCUSSION

The present study is the first research conducted in Maysan Province that explores the association between gallstones and bacterial presence.

In the recent study, a total of forty gallstones were chemically classified. Out of these, 25 were obtained from female participants and 15 from male participants. The gallstones were classified into five different types, as presented in Table 1.

Table (1): Types of gallstone obtained

Types of Gallstone	Number	Percentage (%)
Cholesterol	13	32.5
Bilirubin	8	20
Calcium	7	17.5
Phosphorus	4	10
Calcium- Phosphorus	8	20

Among the various types of gallstones, cholesterol gallstones were the most common in our study, accounting for 32.5% of the cases. These findings are consistent with the results reported by Abd-Alkareem et al. (4), who found that out of 40 gallstones, 25 (62.5%) were cholesterol gallstones, and 15 (37.5%) were pigment gallstones. Similarly, the study by Thomas et al. in 2019 (6) also recorded that 57.1% of the gallstones were cholesterol stones, while 42.9% were pigment stones

Most gallstones are composed of cholesterol. They are formed when cholesterol separates from bile during bile storage in the gallbladder between meals. Approximately 75% of gallstones are cholesterol, while the remaining 25% are pigmented stones. The formation of cholesterol gallstones occurs when the liver secretes bile that is saturated with cholesterol, leading to the precipitation of cholesterol from the solution. Cholesterol is insoluble and requires transportation within the bile using salt micelles and phospholipid vesicles. However, when the amount of cholesterol exceeds the bile's capacity to hold it, cholesterol crystals begin to precipitate. Over time, cholesterol crystals aggregate to form visible gallstones within the gallbladder (10)

In terms of gender, females, and individuals within the age range of 33-50 exhibited a higher incidence of gallstones, as presented in Tables 2 and 3.

Table 2: Incidence of gallstones according to gender

Gender	No. of gallstone	Percentage (%)
Male	23	38.33
Female	37	61.66

Table 3: Incidence of gallstones according to age group

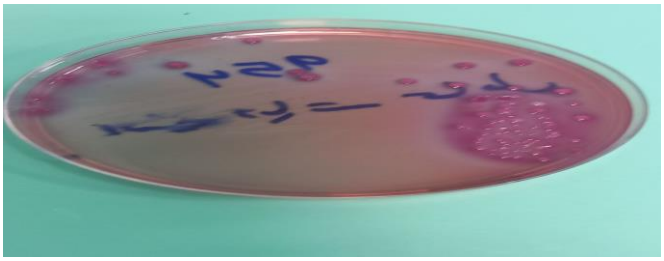
Age period	No. of gallstone	Percentage (%)
20-33	19	31.66
33-50	41	68.33

The present study found that out of sixty patients with gallstones, females accounted for 61.66 % of the total, whereas the male (38.33 %). The most common age group affected by gallstones was between 33 and 50 years old, with a percentage of 68.33%. In contrast, the age group of 20 to 33 years accounted for 31.66% of cases.

These findings align with the findings of Alishi et al. (11) and Aklan et al. (12). Alishi et al. demonstrated that the older age group in their study was significantly associated with the presence of gallstones, with 88.4% of the gallstone group being over 45 years old, compared to 11.6% in the younger age group (30-45 years old). Furthermore, they found that the female gender showed a significant association with gallstones, with 72.1% of females suffering from gallstones compared to 27.9% of males. In the control group, 51% of females were affected by gallstones. Similarly, Aklan et al. reported that females had a significantly higher frequency of gallbladder stones (GBS) compared to males (8.0% vs. 3.2%, $p < 0.001$). The majority of GBS patients in their study were aged 60 years and above (12.9%), while the lowest incidence was observed in the age group below 20 years (1.3%), suggested that older age is a risk factor for GBS, as the prevalence increases with age.

Sex hormones are likely responsible for the increased risk, as estrogen increases biliary cholesterol secretion, leading to cholesterol supersaturation of bile. Therefore, hormone replacement therapy in postmenopausal women and oral contraceptives have also been associated with an increased risk of gallstone disease (13).

Regarding the microbial study, the results revealed various bacterial colonies with different colors and shapes, as shown in Table 5 and Picture 1.

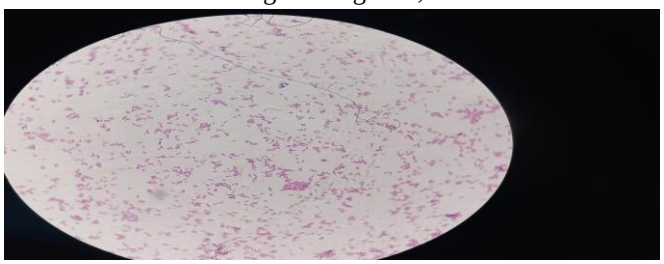


Picture 1: Bacterial colonies

Table 5: Morphological characteristics of selected colonies

Colonies color	Sample no frequency	Gram stain	Microscopic shape
pink to dark pink,	20	-ve	small rod shape
Colorless	7	-ve	rod-shaped
pale or colorless	6	-ve	rod-shaped
greenish-gold fluorescent pigment	6	-ve	rod-shaped
pink-yellow mucoid colonies	5	-ve	rod-shaped
pink colored	5	-ve	rod-shaped
dark red colonies against the light pink background	5	-ve	rod-shaped
glistening pink colonies	3	-ve	rod-shaped
pink-red colonies	2	-ve	rod-shaped
red colonies	1	-ve	Straight rods.

Staining demonstrated that all the selected isolates were gram-negative, as seen in Picture 2.



Picture 2: Gram-negative bacteria

After biochemical identification using the VITEK 2 system, a variety of species were identified, as presented in Table 6.

Table 6: Bacterial species identified by the VITEK 2 system

NO	bacteria isolate	frequency	Percentage (%)
1	*Escherichia coli*	20	33%
2	*Pseudomonas fluorescens*	7	11.60%
3	*Klebsiella pneumoniae*	6	10%
4	*Serratia marcescens*	6	10%
5	*Pseudomonas aeruginosa*	5	8.30%
6	*Kluyvera cryocrescens*	5	8.30%
7	*Proteus vulgaris*	5	8.30%
8	*Raoultella orithinolytica*	3	5%
9	*Klebsiella oxytoca*	2	3.30%
10	*Enterobacter cloacae*	1	1.60%

In the recent study, out of the 60 isolates obtained, *E. coli* was the predominant bacteria, with a frequency of 33%, followed by other bacteria.

The prevalence of *E. coli* among the selected samples in our results agrees with the findings of Al-Zuharri (3), where *E. coli* represented the most common isolates, accounting for 75% in cholecystitis and 79.17% in cholelithiasis. It also aligns with the study conducted by Atiyah et al. (14), which showed that *E. coli* was the predominant isolate among the positive cultures, accounting for 54.55% of the isolates. However, it should be noted that the two studies had differences in other bacteria observed.

Nevertheless, other bacterial genera were prevalent. Hazrah et al. (15) demonstrated that *Klebsiella* spp. was the most common organism isolated, accounting for 18% of cases, followed by *E. coli* (15%), *Enterococcus* spp. (7.5%), and *Enterobacter* spp. (7.5%). *Salmonella* sp. was observed in only 1.5% of cases. *Pseudomonas* spp. (8.8%) was the most common non-enteric pathogen, followed by *Acinetobacter* spp. (7.5%) and *Staphylococcus aureus* (3.8%).

Furthermore, Thapa et al. (16) reported that among positive cultures of patients, *Pseudomonas* was the most commonly cultured organism in 68.4% (29.3% overall). Other isolated organisms included *E. coli*, *Staphylococcus*, *Klebsiella*, Enterococci, and *Acinetobacter*.

Many previous studies have shed light on the role of bacterial presence in gallstone formation. One such study is by Maki (17), who mentioned that both bile stagnation and infection, particularly with *Escherichia coli*, along with dietary deficiencies, appear to induce the formation of calcium bilirubinate stones, which are common in the Asian region. Regarding the mechanism by which calcium bilirubinate separates from bile, it is presumed that the activity of bacterial-origin ft-glucuronidase plays an essential role. This enzyme hydrolyzes bilirubin glucuronide into free bilirubin and glucuronic acid, and the calcium in bile combines with the carboxyl radical of the liberated bilirubin to form calcium bilirubinate (17).

Nakano et al. (18) indicated that most bacterial strains isolated from bile exhibited phospholipase A1 and A2 activity. In contrast, human pancreatic juice and gallbladder epithelial cells contain phospholipase A2. Considering that gallstones are predominantly palmitic acid and must have been cleaved from the first position in the biliary phosphatidylcholine molecule, bacterial phospholipase A1 appears to play a significant role in calcium palmitate formation, which is commonly found in brown pigment gallstones.

Phospholipase A2 plays a role in the formation of cholesterol gallstones by hydrolyzing bile phospholipids into lysolecithin and free fatty acids. Cholesterol crystallization is enhanced in model bile containing hydrophilic species such as soybean or palmitoyl-linoleoyl phosphatidylcholine, which consists mainly of polyunsaturated fatty acids. The enhancement of cholesterol crystallization by phospholipase A2 is observed in bile containing hydrophilic phosphatidylcholine species but not in bile with hydrophobic phosphatidylcholine species. This suggests that the release of polyunsaturated fatty acids through hydrolysis may be responsible for enhanced crystallization. Therefore, the impact of phospholipase A2 on cholesterol gallstone formation depends on the specific phospholipid species present in bile. Thus, selecting phospholipid species during hepatic excretion plays a crucial role in cholesterol stone formation (19).

Several factors influence the stability of supersaturated bile, leading to the formation of cholesterol crystals through nucleation and growth. One of these factors is biliary lipids. The abundance and composition of these lipids impact the composition of biliary vesicles. If these vesicles are rich in cholesterol, they become unstable and serve as precursor particles for the formation of cholesterol crystals. Kinetic factors can influence stability in two ways: they can inhibit cholesterol crystallization, thereby stabilizing the system, or they can promote the nucleation of cholesterol crystals, also contributing to system stability (20).

Regarding the antibiotic sensitivity test patterns, table 7 presents the results of the minimum inhibitory concentration (MIC) for each antibiotic against the isolates. Meanwhile, Table 8 displays the percentages of sensitive, resistant, and intermediate bacteria for each antibiotic used.

Table 7: Minimum Inhibitory Concentration in the VITEK 2 system for antibiotic sensitivity test patterns.

NO	Bacterial species	AM	AMC	CTX	CAZ	FEP	ETP	IMP	MEM	AN	GM	CIP
1	*Klebsiella pneumoniae*	>=32* R	8	<=4*S	>=64	2	2	<=0.5	<=0.5	<=2	<=1	<=O.25
2	*Serratia marcescens*	<=2	6	<=4	<=1	<=1	<=1	4	0.5	4	<=1	<=0.25
3	*Klebsiella pneumoniae*	>=32	8	8	>=64	16	2	<=0.5	<=0.25	<=2	<=2	<=0.25
4	*Esherichia coli*	>=32	>=32	>=128	>=64	>=64	>=64	>=64	>=8	>=16	<=1	>=4
5	*Esherichia coli*	>=32	8	>=64	>=64	>=64	>=64	*I*I	>=16	<=2	<=1	>=4
6	*pseudomonas fluorescens*	0.5	6	8	8	<=1	<=1	>=16	<=0.25	<=2	1	<=0.25
7	*Serratia marcescens*	1	4	>=128	>=64	*1	>=64	<=2	>=16	>=64	>=16	>=4
8	*Raoultell orithinolytica*	>=32	8	<=4	64	*I	2	<=0.5	<=0.5	<=2	<=1	<=0.25
9	*Pseudomonas aeruginosa*	1	4	>=128	>=64	4	>=64	4	>=16	>=64	>=16	>=4
10	*proteus vulgaris*	<=1	>=64	4	>=64	>=64	2	>=64	2	>=2	<=1	0.5

11	*Klutyera cryocrescens*	>=2	>=32	8	>=64	16	*I	<0.25	<=0.25	>=2	>=16	1
12	*pseudomonas fluorescens*	<=1	4	8	>=64	8	8	<=1	<=1	<=0.25	1	<=2
13	*Klebsiella oxytoca*	>=32	<=2	<=4	<=4	<=1	<=1	>=64	0.5	4	1	<=0.25
14	*Esherichia coli*	<=1	>=32	>=128	>=32	*I	>=64	>=8	>016	<=4	<=1	>=4
15	*Pseudomonas aeruginosa*	>=64	<=1	<=2	>=64	*I	2	<=1	2	>=1	>=2	0.5
16	*Esherichia coli*	>=32	>=4	*I	16	16	2	<=0.5	<=025	<=2	<=1	<=0.25
17	*Esherichia coli*	>=32	6	>=32	>=64	>=64	>=64	4	>=16	8	>=16	>=4
18	*Esherichia coli*	>=32	<=1	>=128	>=16	<=4	<=1	*I	<=0.25	<=2	<=1	<=0.25
19	*Klutyera cryocrescens*	16	<=2	<=4	8	<=4	<=1	<=0.5	<=0.25	>=16	<=1	<=0.25
20	*Esherichia coli*	8	2	8	>=64	<=1	>=64	>=16	16	>=32	>=4	4
21	*Klebsiella pneumoniae*	<=8*R	<=16	>=2*S	>=64	2	2	<=0.5	<=0.5	<=0.25	<=16	<=1
22	*pseudomonas fluorescens*	>=8	<=4	<=1	<=1	<=1	<=1	8	0.5	<=0.25	4	<=1
23	*Klebsiella pneumoniae*	>=32	8	>=128	>=64	*I	2	<=0.5	<=0.25	<=0.25	0.5	0.5
24	*Esherichia coli*	>=32	>=16	>=128	>=64	>=64	>=64	>=64	>=16	>=16	<=2	<=1
25	*Esherichia coli*	>=32	>=32	8	>=64	>=64	>=64	*I	>=16	>=16	<=2	<=1
26	*pseudomonas fluorescens*	4	2	>=128	8	<=1	<=1	<=1	<=0.25	1	<=2	<=1
27	*Pseudomonas aeruginosa*	<=1	<=0.5	2	>=64	*1	>=64	2	>=16	>=16	>=64	>=16
28	*Raoultell orithinolytica*	>=32	8	>=128	64	8	2	<=0.5	<=0.5	0.5	<=2	<=1
29	*Serratia marcescens*	16	*I	8	>=64	4	>=64	4	>=16	>=16	>64	>=16
30	*Pseudomonas aeruginosa*	>=64	2	8	*I	>=64	2	8	2	*1	>=2	<=1
31	*Enterobacter cloacae ssp*	<=4	>=32	8	>=64	16	2	<0.25	<=0.25	2	>=2	<=0.5
32	*pseudomonas fluorescens*	<=4	16	<=4	>=64	8	8	<=1	<=1	4	<=0.25	1
33	*Klebsiella oxytoca*	>=32	<=2	>=128	<=4	*I	<=1	8	0.5	>=64	4	1
34	*Esherichia coli*	8	1	<=2	>=32	>=32	>=64	>=8	>=16	1	<=1	<=1
35	*Pseudomonas aeruginosa*	16	*I	<=32	>=64	4	2	<=1	2	4	>=1	>=2
36	*Esherichia coli*	>=32	<=1	>=128	16	*I	2	<=0.5	<=0.25	<=1	<=2	<=1
37	*Esherichia coli*	>=32	>=2	>=128	>=64	>=64	>=64	<=1	>=16	>=2	8	>=16
38	*Esherichia coli*	>=32	<=1	<=4	<=1	<=4	<=1	<=0.5	<=0.25	<=1	>=2	<=1
39	*Klutyera cryocrescens*	16	>=16	16	8	<=4	<=1	<=0.5	<=0.25	>=16	<=1	<=1
40	*Esherichia coli*	8	1	4	>=64	2	>=64	8	4	1	<=0.25	<=4
41	*Klebsiella pneumoniae*	4*R	>=16	<=4*S	>=16	2	2	>=64	<=0.5	<=0.25	<=2	<=1
42	*Serratia marcescens*	>=64	>=64	<=4	<=1	<=1	<=1	<=0.5	0.5	<=0.25	4	<=1
43	*Klebsiella pneumoniae*	>=32	8	8	>=64	16	*I	>=64	<=0.25	<=0.25	<=2	2
44	*Esherichia coli*	>=32	>=32	>=128	>=64	>=64	>=64	<=0.5	>=32	>=16	<=2	<=1
45	*Esherichia coli*	>=32	>=32	>=0.5	>=64	>=64	>=64	>=64	>=16	>=16	*I	<=1
46	*pseudomonas fluorescens*	*I	4	8	8	<=1	<=1	>=8	<=0.25	1	<=2	<=1
47	*Serratia marcescens*	2	>=64	>=128	>=64	*1	>=64	2	>=16	>=16	>=64	>=16

48	*Raoultell orithinolytica*	>=32	16	<=4	64	2	2	< =0.5	<=0.5	<=0.25	<=2	<=1
49	*Proteus vulgaris*	4	8	>=64	>=64	*I	>=64	<=0.5	>=16	>=16	>64	>=16
50	*Proteus vulgaris*	>=64	>=64	8	>=32	>=64	2	>=64	2	*4	>=2	<=1
51	*Klutyera cryocrescens*	16	>=32	8	>=64	16	2	16	<=0.25	>=64	>=2	>=16
52	*pseudomonas fluorescens*	8	4	8	>=64	*I	8	<=0.25	<=1	16	<=0.25	1
53	*Proteus vulgaris*	>=32	>=64	<=4	<=4	<=1	<=1	<=1	0.5	8	4	1
54	*Esherichia coli*	>=64	16	>=128	>=32	>=32	>=64	>=64	>=16	>=64	<=4	<=1
55	*Serratia marcescens*	16	8	<=2	>=64	4	2	>=8	2	16	*I	>=2
56	*Esherichia coli*	>=32	16	2	16	*I	2	8	<=025	16	<=2	<=1
57	*Esherichia coli*	>=32	4	>=128	>=64	>=64	>=32	>=0.5	>=32	4	*I	>=16
58	*Proteus vulgaris*	>=32	>=64	>=128	>=64	<=2	<=1	>=64	<=0.5	>=64	<=2	<=1
59	*Klutyera cryocrescens*	16	*I	<=4	8	<=4	<=2	16	<=0.25	16	>=64	<=1
60	*Esherichia coli*	>=64	8	16	>=64	<=1	*I	8	4	8	>=32	1

*R:Resistant; I:Intermediate; S:Sensetive.AM**:**Ampciillin ;AMC:Amoxicillin clavulanic**;**CTX:Cefotaxime**,**CAZ **:**Ceftazidme**;** FEP**:** Cefepime**;** ETP **:**Ertapenem **;**IMP: Imipenem**;** MEM :Meropenem**;**AN **:**Amikacin **;**GM **:**Gentamicin **;**CIP**:** Ciprofloxac.

Table 8: Percentage of Bacterial Sensitivity to each Antibiotic

NO	Antibiotic name	Bacterial Percentage (%)		
		S	R	I
1	Cefotaxime	71%	26%	1%
2	Amikacin	56%	31%	1%
3	Gentamicin	50%	40%	3%
4	Ciprofloxacin	46%	53%	0
5	Meropenem	40%	60%	0
6	Ceftazidme	35%	18%	1%
7	Imipenem	35%	66%	5%
8	Ertapenem	10%	83%	6%
9	Ampciillin	10%	90%	0
10	Amoxicillin clavulanic	10%	88%	0
11	Cefepime	8%	70%	20%

Our study revealed that Cefotaxime was the most effective antibiotic, with a sensitivity of 71%. Conversely, Cefepime demonstrated the lowest effectiveness, with a sensitivity of only 8%. On the other hand, Ampicillin exhibited the highest resistance among the bacteria, with a resistance rate of 90%.

However, different studies have presented various types of antibiotics. Hamdoon and Abdul-Allah (21) reported that based on the detected antibiogram, Ciprofloxacin was identified as the preferred choice for treating biliary tract infections caused by both gram-negative and gram-positive bacteria. Additionally, Atiyah et al. (14) identified fluoroquinolones (such as Ciprofloxacin and Norfloxacin) as the most effective antibiotics. Similarly, in the study conducted by Thapa et al. (16), Imipenem and Amikacin were found to be the most effective prophylactic antibiotics.

CONCLUSION

The present study investigated the occurrence of gallstones in patients at several hospitals in Maysan province. The study concluded that this disease is often associated with various bacterial species, which may potentially be either associated with or the cause of stone formation. *E. coli* was found to be the predominant species, and Cefotaxime was identified as the most preferred antibiotic used.

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