



# RELATIONSHIP BETWEEN BACTERIAL MICROORGANISMS AND GALLSTONES FORMATION

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## Abstract

This study was conducted for patients with gallstones, and this examination was conducted at the University of Fallujah, College of Medicine, to determine the incidence of gallstones and anti-microbial sensitivity. The examination included both male and female patients (47) who ranged between (22-86) years old, average age (54), (5) the number of male patients, (42) the number of female patients, all of whom were gallstones and use Differential antimicrobials, smoking and diabetes are of varying degrees. and data we obtained by filling out a special questionnaire for patients. In all (47) patients. Where the questionnaire showed that the age group (30-40) years is the group that has suffered most from other gallstones, by determining the resistance of bacteria that constitute stones for some antibiotics such as (ciprofloxacin, ampicillin, erythromycin, critrexone, erythromycin, critrexone, tetracycline and Cefixime), and tests of various chemicals (Catalase production, Oxidase production, Coagulase production, Haemolysis).

**Key words :** gallstones, bacterial susceptibility, biochemical tests.

## Introduction

Gallstones are solid deposits in the digestive fluid that can form in your gallbladder. The gallbladder is a small pear-shaped organ located on the right side of the abdomen just below the liver (Sami *et al.* 2007, Al-Zuhairi *et al.*, 2017). People who have symptoms of gallbladder stones usually need surgical removal of the gallbladder (Zanetti *et al.* 2008). Gallstones that do not cause any signs or symptoms usually do not need treatment. Types of gallstones that may form in the gallbladder include:

Bile cholesterol stones. Often the most common type of gallstones is cholesterol yellow (Madhavi, 2012, Dheeb, 2015). Gallstones are mainly cholesterol that is not dissolved, but may contain other ingredients and Pigment gallstones. These stones are dark brown or black in color when your yellow liquid contains a lot of bilirubin. Gallstones are the most common cause of *cholecystitis* when the gallstone blocks the gallbladder directly (Ali *et al.* 2009, Sana *et al.* 2005). This leads to condensation

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and stagnation of bile, which makes it a fertile environment for the multiplication of types of germs and secondary infection caused by an intestinal organism, especially *E. coli* bacteria. Then the gallbladder wall becomes inflamed, (and in some rare cases the condition may accompany the death of some of the tissues that form the wall and may end up with an explosion of the gallbladder.) In the event of inflammation, it spreads to the neighboring tissues such as diaphragm, colon and liver (Manges *et al.* 2001).

## Materials and Methods

### Culture media-Blood agar

Used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties.-Mueller Hinton Agar : rich medium that support the growth of most microorganism. It is commonly used for antibiotic susceptibility testing. MacConkey agar is differential media because it contains Lactose and Neutral red. Lactose fermenters bacteria will grow in red colonies while non-lactose fermenters will be colorless and clear Mannitol salt agar : is a selective and differential medium used for the isolation of pathogenic staphylococci.

## Identification of bacteria and fungi - Morphological identification of bacteria

Colony morphology observations form a major identifying criterion for fungus and bacteria (Odutayo *et al.* (2005). Biochemical identification of bacteria :Biochemical reactions for identification of the bacteria are discussed below

### Bacitracin test

Bacitracin is an antibiotic interfering with the synthesis of peptidoglycan, a major component of bacterial cell walls. The medium used for growing the bacterial lawn is typically blood agar.

### Optochin test

Optochin differentiation between alpha-hemolytic streptococci as *S. pneumoniae* from *Viridans streptococci*. *S. pneumoniae* are sensitive to the Optochin.

### Catalase Test, Oxidase test

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, Coagulase Test, Voges-Proskauer reaction (Mccrea *et al.* (2007), Indole reaction *Shigella* sp, *Edwardsiella tarda*, *Klebsiella oxytoca* and *Proteus vulgaris* (Del *et al.* (2014), Methyl red test used to identify bacteria producing acids by mechanisms of mixed acid fermentation of glucose. Citrate, Urease [Del *et al.* 2014)], Hydrogen sulfide (H<sub>2</sub>S) H<sub>2</sub>S-producing microbes are *Salmonella*, *Edwardsiella*, *Citrobacter* and *Proteus* sp. (Abdurakhmonov *et al.* 2007, El-Hilali *et al.*, 2016 ), Mannitol Salt Fermentation A pure culture from nutrient agar was sub-cultured on a freshly prepared plate of mannitol salt agar. It was then incubated at a temperature of 37 °C for 24 h. Growth of white colonies surrounded by yellow zones indicated presence of *Staphylococcus aureus* and then confirmed by Gram staining (Lima *et al.* 2007, Nouri *et al.*, 2015) and Urease Test Some bacteria can utilize urea as a non-carbohydrate carbon source using urease enzyme.

### 1. Reagent Cards

The detector cards contain sixty four slits, and each slit performs a specific test and the chemical support structure is changed. The chemicals that are changed measure the various activities related to the treatment and use of foodstuffs such as the conversion of something more than acid, aqueous hydrolysis in the enzyme, growth in the presence of dilution or stopping materials.

### VITEK 2 GN Colorimetric Identification Card

Now four detector cards to define different categories (living organisms) as follows( GN - Gram-negative (alcohol change and alcohol production)

**Table 1:** Suspension Turbidities Used for Card Inoculation.

Product	McFarland Turbidity Range
GN	0.50–0.63
GP	0.50–0.63
YST	1.80–2.20
BCL	1.80–2.20

(chemical alcohol change and production) - GP - YST (living organisms) - BCL -Culture Requirements and Suspension Preparation show in the table 1

## Results and Discussion

The results in table 2 showed that patients with (47) gallstones, ages (22-86) years, average age (54), (5) the number of male patients, (42) the number of female patients, all of them Among them are gallstones, exposure to differential antimicrobials, smoking and asymmetric diabetes. and data we obtained by filling out a special questionnaire for patients. Between the ages of 20 and 30 years, including (0) men and (8) women, of whom (1,7) have collected them. Previous reference. Antibiotics and smoking, respectively, diabetes (0,8), respectively. The group category is (30-40) years old, including (2) men and (10) women, of whom (3,9) are before. Previous reference. Antibiotics, respectively, diabetes and smokers (0,12), respectively. Class group (40-50) years, including (1) men and (6) women, of whom (0,7) are before. Previous reference. Antibiotics, diabetes respectively smoked. The age group (50-60) years, including (0) men and (9) women, of whom (3,6) are. Previous reference. Antibiotics and diabetes, respectively, smokers (0,9), respectively. Class group (60-70) years, including (0) men and (7) women, of whom (1,6) are pre. Previous reference. Antibiotics, respectively, diabetes (2,5), respectively, smokers (0,7), respectively, and age group (70-80) years, including (1) men and (1) women, among them (0,2) They are before. Previous reference. Antibiotics, diabetes and smokers respectively. Age group (80-90) years, including (1) men and (1) women, of whom (0,2) are before. Previous reference. Antibiotics and smokers respectively, diabetes (2,0), respectively. In all (47) patients. Where the questionnaire showed that the age group (30-40) years is the group that suffered most from other gallstones.

The results in table 3 of six types of bacteria in biochemical tests showed that: *Streptococcus pyogenes*: This type of bacteria showed an Sensitive to *Bacitracin*, *Streptococcus viridans*: This type of bacteria showed an Resistance to *Optochin*, *Staphylococcus aureus*: generally negative test showed, *Klebsiella pneumoniae*: It showed negative test for five tests and positive test for five other tests, *E. coli*: A negative test showed six tests

**Table 2 :** Distribution of gallstones patients according to age,sex and different parameters.

Age	Sex		Pre. Op. antibiotic		Diabetes mellitu		Smoking	
	Male	Female	Yes	No	Yes	No	Yes	No
20–30	0	8	1	7	0	8	1	7
30–40	2	10	3	9	0	12	0	12
40-50	1	6	0	7	0	7	0	7
50-60	0	9	3	6	3	6	0	9
60-70	0	7	1	6	2	5	0	7
70-80	1	1	0	2	0	2	0	2
80-90	1	1	0	2	2	0	0	2

**Table 3:** Distribution of bacteria according to the biochemical test.

	<i>Streptococcus pyogenes</i>	<i>Streptococcus viridans</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>E. coli</i>	<i>Streptococcus Spp</i>
Catalase production	-	-	+	+	+	-
Oxidase production	-	-	-	+	-	-
Coagulase production	*	*	+	*	*	*
Haemolysis	+	+	+	-	-	+
Motility	-	-	-	-	+	-
Optochin	*	R	*	*	*	
Bacitracin	S	*	*	*	*	
Indole production	*	*	*	-	+	*
Voges proskauer	-	v	*	+	-	
Methyl red				-	+	*
Simmon citrate	*	*	*	+	-	*
Urease	-	-	+	+	-	-
H <sub>2</sub> S production	*	*	*	-	-	*
Mannitol Salt Fermentation			+		*	*

- : Positive test , +: Negative test , \*: The test was not conducted , V:Variable , R= resistance , S= sensitive

and a positive test in four other tests, *Streptococcus Spp*: It showed a positive test in four tests only.

The results in table 4 showed that most of the isolates (*Streptococcus spp*) were sensitive to all the tested antibacterials (Ciprofloxacin, Ampicillin, Erythromycin, Ceftriaxone, Tetracycline), except (Cefixime). and that antibiotics cannot penetrate the outer membrane that may reduce the permeability of the drug. *Streptococcus spp* resistance to (Cefixime) may be due to the common use of these antibiotics in the treatment of most clinical infections. and that most of the isolates (*Staphylococcus aureus*) were sensitive to most of the tested antibacterials (Ciprofloxacin, Erythromycin, Ceftriaxone, Tetracycline), except (Ampicillin, Cefixime). and that antibiotics cannot penetrate the outer membrane that may reduce the permeability of the drug. *Staphylococcus aureus* resistance to (Ampicillin, Cefixime) may be due to the common use of these antibiotics in the treatment of most clinical infections and that most isolates (*E. coli*) were sensitive to half of the tested antibiotics (Ciprofloxacin, Ceftriaxone, Tetracycline) except for the other half (Ampicillin, Erythromycin, Cefixime) and that antibiotics

cannot penetrate the outer covering of the membrane that may reduces the permeability of the drug. (*E. coli*) resistance (Ampicillin, Erythromycin, Cefixime) may be due to the common use of these antibiotics in treating most clinical infections. Most isolates were sensitive and some were mild to most antibiotics used in the study as shown in table 4, which summarized all the information obtained from the antibiotic sensitivity test.

Dye-negative microbes within the stone components show the efficacy of the urease and the viscous layer they produce. *Streptococcus viridans* are a large group of Gram-positive sympathetic streptococcus Volatility is the most common bacillus in the formation of inflammatory stones, the type mirabilis P.for these types of stones, because they represent more than 70% of the germs isolated from these types of stones, the reason for this is not only because it is the most bacterial species capable of producing urea, but also for its resistance. This study revealed that there is a higher prevalence of resistance to prescribed antibiotics. There is no doubt that there is an urgent need for continuous monitoring of the sensitivity of pathogens in different population groups to commonly

**Table 4:** Distribution of bacteria types according to the antibacterial test.

	<i>Streptococcus spp</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>
Ciprofloxacin	S	S	S
Ampicillin	S	R	R
Erythromycin	S	S	R
Ceftriaxone	S	S	S
Tetracycline	S	S	S
Cefixime	R	R	R

used antimicrobial agents (Bonnet *et al.* 2001, Dheeb, 2013). The data for this study can be used to determine trends in susceptibility to antimicrobials, to formulating local antibiotic policies and in general to assist clinicians in making rational choice of antibiotic therapy to prevent misuse, or excessive use of antibiotics (Gorschluter *et al.* 2001, Abdulbaqi *et al.*, 2018).

### Conclusion

The pathogen that causes multiple gallstones (bacteria). In addition to other factors, humans are the original source of infection, and the distribution of the organism in society. Antibiotics are drugs used to prevent and treat bacterial infections, and resistance to antibiotics occurs when bacteria themselves change in response to the use of these medications. Bacteria are resistant to antibiotics and may cause a more difficult human infection than their counterparts (Lau *et al.* 2000).

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