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Bacterial and Molecular Detection of *Nanobacter Species* from Patients after Cholecystectomy

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ABSTRACT

Background: Bacterial infection of gallbladder play a major role in 50 % of patients with cholelithiasis with or without gallstones and can be caused by any type of bacteria.**Objectives:** To show the role of Nanobacter species in chollangitis and cholelithiasis in patients with cholelithiasis.
Patients and Methods: One hundred and five (105) fluid, stone, or both were taken from Patients with symptoms of cholecystitis and cholelithiasis. They were attending the Consultation Surgical Clinic at Ramadi General Hospital and Heet General Hospital during the period patients after cholecystectomy during the period from November 2019 to April 2020. Patients well examined and diagnosed by a senior surgeon, Specimens of aspirated (fluid and stone) after cholecystectomy were taken from each patient, each stone specimen was preserved in brain heart infusion broth and then sent to the laboratory, in the laboratory each specimen was divided into two parts, one of them was incubated at 37⋄C for 24 hours for bacteriological investigations and the other part stored at -20C for molecular study by polymerase chain reaction (PCR).**Results:** Out of a total of 105 investigated specimens, sixty-two (62,59.0%) specimens gave a positive bacterial culture, and the remaining 43, 41.0% were showing negative bacterial culture. Sixty-two (62) bacterial isolates were isolated from positive culture specimens, including both Gram-positive and Gram-negative bacteria. Gram-positive bacteria found at resembled 15.2% and Gram-negative bacteria were found at a rate of 43.8%.
Conclusion: Meropenem, Imipenem, Levofloxacin, Amikacin, Nitrofurantoin and can be considered a drug of choice to treat Cholelithiasis with sensitivity rate ,85.5%, 71.0%, 64.5%, 67.7%, 62.9% respectively**.**

INTRODUCTION

The presence of stone in the gallbladder is one of the major health problems in many countries in the world, especially in developing countries. The key component of gallstones is cholesterol may consist of calcium carbonate, bilirubin, and bile pigments (Irfan *et al.*,2007). Different bacterial species were isolated from gall bladder patients among them are Gram-negative bacteria particularly *E. coli* and other types of bacteria.

This has been confirmed by many studies conducted in different parts of the world (Ahmed and Syoof,2015). Acute cholecystitis is defined as an acute inflammation of the gall bladder. It is one of the most common inpatient diagnoses at Surgical Departments and in more than 90% of patients, it arises as complications of cholelithiasis (calculous cholecystitis). Bacterial growth in bile is reported in 20% to 70% of patients. The bacterial infection is believed to represent a secondary complication and not the initiating event of the disease. Infection is considered an important negative prognostic factor, and antibiotics are included in treatment recommendations for all grades of severity (Bellows ,2018). Bacterial infection of gallbladder plays a role in 50% of patient with cholelithiasis with or without gallstones and can be caused by any type of bacteria ranging from aerobic Gram-negative to Gram-positive to anaerobic organisms, they reached into gallbladder by ascending from the duodenum, via the hematogenous or via enterohepatic circulation and can survive in gallbladder despite the bactericidal activity of the bile (Arteta *et al.,2017*). One of the most common acute diseases in the Western World is acute cholecystitis, which may range from a mild, painful disorder to a life-threatening illness due to complications. It is usually caused by an infection in an obstructed bile system, associated with gallbladder stones in 90% of cases (Subbaghian *et al.,* 2010). Gram-negative bacteria, mainly Escherichia coli and Klebsiella spp. (together .50%), are the most frequent causative pathogens. Gram-positive bacteria especially enterococci, and anaerobic bacteria (mainly Bacteroides spp. and Clostridium spp.) are also isolated; 30%–80% of cases are caused by more than one pathogen. The pathogenicity of enterococci in this condition is still unclear, as they are almost always detected in mixed infections (Lubasch and Lode,2000).

MATERIALS AND METHODS

Samples Collection, Isolation, and Identification:

This prospective study was carried out to isolate and identify Nanobacter species and other bacteria from bile, inner and outer gallstone after cholecystectomy from hospitalized patients attending Ramadi Teaching Hospital and Heet General Hospital for the period extended from November 2019 to April 2020.

One hundred five (105) samples are collected from the gallbladder. The distribution of samples are as follows; 16 bile fluid, 23 gallstones, and 66 bile fluid and gallstone. In the laboratory, the specimen divided into two parts, one of the parts was incubated at 37 C for 24 hours for microbiological examination by cultured specimen aerobically and anaerobically on suitable culture media. Bacterial isolates are diagnosed routinely and the diagnosis confirmed by using the VITEK 2 system. The antibiotic susceptibility test was done to the *62 bacterial* isolates against 16 commercial common antibacterial agents using standard Kirby Bauer Disc diffusion method using and the results are interpreted according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines 2018. The other part of the specimen stored at -20C for molecular study by polymerase chain reaction (PCR) for the detection of Nanobacteria.

RESULTS AND DISCUSSION

The study revealed that the vast majority of the study sample is within the fourth category of age groups and accounted for (33.3%), this truth comes along with(Ahmad ., et al.,2014), in their study showed that the mean age was (46.13), they also found that the majority of the study sample (72.4%) are females and remaining are males. This result comes along with (Thapa *et al.*,2016). They reported that the study population consisted of (259) symptomatic cholelithiasis,64 (24.71%) male and 195 (75.29%) female with M:F ratio of (1:3) (Table-1).

Table 1: Distribution of Cholecystectomy Patients by Socio-Demographic Characteristics

Socio-demographic characteristic variable	Frequency (N=105)	Percent (%)	Cum. Percent
Age groups			
≤ 34	25	23.8	23.8
35-45	35	33.3	57.1
46-57	31	29.5	86.7
≥ 58	14	13.3	100.0
Statistics Mean ±SD	44.5 ± 8.6(yrs.)		
Gender			
Male	29	27.6	27.6
Female	76	72.4	100.0

Types of Bacterial Isolates:

Table 2 reveled the distribution of Gram-negative bacterial isolates as the following : *Escherichia coli* (16.1 %), *Citrobacter freundii* (11.3 %), *Pseudomonas aeruginosa* (8.1 %), *Achromobacter xylosoxidans* (6.5 %), *Klebsiella oxytoca* (4.8 %), *Enterobacter cloacae* (6.5 %), *Klebsiella oxytoca & Morganella morganii* are (4.8%), *Serratia liquefaciens , Sphingomonas paucimobilis, &Pantoea spp.(* 3.22%), *Yersinia enterocolitica, Serratia marcescens, Aeromonas salmonicida, Raoultella planticola* are (1.61%) .

Table (2): Distribution of Gram-negative Bacterial Species Isolated from the Study Samples.

Gram negative Bacteria	No. Of bacterial isolates	Percentage of Gram-negative bacterial Isolates
Escherichia coli	10	16.1 %
Citrobacter freundii	7	11.3 %
Pseudomonas aeruginosa	5	8.1 %
Achromobacter xylosoxidans	4	6.5 %
Enterobacter cloacae	4	6.5 %
Klebsiella oxytoca	3	4.8 %
Morganella morganii	3	4.8 %
Serratia liquefaciens	2	3.22 %
Sphingomonas paucimobilis	2	3.22 %
Pantoea spp.	2	3.22 %
Yersinia enterocolitica	1	1.61 %
Serratia marcescens	1	1.61 %
Aeromonas salmonicida	1	1.61 %
Raoultella planticola	1	1.61 %
Total	46	74.2 %

While (Table 3) showed distribution of Gram-positive bacterial isolates as follows: *Staph. haemolyticus* (6.5 %), *Strep. Bovis* (4.8 %), *Staph. Epidermidis*, & *Staph. Warneri* are (3.22 %), and *Enterococcus faecium, Lactococcus garvieae, Staph. Xylosus*, and *Staph. Auricularis are (*1.61 %).

Table 3: Distribution of Gram-positive Bacterial Species Isolated from the Study Samples.

Gram positive Bacteria	No. Of bacterial isolates	Percentage of Gram-Positive bacterial Isolates
Staph. haemolyticus	4	6.5 %
Strep. Bovis	3	4.8 %
Staph. Epidermidis	2	3.22 %
Staph. Warneri	2	3.22 %
Enterococcus faecium	1	1.61 %
Lactococcus garvieae	1	1.61 %
Leuconostoc mesenteroides	1	1.61 %
Staph. Xylosus	1	1.61 %
Staph. Auricularis	1	1.61 %
Total	16	25.8 %

N.B. The total number of Gram positive and gram-negative Bacterial isolates are 62.

This result agrees with (Hawar *et al.*, 2019), who found that the Gram-negative bacteria were the predominant cause of gallbladder infection and they represent (61.17%) of their study sample. Also, this result was in agreement with (Nicholson *et al.*, 2012), who found that the enteric microbiota is widespread in soil, water, contaminated meat product, and vegetables and colonize the gastrointestinal tract very early in life, becoming part of the microbiome and almost have been suspected of causing cholelithiasis.

Detection of Nanobacteria by Polymerase Chain Reaction (PCR):

This table (4) summarizes the detection of nanobacteria by polymerase chain reaction (PCR) which is dispersed as follows: positive and they accounted for (67.7%), and remaining negative and they accounted for (32.3%).

Table 4: Distribution of the Observed Frequency, Percentages, and Cumulative Percent with Summary Statistics of Detection of Nanobacteria by Polymerase Chain Reaction (PCR).

No.	Detection of Nanobacteria by PCR	Frequency	Percent %	Cum. Percent
1	**Positive**	**42**	**67.7**	**67.7**
2	**Negative**	**20**	**32.3**	**100.0**

Type of Sample:

The table (5) shows the type of sample which is distributed as follows: fluid only (15.2%), fluid and stone (62.9%), and stone only (21.9%).

Table 5: Distribution of the Observed Frequency, Percentages, and Cumulative Percents with Summary Statistics of Type of Sample.

No.	Type of sample	Frequency	Percent %	Cum. Percent
1	Fluid	16	15.2	15.2
2	Stone	23	21.9	37.1
3	Fluid and stone	66	62.9	100.0

Antibiotics Susceptibility Test for Isolated Bacteria:

The Figure (1) revealed The antimicrobial susceptibility profile of the bacterial isolates revealed that most of the isolates were resistant to penicillin antibiotics such as piperacillin, Ticarcillin, Ticarcillin/ clavulanic acid and piperacillin\ tazobactam with percentage 82.3%, 71.0%, 51.6%, 41.9% respectively, this result agrees with (Abbass and Ahmed 2017), who found that the majority of the organism is resistant to Amoxicillin + Clavulanic acid and Ampicillin. Results showed that the percentage of bacteria isolates exhibited different rates of resistance towards cephalosporin antibiotics such as ceftriaxone (62.9%), cefatoxime (43.5%), and ceftazidime (37.1%), these results disagree with (Ahmad *et al* .,2014) who found that the majority of isolated bacteria were susceptible to Cefuroxime and ceftriaxone and were resistant to Amoxicillin. This result agreed with AL Harbi *et al.,* 2001), who found that isolated bacteria were resistant to all cephalosporins but sensitive to the aminoglycosides, ciprofloxacin, and imipenem. Also, the results revealed that the bacterial isolates exhibited different rates of resistance towards the aminoglycoside antibiotics which include Amikacin and Gentamicin with a percentage (12.4% and 21.9%) respectively, these results in agreement with (Bistgani and Imani 2013), the findings indicated that the most isolated organisms were susceptible to Amikacin and this result was in agreement with(Ahmad *et al.* ,2015), who found that the Cefoperazone with sulbactum and amikacin were the most effective antibiotics. It was found that Fluoroquinolone antibiotics (Ciprofloxacin, levofloxacin) gave good effectiveness towards most of bacteria isolates, the resistance rates were (45.2%, 21.0%) respectively, this result agreed with (Yun and Seo 2018), as the ratio of resistance to ciprofloxacin (23.0%) and levofloxacin (36.4%). Most of the isolates were found to be highly sensitive to Carbapenem antibiotics including Imipenem and Meropenem, where the sensitivity rates were (71.0%,85.5%). At the same time, the percentage of resistance was (19.4 %, 6.4%) respectively, this result agreement with (Thapa *et al.,* 2016), who found that most of the isolated bacteria were sensitive to imipenem and amikacin. The empirical antibiotics used for the treatment of symptomatic cholelithiasis must cover these common bacteria. Imipenem and amikacin must be a part of empirical regime as it will help in reducing the morbidity associated with symptomatic cholelithiasis.it was found that sulfonamides (Trimethoprim/sulfamethoxazole) gave equal effectiveness toward most of bacteria isolates, where the percentage of sensitivity was (50.0%) and the percentage of resistance was (50.0%).

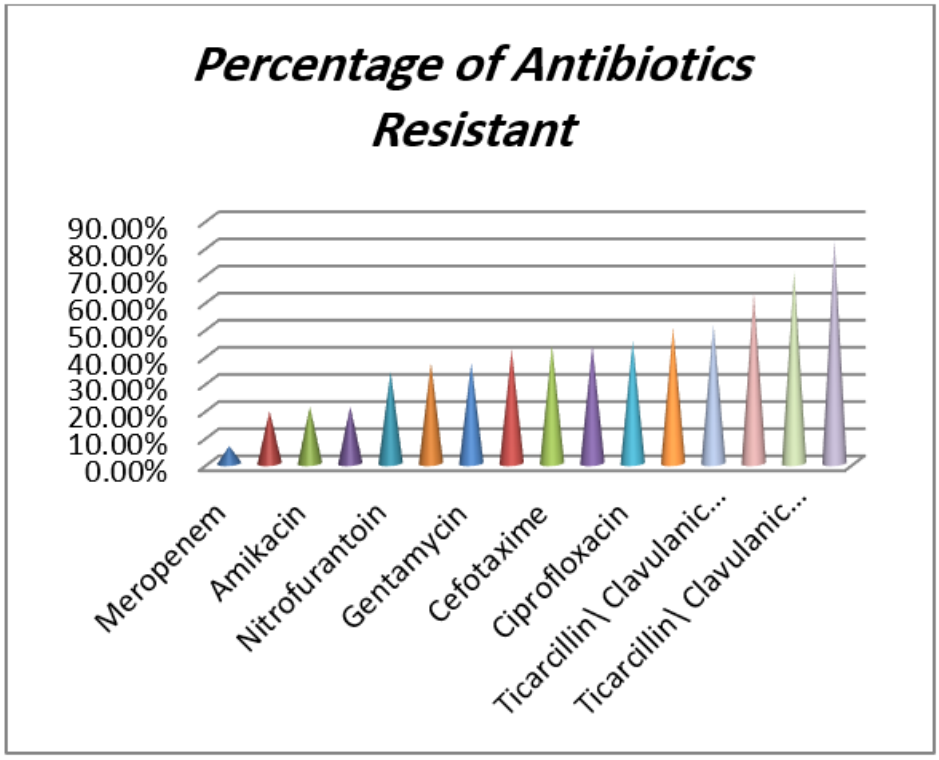

Fig. 1: The The antimicrobial susceptibility profile of the bacterial isolates

REFERENCES

Abbass S. and Ahmed I., (2017). Pattern of Culture and Sensitivity of Micro-Oganism Isolated from Bile after Cholecystectomy, *ISRA Medical Journal*, Volume 9 - Issue 4 | Jul - Aug,PP.(213-217).

Ahmad F., Islahi S., Hingora M. O. and Singh Y.,(2014). Cholelithiasis A Clinical and Microbiological Analysis. *International Journal of Scientific Study*, Vol 2 | Issue 4, PP. (40-45).

Ahmad M, Akhtar MR, Ali A, Ahmad A, Hashmi JS. (2015). Microbiology of bile in symptomatic uncomplicated gallstone Disease. Pakistan Armed Forces Medical Journal;65(4):491-3.

Ahmed Razaq Wajid, Syoof Khowman Alwan (2015). Bacteriological and genetic study on Escherichia coli Causing Acute calculus cholecystitis for Diabetes patients in AL-Diwanyia City; *International Journal of Advanced Research*, Vol. (3), Issue (6), PP.(1374-1382).

AL Harbi M., Osoba O. A., Mowallad A. and Al-Ahmadi K., (2001).Tract microflora in Saudi patients with cholelithiasis, *Tropical Medicine and International Health*, volume 6 no 7 pp (570-574).

Arteta A A, Carvajal-Restrepo H, Sánchez-Jiménez M M, Díaz- Rodríguez S and Cardona-Castro N (2017). Gallbladder microbiota variability in Colombian gallstones patients. *The Journal of Infection in Developing Countries* 11(03), 255-260.

Bellows C. Cholecystitis. BMJ best practice. BMJ Publishing Group; 2018. Available from: https://bestpractice.bmj.com/topics/en-us/78.

Bistgani M.M. and Imani R., (2013). Bile Bacteria of Patients with Cholelithiasis and Theirs Antibiogram, *Acta Medica Iranica*, Vol. 51, No. 11 PP. (779-783).

Irfan S. , Adnan A. , Shahid R.,Zahid M. and Asadullah K ; (2007).Freguency of infection in cholelithiasis . *Journal*

of College of Physicians and Surgeons Pakistan (JCPSP), 17(1): PP. (48-50).

Nicholson J K, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W and Pettersson S (2012). Host-gut microbiota metabolic interactions. *Science*, 1223813.

Sabbaghian S. M., Ranaudo J., Zeng L., Alongi P. A., Perez P. G. and Shamamian p.;(2010). Identification of *Helicobacter* spp. in bile and gallbladder tissue of patients with symptomatic gallbladder disease; *International Hepato-Pancreato Biliary Association (IHPBA),* 12, PP. (129–133).

Taban S. H., Hajir A. Shareef and Najdat B. M., (2019), Isolation and Identification of Aerobic Bacteria from Different Culture Site of Gallbladder in Patients with Cholelithiasis, *Biochemical Cellular Archives* Vol. (19), (No). 1, PP. (281-285), available at www.connectjournals.com/bca.

Thapa B. S., Bajracharya K., Kher R. Y., Pant S. S., Gurung S. and Pudasaini R., (2016).Aerobic Bacteria Associated with Symptomatic Gallstone Disease and their Antimicrobial Susceptibility in Western Nepal, *Journal Lumbini. Medical College* Vol 4, No 2, PP. (50-54).

Yun P.S.and Seo I. H., (2018). Clinical aspects of bile culture in patients undergoing laparoscopic cholecystectomy. *Medicine* 97:26 available at www.md-journal.com.