

Original Research Article

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## Bacterial Screening from Liquid Bile of Patients Underwent Laparoscopic Cholecystectomy Using Chromoginc Agar

Abdulameer Abdullah Al-Mussawi\*, Wisam Hamza Abbas and Zainab Alag Hassan

College of Nursing, University of Basra, Basra, Iraq

\*Corresponding author

### ABSTRACT

The main objective is to evaluate the incidence of bacterial pathogens in the liquid bile of patients underwent laparoscopic cholecystectomy using CHROMagar media. A Total of 34 samples were collected from patients suffering from cholelithiasis who were subjected to laparoscopic cholecystectomy by the same surgeon at Al-Sadr Teaching Hospital in Basra City for the period (31<sup>st</sup> of January 2016 to 30<sup>th</sup> of April 2016). It included 32 (94.1%) females and 2 (5.9%) males, aging between (23 – 61 years). Bile aspirates taken from each gallbladder in the operative room and directly inoculated onto brain heart infusion growth and incubated at 37°C for (24-48 hours). The isolates were identified by routine biochemical tests and CHROMagar media. A total of 34 samples were examined for detection of bacteria showed *E. coli* 11(32.4%), *Enterococcus* spp 7(20.6%), *Pseudomonas aeruginosa* 4(11.8%), *Klebsiella pneumoniae* 3(8.8%), *Salmonella* spp 3(8.8%) and *Staphylococcus aureus* 2(5.9%). A screening procedure using CHROMagar media gives better results and faster in identifying bacteria than conventional methods. Still the stasis secondary to gall stones is the commonest cause for cholecystitis and bacterial infection. Our work wishes to emphasize that *E. coli* presents at high frequency among other bacteria.

#### Keywords

Bacteria, Liquid Bile, Cholelithiasis, Cholecystectomy, CHROMagar media

#### Article Info

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### Introduction

Gallstone disease remains one of the most common medical problems leading to surgical intervention (Romero *et al.*, 2003). It is a significant health problem in developed societies, affecting 10% to 15% of the adult population (Tazuma, 2006). The most prevalent complication of cholelithiasis is chronic cholecystitis usually necessitating cholecystectomy (Vitetta *et al.*, 2000). Abdominal ultrasonography detects the gallstone and sometimes the associated

inflammation. Treatment usually involves antibiotics and cholecystectomy.

Acute cholecystitis is the most common complication of cholelithiasis. Conversely, more than or equal to 95% of patients with acute cholecystitis have cholelithiasis. When a stone becomes impacted in the cystic duct and persistently obstructs it, acute inflammation results. Bacterial infection can supervene. The vicious circle of fluid secretion and inflammation, when unchecked, leads to necrosis and

perforation (Indar, 2002) If acute inflammation resolves then continues to recur, the gallbladder becomes fibrotic and contracted and does not concentrate bile or empty normally-features of chronic cholecystitis (Reda *et al.*, 2016).

The organisms most commonly isolated in biliary tract infections are *Escherichia coli*, *Klebsiella* sp., *Enterococcus* sp. and anaerobes (Reiss *et al.*, 1982; Brook, 1989).

Acute cholecystitis is an infection of the biliary tract, which results from bile stasis due to chronic obstruction. The obstruction is usually attributed to gallstones in 80% of cases. The causes of acalculous cholecystitis include biliary structures, human immunodeficiency virus, cholangiopathy, biliary parasites and primary sclerosing cholangitis. Other causes include complicated cases of burns, trauma, major surgery, diabetes and unusual bacterial infections of the gallbladder (*Salmonella* spp. or *Vibrio cholerae*) and other systemic infections (tuberculosis and syphilis) (Greenberger and Isselbacher, 1998).

In Saudi Arabia, 25% of patients undergoing laparoscopic cholecystectomy for gallstones were bacterial culture positive, and the most common organisms isolated were *E. coli* (28.1%), *E. faecalis* (15.6%) and *P. aeruginosa* (9.4%) (Al Harbi *et al.*, 2001)

Öztürk *et al.*, (2012) founded the most commonly isolated bacteria were *Enterococcus* spp (%26.6%), *Escherichia coli* (20%) and *Enterobacter* spp (20%). Capoor *et al.*, (2008) found. The most common organisms isolated were *Escherichia coli* (29.7%), *Salmonella enterica* serovar Typhi (8.1%), *Klebsiella pneumoniae* (27%). The aim of the current study evaluates the incidence of bacterial pathogens in liquid bile of patients undergoing cholecystectomy.

## Materials and Methods

### Isolation of bacterial species

A total of 34 samples had collected from patients who attended the Al-Sader Teaching Hospital in Basra City in the period (31 January 2016 –30 April 2016). It included 29 (85%) females and 5 (15%) males, aged between (23 – 61 years).

Bile was aspirated during cholecystectomy and collected in a sterile container contain brain heart infusion growths transport media, bile aspirate were directly inoculated onto blood agar and MacConkey agar and incubated at 37°C for (24-48 hours).

### Identification of the isolates

The suspected colonies were examined for their colonial morphology, microscopical examination, biochemical test (Collee *et al.*, 1996) and CHROMagar media.

## Results and Discussion

A total of 34 bile samples aspirated from gall bladders excised laparoscopically by the same surgeon through four ports with the aid of CO<sub>2</sub> inflation and the usage of bipolar coagulation diathermy and titanium clips applied to the cystic artery and duct (Figure 1, 2, 3, and 4), the patients were suffering from cholecystolithiasis.

The bile examined for detection of bacteria showed *E. coli* 11(32.4%), *Enterococcus* spp 7(20.6%), *P. aeruginosa* 4(11.8%), *Klebsiella pneumoniae* 3(8.8%), *Salmonella* spp 3(8.8%) and *S. aureus* 2(5.9%) (Table 1).

In this study the age range was 23-61 years, the median age of the patients was 37 years. 32 (94.1%) females and 2 (5.9%) males and the similar findings have been observed in

previous studies, and agree with local studies, Wajid and Alwan (2015) in Al-Diwaniya city and Al-Zuharri (2011) in Al-Najaf city.

Our study showed the most common isolates was *E. coli* represents 32.4% and this agrees with previous studies (Capoor *et al.*, 2008; Al-Zuharri, 2011). Seven (20.6%) positive samples were identified as *Enterococcus* spp by biochemical tests and confirmed by

CHROMagar medium. ÖZTÜRK *et al.*, (2012) founded the most commonly isolated bacteria were *Enterococcus* spp (26.6%) (Fig. 5).

Agree with Wajid and Alwan (2015) In Iraq, Al-Diwaniya city (88% female and 12% male) Wajid and Alwan (2015) founded 7.2% *E.coli* in bile from cholecystectomy.

**Table.1** Identified of bacterial species by chromogenic medium

Bacterial species	No. (%)	Colony Colour	Colony Size and Morphology
<i>E. coli</i>	11(32.4%)	Dark pink to reddish	Small-sized to medium
<i>Enterococcus spp</i>	7(20.6%)	Blue	Tiny, pinlike
<i>P. aeruginosa</i>	4(11.8%)	Blue-green	Serated edges, diffused
<i>K. pneumoniae</i>	3(8.8%)	Metallic blue	Mucoid
<i>Salmonella spp.</i>	3(8.8%)	Mauve	1.0mm, Raised, smooth
<i>S. aureus</i>	2(5.9%)	Pink	1- 3mm, Mucoid



Fig.(1): Isolation and Identification of the Cystic Artery and Duct



Fig.(2): Double Clipping of the Cystic Duct Proximally and one Clip Distally before Cutting the Duct

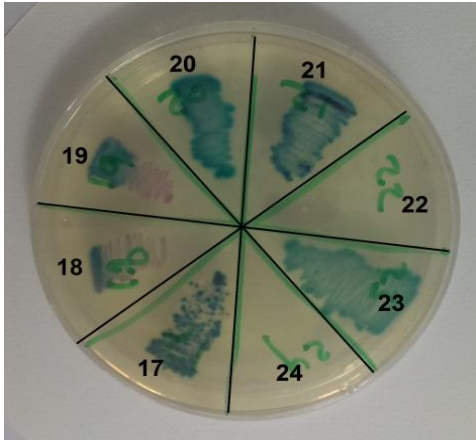


Fig.(3): Cholecystectomy Progress Using Diathermy Coagulation

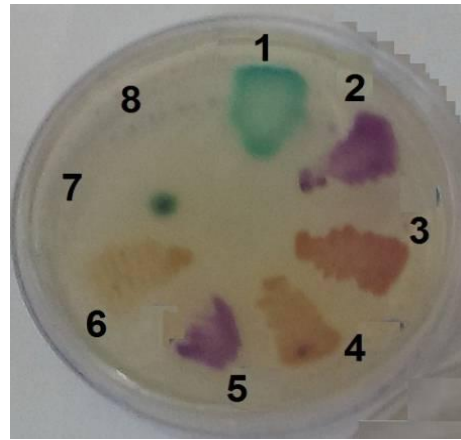


Fig.(4): Cholecystectomy Completed and Ready for Aspiration of Bile Fluid

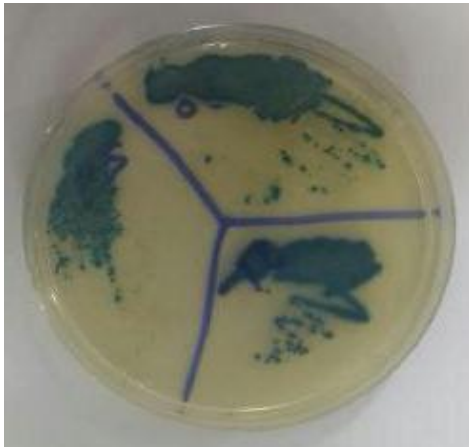
Fig.5 Specific color reactions of microorganisms on CHROMagar media



A. CHROMagar Orientation



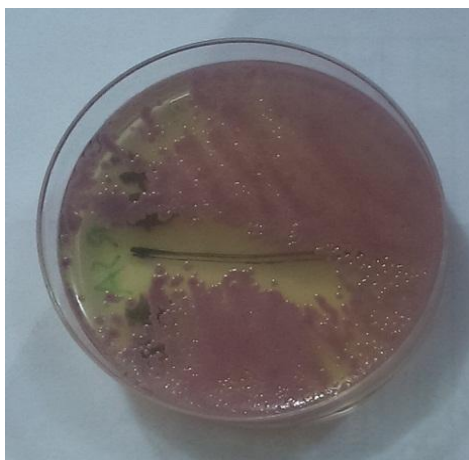
B. CHROMagar Orientation



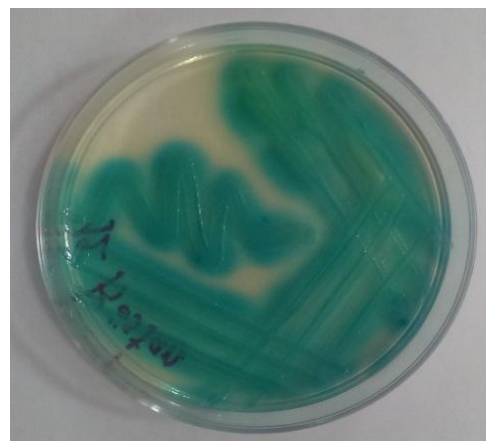
C. CHROMagar *K. pneumoniae*



D. CHROMagar *E. coli*



E. CHROMagar *Staph. aureus*



F. CHROMagar *Pseudomonas*



In one study (Su and Ya, 2015), it has been determined that MRSA can be caused infection in acute cholecystitis.

In an enteric fever endemic country, *Salmonella enterica* serovar *Typhi* and *S. Paratyphi* A are among the major biliary pathogens. Enteric fever persists for many years after convalescence and increases the risk of hepatobiliary malignancy (Vaishnavi *et al.*, 2005; Sharma *et al.*, 2007)

In the present study, CHROMagar was evaluated for the first time in Iraq as a direct isolation medium for bile liquid aspirated directly from the gallbladder at the time of cholecystectomy.

Identification of clinical bacterial samples by using CHROMagar medium give easily and rapid results by color and morphology characteristics to differentiate between the bacterial colonies and this may impact directly on the type and duration of antibiotic choice given for these patients in the future.

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