

6-1975

## The Bacteriology of the Biliary Tract: A preliminary report.

J. A. Sapala

J. L. Ponka

T. R. Neblett

Follow this and additional works at: <https://scholarlycommons.henryford.com/hfhmedjournal>

 Part of the [Bacteria Commons](#), [Life Sciences Commons](#), [Medical Specialties Commons](#), and the [Public Health Commons](#)

---

### Recommended Citation

Sapala, J. A.; Ponka, J. L.; and Neblett, T. R. (1975) "The Bacteriology of the Biliary Tract: A preliminary report.," *Henry Ford Hospital Medical Journal* : Vol. 23 : No. 2 , 81-86.

Available at: <https://scholarlycommons.henryford.com/hfhmedjournal/vol23/iss2/6>

This Article is brought to you for free and open access by Henry Ford Health System Scholarly Commons. It has been accepted for inclusion in Henry Ford Hospital Medical Journal by an authorized editor of Henry Ford Health System Scholarly Commons.

# The Bacteriology of the Biliary Tract

## A preliminary report.

J.A. Sapala, MD\*, J.L. Ponka, MD\* and T.R. Neblett, PhD\*\*

*The bacterial flora of acute biliary tract disease has not been completely elucidated. The organisms identified most often were coliforms. Anaerobic culture technics were not precise enough to provide proper identification. In a 12-month study of 250 patients operated upon for inflammatory gallbladder disease, culture specimens were collected in a most strict and rigorous fashion. It was hoped that a more exact understanding of the relationship between bacteria and inflammatory biliary tract disease might be appreciated. Important technics were developed for gathering specimens, delivery to the laboratory and culturing. More meaningful results are to be expected in the future.*

---

\* Department of Surgery

\*\* Division of Bacteriology-Serology

Address reprint requests to Dr. Ponka at Henry Ford Hospital, 2799 West Grand Boulevard, Detroit MI 48202

THE precise relationship between bacteria and inflammatory gallbladder disease has not yet been clearly defined. This is particularly true in the patient with acute cholecystitis. In over 50% of all gallbladder operations at Henry Ford Hospital during the last five years, culture specimens obtained revealed no growth. We suspected that either specimens were being improperly collected or that bacteria indeed were not present in large enough quantities to yield positive cultures.

A strict, if often inconvenient, method for the collection, transport and plating of all gallbladder cultures was developed to determine if a combined clinical and laboratory effort would yield a consistently higher number of both aerobic and anaerobic cultures. We hoped that a more exact understanding of the relationship between bacteria and inflammatory biliary tract disease might be appreciated. This 12-month study included 250 patients who underwent operation for inflammatory gallbladder disease during all of 1974 at Henry Ford Hospital. Of these, 178 culture specimens were obtained in strict accordance with the following protocol:

Three sterilized glass culture tubes were included in every cholecystectomy set in the operating room (Figure 1).

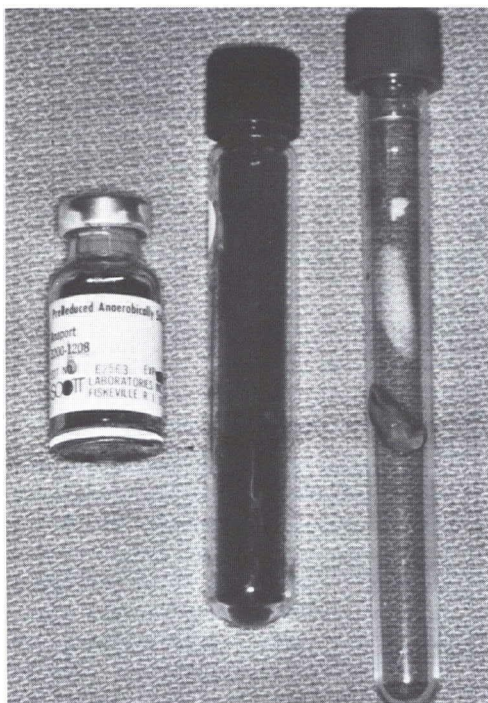


Figure 1

Sterilized glass culture tubes included in cholecystectomy set include tube at right containing portion of gallbladder wall for aerobic culture; middle tube with 2-5 ml of gallbladder bile, and at left PRAS vessel with redox potential low enough to permit survival of most strict anaerobes.

In the first tube, shown on the right, a 1 x 1 cm portion of the gallbladder wall was placed and sent for aerobic culture. The middle tube was inoculated with 2-5 ml of gallbladder bile and also sent for aerobic culture. The third tube, which was similarly inoculated with gallbladder bile, was a pre-reduced anaerobically sterilized vessel (PRAS) with a reduction-oxidation potential (redox) low enough to permit survival of most strict anaerobes.<sup>1</sup>

The redox potential of a given medium is actually a measurement of the ratio of oxidized to reduced bacterial forms.<sup>2</sup> At a given pH and temperature, the redox potential generally increases with the amount of oxygen present in the medium. The more positive the millivoltage potential, the greater the oxygen content. Most aerobes and some aerotolerant anaerobes grow between positive 250 and positive 800 millivolts. The redox potential of the PRAS vessel was set at -150 millivolts, a level on the Eh scale where most strict anaerobes can survive and initiate growth (Figure 2). Because of its greater sensitivity to an oxidized state, Resazurin was chosen as the color indicator to show oxygen contamination over the more commonly used methylene blue. Resazurin will show a color change with very little O<sub>2</sub> contamination, at an Eh range of -42 millivolt. Methylene blue will show a color change at +11 millivolts.

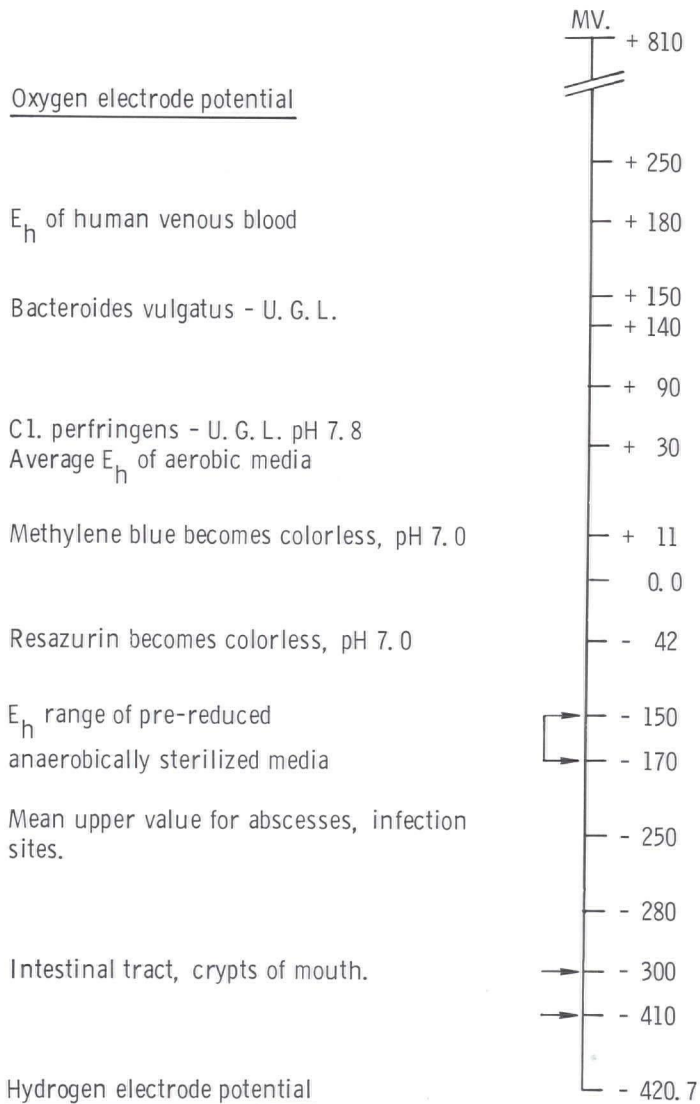
Glass syringes and metal trocars with metal hubs were used in place of disposable syringes and needles because the latter were found to show significant permeability to oxygen. In addition, polyvinyl and polyethylene plastics may oxidize bacterial media on contact by precipitating on their surface an exchange of electrons, thus raising millivoltage potential.<sup>3</sup>

The trocar was placed into the intact specimen prior to drainage, as in the case of acute gangrenous cholecystitis. If the specimen was removed intact, the trocar was then placed and bile aspirated. Once inoculated, the three tubes were taken directly to the bacteriology laboratory for immediate plating. No cotton swabs were used, and no specimens were refrigerated for future plating. Aerobic specimens, including a portion of the excised gallbladder wall, were plated directly onto the appropriate agar medium.

The PRAS vessel containing the specimen for anaerobic cultures was plated on a PRAS roll streak tube, provided that the Resazurin color indicator did not reveal any oxygen contamination. The plating was performed

## The Bacteriology of the Biliary Tract

### $E_h$ SCALE SHOWING VARIOUS SIGNIFICANT VALUES



\* Upper growth limit

**Figure 2**  
Redox potential of the PRAS vessel was set at -150 millivolts.

using the VPI inoculator (Figure 3 ) which gave a continual 120 cc/min flow of nitrogen, CO<sub>2</sub> and hydrogen to displace any circulating oxygen at the mouth of the roll streak tube.<sup>4</sup> The tube rotates on its vertical axis so that plating can be facilitated, and so that there will be an adequate distance between the growth of colonies. Once plated, the roll streak tube was sealed and incubated at 35°C until final speciation of any anaerobic growth was completed, usually in 2-5 days.

Of the 178 culture specimens obtained, 43 (25%) were positive, (Figure 4). Thirty-five were aerobes and 8 were anaerobes. At first glance, this would seem to indicate that the percentage return for our effort was quite low. However, when we analyzed all cultures relative to the kind of surgical pathology found at operation, we noted some very interesting findings.

Figure 4

POSITIVE CULTURES 178 PATIENTS	
Total Positive Cultures	43 (25%)
Aerobes	35 (approx. 20%)
Anaerobes	8 (approx. 5%)

Patients were divided into three groups according to their surgical pathology (Figure 5). Type I patients consisted of 129 individuals who had chronic uncomplicated cholecystitis. Of these, only 10% yielded positive cultures. Excluding probable skin contaminants such as *S. epidermidis* and alpha streptococci, only 3% were positive cultures. Therefore, it seems almost futile to obtain routine gallbladder cultures from chronically inflamed gallbladders without other complications. Type II patients consisted of 27 individuals with evidence of acute changes on the final pathology report or with frank suppuration at the time of operation, as in the patient undergo-

ing cholecystostomy. In this group, culture results were far more encouraging, ranging just over 70%. In the last group of patients, Type III, 22 persons with either acute or chronic cholecystitis were found to have concomitant evidence of biliary tract obstruction, either stones or a neoplastic process. Here, 11 positive cultures represented a 50% return.

With the possible exception of *E.coli*, no single predominating organism of the various aerobic species was grown (Figure 6). Most were of enteric origin consisting predominately of *E.coli* and the various *Enterobacteriaceae*. No *Salmonella* were isolated.

The anaerobic species included the *Clostridium*, *Bacteroides*, *Propionibacterium* and *Peptococcus magnus* (Figure 7). Final speciation of *Lactobacillus* and *Peptostreptococcus* genera has not yet been worked out in our laboratory and, therefore, they are listed only as species.

In summary, the bacteriology of the inflamed gallbladder remains complex. Nevertheless, by implementing a strict method of collection and a precise technique of plating all culture specimens, definite patterns of microbial growth become apparent. In uncomplicated chronic cholecystitis, bacteria do not flourish in great numbers in the biliary tract. In acutely inflamed states, or when there is biliary tract obstruction, the number of positive cultures rises greatly. In this stage, anaerobic bacteria have begun to be isolated in increasing numbers. No specific organism appears to be the predominant in this study. Most appear to be of enteric origin. It is hoped that further study will confirm these general observations and will eventually result in improved management of patients with acute biliary tract disease.

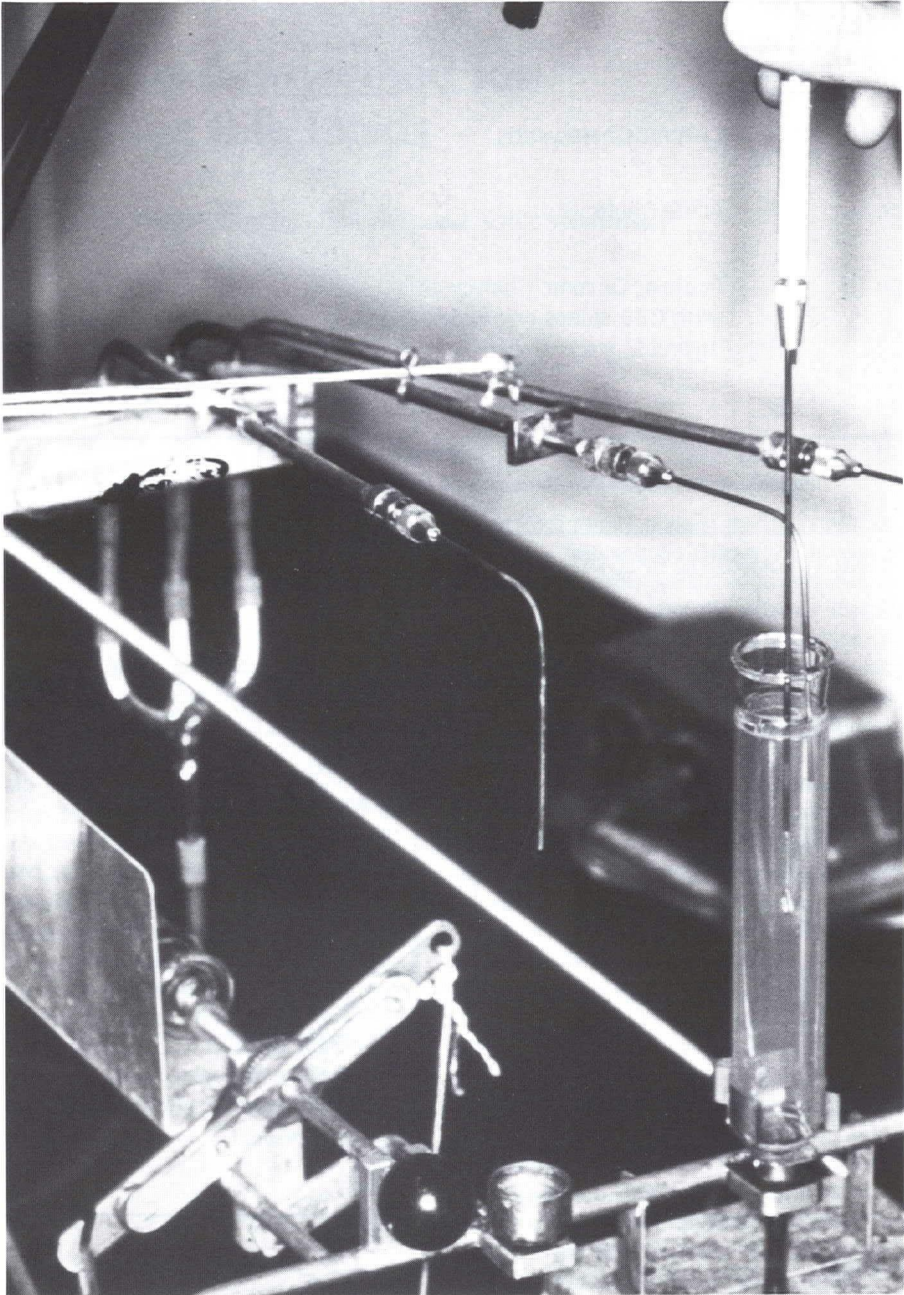


Figure 3  
VPI inoculator used for plating.

Sapala, Ponka, and Neblett

	<u>No. of Patients</u>		<u>Number + Cultures</u>		<u>Aerobes</u>	<u>Anaerobes</u>
Type I	129	Chronic Cholecystitis	13	10%	11	2
Type II	27	Acute Cholecystitis	19	70%	16	3
Type III	22	Acute or Chronic Cholecystitis (with CBD stones or neoplasia, hydrops, etc.)	11	50%	8	3
TOTAL	<u>178</u>		<u>43</u>	<u>25%</u>	<u>35</u>	<u>8</u>

Figure 5

Patients were divided into three groups according to their surgical pathology.

<u>STRICT ANAEROBIC SPECIATION</u> (8 cultures)	
<u>Organism</u>	<u>No. of Cultures</u>
Clostridium perfringens	1
Bacteroides fragilis	2
Propionibacterium acnes	2
Peptococcus magnus	1
Lactobacillus sp.	1
Peptostreptococcus sp.	1
TOTAL	<u>8</u>

Figure 6

<u>AEROBIC AND FACULTATIVE ANAEROBIC SPECIATION</u> (35 cultures)	
<u>Organism</u>	<u>No. of Cultures</u>
Alpha hemolytic streptococcus (2 group D enterococci)	7
Staphylococcus aureus	2
Staphylococcus epidermidis	2
Escherichia coli	8
Streptococcus faecalis	3
Klebsiella pneumoniae	5
Pseudomonas aeruginosa	1
Pseudomonas maltophilia	1
Proteus morgani	1
Enterobacter aerogenes	1
Enterobacter cloacae	1
Serratia liquifaciens	1
Citrobacter freundii	2
TOTAL	<u>35</u>

Figure 7

References

1. Holdeman L and Moore WEC: Roll-tube technique for anaerobic bacteria. *Amer J Clin Nutr* 25:1314-17, Dec 1972
2. Hentges DJ and Maier BR: Theoretical basis for anaerobic methodology. *Amer J Clin Nutr* 25:1299-1305, Dec 1972
3. Holdeman L and Moore WEC: Personal communication
4. Anaerobe Laboratory Manual, Virg Polytechnic Instit and State Univ, Blacksburg, VA, 1974