Original Article

Isolation And Identification Of Bacteria That Cause Cholecystitis

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Abstract

Objective: Isolation and identification of bacteria that cause cholecystitis by cold centrifuge method and brain heart infusion broth method.

Background: This study includes isolation and identification of the microorganisms that cause cholecystitis in man, five different microorganisms isolated from forty samples taken from 40 patients after removing the infected gallbladder.

Material and method: Two isolates of Staphylococcus aureus, two isolates of Listeria monocytogenes and five isolates of Escherichia coli. These microorganisms isolated by cold centrifuge method. Just one isolate of Salmonella typhi and one isolate of Shigella sonnei isolated by placing the stones found in infected gallbladders in Brain heart infusion broth for ten days from incubating at 37 Cº, Shigella sonnei isolated in the seventh day while Salmonella typhi isolated in the tenth day of incubation.
To identify a bacteria; cultural, microscopic examination and Biochemical tests were done in addition to api system and antisera for diagnosis of Salmonella typhi and Shigella sonnei.

Results: Samples taken from forty patients post cholecytectomy operations, five species of bacteria isolated by two methods (brain heart infusion broth method and cold centrifuge method) and cultured and identified on Macconkey, Blood, S.S, EMB, XLD, Mannitol salt, in addition to other determination tests, the isolated species of bacteria.

Conclusion: Different pathogenic microorganisms from the human bile are able to cause cholecysititis and there was difficulty to isolate Salmonella typhi and Shigella sonnei from human bile because of the biofilms formed by bacteria in the gallstones.

Keywords:Isolation,Identificationf, Bacteria, Cholecystitis

INTRODUCTION:

The pathogenic microorganism is defined as one of the causes or is capable of causing disease. Virulence factors are those characteristics of a bacterium that enhances it`s pathogenecity, i.e. it`s ability to cause disease (1).
Listeria monocytogenes is the agent of Listeriosis is a serious infection caused by eating food contaminated with the bacteria. The disease affects primarily pregnant women, and adults with weakened immune systems, (2).
Staphylococcus aureus is one of the most common causes of bacterial infection and is an important cause of food poisoning and toxic shock syndrome. The majority of the diseases caused by Staphylococcus aureus pathogenesis depend on the combined action of several virulence factors (1).
Salmonella typhi is the causative agent of typhoid fever, the genus Salmonella is found in the intestine of man, animals and birds. Sometimes food, (egg and meat) may be contaminated with this organism. It may cause enteric fever, gastroenteritis and septicemia, (3). Shigella sonnei causes an enterobacterium disease called shigellosis. Shigella invades and destroys the mucosa of the large intestine. Infection rarely penetrates to deeper layers of the intestine, and dose not lead to Shigella becteremia.
Escherichia coli are responsible for sever acute hemorrhagic diarrhea and abdominal cramps, (4).
Presence of gallstones in the gallbladder may lead to acute cholecystitis; an inflammatory condition characterized by retention of bile in the gallbladder and often secondary infection by intestinal microorganisms, predominantly E.coli and Bacteroides species. Typhoid fever bacteria can live on gallstones in people who carry the disease without showing any symptoms, (5).

Typhoid bacteria take coven on gallstones

Typhoid fever bacteria can live on gallstones in people who carry the disease without showing any symptoms. This could explain how the disease, that causes fever, headach, nausea, loss of appetite and diarrhoea-cotinues to be spread despite having no environmental reservoir.

It has been known for decades that Salmonella enterica typhi can accumulate in the gallbladders of people who have recovered from typhoid fever and sometimes in people who have never experienced any symptoms.
The authors observed the formation of biofilms in gallstones of mice infected with a version of typhoid fever and founded that they had more Salmonella typhi in their gallbladder tissue, bile and fecal matter than gallstone – free mice.
These biofilms were also observed on the gallstones of patients in Mexico City where typhoid is common; those patients were found to be carrying typhoid when they had their gallbladders removed (6).

Bacterial biofilms:

Bacterial biofilms are clusters of bacteria that are attached to a surface and/or to each other and embedded in a self-produced matrix. The biofilm matrix consists of substances like proteins (e.g., fibrin), polysaccharide (e.g., alginate), as well as eDNA. In addition to the protection offered by the matrix, bacteria in biofilms can employ several survival strategies to evade the host defense systems (7, 8). By staying dormant and hidden from the immune system, they may cause local tissue damage and later cause an acute infection. Within the biofilm, the bacteria adapt to environmental anoxia and nutrient limitation by exhibiting an altered metabolism, gene expression, and protein production, which can lead to a lower metabolic rate and a reduced rate of cell division (9, 10). In addition, these adaptations make the bacteria more resistant to antimicrobial therapy by inactivating the antimicrobial targets or reducing the requirements for the cellular function that the antimicrobials interfere with. During a biofilm infection, simultaneous activation of both innate and acquired host immune responses may occur; neither of which are able to eliminate the biofilm pathogen, but instead accelerate collateral tissue damage. Consequently, biofilm-related diseases are typically persistent infections that develop slowly, are rarely resolved by the immune system, and respond inconsistently to antimicrobial treatments (11).

MATERIAL AND METHOD:

40 Samples were collected from 40 patients suffering of cholecystitis from the Hussein Teaching Hospital in samawa city in Iraq, after cholecytectomy operations, the gallbladder transferred to the laboratory under cool conditions, the bladder juice evacuated using disposal syringe.
First :- cold centrifuge method, after draw the bile, put in sterile test tube and operation making of the centrifugal by used cold centrifuge system quickly at 10000 rpm for (30 – 15) min, and hence take the sediment and culture on Blood agar, MacConkey agar, Nutrient broth, Trypton soya broth and other media.
Second: - Brain heart infusion broth method: stones in the gallbladder may lead to acute cholecystitis, the stones transmitted to sacrotubes containing 25 ml of Brain heart infusion broth, and incubated in 37 Cº for 24 hr, the mixture then cultured on Blood and MacConkey agar, and incubated for ten days.

RESULTS:

Bacteriological examinations included the isolation of bacteria from human gallbladder and identifying them in respect to the microscopic, culture, api system, biochemical tests, Anti sera, and other examinations.

1. Collection & Samples isolation

Samples taken from forty patients post cholecytectomy operations, five species of bacteria isolated and cultured and identified on Macconkey, Blood, S.S, EMB, XLD, Mannitol salt, in addition to other determination tests, the isolated species of bacteria shown in Table (1).

Table 1. Number of each isolated bacterial species.

NO.	Bacterial species	Isolates number
1	*Staphylococcus aureus*	**2**
2	*Listeria munocytogenes*	**2**
3	*Salmonella typhi*	**1**
4	*Shigella Sonnei*	**1**
5	***Escherichia coli***	**5**

Identification of bacteria:

Staphylococcus aureus:

Cultural characteristics:

Detection of S. aureus colonies were recognized first on blood agar as golden yellow colonies with clear zone surrounding them due to RBCs hemolysis, while it failed to grow on Macconkey agar due to its content of bile salt and crystal violate which inhibit the growth of gram positive bacteria.
The growth on Mannitol salt agar was recognized by changing the color of the agar from red to yellow due to fermentation of mannitol in the media. A wide zone of beta hemolysis on blood agar can be seen in figure (1)

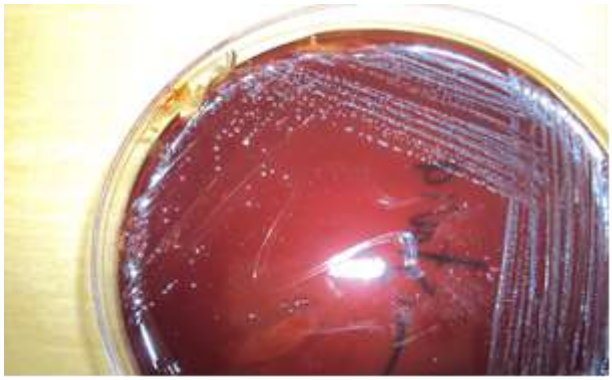

Figure (1) *Staphylococcus aureus* growth on blood agar

LISTERIA MONOCYTOGENES :

Cultural Characteristics :

L.monocytogenes colonies were recognized on blood agar as smooth smell, translucent and bluish – grey in colour. On blood agar a zone surrounding the beta hemolysis, as seen in figure (2)

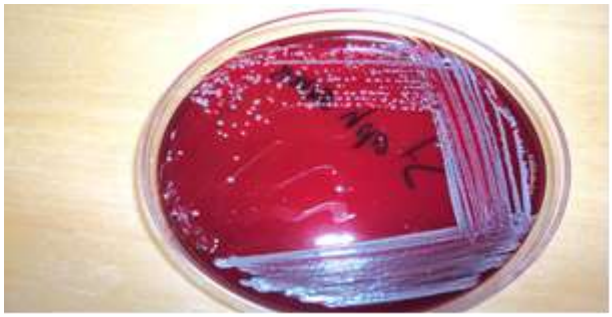

Figure (2) showing the growth of L.monocytogenes on the blood agar
Key: (+) positive reaction; (**–**) negative reaction

SALMONELLA TYPHI:

Culture Characteristics:

After 24 hrs of incubation at 37 Cº, the bacterium showed convex (2 – 4) mm in diameter, and smooth colonies. On Macconkey agar, they looked pale due to their inability for fermenting lactose, but on S – S agar, they were colorless, on Xylose – Lysine – desoxycholate (XLD) agar, the colonies were red in color with black colony center, while on Nutrient agar, the colonies were gray – white in color.

SHIGELLA SONNEI :

Cultural Characteristics:

After 24 hrs of incubation at 37 Cº colonies of this bacterium were facultative anaerobes, convex, circular and transparent, ferment glucose. On Macconkey agar, the colonies were pink in color (weak), but on S – S agar, they were colorless and on Xylose – Lysine – desoxycholate (XLD) agar, the colonies were red in color without black center.

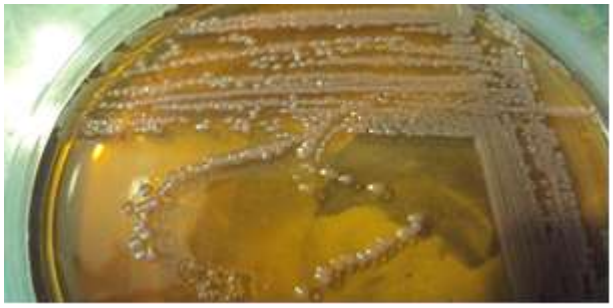

Figure (3) showed *Shigella sonnei* growth on S.S agar.

ESCHERICH COLI :

Cultural Characteristics :

E. coli was first recognized on MacConkey agar, their colonies appeared as flat smooth and pink in color as a result of lactose fermentation, while on blood agar it gave dark, convex colonies. It seems as a dark red to brown color with a greenish metallic sheen on EMB agar, which differentiated from other types.

DISCUSSION:

Each isolated bacterial species are showed that just two isolates (5%) of Staph. aureus from the total number of the patients after removing the gallbladder, this result agree with that done by (12), who obtained Staph. aureus isolates from 2.6% of samples , and from 9.7% of samples in the study of , (13).

Listeria monocytogenes isolated from bile at 5% being obtained in two isolates from the total number of the patients, this result agreed with those achieved by, (14). Some of the patient considered as carriers for the pathogens in human gallbladder without demonstrating symptoms.

The current study we demonstrate that initial attachment of Listeria monocytogenes cells with plastic surfaces followed with increasingly growth in the presence of bile. Improved biofilm formation was confirmed by crystal violet staining. Enhanced biofilm formation in response to bile may influence the ability of L. monocytogenes to form biofilms in vivo during infection and may contribute to survival of this important pathogen in the human gastrointestinal tract and gallbladder.

one isolates 2.5% of Salmonella typhi from the total number of the patients, this result agreed with that got by, (15).

Salmonella typhi isolated from human gallbladder at a rate of 2.5% by placing the stones of the gallbladder which taken from gallbladder in sacrotubes containing 25 ml from brain heart infusion broth, the microorganism isolated after 10 days of incubation showing a difficulty in isolating, therefore the enrichment media must be secured for 10 days , Salmonella typhi can live on gallstones in people who carry the disease without showing any symptoms, therefore that microorganism situated on the outer surfaces of the gallstone , and can be isolated after placing the stones in a brain heart infusion broth, these result agree with those of (6), who isolated Salmonella typhi from the stones which founded in human gallbladder and observed the formation of biofilms in gallstones of mice infected with a version of typhoid fever and found that they had more Salmonella typhi in their gallbladder tissue, bile and fecal matter than gallstone-free mice.

just one isolate 2.5% of Shigella sonnei detected from the total number of the patients under went the study. Shigella sonnei isolated after placing the stones which taken from gallbladder in sacrotubes containing 25 ml from brain heart infusion broth, Shigella sonnei isolated after 7 days of incubation, difficulties experienced in isolating the microorganism so the same methods as those used in detection the Salmonella typhi were followed . Shigella are typically spread from one person to other, with contaminated stools serves as a major source of organisms. Flies and contaminated food or water can also transmit the disease, (1).

E. coli isolated also from the bile in a rate of 12.5% , five isolates from the total number of the 40 samples , this result come in agreement with those obtained by, (13), who got the microorganism in a rate of 25%. E.coli isolated from gallbladder by, (16), at a rate of 29.7% , the same team isolated Salmonella typhi at a of 3.8% , they contributed the variation in the isolating rates to the geographical sites , environmental factors and the isolating methods, in addition to the nutritional factor. Bacterial isolates were found in pigment stone-containing bile, Non-lithogenic bile revealed no bacteria, explaining an association between gallstone formation and the presence of bacteria in bile (17).

REFERENCES:

1. Richard, A; Pamela, C; and Bruce, D. Microbiology, 2nd edition. 2007.
2. Todar, K. Salmonella and Salmonellosis, Online textbook of bacteriology. 2008.
3. Gupte, S. Medical Microbiology. The short textbook. 2010.
4. Wei , H. L.; Wang , Y.W; Li , C. C; Tung, S. K.; and Chiou , C. S. "Epidemiology and evolution of genotype and antimicrobial resistance of an imported shigella sonnei clone circulating in central Taiwan." Diagnostic Microbiology and infection Diseases. 2007.
5. Channa, N.A.; Khand, F.D.; khand, T.U., Leghari, M.H., and Memon , A.N" Anglysis of human gallstones by fourier Tranform infrared (FTIR)." Pakistan Journal of medical sciences. 2007. 23 (u): 546 – 50.
6. Daniela, H. Science and Developent Network. Typhoid bacteria take cover on gallstones. 2010.
7. Høiby N. A short history of microbial biofilms and biofilm infections. APMIS. 2017;125:272–275.
8. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS. 2013;121:1–58.
9. Stoodley P., Hall-Stoodley L. Evolving concepts in biofilm infections. Cell. Microbiol. 2009;11:1034–1043.
10. Donlan R.M., Costerton J.W. Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. Clin. Microbiol. Rev. 2002;15:167–193.
11. Moser C., Pedersen H.T., Lerche C.J., Kolpen M., Line L., Thomsen K., Høiby N., Jensen P. Østrup Biofilms and host response-helpful or harmful. APMIS. 2017;125:320–338.
12. Garcia, J. M.; Iglesias, L., Vallejo, M., Garcia, N., Gomez, J.A., Garcia, V., Alcala, L., pelaez, T. and Bouza, E. Inter science conference on Antimicrobial Agents and chemotherapy. 2003.
13. Bae, W.K.; Moon, Y.S., kim, J.H., Lee, S.H. and cho, C.R. Department of internal medicin. lison paik hospital , Inje university college of medicine, Goyang, korea. 2008.
14. Hardy, J., Francis, K.P.; Doeboer , M.; Chu, P.; Gibbs, K.; and Contag, C.H. Extra – cellular replication of Listeria monocytogenes in the murine gallbladder. Science. 2004. 303: 851 – 853.
15. Tewari, M., Mishra, R. R., and shukla, H. S. Department of surgical oncology, Institute of medical sciences, Banaras Hindu University. 2010.
16. Capoor, M.R.; Nair, D., Rajni, khanna, G., Krishna, S.V., chintamani, M.S., and Aggarwal, P. Department of Microbiology. Vardhman mahveer medical college and Safdarjung Hospital, New Delhi, India. 2008.
17. Abeysuriya, V., Deen, K.I, wijesuriya, T., and salgado, S.S. Department of clinical Anatomy, faculty of medicine. 2008…2008