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BACTERIAL INFECTION OF CARCINOMA IN LIVER DISEASES

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ABSTRACT

The development of cancer is associated with several genetic and environmental factors. In addition, many studies showed an association between the development of cancer and bacterial and viral infections for decades. In this study, we aimed to collect and identify various types of bacterial species isolated from patients diagnosed with hepatic carcinoma using different isolation and identification techniques as a preliminary step towards setting of complete profile and thus an early alarm for all possible bacterial infections associated with liver diseases. 204 blood samples were collected from patients diagnosed by hepatocellular carcinoma attending medical labs from 2014to 2015. Different media types were used for isolation of pathogenic and potential pathogenic bacteria associated with hepatocellular carcinoma. Bacterial isolates were purified by sub-culturing and identified using analytical profile index (API) test kits. Specimens were also subjected to some biochemical tests including liver enzymes, glutamic pyruvic transaminase (GPT), glutamic oxalo acetic transaminase (GOT), alkaline phosphatase (ALP) activities, and albumin and Alfa-fetoprotein (AFP) levels as a tumer marker. Hundreds of bacteria were isolated on different Isolation media; however, the most distinguished species were *Staphylococcus aureus*, *Enterobacter aerogenes*, *Klebsiella sp* and *Salmonella typhi* confirmed with 16S r RNA technique. Based on these results, hepatocellular carcinoma patients are more susceptible to be infected with these bacterial strains. Further studies on a larger scale are required to can set a clinical profile pointing-out the most probable bacterial strains affecting those patients and the best strategies for prevention and treatment.

KEYWORDS: Bacteria, carcinoma, Liver, infections.

INTRODUCTION

The relationship between different bacterial and viral infections and malignancy has been studied extensively. It is estimated that over 15% of malignancies worldwide can be attributed to infections or about 1.2 million cases per year.[1] Many viruses can integrate into the human genome and directly initiate tumor, such as human papilloma virus (HPV) in cervical cancer and herpes-virus in Kaposi's sarcoma.[2,3] In other cases, bacteria can develop cancer indirectly, for instance with *Helicobacter pylori*, which contributes in both gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma due to chronic inflammation caused by the bacteria.[4,5] In the other hand, several studies have reported different indigenous species of bacteria being isolated from cancer tissues in patients.[6,7] Furthermore, recent data promote the paradigm that gut bacteria can influence cancer risk in extra-intestinal organ.[8,9] The means by which this occurs is not fully elucidated, but is thought to involve both immunity and metabolism as key factors in tumor promotion by intestinal bacteria.[10]

Studies and clinical trials showed that bacteria are naturally capable of homing and replication especially within tumors.[11,12,13,14] The presence of bacteria within tumors could be due to infection via the vasculature and their ability to survive and grow due to the presence of nutrients within the hypoxic region of the tumor at a later stage in tumor growth. For example, sampling from humans has indicated bacterial translocation of Gastro-Intestinal Tract (GIT) - associated bacteria may be a phenomenon that occurs in healthy individuals representing a normal physiological event without deleterious consequences.[15] It may be that bacteria egress from the GIT at very low numbers, and normally are quickly eliminated by the immune system. However, the phenomenon of bacterial replication within tumors results in dramatic increases in bacterial numbers within a confined region.

Bacterial infections are a common cause of morbidity in patients with hepatic cirrhosis. Various bacterial infections can decompensate liver status and lead to death in patients with cirrhosis.[16,17] Moreover, bacterial infections have been acknowledged as a potential trigger factor in many complications of cirrhosis, including variceal bleeding, hepatic encephalopathy, renal failure and impairment in clotting factors.[18] Infection significantly increases the mortality rate. Various infections are directly responsible for 30–50% of deaths in patients with cirrhosis.[17,19] About 50% of the infections present atypical clinical appearance and some disease specific characteristics of this patient population make identification of infectious episodes even more difficult.[20] An appropriate clinical approach would include alertness for possible infections, and its systematic diagnostic examinations.

In this study, we aimed to collect and identify various types of bacterial species isolated from patients diagnosed with hepatic carcinoma using different isolation and identification techniques as a preliminary step towards setting of complete profile and thus an early alarm for all possible bacterial infections associated with liver diseases.

MATERIALS AND METHODS

- Patients and samples collection

The total number of 204 patients already diagnosed with hepatocellular carcinoma. All patients were attending in “Cairolab”analysis laboratories for regular testing during the period from 2014 to 2015.
Blood was withdrawn from the patients aseptically by vein puncture and 3 ml of each sample were mixed with disodium EDTA (pH 6.5).

- Culture media

Different media types were used to fulfill the requirements of bacteria associated with hepatocellular carcinoma. All media types were ready made viz. Azide agar (Difco) + 5% (w/v) blood, Staphylococcus medium 110 (Difco), Brain heart infusion (Sigma) and Tryptose agar (Difco) + 5% (w/v) blood, in addition to blood agar medium.

Subculture and purification of bacterial isolates The bacterial isolates were subcultured on blood agar medium plates. The best single and pure growing colony, and the most characteristically were picked up by a sterile loop and subjected for purification on the same isolation media. Agar streak method was used for purification process. A well separate colony from each isolate was picked up on histamine decarboxylase agar slants and incubated at 37oC for 24 hrs. Purity was checked by microscopic examination of the isolates using Gram stain.

- Identification of isolates

The identification of all histamine producing bacteria were performed using API strips inoculated and incubated as described by the manufacturer (bio MerieuxVitek System, France). Examination of the strips was conducted after 18-24 h, and the results from the 24 h analysis were used. The results were read and analyzed using an analytical profile index, a mini-API instrument (bio MerieuxVitek Systems).[21]

Biochemical analysis

Liver enzymes for the patients were measured including GPT, GOT and ALP activities by IFCC method. Also, Albumin levels were analyzed for the patients. Tests were conducted using Randox® reagents (USA) in accordance with kits procedures.

In addition, Alfa-fetoprotein as a tumor marker was measured by fully automated analyzer DRG: HYBRiD-XL® using AFP kit (Cat. No. HYE-5337).[22]

16S rRNA determination

The identification of the bacteria were performed using the 16S rRNA gene was determintion by using the universal primers, automated DNA sequencer sequenced by the dideoxy-chain termination method[23] at clinilab. (GenBank) were searched for sequences similarity analysis of the 16S rRNA sequence obtained.

RESULTS AND DISCUSSION

Two hundred and four specimens were used for isolation of pathogenic and potential pathogenic bacteria from patients attending medical Labs during 2014 and 2015. Patients attended for symptomatic features of hepatocellular carcinoma. Specimens collected from 78 females and 126 males. The patients' age were ranged between 25 and 77 years old. All specimens given decoded numbers for consent and medication history as well as the names of the patients were hidden following rules of Lab administration.

Bacterial isolates

Specimens were cultured on different types of media for isolation of bacteria associated with hepatocellular carcinoma. Blood agar exhibited the highest colony forming units/ml (CFU/ml) of bacteria (5.6 x 104) while Azide blood agar recorded the lowest CFU/ml (0.1 x 102) as shown in (table 1).

Table 1: colony forming units / ml (CFU/ml) resulted from culturing specimens on different types of culture media.

Culture Medium	CFU/ml
Azide blood agar	0.1×10^2
Staphylococcus medium 110	0.4×10^2
Brain heart infusion	2.7×10^4
Tryptose blood agar medium	2.0×10^3
Blood agar	5.6×10^4

Identification of isolates

As mentioned previously, all isolated bacteria were identified by API strips. Several hundreds of bacteria isolated on different Isolation media, but the identification process was preceded only on representative colonies, which were noticed to grow vigorously. In addition, each identified colony was not similar morphologically to each other as much as we can i.e., to get different types of bacteria coverage range of bacteria associated with hepatocellular carcinoma.

However, the bacteria obtained in this study were 4 species; a Genera of Gram-positive bacterium was *Staphylococcus aureus* and Genera of Gram-negative were *Enterobacter aerogenes*, *Klebsiella sp* and *Salmonella typhi*. (Figure 1).

Figure : *Klebsiella Pneumoniae* culture on tryptose blood agar medium

Biochemical analysis

Upon measuring GPT, GOT, ALP enzymes activities, and Albumin levels, results showed that out of 119patients, only four patients recorded abnormal levels of all tests. A number of three patients showed elevated levels of GPT with normal GOT enzymes activities levels and 21 cases showed elevated GOT levels with normal GPT. enzymes activities Mean while, 32 samples showed high levels of both GPT and GOT enzymes activities above normal. Twenty patients showed high ALP activities and for Albumin levels; 31 patients showed elevated test results.

Alfa-fetoprotein (AFP) used as tumor marker for hepatocellular carcinoma was analyzed for 204 patients' samples. Results showed that 73 cases were above normal level (10ng/ml) while seven cases showed extremely high levels of Alfa-fetoprotein (above 500ng/ml).

Bacterial identification by 16S rRNA gene

In order to confirm the identification of bacteria by 16S **rRNA** partial sequence sequence was determined. The sequencing result showed that 16S **rRNA** sequence from *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella typhi* and *Klebsiella sp*. (Fig. 2). Showed The GenBank database was used to compare the partial sequence of 16S rRNA sequence from one isolate and the highest nucleotides identity were 99% with *Klebsiella pneumoniae.* GenBankGraphics Next Previous Descriptions *Klebsiella pneumonia* strain TW-6 16S ribosomal RNA gene, Partial sequence. Sequence ID: KT887952.1Length*:* 1408 Number of Matches: 1 Related Information.

Range 1: 194 to 469GenBankGraphics Next Match Previous Match First Match.

Alignment statistics for match #1					
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Features:

F-sequence

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Query  1
TAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGC  60

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Sbjct  194
TAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGC  253

Query  61
CACACTGGAACTGAGACACGGTCCTGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA     120


  |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct  254
  CACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA     313

  Query  121
  CAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAA     180

  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  Sbjct  314
  CAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAA  373
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Query  181
AGCACTTTCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTTGTCGATTGACGTTACCCG      240

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  374
AGCACTTTCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTTGTCGATTGACGTTACCCG   433

Query  241   CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCG   276

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Sbjct434     CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCG   469
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Klebsiella pneumoniae strain GXNN3 16S ribosomal RNA gene, partial sequence
Sequence ID: KU936064.1Length: 1504Number of Matches: 1
Related Information
Range 1: 7 to 337GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match

Score	Expect	Identities	Gaps	Strand	Frame
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Features:
R-sequence

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Query  1
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Sbjct  337
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Query  61
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Sbjct  277
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Query  121
TGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCT      180

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Sbjct  217
TGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCT   158

Query  181
ACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCC      240


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Query  301  TGCCGCCAGCGTTCAATCTGAGCCAGGATCA  331
            |||||||||||||||||||||||||||||||
Sbjct  37   TGCCGCCAGCGTTCAATCTGAGCCAGGATCA 7
```

Klebsiella pneumoniae strain GXNN3 16S ribosomal RNA gene, partial sequence
Sequence ID: KU936064.1Length: 1504Number of Matches: 1

Related Information
Range 1: 7 to 337GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match					
Score	**Expect**	**Identities**	**Gaps**	**Strand**	**Frame**
612 bits(331)	2e-171	331/331(100%)	0/331(0%)	Plus/Minus	

CONCLUSIONS

Based on these results, hepatocellular carcinoma patients are more susceptible to be infected with these bacterial strains. Further studies on a larger scale are required to set a clinical profile pointing-out the most probable bacterial strains affecting those patients and the best strategies for prevention and treatment.

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