Chapter

Role of Gut Microbiome and Enteric Bacteria in Gallbladder Cancer

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Abstract

Gallbladder cancer (GBC) is associated with a sinister prognosis, a short survival time, and early metastasis to distant sites. Chronic inflammation of the gallbladder due to gallstone disease and biliary bacteria remain key factors in the pathogenesis of GBC. The association of chronic bacterial infections with the development of GBC has provided a new perspective on the causation of GBC. A strong link between chronic Salmonella infection and enterohepatic strains of Helicobacter species with GBC has been suggested. It is believed that many other enteric bacterial strains, predominantly the Enterobacteriaceae species, are associated with the development of GBC. However, the available literature mainly comprises observational studies and small meta-analyses necessitating the requirement of a higher level of evidence. This chapter discusses the role of the gut microbiome, dysbiosis and its association with carcinogenesis, and the organisms associated with the causation of GBC.

Keywords: gallbladder neoplasm, dysbiosis, gut microbiome, brain-gut axis, gastrointestinal microbiome

1. Introduction

Gallbladder cancer (GBC) is the most common malignancy of the biliary tract with an aggressive clinical course and short median survival [1]. While being a rarity in the western world, GBC is one of the major causes of cancer-related morbidity and mortality in South Asian and Southeast Asian countries [2]. Females are more commonly affected than males. According to the cancer statistics of 2020, GBC accounts for 0.6% of the total cancer cases and is associated with 0.9% of total cancer-related deaths [3]. Around 10% of the global GBC burden is contributed by India, with the Northern, Central, and North-eastern parts as the highest contributors [4]. Only 10% of cases present at an early stage which can be owed to the aggressive tumor biology of this cancer and the lack of effective screening techniques for its early detection [5]. Chronic inflammation of the gallbladder remains a major factor in the pathogenesis of GBC, although the causes are multifactorial. Gall stones, heavy metals, environmental toxins, and carcinogens have

all been implicated in chronic irritation of the gallbladder mucosa, thereby leading to dysplasia and subsequent development of neoplasia.

The landscape of the microbiome populating our digestive tract has received a lot of scientific attention in recent years [5]. There is ample evidence linking the human microbiome and its metabolites to carcinogenesis. It is proven that balanced flora or microbial eubiosis is related to health while dysbiosis or unbalanced flora can lead to various diseases, including cancers [6, 7]. There can be multiple triggers causing dysbiosis, including fluctuations in the environment, inflammation, infection, medications, dietary changes, or genetic predisposition. The International Agency for Research on Cancer labeled ten microbial species as carcinogens [8]. Around 15–20% of cancers are linked to microbial pathogens, with *Helicobacter pylori* (*H. pylori*), human papillomavirus, Hepatitis B virus, and hepatitis C virus being the four predominant species, driving 90% of infection-associated cancers [6, 9, 10]. However, there is very limited information available on the microbial species inhabiting the human gall bladder, except for a few cultivable species of bacteria associated with cholelithiasis [11, 12]. It was seen that the biliary tract has an abundance of Enterobacteriaceae [13]. Microorganisms in the common bile duct of patients having gallstones were more commonly those that inhabited the human respiratory tract and oral cavity rather than intestinal microbes [14]. Very recently, culture-negative bile samples acquired from normal gallbladders were evaluated using 6S ribosome gene analysis. A very simple and less diverse bacterial flora was found comprising the Firmicutes, Proteobacteria, and Actinobacteria phyla [15].

Detection of some bacteria does not indicate its causality in inflammation or cancer. However, recent amassing evidence indicates that microbiota dysbiosis and chronic inflammation contribute to carcinogenesis [16]. Several reports point towards strains of Salmonella and Helicobacter colonizing the gall bladder and are linked to an escalated risk of developing GBC [17, 18]. Premalignant lesions were found to be coexisting with chronic Salmonella infestation, despite the absence of gallstones [19]. Various experimental studies and epidemiologic data support the induction of carcinogenesis due to dysbiosis of the gallbladder microbiome. However, results indicating only cultivable species limit these claims. Also, despite the proximity of a large diverse microflora reservoir in the gut, little is known about its impact on the human bile microbiome. In this chapter, we aim to provide a comprehensive review of all the available literature on the gut and biliary microbiome and their association with GBC.

2. Understanding the human gut microbiome

The term microbiome has been derived from two words, “micro” and “biome”, meaning, a specific microbial community with distinct physiological and chemical properties, residing in a well-defined habitat which is their “theatre of activity”. This definition was proposed by Whipps while working on mycoparasites [20]. The term “gut microbiome” or “human microbiome” was coined by Joshua Lederberg in 2001 and since then it has been a topic for debate among researchers [21]. The human microbiome can be defined as a specific community of commensal, symbiotic and pathogenic micro-organisms that reside within our body spaces [22]. These include gut bacteria, eukaryotes, archaea, and specific viruses [23, 24]. In a healthy individual, these bacteria are responsible for various synthetic and metabolic functions and detoxification of various xenobiotics [25]. They form an integral part of the “gut-brain axis” which is bidirectional communication between the gut and the

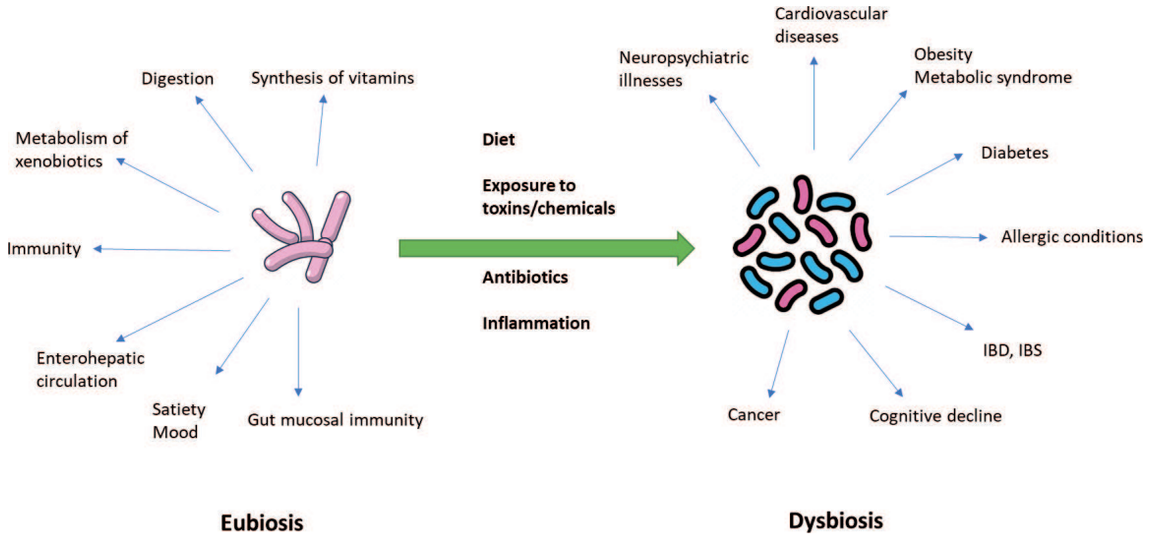

Figure 1.
Importance of gut bacterial microflora. The figure illustrates the role of enteric bacteria in the maintenance of homeostasis. The "eubiotic" bacteria display a complex interaction with the various synthetic and metabolic functions of our body as well as in the "gut-brain crosstalk". Alteration or "dysbiosis" due to any factor (diet, chemicals, antibiotics, inflammatory conditions) may lead to "blooms" of harmful bacteria. The dysbiotic bacteria have now been linked to various cardiovascular, metabolic, neuropsychiatric diseases, including cancer.

cognitive and emotional centers of the brain. This link is responsible for satiety and appetite regulation, elevation of mood, cognitive development, and neuroprotection [26]. Studies have also found a link between the gut microbiome and immune homeostasis. The complex and bidirectional interaction between the gut microbiota and the host immune system is responsible for the development of both innate and adaptive immunity, thus preventing the body from pathogenic organisms [27, 28]. Moreover, the microbiota is also responsible for the maintenance of gut mucosal integrity and prevents the overgrowth of pathogenic organisms, thus maintaining the first line of defense against the pathogens [29]. Therefore, any imbalance in the gut microbiota may lead to the development of various autoimmune diseases. This concept of "dysbiosis" or "imbalance" in the gut microbiota may result in relative "blooms" of harmful bacteria, especially Enterobacteriaceae [30, 31]. Dysbiosis can be caused by a variety of factors, namely, dietary changes, inflammatory conditions, exposure to drugs, and toxins [32, 33] (**Figure 1**). The gut bacteria have been linked to a wide variety of cardiovascular diseases [34, 35], obesity [36, 37], inflammatory bowel disease [38], irritable bowel syndrome [39], and some neuropsychiatric diseases like depression [40]. But what has intrigued the researchers is the role of gut microbiota in the development of cancer.

3. Mechanism of carcinogenesis

To ascertain the role of gut microbiota in the development of cancer, we need to look at the mechanisms responsible for carcinogenesis. The normal cells get altered into cancerous cells, by changes at the cellular, genetic or epigenetic levels. This process is known as "cell transformation" [41]. TP53 is a tumor suppressor gene that encodes the protein P53. P53 acts as a tumor suppressor which causes a transient cell cycle arrest, allowing the cells to repair the damage caused to the DNA before the cell divides. The cells that are unable to repair the damage undergo apoptosis. This ensures that the

potentially oncogenic mutations are not propagated [42]. Chronic inflammation causes alteration in the TP53 gene, leading to its inactivation. This results in an unregulated cell cycle and cell division, leading to the accumulation of mutations and uncontrolled cellular proliferation. TP53 alterations were seen in biliary epithelia of patients with gallstone disease with an increased frequency with the disease progression from metaplasia to carcinoma [43]. TP53 was the most commonly mutated gene, followed by PIK3CA, SMAD4, ARID1A, KRAS [44–46] and amplification of ERBB2 [47].

The study of these genetic alterations and mechanisms of carcinogenesis has been made possible with the development of various ex vivo and in vivo animal models. These models have been used extensively to decipher the etiopathogenesis of GBC and to develop and test the treatment protocols [48]. Ex vivo models use cell lines to study the tumor characteristics and cellular and genetic abnormalities. But it was seen that different cell lines yielded different tumor characteristics for the same type of tumor, thus complicating the interpretation [49]. In vivo models were superior to the cell lines as they used genetically engineered animals that could retain the genetic mutations and could undergo cellular differentiation. The tumor cells with mutated or amplified genes were inoculated in them and studied for the development of cancer [49–51]. The drawback of these models was the lack of innate immunity which led to altered results as the cancer was not strictly recapitulated [51].

Although these models indicate a causal relationship between the risk factors and carcinogenesis, the human body reacts quite differently as compared to an animal model, thus necessitating the need for the development of an ideal human model.

4. Role of gut microbiota in cancer causation

There has been an ongoing debate among researchers on the role of gut microbiota in the causation of cancer as cancer is neither a contagious nor an infectious disease [16]. The first proposition of the possible role of gut microbiota in cancer causation was given by Russel in 1890, which was supported by positive results over the subsequent years [52–54]. However, in 1963, a group of scientists from NCI, USA claimed that the bacteria found in the cancer tissues were probably contaminants [16]. This subject remained controversial until Marshall, in his study, proved the association between *H. pylori* and gastric adenocarcinoma [55]. This was a breakthrough study in this aspect and since then, a number of bacteria have been linked to a variety of cancers [56–58]. However, the mechanism by which the microbiota cause cancer is still unclear. While there is no concrete evidence supporting the causation of cancer, there may be a role of the bacteria in its progression [59].

Microbiota may act as a carcinogen in two ways, either by inducing a chronic inflammatory state or direct injury by material toxins and metabolites [16, 60–62]. Release of pro-inflammatory mediators like TNF-α and IL-1 and generation of reactive oxygen species (ROS) stimulates lymphoepithelial proliferation and cell division. This leads to immune dysregulation, thereby leading to tumorigenesis [27]. It causes alteration in the cell cycle leading to immunosuppression [63]. It also results in genetic and cellular damage and genomic instability which preclude carcinogenesis [64]. The bacterial toxins are genotoxins that cause DNA damage and may lead to the development of cancer [65, 66]. Thus, chronic bacterial infections demonstrate a dual role in carcinogenesis by both stimulating and inhibiting the immune system.

5. The biliary microbiome

According to traditional thinking, the biliary tract has always been considered sterile. This is because of the anti-microbial properties possessed by bile which affects the bacterial membrane and DNA [67]. However, inflammatory conditions of the biliary tract, like acute cholecystitis and cholangitis have frequently cultured bacterial colonies commonly found in the human gut; the common organisms being, *Escherichia coli*, Enterobacter, Pseudomonas, and Citrobacter spp. [68]. This can be explained by the pathophysiology of these diseases, which is, biliary obstruction and gut bacterial translocation. However, recent studies have indicated that even under nonpathogenic circumstances, the human bile comprises a rich diversity of microbial flora which is actively involved in the regulation of the size and composition of the bile acid pool as well as the metabolism of bile acids [69, 70]. However, this normal biliary microbiome mainly included Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes which were also found in the human gut [70, 71]. This can partly be explained by the close association of the human gut and the biliary tract and the involvement of enteric bacteria in enterohepatic circulation but the evidence is largely limited to animal models and an ideal human experimental model is required [72].

Dysbiosis of the gut bacteria has been implicated in the development and progression of various cancers, including gastric [73, 74], colorectal [75], and oral cancers [76, 77], however, their association with causation or progression of the hepatobiliary cancers is still in question. The natural synergy that exists between the bile acid metabolism and the biliary microbiome reaffirms the proposition that biliary microbial dysbiosis may lead to various biliary tract diseases including gallstone formation and the development of cancer.

6. Mechanism of carcinogenesis in the biliary tract

Cholangiocytes are considered the potential cells of origin for biliary tract cancers, including gallbladder cancer [78]. Any insult to the cholangiocytes leads to the release of pro-inflammatory mediators like IL-6 and IL-1β which results in the differentiation of T helper cells (Th-17 cells). The cholangiocytes interact with Th-17 cells leading to their activation and proliferation, in order to compensate for the cell loss [79]. Moreover, the bacteria and their products are recognized by the cholangiocytes through the Pathogen Associated Molecular Patterns (PAMPs) present in the bile, which interacts with the pattern recognition receptors, that are, the Toll-like receptors (TLRs) and the NOD-like receptors (NLRs), leading to their activation [80]. This results in collagen deposition and fibrosis. The resultant cholangiopathy may lead to ductopenia, dysplasia, and malignant transformation [81]. Chronic inflammation leads to the release of mediators like IL-17, TNF-α, and TGF-β which cause genetic alterations in the tumor suppressor genes and the proto-oncogenes resulting in cell transformation [82]. These mediators are among the few which have been implicated in the causation of carcinogenesis [83–85].

7. Enteric bacteria and gallbladder cancer

Gallbladder cancer is the most common biliary tract cancer and the etiopathogenesis is multifactorial [86]. However, chronic inflammation [87] and gallstone disease represent the most important aetiologies in the development of GBC and are supported by Level II

evidence [88]. The recent development of culture and culture-independent techniques have identified various organisms which are associated with the formation of both pigmented as well as cholesterol gallstones [89, 90]. These dysbiotic organisms are mainly enteric bacteria that have the ability to form a biofilm, thereby resisting cellular and DNA damage caused by bile. They are namely, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus spp*, *Acinetobacter spp* which were associated with the patients presenting with gallstones [91–93]. *Clostridium*, *Bifidobacterium*, *Peptostreptococcus*, *Bacteroides* were among the other bacteria leading to the formation of gallstones by interfering with the enterohepatic circulation [94, 95]. With the development of Polymerase chain reaction—denaturing gradient gel electrophoresis (PCR-DGGE), various uncultivable bacteria like *Staphylococcus hemolyticus*, *Enterobacter or Citrobacter spp*, *Morganella spp*, *Salmonella spp.*, *Capnocytophaga spp*, *Lactococcus species*, *Bacillus spp*, and *H. Pylori* have been isolated in different compositions [72, 96]. Some pathogens of the oral cavity have also been implicated in the formation of gallstones by affecting the motility of the gallbladder and the production of mucin [97]. These bacteria can be indirectly linked with GBC. Recent studies have demonstrated positive cultures of enteric bacteria in GBC patients projecting a direct association of the gut flora with GBC, however, the level of evidence is low [91].

Although various bacteria have now been identified and linked with the development of gallstones and their theoretical association with GBC, *Helicobacter species* and *Salmonella typhi* have been extensively studied and are strongly implicated in the development of GBC [98, 99].

8. The Helicobacter species and gallbladder cancer

The *Helicobacter species*, especially *H. pylori*, have been largely implicated in the causation of gastric as well as intestinal cancers by the mechanism of inflammation-induced tumorigenesis and are now being associated with the development of various hepatobiliary cancers [100].

H. pylori induces a chronic inflammatory state by resulting in the release of various pro-inflammatory mediators like TNF-α, IL-1, IL-6, and other vasoactive substances [101]. They also prevent cell adhesion and lead to the migration of the mutated epithelial cells [102]. Free radicals produced cause oxidative damage to the biliary epithelium [103]. IL-8 production may also promote inflammation and alter cellular proliferation and apoptosis [87]. The Cag- A protein secreted by *H. pylori* is one of the most extensively studied virulence factors responsible for producing a chronic inflammatory state as seen in gastric epithelial cells and increasing the risk of gallstones [90]. Some strains possess pathogenicity islands which produce a type IV secretion system and also result in a "hummingbird" epithelial phenotype of epithelial cells which are implicated in rival cell death, thus resulting in the death of the normal biliary microbiota and producing a Helicobacter "bloom" [104, 105]. This "bloom" of Helicobacter results in chronic inflammation of the biliary epithelium by the various mechanisms mentioned, leading to dysplasia and subsequent neoplasia.

Since the first evidence of *H. pylori* in gallbladder mucosa in a patient with cholecystitis was detected by Kawaguchi et al. [106] in 1996, ample studies have shown an association of Helicobacter species, especially *H. pylori*, with the formation of gallstones [107–109] which have also been reiterated by recent meta-analyses [110]. Another study by Kuroki et al. [111] reported a higher biliary epithelial proliferation rate in patients infected with *Helicobacter species* as compared to the control group.

The isolation of bacteria in these studies was done using various next-generation sequencing techniques. These techniques are being utilized to establish an association between the *Helicobacter species* and GBC. While some have shown promising results [112, 113], others have negated this theory [114, 115]. Apart from *H. pylori*, attempt at isolating other enterohepatic strains like *H. bilis*, *H. hepaticus,* and *H. pullorum* have been done in a number of studies [116]. Dewhirst and Fox [117] identified 5 strains of *H. bilis*, 2 strains of *Flexisipira rappini*, and one strain of *H. pullorum* using PCR analysis in patients with gallbladder diseases and GBC. Various studies have demonstrated high positivity of *H. bilis* in patients with biliary tract and gallbladder cancer, suggesting an association of *H. bilis* with GBC [118–120].

There have been several meta-analyses suggesting an association between Helicobacter infection and cancer of the biliary tract with conflicting results [121, 122]. However, a recent meta-analysis has suggested a significant association between *H. pylori* infection and biliary tract cancer [98]. The available research has suggested a strong association between *Helicobacter species* and GBC; however, these studies are largely limited to observational studies or small meta-analyses necessitating the need for a higher level of evidence in order to establish a general consensus.

9. *Salmonella typhi* and gallbladder cancer

Salmonella enterica serovar typhi is a gram-negative, flagellated, rod-shaped bacteria which is the causative agent of typhoid fever. It resides in the gallbladder and results in chronic inflammation of the gall bladder mucosa leading to the formation of gallstones [123]. It also produces a biofilm that prevents it from the anti-bacterial action of the bile and thus results in its persistence in the gallbladder leading to a chronic carrier state.

Salmonella typhi has been strongly associated with the development of gallstone disease and chronic infection with *S. typhi* is now being linked to GBC. The earliest evidence dates back to 1964 when Cargill et al. suggested a probable association between chronic typhoid and paratyphoid carriers and GBC [76]. In 1971, Axelrod et al., also reported a similar association between *S. typhi* and GBC. Since then, several studies have reiterated the results [124–126]. There are certain proposed mechanisms by which Salmonella may result in a chronic inflammatory state and subsequent development of cancer. The typhoid toxin is carcinogenic and causes alterations in the cell cycle and DNA damage [127]. AvrA, is an effector protein synthesized by Salmonella pathogenicity island 1 via Type III secretion system which subdues the host inflammatory response and prevents autophagy, thus resulting in the persistence of the bacteria and the chronic carrier state [128, 129].

Typhoid fever and GBC are endemic in the Gangetic belt and the northern states of India which provides strong evidence to suggest an association between *S. typhi* and GBC [7, 130, 131]. A study conducted in Northern India demonstrated that patients with gallbladder cancer had a higher Vi polysaccharide as compared to the control group and the risk of developing GBC in typhoid carrier patients was 8.47 times higher than the non-carrier group, thus concluding the chronic typhoid carrier state as a risk factor for GBC [130]. This has been reinforced by a number of recent studies [127, 132]. Although there is emerging evidence suggesting a positive association between *S. typhi* and GBC but the data is limited, with conflicting results, thus requiring larger epidemiological studies to establish a consensus [99, 133].

10. Miscellaneous bacteria and gallbladder cancer

Gene fragments of *Collibacillus*, *B. fragilis*, *Klebsiella*, *C. perfringens* and *Clostridium* spp. have been identified in the bile and gallbladder tissue of patients with GBC [134]. A positive correlation between the bacterial species of *E. coli*, *E. fecalis*, *Klebsiella*, and *Enterobacter spp. B10* along with *Peptostreptococcus stomatis*, *Fusobacterium*, *Firmicutes nucleatum*, and *Enterococcus faecium* with the development

Study/year	Sample	Bacterial strain	Isolation technique	Inference
Welton et al. [125]	Deceased typhoid carriers	S. typhi	Record registers	Chronic typhoid carriers are 6 times more likely to die of hepatobiliary cancer than controls ($P < 0.001$)
Caygill et al. [124]	Chronic typhoid carriers	S. typhi	Record registers	167-fold higher risk of GBC in chronic typhoid carriers Chronic, and not acute infection is a risk factor for GBC
Csendes et al. [137]	Tissue, bile	E. coli, E. faecalis, Klebsiella Enterobacter	Culture	Both aerobic and anaerobic gram-negative bacteria were found and may have a role in GBC
Shukla et al. [130]	Serum	S. typhi	IHA Vi antigen	Significantly high Vi positivity in patients with gallbladder carcinoma compared to controls Risk of developing GBC is 8.47 times more in culture-positive typhoid carriers than the noncarriers
Dutta et al. [131]	Serum	S. typhi	ELISA Vi antigen	Chronic typhoid carrier state is a risk factor for GBC
Dewhirst et al. [117]	Multiple sources: animal and human tissue, blood, stool, fetus	H. bilis, Flexisipira rappini, H. pullorum	PCR (16S rRNA)	Correlation of Helicobacter species with GBC and other biliary tract diseases Identified 5 strains of H. bilis, 2 strains of Flexisipira rappini, and one strain of H. pullorum
Matsukura et al. [118]	Bile	H. bilis	PCR (16S rRNA)	H. bilis infection in bile was associated with gallbladder cancer in Japanese and Thai patients
Fukuda et al. [112]	Bile, tissue	Helicobacter	PCR, Histology, IHC	Significantly high positivity of Helicobacter DNAs in 52.6% of patients with hepatobiliary cancer than that in the benign cases ($P = 0.03$)
Lu et al. [134]	Tissue	Colibacillus B. fragilis, Klebsiella C. perfringens Clostridium	PCR 16S rRNA	Possible association of both aerobic and anaerobic bacteria with GBC
Murata et al. [119]	Tissue	H. bilis	Nested PCR (16S rRNA)	4 out of 14 cases with biliary tract cancer were positive for H. bilis which may indicate their role in GBC

Study/year	Sample	Bacterial strain	Isolation technique	Inference
Kobayashi et al. [120]	Bile	H. pylori H. hepaticus H. bilis	PCR	Helicobacter DNA was detected in bile of 86% of malignant biliary diseases DNA fragments of Helicobacter species other than H. pylori, H. hepaticus, and H. bilis were commonly detectable
Bohr et al. [115]	Tissue	Helicobacter spp.	Culture, IHC, PCR (16S rRNA)	Helicobacter species do not play a predominant role in the pathogenesis of GSD and GBC in the German population
Shimoyama et al. [113]	Blood	H. hepaticus	ELISA	H. hepaticus-specific antigen was significantly higher in patients with biliary tract cancer ($P < 0.05$)
Iyer et al. [132]	Tissue	143 HPV S. typhi Ty2 S. typhi CT18 S. typhimurium-LT2 S. choleraesuis-SCB67 S. paratyphi-TCC S. paratyphi SPB7	PCR analysis	Association of non-typhoidal Salmonella species with GBC along with typhoidal strains Chronic carrier state is a risk factor for GBC
Tsuchiya et al. [114]	Blood	H. pylori	ELISA	No significant differences in antibody titers or H. pylori infection positivity rates between cases and controls
Song et al. [135]	Tissue	Peptostreptococcus stomatis Enterococcus faecium	DNA extraction and metagenomic sequencing	Existence of an altered microbiota in GBC

Table 1.
Studies show the association of the gut microbiome with gallbladder cancer.

of GBC has also been found in recent studies [135–137]. These bacteria were commensals of the gut and have been associated with colorectal cancer [138], gastric cancer [139], and metastatic melanoma [140]. Thus, their presence in gallbladder tissue and bile may indicate their association with GBC. **Table 1** summarizes the various studies showing an association of gut microbiota with GBC.

11. Therapeutic perspective: "microbial therapeutics"

There is a complex interplay between the human body and its microbiome. While a normal gut flora is essential for homeostasis, dysbiosis may lead to a multitude of diseases. Several mechanisms associated with carcinogenesis are now being utilized in its prevention. GBC has been associated with chronic inflammation and chronic typhoid carrier state; thus, many animal models have been developed to study the role of antibiotics in the eradication of Salmonella, thereby reducing the chances of development of GBC. But the results have been conflicting [128] and Cholecystectomy remains the only definitive treatment for eradication of the carrier state of Salmonella [128]. There was

a rise in the number of prophylactic cholecystectomies owing to this but it also saw an increase in the number of colorectal malignancies due to gut bacterial dysbiosis, thereby emphasizing their role in the development of cancer [141].

The role of the gut microbiome in the maintenance of homeostasis encouraged the researchers to utilize their potential in the therapeutic management of the disease. Microbiome therapeutics consist of additive therapy, subtractive therapy, and modulatory therapy. Additive therapy with genetically engineered or natural probiotic agents has shown some benefit in colorectal cancer and is now being utilized in GBC. There is emerging evidence regarding the association of probiotics and dietary changes with a decreased incidence of gallstone disease, thereby reducing the chances of GBC, thus additive therapy with natural or genetically engineered probiotic organisms may prove beneficial. However, there is still a dearth of evidence in this aspect [142, 143]. Subtractive therapy is being utilized by genetically engineered *E. coli* strains with a cloned antibiofilm protease Deg P gene or a cloned Lysine and Pyosin gene which results in inhibition of growth of pathogenic bacteria in the gut, thereby preventing dysbiosis [144, 145]. *E. coli* strains with a cloned antibiotic Microcin H47 gene may also help in inhibiting and displacing Salmonella from the gut [146]. These two methods can be utilized in patients with a Chronic salmonella carrier state and may be used as an alternative to Cholecystectomy. This may also reduce the incidence of GBC in these patients. Apart from this, genetically engineered bacteria are also being used to test the effect and toxicity profile of chemotherapy [147, 148], develop cancer vaccines and targeted biological therapies [149].

12. Conclusion

The gut microbiome forms an integral part of the human body and is often referred to as the "forgotten organ". Its role in health and disease has been studied extensively over the past two decades but the possibility of its role in cancer causation has caught the eye of researchers. The association between the gut microbiome and cancer has provided new insight into understanding the pathophysiology of cancer and planning the management strategies. There is a strong correlation between gut microbial dysbiosis and the development of colorectal and gastric adenocarcinomas, however, their role in hepatobiliary cancers, especially GBC remains poorly understood. This can be owed to the short survival of GBC resulting in vast unexplored domains of this disease and the difficulty to isolate the bacteria involved via routine culture methods. Moreover, the lack of an ideal animal or a human model has greatly limited the research. The advent of the next-generation sequencing methods has seen emerging evidence linking various bacteria to the etiopathogenesis of GBC, but causality is far from proven. A higher level of evidence either in the form of larger meta-analyses or larger epidemiological studies is needed to establish a consensus.

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