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Characterisation of Gastric Microbiota involved in Gastric Carcinogenesis Current Findings and Challenges.

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CONTENTS

1. ABSTRACT

Aim- to systematically review the current data regarding the characterization of gastric microbiota involved in gastric carcinogenesis.

Methodology- Research databases such as PubMed, NCBI, SCIENCEDIRECT and google scholar were utilised. The different search terms used to make the primary search include: “Gastric carcinogenesis”, “Healthy gastric microbiota”, “microbiota metabolism” and “future challenges in gastric microbiota research”. The articles were initially reviewed based on the year of publication (2010-2023), the abstract’s content and the title. After extensive reviewing, several articles were considered appropriate. Finally, the remaining articles were assessed based on their relevance to the topic through thorough analysis. This yielded 74 articles of which 6 were identified for analysis of normal and pathological gastric microbiota. 39 articles were used to record analysis of metabolites, and its association between microbiota and gastric cancer.

Results- from the 6 selected articles 4 were used to analyse normal and abnormal microbiota respectively including their association during different stages of Correa’s cascade. While 10 studies were used to draw conclusions of the potential effects of metabolites on gastric carcinogenesis.

Conclusion- From the following literature review it is clear that the current understanding of the effects on gastric cancer because of variability in microbiota and vice versa remains unclear, there are various factors at play that affect these studies, present-day understanding of direct and indirect mechanisms by microbes that lead to different metabolic outcomes in gastric cancer remains limited. Hence large-scale meta-analyses considering these challenges should be considered.

1. SANTRAUKA

Tikslas – sistemingai peržiūrėti dabartinius duomenis, susijusius su skrandžio mikrobiotos, dalyvaujančios skrandžio kancerogeneze, apibūdinimu.

Metodika – buvo naudojamos tyrimų duomenų bazės, tokios kaip PubMed, NCBI, SCIENCEDIRECT ir google scholar. Įvairūs paieškos terminai, naudojami atliekant pirminę paiešką, yra: „Skrandžio kancerogenezė“, „Sveika skrandžio mikrobiota“, „mikrobiotos metabolizmas“ ir „būsimi iššūkiai atliekant skrandžio mikrobiotos tyrimus“. Iš pradžių straipsniai buvo peržiūrėti pagal išleidimo metus (2010-2023), santraukos turinį ir pavadinimą. Po išsamios peržiūros buvo nuspręsta, kad keli straipsniai yra tinkami. Galiausiai, likę straipsniai buvo įvertinti pagal jų aktualumą temai, atliekant išsamią analizę. Gauti 74 straipsniai, iš kurių 6 buvo nustatyti normalios ir patologinės skrandžio mikrobiotos analizei. 39 straipsniai buvo panaudoti norint įrašyti metabolitų analizę, jos ryšį tarp mikrobiotos ir skrandžio vėžio.

Rezultatai – iš 6 atrinktų straipsnių 4 buvo panaudoti normaliai ir nenormaliai mikrobiotai atitinkamai analizuoti, įskaitant jos ryšį įvairiais Correa kaskados etapais. Tuo tarpu 10 tyrimų buvo panaudoti norint padaryti išvadas apie galimą metabolitų poveikį skrandžio kancerogenezei.
Išvada. Iš šios literatūros apžvalgos aišku, kad dabartinis supratimas apie poveikį skrandžio vėžiui dėl mikrobiotos kintamumo ir atvirkščiai lieka neaiškus, yra įvairių veiksnių, turinčių įtakos šiems tyrimams, šiandieniniam tiesioginių ir netiesioginių mechanizmų supratimui. mikrobų, dėl kurių skrandžio vėžys sukelia skirtingus metabolinius rezultatus, išlieka ribotas. Taigi, atsižvelgiant į šiuos iššūkius, reikėtų apsvarstyti didelio masto metaanalizes.

2. ACKNOWLEDGEMENT

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3. CONFLICT OF INTEREST

The Author reports no conflict of interest.

4. ETHICS COMMITTEE CLEARANCE

Self-assessed ethical clearance was submitted and approved for the following review.

5. INTRODUCTION

Gastric cancer (GC) is a multifactorial disease, where many factors can influence its development, both environmental and genetic factors [1]. Present-day statistics indicate gastric cancer as the fourth leading cause of cancer deaths worldwide, where the rate of average survival is less than 12 months for the advanced stage [2]. Each year around 990,000 people are diagnosed with Gastric cancer worldwide, out of which approximately 738,000 die. Incident rates for gastric cancer are highly variable based on sex and geography whereas men are two to three times more likely to be affected by Gastric cancer [3].

Gram-negative *Helicobacter pylori* have been described as a class I carcinogen of Gastric cancer development by the World Health organisation[4]. Another well-known infectious factor, Epstein-Barr virus is present in about 10 percent of gastric cancers. However, there is lack of evidence proving a distinct ecological link between the role of the Epstein-Barr virus and Gastric cancer development [5]. Hence the possibility of a relationship between gastric microbiota and its role in the development of gastric cancer and vice versa is worth investigating.

Complex microbial communities are part of distinct habitats that exist within the human body; and its metabolites have both physiological and pathological effects on homeostasis maintenance and disease development [6]. In recent decades, interest in the relationship between the human microbiome and disease has increased. Although there is evidence that disruption of the microbiome-host balance in the stomach may promote the development of gastric cancer, the mechanism is not clear. To gain a better understanding of the relationship between the gastric microbiome and gastric carcinogenesis, we focus specifically on the possible mechanisms of the gastric microbiome in the development of gastric cancer including challenges.

6. AIM AND OBJECTIVES

AIM- to systematically review the current data and challenges regarding the characterisation of the gastric microbiota involved in gastric carcinogenesis.

Objectives:

1) to analyse data on the gastric microbiota in healthy individuals.
2) to analyse data on the features of gastric microbiota at different stages of Correa's cascade.
3) to analyse on metabolic effects of gastric microbiota in gastric carcinogenesis.
4) to outline challenges in gastric microbiota and carcinogenesis research.

7. LITERATURE REVIEW

7.1. Microbial Diversity in the Human stomach

In the past, the stomach was thought to be a sterile organ because of its highly acidic environment. However, the discovery of H. pylori in the stomach of patients with gastritis and peptic ulcer by Marshall and Warren in 1982 disproved this assumption [7].

Research on the microbiota of the stomach lay dormant for many years, largely due to the dogma that the stomach is a sterile "organ" inhospitable to bacteria because of its acid production. In addition, the reflux of bile acids in the stomach, the thickness of the mucus layer, and the effectiveness of gastric peristalsis may have prevented bacterial colonisation of the stomach. In addition, nitrate present in saliva and food is converted to nitrite by lactobacilli present in the mouth, which is converted to nitric oxide in the stomach by the gastric juice, a potent antimicrobial agent. All of these factors, along with the technical difficulties in collecting samples for analysis and the lack of simple, reliable diagnostic tests, have complicated the challenging study of the gastric microbiota [9,10].

Recent advancements in microbiology along with invasive sampling techniques have led to massively parallel sequencing of the 16S rRNA gene which remains the gold standard for microbiota studies for more than a decade after its initial publication. A major advantage of this technology is that the presence of human DNA in the sample does not need to be considered, as 16S RNA is only present in prokaryotic cells. Consequently, samples in which human DNA is dominant, such as in biopsy material, can be analysed for bacterial content only [11]. Gastric samples from healthy people and patients with gastritis were subjected to 16S rRNA gene amplicon sequencing by Li et al. They found that Prevotella, Streptococcus, Veillonella, Rothia, and Haemophilus species dominated the population [12]. Similarly using 16S rRNA gene amplicon sequencing, Bik et al. discovered that the dominant genera were Streptococcus, Prevotella, Rothia, Fusobacterium, and Veillonella.[8,12].

The human gastric microbiota is subject-specific and includes several bacterial species, with H. pylori frequently detected. Several bacterial species belonging to Prevotella, Streptococcus, Veillonella, Neisseria, Fusobacterium, and Haemophilus are detected more frequently than H pylori in gastric specimens. With the exception of H. pylori-induced conditions, the global characteristics of the gastric microbiota cannot be determined in health or disease [8].

To obtain a comprehensive picture of the human gastric microbiota, analysed data from 36 publications (the initial search yielded 195 publications based on the abstract, reduced to 50, and finally to 36 after

detailed reading; Tables 1-3; Table S1). In total, the gastric microbiota signatures of 1479 individuals were examined. Most of these subjects were patients suffering from various gastrointestinal diseases, whereas 328 subjects were healthy volunteers (controls). In several studies, patients suffering from different diseases were treated as controls (e.g., patients with gastritis were compared with cancer patients).

In general, differences in the gastric microbiota between the compared groups were studied in terms of the abundance of certain microbial characteristics. Certain groups of microorganisms were rarely found exclusively in one diagnostic group [8].

TABLE 2 Studies assessing gastric disease (excluding gastric cancer)

Reference	Gastric sample	# Subjects	Diagnosis	Country	Age group	Relevant finding
Chen et al[54]	Biopsy	18	Gastric ulcer,[9] duodenal ulcer[9]	China	Adult	*Helicobacter, Prevotella, Neisseria* and *Streptococcus* found in gastric and duodenal mucosa, HP dominates gastric mucosa, which is linked with reduced diversity
Parsons et al[22]	Biopsy	95	Autoimmune atrophy[21]	UK	Adult	Autoimmune atrophy has similar bacterial diversity and higher bacterial abundance particularly of *Streptococcus*
Llorca et al[41]	Biopsy	51	Dyspepsia	Spain	Paediatric	HP positive children have lower diversity. HP negative dominated by *Pseudomonas, Escherichia, Clostridium, Staphylococcus* and *Rothia*
Paroni Sterbini et al[44]	Biopsy	24	Dyspepsia	Italy	Adult	HP presence did not influence diversity of gastric microbiota, while abundance of several genera was affected. *Streptococcus* was most strongly promoted with PPI use.
Delgado et al[12]	Biopsy	4	Dyspepsia	Spain	Adult	*Propionibacterium, Lactobacillus, Streptococcus* and *Staphylococcus* are the predominant bacterial groups based on sequencing and cultivation.
Das et al[107]	Biopsy	39	HP infection suspected	India	Adult	HP abundance negatively affecting diversity. *Lactobacillus, Halomonas* and *Prevotella* abundant. Geographical impact on microbiota.
Nazrollazhadeh et al[55]	biopsy	91	Healthy,[19] gastritis,[18] oesophagitis,[17] oesophagus dysplasia and oesophagus carcinoma[37]	Iran	Adult	Presence and increased abundance of Clostridiales and Erysipelotrichales in gastric samples was associated with early oesophageal cancer and oesophageal dysplasia
Zhao et al[108]	Biopsy	80	Gastritis	China	Adult	Cytotoxin-associated gene A-positive. HP in the stomach of gastritis patients induces modest changes in the tongue coating microbiota.
Yang et al[109]	Biopsy	16	AIDS	USA	Adult	Immunosuppressive therapy in AIDS patients promoted environmental bacteria (*Burkholderia* and *Bradyrhizobium*) in the upper GI tract. These bacteria are mutual exclusive with *Lactobacillus* that was depleted in AIDS *Prevotella, Ralstonia* and *Fusobacterium* were enriched in patients
Gall et al[50]	Biopsy and brush	12	GERD,[2] metaplasia,[5] dysplasia [5]	USA	Adult	Within individuals, bacterial communities of the stomach and oesophagus show overlapping community. In BE cohort, predominance of *Streptococcus* and *Prevotella* species.
Schulz et al[9]	Biopsy and fluid	24	Gastritis	Germany	Adult	Gastric, oral and duodenal microbiota are similar and individual specific. HP presence in the stomach influences oral and duodenal microbiota too. Streptococcus dominant in HP negative subjects
Ye et al[10]	Fluid	6	Choledocholithiasis	China	Adult	Gastric microbiota has higher degree of similarity to bile microbiota than saliva but lower than duodenal microbiota. Genera prevalent in all sites include *Streptococcus, Veillonella, Prevotella* and *Rothia*.
Lamarche et al[110]	Fluid	34	ICU critically ill	Canada	Adult	In contrast to endotracheal aspirate, gastric aspirate microbiota was not linked to hospital mortality in ICU patients
Vonaesch et al[8]	Fluid	57	Children with stunted growth	47 CAR; 10 Madagascar	Paediatric	Gastric and duodenal microbiota are highly similar and dominated by *Haemophilus, Neisseria, Prevotella, Alloprevotella, Streptococcus, Veillonella, Actinobacillus, Moraxella, Leptotrichia* and *Fusobacterium*

TABLE 2 (Continued)

Reference	Gastric sample	# Subjects	Diagnosis	Country	Age group	Relevant finding
Amir et al[52]	Fluid	34	15 GERD, 13 oesophagitis, 6 BE	Israel	Adult	Significantly increased Enterobacteriaceae and Methylobacteriaceae in gastric fluid of oesophagitis and BE patients, significantly reduced Pasteurellaceae and Porphymonodaceae
Von Rosenvigne et al[14]	Fluid	25	various pathologies (including AIDS)	USA	Adult	Immunosuppression correlates with decreased abundance of *Prevotella*, *Fusobacterium* and *Leptotrichia* (6%) and increased abundance of *Lactobacillus*. *Campylobacter* significantly more active than abundant.
Rosen et al[111]	Fluid	116	chronic cough	USA	Paediatric	*Streptococcus* stimulated by PPI. Lung bacteria (*Pseudomonas*, *Proteus*, *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus Bacteroides* and *Acinetobacter*) found in gastric fluid and not the oropharyngeal fluid, suggesting exchange of species during chronic cough.

Abbreviations: BE, Barrett's oesophagus; CAR, Central African Republic; GERD, Gastro-oesophageal reflux disease; GI, Gastrointestinal; HP, *Helicobacter pylori*; ICU, Intensive care unit; PPI, proton pump inhibitor.

Approximately two-thirds of the papers described detected Prevotella, Streptococcus, Veillonella, Neisseria, Fusobacterium, and Haemophilus species and stated that the microbiota is subject-specific and varies between individuals. More than 65% of the bacterial groups found in the stomach have also been identified in the human oral cavity, and many of the bacteria found in the stomach may have originated in the oral cavity or as intestine reflux [8,9,12].

The composition of gastric microbiota at the genus level is dynamic and is influenced by factors such as dietary habits, medication use, gastric mucosa inflammation, and, of course, H. pylori colonisation. While many studies document the effect of diet on gut microbiota composition in humans, there are only a few with evidence addressing the effects of diet on gastric microbiota, which is mostly limited to animal model studies. An in vivo study found higher levels of total aerobes, total anaerobes, and Lactobacilli in the stomachs of mice fed a non-purified diet (food derived from natural sources) compared to mice fed a purified diet (refined food). This rise is associated with lower levels of Toll-like receptor 2 (TLR-2) mRNA in the stomach [13].

The long-term use of proton pump inhibitors (PPIs) and H2-antagonists, as well as atrophic gastritis, affects the composition of the gastric microbiota; this is not surprising, considering that gastric microbiota depend on gastric acid secretion. Bacterial overgrowth occurs when the gastric pH was >3.8.23 Oro-pharyngeal-like bacteria and faecal-like bacteria are significantly more abundant in patients on PPI therapy than in patients on H2-antagonists and untreated control subjects.24 Treatment for 2 weeks with

PPI reduces gastric acid secretion by 75% and this was sufficient to permit bacterial colonisation of the stomach in healthy volunteers. Omeprazole (40 mg/day) for 3 months induced gastric bacterial overgrowth in 10 of 30 patients, compared with 1 of 10 control subjects; however, after only 14 days of PPI treatment (omeprazole 30 mg/day), the total number of gastric bacteria had increased to a significant level [8,13]

Helicobacter pylori, a spiral-shaped member of the Gram-negative Epsilonproteobacteria, is the most common component of the gastric microbiota in more than half of all humans. Infection with H. pylori has a significant impact on gastric physiology and, as a result, the properties of the gastric mucosa as an ecological niche for other bacteria[9]. Infection is usually life-long unless treated, in the elderly *H.pylori* load diminishes with increasing mucosal atrophy [9] .

Gastric cancer is frequently the result of a series of events that occur in patients colonised with *H. pylori*. A variety of host and environmental factors influence disease development, with gastric cancer typically developing after *H. pylori-induced* gastric mucosal atrophy with achlorhydria or hypochlorhydria. Changes in the intragastric environment affect the composition and function of the normal gastric microbiota. The relative contribution of bacteria vs. other components in the development of gastric cancer is unknown. Although the importance of *H. pylori* is widely acknowledged, there is no agreement on whether or not specific non-*H. Pylori* bacteria may play important roles in the pathogenesis of gastric cancer [11].

7.2.Different stages of Correa's cascade and gastric microbiota.

7.2.1 Correa's cascade.

Correa's cascade process is characterised by dynamic pathological changes of normal gastric mucosa. Lauren's classification (1965) divides GC into three types: enteric, diffuse, and mixed. In Correa's cascade intestinal-type Gastric cancer, chronic atrophic gastritis, and intestinal metaplasia begin from the antrum-corpus junction and slowly spread to other areas of the gastric mucosa[14].

Genetic instability is a key feature of tumour cells, accelerating tumorigenesis and causing irreversible lesions. DNA methylation, an epigenetic modification, is an important molecular marker for the degree of gastric mucosal atrophy and the risk of Gastric cancer, and it plays a critical role in Correa's cascade. Patients with Intestinal metaplasia have higher levels of DNA methylation than those with chronic atrophic gastritis during this process. A key therapeutic point in Correa's cascade, involving irreversible pathological and genetic changes, may exist and may be a critical step in the development of Gastric cancer. [14,15].

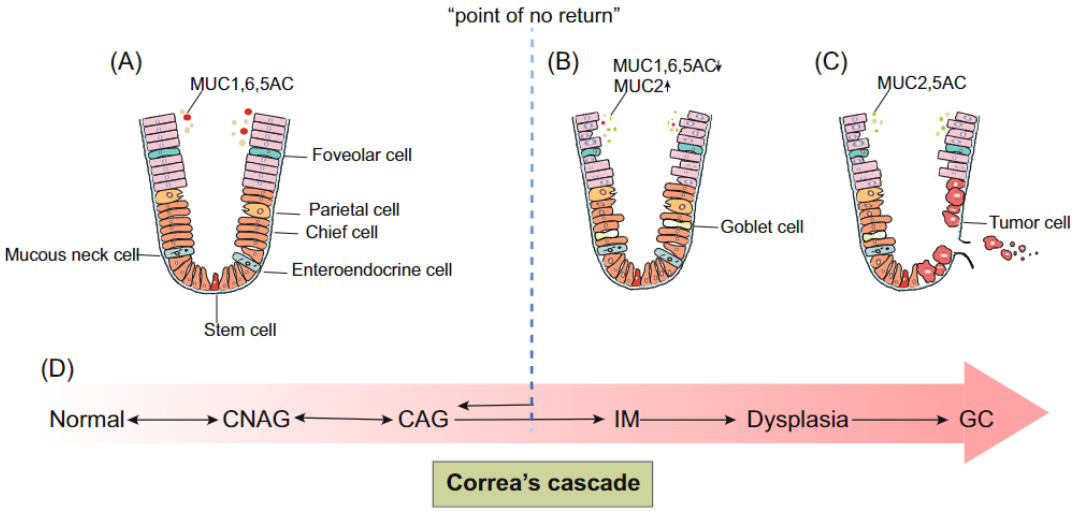

FIGURE 1 Gastric pathological features at different stages of Correa's cascade. (A) Normal gastric mucosa has intact glands, neatly arranged host cells and a normal nucleocytoplasmic ratio; mucin secretion is dominated by MUC1, MUC5AC and MUC6. (B) The gastric mucosa of IM patients has atrophied glands, disorganised host cells, increased nuclear-cytoplasmic ratio, different size of nuclei and goblet cells, reduced secretion of mucins MUC1, MUC5AC and MUC6, and increased secretion of MUC2. (C) The glands in the gastric mucosa of GC patients are destroyed, and tumour cells replace normal cells to form glands and break through the base. Mucin secretion is dominated by MUC2 and MUC5AC. (D) Correa's cascade is a process of gastric carcinogenesis, and there may be a "point of no return" or key therapeutic point between the CAG and IM disease stages with characteristic pathological changes. CAG, chronic atrophic gastritis; CNAG, chronic nonatrophic gastritis; GC, gastric cancer; IM, intestinal metaplasia [Color figure can be viewed at wileyonlinelibrary.com]

The various stages of Correa's cascade displayed distinct pathological characteristics (Figure1). In patients with gastritis, monocytes and polymorphonuclear neutrophils accumulate primarily in the lamina propria of the gastric mucosa, as opposed to the normal gastric mucosa. Chronic atrophic gastritis patients may also have gastric mucosal intrinsic gland atrophy and lamina propria fibrosis [14,16]. As the severity of the chronic atrophic gastritis progresses mucosal epithelial cells are replaced by goblet cells and intestinal cells hence over time acquire intestinal pattern as a whole [16]. Gastric Mucin expression gradually decreases whereas non-gastric mucin expression increases as Correa’s cascade progresses towards gastric cancer [14,15,16]. It is established that Intestinal type Gastric cancer is caused by *H.pylori* infection, glandular pattern tumour cells break through the base [14]. Hence distinct microbiota at various stages of Correa’s cascade is a point of further discussion.

7.2.2 Correa's cascade and respective gastric microbiota

TABLE 1 Gastric microbiota characteristics at different stages of Correa's cascade

References	Sample type	Sample grouping (size)	Sequencing methods	Country	Gastric microbiota composition at different Correa's cascade stage	Main conclusions
Eun, 2014[9]	Gastric biopsies	A: CAG (10) B: IM (10) C: Noncardia GC (11)	16S rRNA gene Sequencing using 454 GS-FLX Titanium	South Korea	From A to C: Increased bacterial richness and diversity gradually, the enrichment of *Streptococcaceae* and the depletion of *Helicobacteraceae*	There were significant differences in gastric microbiota composition between patients with GC, IM and chronic gastritis
Aviles-Jimenez, 2014[74]	Gastric biopsies	A: NAG (5) B: IM (5) C: GC (5)	16S rRNA gene Sequencing	Mexico	From A to C: Decreased bacterial diversity, especially between A and C. The depletion of *Porphyromonas*, *Neisseria*, TM7 group and *S. sinensis*, as well as the enrichment of *L.coleohominis* and *Lachnospiraceae*	Gastric microbial diversity changes from patients with NAG to patients with IM and to patients with GC. This research revealed a significant separation of GC from NAG, whereas IM did not separate from either NAG or GC
Wang, 2016[75]	Gastric biopsies	A: CAG (6) B: GC (6)	16S rRNA gene Sequencing using 454 GS-FLX Titanium	China	B vs A: The enrichment of *Lactobacillus*, *Escherichia–Shigella*, *Nitrospirae*, *Burkholderia fungorum*, *Lachnospiraceae*	The gastric microbiota of GC changes, the number and diversity of microorganisms increase, and the number of microorganisms with potential cancer-promoting activities increases
Castaño-Rodríguez, 2017[14]	Gastric biopsies (antral)	A: Functional dyspepsia (20) B: GC (12)	16S rRNA gene Sequencing using Illumina MiSeq	Singapore	B vs A: Increased bacterial richness, phylogenetic diversity and co-occurrence interactions but not Shannon's diversity and evenness	Three mechanisms by which GC occurs: enrichment of proinflammatory oral microbiota, increased abundance of lactic acid producing bacteria and increased production of short-chain fatty acids
Ferreira, 2018[18]	Gastric biopsies	A: CAG (81) B: GC (54)	16S rRNA gene sequencing using Ion PGM Torrent	Portugal	From A to B: Decreased bacterial diversity and relative abundance of *Helicobacter pylori*, the enrichment of non-*H. pylori* or conditional pathogens	A genotoxic nitrosating microbial community has been identified in GC patients
Coker, 2018[15]	Gastric biopsies	A: SG (21) B: AG (23) C: IM (17) D: GC (20)	16S rRNA gene sequencing using Illumina MiSeq	China	D: The enrichment of *Peptostreptococcus stomatitis*, *Streptococcus anginosus*, *Parvimonas Micra*, *Slackia exigua* and *Dialister pneumosintes* C, D vs A, B: Decreased bacterial abundance	Dysbiosis exists during gastric carcinogenesis, and the composition of oral microbiota can be used to distinguish GC from AG
Hu, 2018[76]	Gastric wash samples	A: SG (5) B: GC (6)	Shotgun Metagenomic sequencing using Illumina HiSeq X10	China	B vs A: The enrichment of *Neisseria*, *Alloprevotella*, *Aggregatibacter* and the depletion of *Sphingobium yanoikuyae*	Gastric microbiota composition and function can be used to predict the prognosis and diagnosis of GC and to distinguish SG from GC

Table 1. [14]

Abbreviations: AG, atrophic gastritis; EGN, early gastric neoplasia; GIN, gastric intraepithelial neoplasia; IN, intraepithelial neoplasia; NAG, non-atrophic gastritis; SG, superficial gastritis.

TABLE 1 (Continued)

References	Sample type	Sample grouping (size)	Sequencing methods	Country	Gastric microbiota composition at different Correa's cascade stage	Main conclusions
Hsieh, 2018[77]	Gastric biopsies	A: Gastritis B: IM C: GC	16S rRNA gene sequencing using Illumina MiSeq	Taiwan	A, B: Similar gastric microbiota composition between these two groups C vs A, B: The enrichment of *Clostridium*, *Fusobacterium*, *Lactobacillus* and the depletion of *H. pylori*	Three enriched candidate bacteria could serve as specific bacteria for predicting GC
Liu, 2019[78]	Gastric biopsies	A: Normal (230) B: Peritumour (247) C: Tumour (229)	16S rRNA gene sequencing using Illumina MiSeq	China	B, C vs A: Decreased bacterial richness, simplified correlation network of abundant gastric microbiota	The composition and function of the gastric microbiota are mainly affected by the gastric microenvironment and have nothing to do with the staging and typing of GC
Gunathilake, 2019[79]	Gastric biopsies	A: Control (288) B: GC (268)	16S rRNA gene sequencing using Illumina MiSeq	South Korea	B vs A: Increased relative abundance of *H. pylori*, *Propionibacterium acnes*, *Prevotella copri*, Low relative abundance of *Lactococcus lactis*	A combination of four candidate bacteria can be used as a predictive marker in Korean GC patients
Park, 2019[80]	Gastric biopsies	A: *Helicobacter pylori*- CNAG (48) B: *H. pylori*- IM (9) C: *H. pylori*- GC (23) D: *H. pylori*+ CNAG (14) E: *H. pylori*+ IM (12) F: *H. pylori*+ GC (32)	16S rRNA gene sequencing using Illumina MiSeq	South Korea	From D to E: Increased relative abundance of *Rhizobiales* A vs B, C: Increased relative abundance of Firmicutes and Cyanobacteria	The gene encoding T4SS protein in IM patients may be transferred from gastric bacteria such as *Rhizobium* and *Neisseria* to *H. pylori* to promote the occurrence of GC
Wang, 2020[17]	Gastric biopsies	A: Normal (30) B: CNAG (21) C: IM (27) D: IN (25) E: GC (29)	16S rRNA gene Sequencing using Illumina MiSeq	China	From A to E: Decreased bacterial richness and diversity gradually, especially during the IN stage	IN is considered a critical stage between atrophic/IM lesions and cancer, and alterations in the composition of the gastric microbiota may precede IN
Ndegwa N, 2020[81]	Cytology brush samples	A: Normal (171) B: CNAG (33) C: CAG (12)	16S rRNA gene sequencing using Illumina MiSeq	Sweden	B, C vs A: Decreased bacterial diversity	From the normal to the precancerous stage, the number of pathogenic microorganisms increased continuously, and there were significant differences in gastric microbiota composition
Gantuya, 2020[82]	Gastric biopsies	A: Normal (20) B: gastritis (20) C: atrophy (40) D: IM (40) E: GC (48)	16S rRNA gene sequencing using Illumina MiSeq	Mongolia	From A to E: The highest overall bacterial alpha diversity metrics in the group A, followed by D and E. The group B and C had the least diversity D, E vs A, B: The enrichment of Firmicutes	Microbial factors other than *H. pylori* may play a role in Mongolian GC. This study identified novel associations between GC and the genera Enterococcus, Lactobacillus, Carnobacterium, Glutamicibacter, Paeniglutamicibacter, Fusobacterium and Parvimonas
Zhang, 2021[16]	Gastric biopsies	A: SG B: AG C: IN D: GC	16S rRNA gene sequencing using Illumina MiSeq	China	From A to D: The enrichment of *Slackia*, *Selenomonas*, *Bergeyella* and *Capnocytophaga*	Oral microbiota was enriched in the gastric mucosa of GC patients, and intestinal microbiota was enriched in the gastric mucosa of IN patients
Png, 2022[83]	Gastric biopsies (antral)	A: Normal B: IM C: EGN	16S rRNA gene sequencing using Illumina MiSeq	Singapore	C vs A, B: The enrichment of Proteobacteria and depletion of Bacteroidetes B, C: The depletion of *Lactobacillus* and *Bifidobacteria*	Identify a constellation of six bacterial taxonomic markers, that accurately classify patients who would develop EGN

There is no doubt that a complex interplay between the host gastric microbiota and the gastric mucosa promotes health and gastric homoeostasis, and that an imbalance contributes to inflammation, susceptibility to pathogens, and disease (cancer included). *H. pylori* replication in the human stomach is widely acknowledged to play a critical role in the early stages of gastric disease development by causing increased inflammation and progressive changes in the gastric mucosa, including changes in mucin expression [8,14,15,16]. However, after a certain point in the cascade, the development of gastric cancer may be *H. pylori* independent, because colonisation decreases in later stages of carcinogenesis, particularly in patients with intestinal metaplasia and dysplasia, and *H. pylori* are frequently absent/undetectable at the cancer stage. Furthermore, in some patients, progression to malignancy can occur after *H. pylori* eradication. All of this suggests that other factors, such as mucin expression and other components of the gastric microbiota, are involved in the carcinogenesis process. [8,15].

From Table 1 following can be concluded, Some studies have shown that patients with gastric cancer have significant *H. pylori* depletion as well as significant enrichment of cancer-promoting *non-H. pylori* microbiota. [8,14]. Some studies indicate a marked increase in *H.pylori* colonisation in gastric cancer patients, also Enrichment of oral microbiota such as *Fusobacterium, Veillonella, Peptostreptococcus, Streptococcus, Slackia, Parvmonas,* and *Haemophilus* was found in patients with gastric cancer and was linked to the occurrence of gastric cancer, additionally *Slackia, Bergeyella, Selenomonas* and *Capnocytophaga* was increased throughout Correa's cascade. [8,14,17].

However, with the progression of Correa's cascade and spontaneous clearance of H. pylori, H. pylori colonisation in the stomach mucosa gradually diminishes, followed by changes in the quantity of non-H. pylori microbiota [20]. Lactic acid bacteria were more abundant in gastric cancer patients which were previously deemed absent or not present in previous studies [14]. Composition of the gastric mucosa in Patients with intraepithelial neoplasia and gastric cancer had significantly higher levels of *Actinobacteria, Bacteroidetes, Firmicutes,* and *Fusobacteria.*[14,17]. Hence changes in the gastric microbiota can be used to determine the progression of gastric cancer. At a later stage, the *non-H. pylori* microbiota dysfunction alters the microbiota-host balance and worsens the severity of gastric mucosal lesions, encouraging gastric cancer advancement [21]. The proportions of Acidobacteria and Proteobacteria were lower in the intraepithelial neoplasia and gastric cancer groups compared to other precancerous lesion categories [22].

Butyric acid-producing bacteria such as *Lachnospiraceae* and *Ruminococcaceae* were depleted in GC patients, resulting in decreased butyrate generation and accelerated gastric cancer development based on the data collected from rats [23].

Although The Interaction between *H.pylori* and *non-H.pylori* microbiota remains contentious in correa's cascade several studies in vitro studies have shown promising results where *non-H.pylori* interaction with *H.pylori* causes inhibition of the correa's cascade additionally Various studies have shown that H. pylori infection affects the structure of the gut microbiota population. In contrast, some have reported that gut microbiota affects H. pylori colonization. [24, 25]. This is also evident in the interaction between lactic acid bacteria and *H.pylori*, it protects from the damaging effects of *H.pylori's* inflammatory factors also preventing its colonisation [24]. Hence the composition of microbiota at different stages of Correa's cascade can be of clinical significance.

7.3. Metabolic effects of gastric microbiota in gastric carcinogenesis.

7.3.1 Effects of *H.pylori* metabolism.

Changes in the gut microbiota are linked to a variety of inflammatory and metabolic diseases. Although many studies have looked at the relationship between gastric microbiota and *H. pylori,* little is known about its effect on downstream gut microbiota [26]. Microbiome changes are frequently followed by changes in microbial functions. The relative abundance of 19 gut microbial pathways differs significantly between subjects with and without *H. pylori* [27]. Aside from the impact on host microbes, persistent H. pylori infection can cause harmful inflammatory processes [28]. The dysbiosis of the intestinal microbiota caused by H. pylori infection can affect Vit.B12 production. A cobalt corrinoid is Vit.B12. Because humans cannot produce Vit.B12, it is produced solely by microorganisms, particularly anaerobes [28].

Further emphasising the fact Wang et al. discovered that the levels of plasma Vit.B12 and gut microbial Vit B12 biosynthesis were significantly lower in subjects with positive *H. pylori* compared to subjects with negative *H. pylori*. Lower Vit.B12 biosynthesis module levels were linked to lower Vit.B12 concentrations in subjects with *H. pylori* infection, indicating that *H. pylori* infection-related gut microbiota dysbiosis increases the risk of Vit.B12 deficiency. This demonstrates that some changes in gut microbial species and functions correlate with *H. pylori* infection, implying that the gut microbial shift in patients with *H. pylori* infection may indirectly increase Vit.B12 deficiency [29].

Furthermore, previous research has suggested that *H. pylori-induced* gastric sinusitis may result in type B chronic gastritis, followed by decreased gastric acid secretion, resulting in Vit.B12 malabsorption [30]. Thus, *H. pylori* infection can reduce both absorption capacity and Vit.B12 production, increasing the risk of Vit.B12 deficiency. Reduced Vit. b levels are also linked to a significant elevated risk for non-cardia gastric adenocarcinoma [31]. *H.pylori* infection has also been linked to food-bound vitamin B12 malabsorption, possibly due to the induction of atrophic gastritis, which is accompanied by achlorhydria (increased gastric pH). Furthermore, vitamin B12 absorption requires acid-producing gastric mucosa, which allows vitamin B12 to be released from its binding proteins [32,33].

It has also been demonstrated that gastritis and *H. pylori* infection alter the body's basal metabolic function additionally seventeen KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways revealed significant differences between the H. pylori-infected group and the healthy control group. The findings of this study revealed a significant increase in activity in *H. pylori*-positive children's metabolic pathways [34]. As metabolism-related pathways (fatty acid metabolism, LPS biosynthesis, beta-lactam resistance, xenobiotics

metabolism by cytochrome P450, glycosphingolipid biosynthesis-ganglio series, glycosphingolipid biosynthesis-globo series, N-glycan biosynthesis, and glycosaminoglycan degradation) were enriched in the *H. pylori*-positive group, peptidoglycan biosynthesis was depleted [34]. Based on the functional analysis of the microbiome, the lipid metabolism pathway was increased in the gastritis group, indicating that the gut microbiome also affects *H. pylori*-induced gastritis.

7.3.2 Gut microbiota its effect on Metabolites and Carcinogenesis.

The role of commensal and pathogenic bacteria in the pathogenesis of cancer has been confirmed in recent years. Numerous aspects of cancer, including prevention, induction, response to treatment, and development of resistance, are influenced by bacteria. In addition to bacterial genotoxins (like colibactin, CagA, VirA, P37, and IpgD), common byproducts of bacterial metabolism can also cause these effects. The impact of gut microbial metabolites, such as short-chain fatty acids (SCFAs), polyamines, and byproducts of polyphenol and tryptophan catabolism, on the onset and progression of cancer has been demonstrated by a number of studies. Bacterial metabolites play a significant role in the development of cancer because they can alter the cell cycle and control the immune system through transcriptional and epigenetic metabolites.However, the mechanism underlying these effects is still poorly understood [35,36].

Gut microbiota produces short-chain fatty acids such as butyrate, propionate, and acetate from fermentable non-digestible carbohydrates, Bacteroidetes are the primary producers of acetate and propionate, while Firmicutes are the primary producers of butyrate [35,36]. Bacterial concentrations can be altered by diet, age and diseases, or other conditions [63]. Short chain fatty acids can activate a variety of cellular mechanisms, the majority of which are involved in the prevention of carcinogenesis. This effect is linked to the regulation of cellular pathways (e.g., Akt/mTOR and MEK/ERK signalling pathways), transcription factors (downregulation of NF-kB), and epigenetic regulation (e.g., inhibition of HDACs-histone deacetylases-activity, DNA methylation, histone phosphorylation and methylation), resulting in cell cycle, apoptosis, and immune response regulation [38,39]. Multiple studies have confirmed that butyrate plays an important role in the human body and is the only short-chain fatty acid with anticarcinogenic activity [38,40]. *Faecalibacterium prausnitzii*, a member of the next-generation probiotics group, is the main

butyrate producer[35]. It provides energy to colonocytes, boosts gastrointestinal immunity, and keeps the intestinal barrier intact [41,42]. Butyrate, on the other hand, may promote carcinogenesis by increasing aberrant epithelial cell proliferation [43].

Matthews et al. looked at how two short chain-fatty acids, propionate and butyrate, affected cell viability and cell cycle regulation in a human gastric cancer cell line (Kato III) [44]. Short chain fatty acids were applied to the cell lines for 24, 48, and 72 hours. Flow cytometry was used to assess apoptosis induction and cell cycle changes. In Kato III cells, Short chain fatty acids caused apoptosis and necrosis. It was also discovered that the effect obtained after using butyrate was significantly greater than that obtained after using propionate. Sodium butyrate, interestingly, has the ability to inhibit cell proliferation and induce differentiation in a variety of cancer cells[45]. It implies changes in the proliferation of apoptosis-related genes in human gastric cell lines, with FAK (focal adhesion kinase) expression decreasing and DAPK1/2 (death-associated protein kinase) expression increasing, which induces apoptosis. Sodium butyrate treatment causes p53 acetylation, which induces p21 {cyclin-dependent kinase inhibitor 1A (CDKN)}, which inhibits the activity of cyclin-dependent kinase 2 (CDK2) in G1/S phase, resulting in cycle arrest in G1 [46]. Therefore these effects indicate short chain fatty acids could have anti-cancer effects and enhance the efficacy of the chemotherapeutics used to treat gastric cancer [44].

In the gut, *Firmicutes sp*. primarily produces polyamines (PAs), which include putrescine, cadaverine, spermidine, and spermine[47]. The Functions of polyamines include protecting against acids and free radicals, producing siderophores, and preserving the stability of cell walls [48]. Studies using cell culture and animal models have demonstrated that increased intracellular polyamines levels and changes in its metabolism are related to a variety of malignancies [49]. Key enzymes involved in the production of polyamines include ornithine decarboxylase (ODC) and adenosylmethionine decarboxylase 1 (AMD1). The development of colon cancer has been linked to higher Ornithine decarboxylase (ODC) activity and, consequently, higher polyamines concentration [50].In contrast, a diet high in probiotics (Bifidobacterium sp., Lactobacillus sp., and Streptococcus sp.) had an anticancer impact by lowering the content of Polyamines in a mouse model [51]. adenosylmethionine decarboxylase 1 (AMD1) potential role in human stomach malignancies is Unknown. Recently, Xu et al. demonstrated that knocking down AMD1 in a tumour xenograft model inhibited tumour growth in vivo and that inhibiting AMD1 with the inhibitor SAM486A {SAM486A (CGP 48664) is a new inhibitor of the polyamine biosynthetic enzyme S-adenosylmethionine

decarboxylase (SAMDC)} stopped cell cycle progression during the G1-to-S transition in human gastric cancer cells [52].

The importance of N-nitroso compounds (NOC) in gastric carcinogenesis is well established [53]. N-nitroso compounds (NOCs) are produced both naturally and as a result of endogenous synthesis, such as in the case of processed meat, smoked fish, and some vegetables [54]. promoting the formation of NOCs, several bacteria, including *Veillonella, Clostridium, Haemophilus, Staphylococcus, Neisseria, Lactobacillus,* and *Nitrospirae,* contribute to gastric carcinogenesis [55]. Patients with gastric cancer had greater NOC levels than healthy persons, according to epidemiologic studies. Although there was no statistically significant difference between gastric cancer patients and controls when it comes to nitrosating or nitrate-reducing bacteria, which were found to be more prevalent in gastric cancer patients, although not statistically significant [56]. At the same time, nitrate and nitrate reductase activity was elevated in gastric cancer than in chronic gastritis [57].

Lactic acid bacteria stimulate generation of free radicals which are responsible for the formation of N-nitroso compounds that induce mutagenesis, angiogenesis, and protooncogene expression additionally inhibiting apoptosis, demonstrated in both in vitro and in vivo studies [58].

Lactic acid bacteria appear to be abundantly increased in gastric cancer patients which metabolise lactate as a source of energy to cancer cells additionally playing a regulatory role in various aspects of carcinogenesis which includes tumour angiogenesis, immune escape, tumour cell migration, and metastasis [59,60]. In patients with gastric cancer, higher levels of L- and D- lactate and lactate dehydrogenase were found than in those with gastric ulcers and healthy patients [61].

Metabolites such as polyphenols and tryptophan can contribute to carcinogenesis, polyphenols are mostly produced by gut bacteria *Clostridium sp*. and *Eubacterium sp.* And by probiotic bacteria such as *Bifidobacterium,* and *Lactobacillus* [62,63]. Polyphenols can exert anticarcinogenic effects by impacting the cell cycle and inducing apoptosis and inhibition of proinflammatory cytokines production.

Tryptophan and its metabolism on the other hand, has an opposite effect by playing an essential role in the suppression of anticancer immune response leading to an increase in malignancy and tumour progression of the cancer cells [64]. Tryptophan is primarily metabolised by *Firmicutes (Clostridium sporogenes, Ruminococcus gnavus and Lactobacillus sp.)* along with some opportunistic bacteria [65]. Even despite clear evidence about the effect of these metabolites and carcinogenesis, data available on the role of bacteria is scarce hence further studies could provide a clear understanding of the relationship between diet, gut microbiota and carcinogenesis, proving beneficial for gastric cancer patients in the long term.

7.4. Challenges in Gastric carcinogenesis and its microbiota research.

7.4.1 Key factors affecting microbiota research.

One of the main factors influencing the composition of the gut microbiome is the host genotype. According to Zoetendal et al., Monozygotic twins have greater microbial similarities than dizygotic twins or unrelated individuals [66]. Therefore the Indian population can serve as a good example of genetically diverse human populations. Because of the high genetic diversity in the population, there is a large and difficult population basis for defining the core gut microbiome [66,67]. Studies examining the effect of geographic location on the gut microbiome have revealed significant differences between individuals' gut microbiomes [68]. Yatsunenko et al. investigated the difference in gene content harboured by the microbiome in various populations in addition to microbiome composition [68]. The urease gene was one of the main genes that varied according to the geographic location of the individuals. When compared to the US population, the Malawian and Amerindian baby microbiomes had a substantially higher representation of this gene [68]. A study of the gut microbiome of Korean people revealed a unique gut microbiome composition when compared to people from the United States, Japan, and China [69]. Thus proving that microbial diversity is directly linked with the geographical differences among a certain population.

The dietary habits have also shown to affect the composition of the gut microbiota, diversity has increased in present-day modern humans, hence adding a layer of complexity in microbiota studies. The effects of diet on the host-microbe interactions are complex and the effect duration of specific dietary components remains difficult to study, hence playing a key role in influencing the abundance of gastrointestinal microbes both short and long-term along with its metabolites [70]. Although the precise process underlying the development of gastric cancer is still unknown, a combination of genetic, dietary, and lifestyle variables are involved. The development, progression, and survival of patients with carcinomas have long been considered to be influenced by nutritional status before, during and after cancer diagnosis [71].

7.4.2 Challenges in Research.

Most Studies investigated the diversity of the stomach microbiota between patients with gastric cancer and those who had the disease at different stages of development. More recently, Liu et al. investigated the diversity and richness of bacteria in particular gastric microhabitats in connection to the development of gastric cancer in 276 individuals [72]. Researchers observed less diversity and richness in peritumoral and tumoral microhabitats compared to normal gastric tissue after evaluating normal, peritumoral, and tumoral tissues from individuals with gastric cancer[72]. According to this finding, patients with gastric cancer may have different gastric microbiota richness depending on their individual stomach microhabitat rather than just when they advance from atrophic gastritis to gastric cancer.

Research on the human microbiota has vastly improved recently as the role of microbes in various diseases becomes clear, traditionally gastric microbiota research has been limited by acidic conditions and a lack of advancement in culture techniques, but recent improvements in genetic sampling and genomic testing has led to renewed interest in gastric microbiota research [73].

The fact that all of the data in these studies are retrospective and correlational in character is a significant drawback of the research on the relationship between the stomach microbiota and the development of gastric cancer. It was not practical to follow the same people throughout the process because the continuum of stomach cancer development spans decades. Each study employed a cross-sectional study design to compare variation among patients with various characteristics at a particular time. Therefore, investigations that are both prospective and longitudinal are required to evaluate the changes in gut microbiota over time. These studies are rather unrealistic because only 3% of those with H. pylori infection go on to develop stomach cancer [74]. Moreover, earlier studies without first determining the baseline makeup of the gastric microbiota in each individual, prior research has compared the microbiota of patients with various stages of gastric disease to one another. As a result, it is impossible to take into account how the patients' diets, past illnesses, or ethnic differences may have affected their stomach microbiome in this research. Additionally, any subsequent medical interventions or alterations in lifestyle may potentially have an impact on the stomach microbiome. It is therefore still hard to prove a causal relationship between the microbiome and stomach cancer. Confounding influences may be reduced with the use of data collection on therapies and diets [71].

8. METHODOLOGY

This literature review was conducted according to the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) to investigate and analyse data on gastric microbiota,
Its metabolic effects and present and future challenges as part of such studies.

Initial search across various databases such as PUBMED, SPRINGER, WILEY ONLINE LIBRARY, resulted in a total of 988 studies related to the topic of concern. After applying the criteria highlighted in the table, resulting in 170 articles including Free full text, Clinical Trial, Meta-Analysis, Randomised Controlled Trial, and Review, of which were extensively reviewed after which, reminders were deemed appropriate to be included in the literature review.

Table 3: Inclusion and Exclusion criteria

Inclusion	**Exclusion**
Free full text	Textbook references or articles
Published literature	Restricted Literature
Studies based on humans	Animal studies
Studies published 2010-2023	Studies prior year 1990

9. RESULTS

In various studies among normal individuals, two-thirds of overall papers described detecting Streptococcus, Veillonella, Neisseria, Fusobacterium, and Haemophilus species. There is a wide range of variability across different individuals [8,9,12]. Gastric cancer is frequently associated with *H.pylori* infection [11]. Some studies have shown that patients with gastric cancer have significant *H. pylori* depletion as well as significant enrichment of cancer-promoting *non-H. pylori* microbiota. [8,14]. Some studies indicate a marked increase in *H.pylori* colonisation in gastric cancer patients, also Enrichment of oral microbiota such as *Fusobacterium, Veillonella, Peptostreptococcus, Streptococcus, Slackia, Parvmonas,* and *Haemophilus* was found in patients with gastric cancer and was linked to the occurrence of gastric cancer, additionally *Slackia, Bergeyella, Selenomonas* and, *Capnocytophaga* was increased throughout Correa's cascade. [8,14,17].

Progression of Correa's cascade and spontaneous clearance of *H. pylori*, *H. pylori* colonisation in the stomach mucosa gradually diminishes, followed by changes in the quantity of *non-H. pylori* microbiota [20]. But through various studies, the composition of the gastric mucosa in Patients with intraepithelial neoplasia and gastric cancer had significantly higher levels of Actinobacteria, Bacteroidetes, Firmicutes, and Fusobacteria.[14,17]. It is plausible to suspect that changes in the gastric microbiota across various stages of Correa's cascade can be used to determine exact progression. However interaction between *H.pylori* and *non-H.pylori* microbiota remains contentious in Correa's cascade.

H.pylori has been shown to alter basal metabolic function between infected and healthy groups with a significant increase among children [34]. Dysbiosis caused by the *H.pylori* infection can cause a significant decrease in vitamin B12 production [29]. Dysbiosis has also demonstrated disturbances to bacterial metabolism in the stomach inducing differentiation in a variety of cancer cells [35,38,43,44,45,]. Studies demonstrating the link between N-nitroso compounds and free radicals have established potential negative effects for Gastric carcinogenesis [54,55,56,57,58].

Bacterial metabolites have been known to play a significant role in the development of cancer as they have the ability to alter the cell cycle and control the immune system through transcription and epigenetics, regardless the mechanics are poorly understood [35,36]. Multiple studies have confirmed that butyrate, one of the metabolites, plays an important role in the human body and is the only short chain fatty acid with anticarcinogenic activity, promoting carcinogenesis through epithelial cell proliferation and differentiation of cancer cells [38,40 43]. Another metabolite Short chain fatty acids caused apoptosis and necrosis in

cancer cells, this indicated its potential for anticancer effects and enhancing the effects of chemotherapeutics, demonstrated by Matthews et al. using flow cytometry [44].

N-nitroso compounds are known to contribute to Gastric carcinogenesis produced by several microbes. Patients with gastric cancer had Higher levels of N-nitroso compounds than the control group, based on epidemiological studies [54,55,57].

10.CONCLUSION

1) Across various studies, there is a wide variability in healthy gastric microbiota detecting Streptococcus, Veillonella, Neisseria, Fusobacterium, and Haemophilus species.
2) Throughout Correa's cascade, the number of *H.pylori* declines, and other microbes become dominant as the cascade progresses.
3) *H.pylori*-induced dysbiosis causes metabolic disturbance in other microbes resulting in a variety of cancer cells, potentially adding to the negative consequences for the patient by altering carcinogenic effects.
4) Despite modern advancements in microbiological studies and diagnostic techniques, the level of evidence remains conflicting as various factors such as diet, lifestyle, epigenetics along with metabolic changes. preexisting pathologies play a significant role in the outcome of these studies. Therefore comprehensive accounting for various factors is necessary in order to draw significant conclusions.

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