

# Gut microbiota and oesophageal disease – an update

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**Abstract:** It is a matter of common knowledge that incidence rates of inflammatory and neoplastic conditions of the distal oesophagus, such as eosinophilic oesophagitis, Barrett's oesophagus, oesophageal adenocarcinoma (OAC), and their common main risk factor gastro-oesophageal reflux disease (GORD) are on the rise. All conditions were linked to lifestyle-related risk factors such as diet or medication without a comprehensive understanding of the pathophysiological mechanisms involved. It is a very recent knowledge that the oesophagus harbours a specific and unique microbiome that changes in correlation with disease conditions. Recent epidemiological data indicate potential causative relationships between disease-specific risk factors, changes in the gastrointestinal microbiota composition, and disease progression, as already reported in other gastrointestinal diseases such as colorectal cancer. It is plausible that alterations in the microbial community might account for inflammatory or potential carcinogenic changes in the microenvironment via interference with the immune system, bacterial defence, and metabolic pathways. It is of crucial importance to accomplish a more comprehensive and systemic understanding of the complex mechanistic interactions between host, lifestyle, environment, and microbiota to elucidate the onset, progress, and potential therapy of oesophageal diseases, as well as their association with other diseases of the gastrointestinal tract.

**Keywords:** Barrett's oesophagus, Eosinophilic oesophagitis, Intestinal metaplasia, High fat diet, Toll-like receptor, Oesophageal microbiota.

## INTRODUCTION

Over the past 40-50 years, incidence rates of diseases of the distal oesophagus increased significantly. This included gastro-oesophageal reflux disease (GORD), Barrett's oesophagus, oesophageal adenocarcinoma (OAC), and eosinophilic oesophagitis, whereas incidence of oesophageal squamous cell carcinoma (OSCC) and its precursor, oesophageal squamous dysplasia (OSD), declined. Oesophageal pathology often shares common risk factors such as smoking, alcohol consumption and NSAID intake, as well as dietary based risk factors<sup>1-3</sup>. Exposure to food antigens plays a role in onset of eosinophilic oesophagitis, GORD, Barrett's oesophagus and OAC. During the past decades, dietary factors changed drastically in western countries leading to constantly increasing obesity rates. Obesity as risk factor is often associated with a "western diet" with high intake of meat and processed food when compared to a "healthy" diet rich in polyunsaturated fats, omega-3 fatty acids, fibre, and micronutrients<sup>3-6</sup>. The lower incidence of OSCC is most likely a result of a reduced consumption of both alcohol and tobacco in most Western populations<sup>7,8</sup>.

Some studies<sup>9-14</sup> provide evidence for a potential interaction between the abovementioned risk factors and subsequent changes in the microbiome composition in oesophageal disease. The gastrointestinal microbiota are greatly contributing to food digestion, energy recovery, and metabolism, e.g., fermentation of polysaccharides to short-chain fatty acids (SCFA)<sup>15,16</sup>. The gut microbial community and its functional potential are mainly shaped by diet composition. The microbial profile of the gastrointestinal tract changes in response to increased fat or sucrose intake. This can contribute

to the development of metabolic disorders such as obesity and diabetes along with immune system activation and systemic inflammatory effects<sup>16,17</sup>. The microbiome educates adaptive immunity to maintain a balanced microbial homeostasis and functional host barriers<sup>18-20</sup>. Microbiome diversity and colonization of the gut with commensal microbiota is beneficial and prevents niche formation that would enable pathogen overgrowth<sup>21</sup>. The composition of the microbiota in each segment of the gastrointestinal tract is further influenced by factors like oxygen, pH, SCFA and bile acid levels, as well as the composition of the local mucus and presence of antimicrobial proteins<sup>22,23</sup>. These factors favour the development of very specific microbial communities for every gut segment.

The oesophageal microbiome has not been known for a long time. First studies of the oesophageal microbiome were initiated in the 1980s using culture dependent methods showing mainly the existence of a non-temporary oesophageal microbiome similar to the one of the oral cavity<sup>24-26</sup>. A characterization of the specific oesophageal microbiota was possible with more advanced sequencing approaches as conducted first by Pei et al<sup>27</sup> in 2004. They detected members of six phyla: *Firmicutes*, *Bacteroidetes*, *Actino-*, *Proteo-*, and *Fusobacteria* and *TM7*. The absence of members of the *Spirochaetes*, which typically colonize the oral cavity provided evidence of a specific oesophageal microbiota<sup>27</sup>. Subsequent studies<sup>11,28</sup> confirmed these initial findings and showed prevailing colonization of the oesophagus with gram-positive bacteria. Deshpande et al<sup>29</sup> investigated the oesophageal microbiota of 106 patients with different oesophageal conditions. They confirmed shifts in the microbiota related to different pathological conditions and disease stages, but also confirmed age as an influencing factor, as well as host SNPs in genes such as *NOTCH2* and *NREP*<sup>29</sup>. Affected was not only the composition of the local bacterial community, but also of fungi (e.g., *Candida*) and bacteriophages.

## THE OESOPHAGEAL MICROBIOME IN DIFFERENT DISEASE CONDITIONS

### Changes in Reflux Oesophagitis and Barrett's Oesophagus

IN 2009, Yang et al<sup>9</sup> published a large scale study characterizing the healthy oesophageal microbiome and shifts in GORD and Barrett's oesophagus by analysing distal oesophageal biopsy samples with bacterial 16S sequencing<sup>9,27,30</sup>. They defined two different types of microbiome, with type I microbiome characterized by gram-positive bacteria, mainly *Firmicutes* of the *Streptococcus* genus (78%) and type II microbiome by decreased *Streptococci* (29%) and increased amounts of gram-negative, anaerobe/microaerophile bacteria, mainly *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Spirochaetes*. While the type I microbiome was correlated to the healthy oesophagus (92% of healthy individuals), the type II microbiome was strongly correlated with GORD and Barrett's oesophagus resulting in an odds ratio (OR) of 15.4 (95% confidence interval [CI]: 1.5-161.0) and 16.5 (95% CI: 1.5-183.1), respectively<sup>9</sup>. Lipopolysaccharides (LPS), which are part of gram-negative bacterial membranes, are suspected to facilitate reflux by oesophageal sphincter relaxation and support chronic inflammation (via activation of the toll-like receptor (TLR) 4 and NF- $\kappa$ B pathway) with increased production of proinflammatory cytokines<sup>11</sup>. A comparable study in Japanese patients confirmed these findings. Normal oesophageal and GORD samples yielded mainly bacteria of the *Streptococcus* genus, while in Barrett's oesophagus the gram-negative, obligate anaerobic *Veilonella* dominated. Bacteria of the also gram-negative, obligate anaerobic phylum *Fusobacterium* were only found in patients with reflux oesophagitis and Barrett's oesophagus<sup>31</sup>. In a study performing 16S RNA pyrosequencing, significantly increased *Enterobacteriaceae*, specifically *Escherichia* levels were detected in gastric refluxates of patients with oesophagitis and Barrett's oesophagus compared to non-reflux control samples (30.3% vs. 5.6%). *Methylobacteriaceae* were only found in disease conditions while *Pasteurellaceae* and *Porphyromonaceae* were more abundant in control samples<sup>32</sup>. *Enterobacteriaceae* trigger inflammation in inflammatory bowel disease and might act similarly in the upper gastrointestinal tract<sup>33</sup>. The dominance of gram-negative bacteria in reflux esophagitis was again confirmed in a most recent qPCR-based study published by Dogan et al<sup>34</sup> in 2019. The dominant genera detected in patients with reflux symptoms were *Veilonella parvula*, *Fusobacterium nucleatum*, *Neisseria meningitis*, and *Prevotella intermedia*, all gram-negative with substantial virulence potential<sup>34</sup>. *Fusobacterium nucleatum* has been shown to trigger colorectal cancer via its adhesin (FadA)<sup>35,36</sup>. *Streptococcus pyogenes* and *Lactibacillus acidophilus* were detected each in 15% of the normal controls vs. 6% and 3%

of the reflux patients, respectively, with *Lactibacillus acidophilus* displaying positive effects in diarrhoea and gastritis<sup>34</sup>. A recent study on Chinese patients with erosive oesophagitis showed a trend towards overall lower diversity with less abundance of *Bacteroidetes*. Interestingly, there was depletion of *Prevotella*, *Moraxella*, and *Helicobacter*<sup>37</sup>.

### Changes in OAC

To date, most studies examining the role of the microbiome in oesophageal cancer do not distinguish between OSCC and OAC. Thus, little information is available characterizing OAC specifically. Blackett et al<sup>38</sup> combined cultivation- and 16SRNA sequencing-based evaluation of biopsies. They found expansion of gram-negative bacteria in disease, with members of the *Campylobacter* genus to be markedly increased in GORD and Barrett's oesophagus and decreased in OAC, indicating an association to reflux. Abundance of most phyla in OAC more closely resembled features present in non-reflux patients, rather than the GORD or Barrett's oesophagus microbial phenotype. When OAC was compared to healthy controls or other patient groups, no specific microbial shift was found<sup>38</sup>. The fact that the oesophageal microbiota in patients with OAC is more similar to "healthy" subjects rather than patients with GORD or Barrett's oesophagus might suggest an influence rather early stages of oesophageal disease. Zaidi et al<sup>39</sup> studied the OAC associated microbiota in a surgically induced rat model and on human samples. In the rat model, *E. coli* was dominant in Barrett's oesophagus and OAC (60% and 100%) and triggered inflammation by elevation of IL6 levels. In human samples, abundance of *Streptococcus pneumoniae* decreased in dysplasia and OAC compared to normal epithelium and Barrett's oesophagus<sup>39</sup>. *Streptococci* trigger inflammation via cytokine induction and are suspected to invade the epithelium during disease progression until carcinogenesis induces excessive tissue damage<sup>40</sup>. Peters et al<sup>41</sup> investigated oral wash samples and demonstrated microbial shifts that were uniquely associated with OAC and OSCC. They postulated an association of the periodontal pathogen *T. forsythia* and OAC risk (OR 1.21 95% CI: 1.01-1.46). Also the oral species *Actinomyces cardiffensis*, *Selenomonas* oral taxon 134 and *Veillonella* oral taxon 917 were identified to be related to increased OAC risk<sup>41</sup>. This is not surprising as an association between oral health and oesophageal cancer risk has been reported previously<sup>42</sup>. A lower risk for OAC development was associated with members of the phyla *Firmicutes* (*Lachnoanaerobaculum umeaense*, *Oribacterium parvum*, *Solobacterium moorei*), and *Proteobacteria* (*Neisseria sicca*, *Neisseria flavescens*, and *Haemophilus* oral taxon 908), as well as *Corynebacterium durum*, *Prevotella nanceiensis* and, in concordance with the findings by Zaidi et al<sup>39</sup>, *Streptococcus pneumoniae*. Some of these results were only confirmed under certain epidemiological conditions, like a lower risk of OAC with depletion of *Neisseria* in smokers<sup>41</sup>. Another study using the non-invasive Cytosponge™ for sampling, found that *Lactobacilli* were dominating in half of the cancer samples, with *Lactobacillus fermentum* significantly enriched in OAC samples. Overall, bacterial richness was decreased in high grade dysplasia<sup>43</sup>. *Streptococci* and *Lactobacilli* are capable of surviving in a low pH environment which might support malignant progression via competitor growth inhibition, induction of TLRs and tissue damage by toxin release.

Epidemiological evidence indicates an inverse relationship between the eradication of *Helicobacter pylori* (*H. pylori*) and incidence of OAC, potentially linked to a shift in the microbial community<sup>44-46</sup>. There are numerous studies with conflicting results and there is still need for further data to address this conclusively. However, a recent meta-analysis of 72 studies including 84,717 cases and 390,749 controls confirms the abovementioned inverse relationship (OR 0.68; 95% CI: 0.58-0.79,  $p < 0.001$ ), with the results being even more evident for dysplastic (OR 0.37; 95% CI: 0.26-0.51) or long-segment (OR 0.25; 95% CI: 0.11-0.59) Barrett's oesophagus<sup>47</sup>.

Diet is a key environmental factor influencing the gut microbiota while vice versa microbes influence the effects of diet via metabolic pathways<sup>48-50</sup>. The increase in incidence of Barrett's oesophagus and OAC over the last decades was preceded by a shift towards a "western diet" in North America and Europe, which is rich in refined fat, sugars, carbohydrates, artificial food supplements, and low in plant-based fibre<sup>51,52</sup>. Diet and obesity are the main risk factors for GORD, Barrett's oesophagus and OAC<sup>3-5</sup>. Western diet favours obesity in humans, while mice raised under germ free conditions are protected from excessive weight gain<sup>50,53,54</sup>. In a genetic mouse model of Barrett's oesophagus, treatment with a high fat diet accelerated carcinogenesis via alterations of the gut microbiota and induction of pro-inflammatory IL8 pathways. This was the first study to show a link between diet, obesity and malignant progression from Barrett's oesophagus to OAC<sup>55</sup>. Interestingly, changes induced

in the lower intestinal microbiota were associated with disease progression indicating an influence from the distance by systemic metabolites. This is in line with a study in human subjects showing that fat intake had no major influence on the oesophageal microbiota, but that these change depending on the fibre-content of the diet<sup>56</sup>. High fibre diet leads to relative abundance of *Firmicutes* and decrease of gram-negative bacteria, whereas low-fibre intake results in increase of gram-negative bacteria, mainly *Prevotella*, *Neisseria* and *Eikenella*, also changing metabolic pathways.

Excessive antibiotic treatment of patients and animals bred for meat production started in the 1950s and was associated with an accelerated loss of microbial diversity<sup>57</sup>. A case-control study found a dose-dependent relationship between penicillin treatment and an increased risk of several cancer types, including oesophageal cancer<sup>58</sup>. Penicillin acts effectively against *Staphylococci* and *Streptococci*, with the latter being associated with the “healthy” oesophageal microbiota<sup>9</sup>. Further studies both in humans and in animal models delivered conflicting results, so that more data is required to draw appropriate conclusions with regards to this matter.

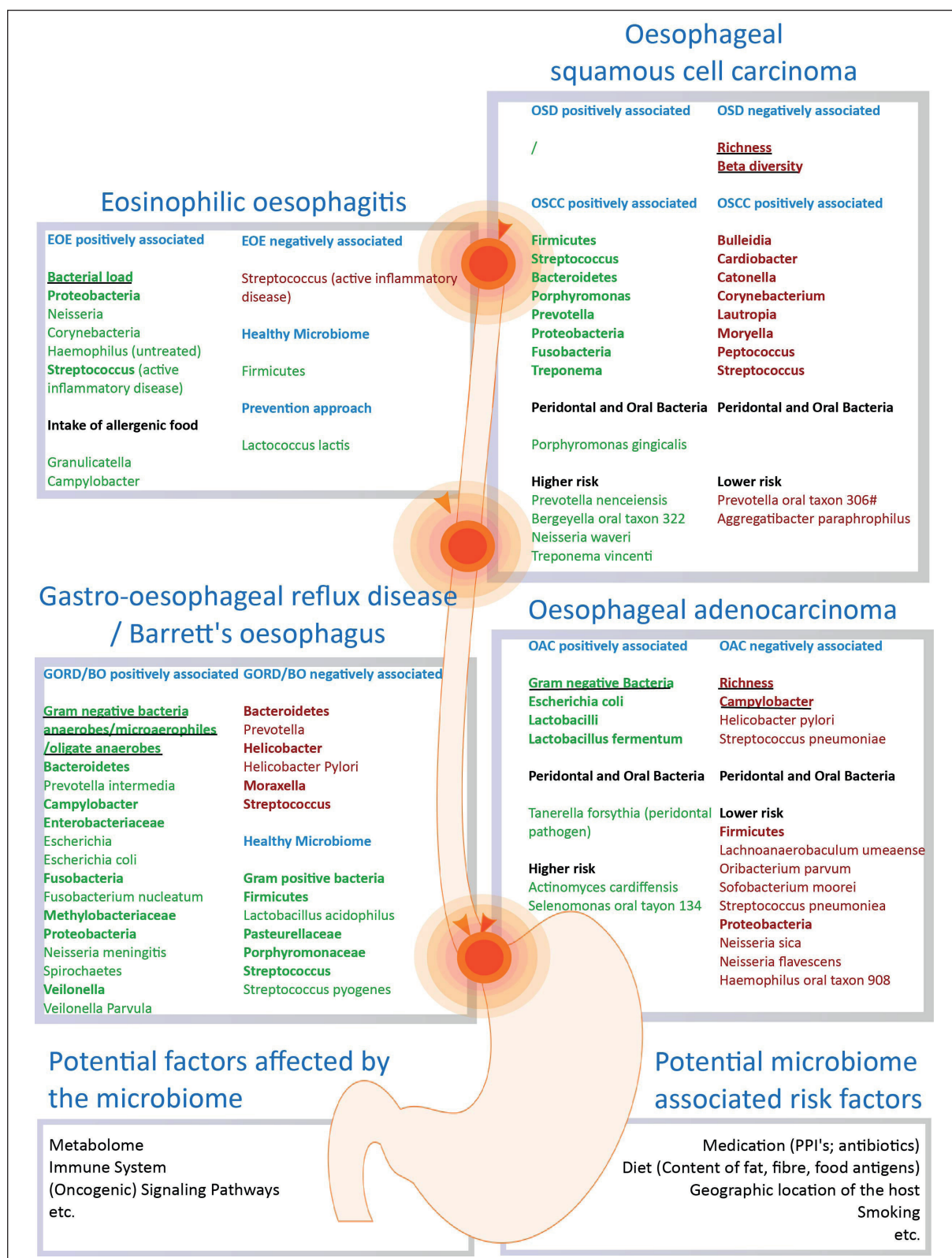
### Changes in OSD and OSCC

Yu et al<sup>14</sup> examined the impact of the microbiota on OSCC and its precursor OSD in samples collected with a rubber balloon or cytomech. They found lower microbial richness, as well as lower beta-diversity to be significantly associated with OSD. However, there was no significant association between single bacterial taxa and OSD or OSCC. This was confirmed in 2015 by Chen et al<sup>59</sup>, also reporting a lower abundance of bacteria of the genera *Bulleidia*, *Cardiobacterium*, *Catonella*, *Corynebacterium*, *Lautropia*, *Moryella*, and *Peptococcus*, and higher abundance of *Prevotella*, *Streptococcus* and *Porphyromonas* in OSCC compared to non-OSCC cases<sup>59</sup>. Shao et al<sup>60</sup> recently described a local tumour environment of *Firmicutes*, *Bacteroidetes* and *Proteobacteria*, with more presence of *Fusobacteria* and less *Streptococcus* in the actual tumour tissue compared to adjacent non-tumour samples. Peters et al<sup>41</sup> found a trend towards an association between the periodontal pathogen *P. gingivalis* and OSCC risk. *Prevotella nanceiensis*, *Bergeyella* oral taxon 322, *Neisseria weaveri*, and *Treponema vincenti* were associated with higher OSCC risk, while the risk was lower in presence of *Prevotella* oral taxon 306 and *Aggregatibacter paraphrophilus*<sup>41</sup>. *P. gingivalis* abundance was confirmed in OSCC tumour tissue, and was associated with lymph node metastases and decreased survival in another study<sup>61</sup>. Data from 45 patients was used to analyse the prognostic relevance of the oesophageal microbiota in OSCC<sup>62</sup>. *Prevotella* and *Treponema* were associated with nodal-positive disease and *Streptococcus* with advanced T-stage. The combination of *prevotella* and *Streptococcus* was confirmed as independent prognostic factor.

For studies on OAC and OSCC, it has to be considered that tumour tissue is characterised by instability and disintegration, which is also likely to affect the microbial profile of these samples.

### Changes in Eosinophilic Oesophagitis

Eosinophilic oesophagitis is a chronic inflammatory disorder with an increase of intramucosal eosinophil granulocytes. It was defined as disease only 20 years ago. In 2015, Harris et al<sup>63</sup> examined mucus from paediatric and adult patients with eosinophilic oesophagitis by 16S RNA sequencing. They were the first to confirm that the bacterial load was significantly increased in eosinophilic oesophagitis compared to control samples. Untreated eosinophilic oesophagitis patients showed a characteristically high abundance of *Haemophilus*, while treatment with PPI leads to a decrease of *Streptococcus*<sup>63</sup>. The abundance of *Haemophilus* in eosinophilic oesophagitis was also found in a recent study examining the salivary microbiota of paediatric patients. In this study, *Streptococcus* was found to be associated with active inflammatory disease<sup>64</sup>. Members of the *Haemophilus* family can trigger a number of inflammatory conditions (e.g., meningitis) while *Streptococci* show invasive behaviour and trigger inflammation via cytokine production. In another study, oral swabs and biopsies of paediatric patients with eosinophilic oesophagitis were examined, confirming a shift of the oesophageal microbiota in this condition with enrichment of members of the *Proteobacteria*, mainly *Neisseria* and *Corynebacteria* whereas *Firmicutes* were dominant in control subjects. Since food antigens play a major role in the pathogenesis of eosinophilic oesophagitis, also patients with and without dietary intervention were examined. Consumption of highly allergenic food items led



**Figure 1.** Known changes of the oesophageal microbiota in relation to disease. Subdivision takes place in positive and negative association to the disease or respective characteristics of a healthy microbiome. Positively associated microbiota are depicted in green, negatively associated microbiota in red; with key features underlined. Bacterial associations on genus level are displayed in bold with related lower taxonomic classes arranged below. Further are shown factors potentially affected by the microbiota and disease associated risk factors potentially associated to the microbiota. (EoE: eosinophilic oesophagitis; OSD: oesophageal squamous dysplasia; OSCC: oesophageal squamous cell carcinoma; GORD: gastroesophageal reflux disease; BO: Barrett's oesophagus; OAC: oesophageal adenocarcinoma; PPI: proton pump inhibitor).

to enrichment of members of the *Granulicatella* and *Campylobacter* genera<sup>65</sup>. Arias et al<sup>66</sup> recently published data on the impact of the “6-food elimination diet” on the oesophageal microbiota also including assessment of the expression of TLRs, MyD88, NFκB, and proinflammatory cytokines. The bacterial load decreased under diet alongside a downregulation of TLR expression and normalisation of (initially increased) cytokines. Interestingly, Jensen et al<sup>67</sup> found a six-fold increased risk for eosinophilic oesophagitis in children treated with antibiotics during their first six months of life, in line with data for other allergy-associated conditions. Holvoet et al<sup>68</sup> investigated the impact of probiotics in a genetic murine model of eosinophilic oesophagitis and reported a beneficial effect of *Lactococcus lactis* NC2287 on inflammation and disease outcome<sup>68</sup>.

## SUMMARY AND CONCLUSION

Parallels can be drawn between the rapidly increasing incidence rates of diseases of the lower oesophagus and epidemiological risk factors for these conditions. The most important common risk factors for eosinophilic oesophagitis, GORD, Barrett’s oesophagus and OAC, namely diet, obesity and drug therapy, all selectively induce changes of the oral, oesophageal and/or gastrointestinal microbiota. These oesophageal diseases are in turn associated with distinctive shifts in the microbiota (Figure 1). The microbiota play a critical role in modulation of metabolic and inflammatory pathways triggering disease and carcinogenesis in the upper gastrointestinal tract and might be the missing link to explain how certain epidemiological risk factors influence disease development. Many microbiome studies in coherence with oesophageal disease states were conducted in a descriptive manner, failing to prove direct functional causalities. Thus, it seems of critical importance to further examine the complex interrelationships between epidemiological, environmental and genetic factors, and changes in the gastrointestinal microbial communities in view of causal connections regarding disease onset and development, as well as protection.

## Conflict of Interest

The Authors declare that they have no conflict of interests.

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