ACCSCIENCE PUBLISHING

Microbes & Immunity

ORIGINAL RESEARCH ARTICLE

Impact of dietary emulsifiers on the presence of adherent-invasive *Escherichia coli* in Crohn's disease

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Citation: Lin Y, Dong X, Tun HM, *et al*. Impact of dietary emulsifiers on the presence of adherent-invasive *Escherichia coli* in Crohn's disease. *Microbes & Immunity.* 2025;2(4):67-78.
doi: 10.36922/MI025230051

Received: June 6, 2025

Revised: July 18, 2025

Accepted: August 1, 2025

Published online: August 21, 2025

Abstract

Adherent-invasive *Escherichia coli* (AIEC) has been implicated in Crohn's disease (CD) pathogenesis. We aimed to evaluate the impact of dietary factors on the presence of AIEC in patients with CD and to identify AIEC-associated mucosa microbial signatures in regions with different urbanization levels. A total of 112 CD patients and healthy controls were recruited from a rural area in China (Yunnan). Clinical demographics, food additive questionnaires, and ileal biopsies were collected from subjects in rural China. AIEC was isolated from biopsy samples by an antibiotic protection assay. Correlation between AIEC presence and food additives was evaluated using multivariate logistic regression. In addition, a secondary dataset of an urban CD cohort (Hong Kong) was included for microbiome analysis. AIEC was detected in the ileal mucosa in 20.83% of patients with CD in rural China. Multivariate analysis showed that living in an urban area was associated with the presence of AIEC in CD patients. Carrageenan consumption was positively correlated with AIEC presence in CD. AIEC-positive CD patients with primary education consumed more carrageenan than AIEC-negative CD patients (p=0.008). AIEC presence in CD patients was associated with 23 microbial genera in both urban and rural areas. AIEC-positive CD patients showed a decrease in anti-inflammatory pathways. AIEC colonizes the gut mucosa of CD patients in a rural area of China, with its presence significantly

associated with higher carrageenan consumption. These findings suggest a potential link between dietary emulsifiers, microbial dysbiosis, and AIEC-related CD pathogenesis.

Keywords: Adherent-invasive *Escherichia coli*; Crohn's disease; Dietary emulsifiers

1. Introduction

Crohn's disease (CD) is an intestinal inflammatory disorder that predominantly affects the distal small intestine. The pathogenesis of CD involves a complex interplay between environmental factors, genetic variants, and abnormal gut microbiota, associated with a dysregulated immunological response.[1] Studies have shown that patients with CD had a reduced abundance of beneficial microbes such as *Faecalibacterium, Roseburia,* and *Clostridium* and an increased abundance of pathogenic commensals, including *Fusobacterium*, *Shigella*, and *Escherichia,* compared to healthy controls.[2,3] In particular, an increased abundance of adherent-invasive *Escherichia coli* (AIEC) is commonly detected in the terminal ileum of patients with CD.[4] AIEC has the ability to bind to the adhesion molecule receptor CEACAM6 on the membrane of enterocytes through type 1 pili,[5] and can invade and replicate in the intestinal epithelial cells. Moreover, the CEACAM6 receptor has been shown to be overexpressed in patients with CD, further facilitating AIEC adhesion in the intestinal epithelial cells.[6] Mechanistic studies have revealed that AIEC can infect macrophages and lead to the release of proinflammatory cytokines, including tumor necrosis factor-alpha and interleukin (IL)-1β.[7] AIEC could also prevent the restoration of normal gut microbiota in dextran sodium sulfate-induced colitis mice models after fecal microbiota transplantation.[8] Furthermore, AIEC is involved in the synthesis of propanediol dehydratase, which can increase the fermentation of propanediol and trigger T cells-induced intestinal inflammation in mice model.[9] Altogether, these findings suggest that AIEC can potentially aggravate abnormal immune responses in CD and contribute to chronic mucosal inflammation.

CD incidence has substantially increased in newly industrialized countries over the past few decades in parallel with rapid urbanization in these regions.[10] Some of the major culprits include early life exposure, consumption of highly processed foods, and changes in hygiene and socioeconomic status.[11-13] The interaction between the host and environment during urbanization may play a role in initiating CD.[14] A migrant study from Canada reported that immigration from developing countries at a younger age was associated with an increased risk of inflammatory bowel disease (IBD), suggesting that early exposure to an urbanized environment may contribute to the development of IBD.[15] Food additives in processed foods, such as emulsifiers, have recently been shown to induce chronic intestinal inflammation in rodents and may potentially play a role in the development and exacerbation of IBD in humans.[16] Common food emulsifiers, such as polysorbate-80 and carboxymethylcellulose, promoted gut inflammation in gnotobiotic mice colonized by AIEC.[17] Another emulsifier, carrageenan, was associated with altered gut microbiome composition and increased expression of pro-inflammatory molecules in an *in vitro* cultivation system.[18]

Importantly, despite experimental evidence linking emulsifiers to AIEC pathogenicity, no human population studies have examined this interaction. Our work addresses this gap by providing the first human evidence linking dietary carrageenan to AIEC prevalence in CD patients and uncovering diet-microbe-pathogen interactions in CD pathogenesis. We further identify AIEC-associated mucosal microbiota signatures, providing novel insights into how urban environmental exposures and diet may interact with microbial factors in the pathogenesis of CD. These findings offer translational insights into how urbanization and diet may synergistically promote AIEC-driven CD.

2. Materials and methods

2.1. Study population

The study population consisted of patients with CD residing in urban and rural areas of China and their corresponding controls. The rural cohort was recruited from the First Affiliated Hospital of Kunming Medical University in Yunnan (population density <1000/km^2)[19] between August 2018 and January 2019. Patients with CD were diagnosed based on endoscopic, radiological, and histological examinations. Healthy controls were subjects who underwent colonoscopies without gastrointestinal diseases in the same hospital. All subjects filled out questionnaires that measured the social demographics and clinical characteristics, and questionnaires that recorded the consumption of food additives. The estimation of food additive intake in each subject was described in the Supplementary Methods section

(Supplementary File). The food additives questionnaire was validated in a CD survey across Australia, Hong Kong, and mainland China to identify the exposure to food additives in CD patients and healthy controls.[11,20] Our study also incorporated a secondary dataset of CD patients and healthy controls from Hong Kong as an urban cohort.[8] The subjects in the urban cohort were recruited from the Prince of Wales Hospital in Hong Kong (population density = 6582.6/km^2).[21] All participants had not been exposed to antibiotics, probiotics, or prebiotics in the past three months before enrollment. All participants gave informed consent, and the study was conducted in accordance with the Declaration of Helsinki. The identification of AIEC presence, sample DNA extraction, and 16S amplicon sequencing were detailed in the Supplementary Methods section. The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Kunming Medical School (reference no. 2017.L.15-1).

2.2. Statistical analysis

Characteristics of CD patients with and without AIEC presence were reported. Data were presented as counts for categorical variables with percentages, and the mean or median for continuous variables with standard deviation or interquartile range. In univariate analysis, the Wilcoxon rank sum test was applied to determine the statistical significance for continuous variables, and Pearson's Chi-squared test was used to identify the statistical difference for categorical variables. The food additives difference between CD patients with and without AIEC presence was calculated using the smd package to obtain the Standardized Mean Difference. Multivariate logistic regression was used to assess the relationship between the risk factors and outcome, with confounders adjusted. We evaluated the association between CD and AIEC presence and the association between AIEC presence and consumption of food additives in the rural cohort. Finally, the impact of urbanization on AIEC prevalence was analyzed in CD patients from rural and urban regions.

2.3. The 16S amplicon sequencing analysis

The taxonomy annotation and functional prediction for the mucosal microbiome sequencing data are detailed in the Supplementary Methods section. For alpha diversity analysis, Shannon diversity and Observed Features were calculated using the operational taxonomic units table that was rarefied to 10,000 sequences per sample. In addition, Bray–Curtis distance was calculated for all samples, and the analysis of similarities (ANOSIM) test was used to identify the statistical difference in beta diversity. The explanation of host factors on the microbiome composition variation was identified by the permutational multivariate analysis of variance (PERMANOVA) test. Differentially abundant taxa and functional modules were identified using a linear mixed model with the geographic region as a random effect. The batch effect of microbiome data was adjusted by the MMUPHin method.[22] A sensitivity analysis was performed to verify the robustness of differentially abundant taxa using the adjusted microbiome data. After selecting the significantly different genera, we validated the discrimination ability of selected microbial genera using the random forest model. Five-fold cross-validation was applied during the model training. The model was trained in 80% of CD patients from two cohorts and validated on the remaining 20% of CD patients and those patients from different regions. We also compared the functional differences in CD patients with and without AIEC presence using a linear mixed model.

3. Results

3.1. The presence of AIEC was significantly associated with CD risk and carrageenan intake

The study design is illustrated in the Graphical Abstract. A total of 112 subjects, including 72 CD patients and 40 healthy controls from a rural area (Yunnan, China), were recruited (Figure S1). AIEC was detected in 20.83% of CD patients and 12.50% of healthy controls (p=0.270, Table 1). Among CD patients, AIEC presence was significantly associated with lower educational attainment (p=0.023), with 33.0% of AIEC-positive patients having no formal education compared to 7.0% of AIEC-negative patients (Table 2). Multivariate logistic regression showed a significant association between AIEC presence and increased CD risk (adjusted Odds Ratio [aOR] = 7.50, 95% confidence interval [CI]: 1.04–54.23, p=0.046, Figure 1A and Table S1). Low education level (middle school) was positively associated with CD risk (aOR = 8.20, 95% CI: 1.35–49.71, p=0.022), whereas body mass index (BMI) was negatively associated with CD risk (aOR = 0.77, 95% CI: 0.65–0.9, p=0.001). CD patients consumed more aluminum silicate (8382 mg/year vs. 2092 mg/year, p=0.038) and titanium dioxide (127151 mg/year vs. 29664 mg/year,

Table 1. AIEC prevalence in the urban and rural cohorts

Area	AIEC-positive rate (%)	AIEC-positive	AIEC-negative	p-value†
Rural CD	20.83	15	57	0.270
Rural HC	12.50	5	35	
Urban CD	30.00	18	42	0.003
Urban HC	7.14	4	52	

Notes: †p-value was calculated according to Pearson's Chi-squared test. Abbreviations: AIEC: Adherent-invasive *Escherichia coli*; CD: Crohn's disease; HC: Healthy controls.

Table 2. The characteristics of CD patients in AIEC-positive and AIEC-negative groups in the rural cohort

Characteristic	AIEC-positive (*n*=15)	AIEC-negative (*n*=57)	*p*-value
Age[†]	45 (20)	39 (23)	0.220
BMI[†]	20.8 (4.6)	20.8 (5.3)	0.792
Surgery history[‡] (%)	9 (60.0)	29 (50.9)	0.735
Education level[§] (%)			
College/University	6 (40.0)	18 (31.6)	0.023
Middle school	2 (13.3)	24 (42.1)	
No formal schooling	5 (33.3)	4 (7.0)	
Primary school	2 (13.3)	11 (19.3)	
Smoker status[§] (%)			
Ex-smoker	3 (20.0)	5 (8.8)	0.400
Non-smoker	11 (73.3)	42 (73.7)	
Smoker	1 (6.7)	10 (17.5)	
Alcohol consumption[§] (%)			
Current drinker	2 (12.3)	2 (3.5)	0.481
Former drinker	1 (6.7)	7 (12.3)	
Lifetime abstainer	12 (80)	47 (82.4)	
Other	0 (0)	1 (1.8)	
CD Location[§]			
L1	2 (13.3)	6 (10.5)	0.476
L2	4 (26.7)	8 (14.0)	
L3	9 (60.0)	38 (66.7)	
Other	0 (0)	5 (8.8)	

Notes: The number in each cell denotes the median (interquartile range) for the continuous variables or *n* (%) for the categorical variables. CD Location indicates areas in the gastrointestinal tract affected by CD: ileal (L1), colonic (L2), ileocolonic (L3), and others. [†]Wilcoxon rank sum test; [‡]Pearson's Chi-squared test; [§]Fisher's exact test.
Abbreviations: AIEC: Adherent-invasive *Escherichia coli*; BMI: Body mass index; CD: Crohn's disease.

p=0.035) than healthy controls (Table S2). Among CD patients, AIEC presence was associated with higher carrageenan consumption (aOR = 4.49, CI: 1.28–15.75, *p*=0.019, Figure 1B and Table S3), with the largest observed difference among patients with primary education (Standardized Mean Difference, SMD = 3.810, *p*=0.008; Table S4 and Figure S2).

3.2. The presence of AIEC was associated with urbanization and mucosal microbiota dysbiosis

To assess the impact of urbanization on mucosal microbiota and AIEC prevalence, we compared data from the current rural cohort with our previous cohort of 116 patients with CD and healthy controls recruited from an urban area (Hong Kong).[8] We found that the prevalence of AIEC was significantly higher in patients with CD only in the urban area (*p*=0.003) but not in the rural area (*p*=0.31) (Table 1). Multivariate analysis showed a positive association between AIEC risk and living in an urban area in CD patients (aOR = 2.56, 95% CI: 1.03–6.38, *p*=0.04, Table S5). Principal coordinates analysis showed that there were two distinct clusters of individuals living in urban and rural areas (ANOSIM test R = 0.22, *p*=0.001, Figure 2A). PERMANOVA test indicated that geographic regions accounted for larger differences in the gut microbiome composition and functional pathways than CD diagnosis and the presence of AIEC (Figure 2B). For the alpha diversity, Shannon and Observed Features were significantly decreased in AIEC-positive CD patients in the urban area (*p*=0.011) but not in the rural area (Figure S3A and B). A linear mixed model identified 23 microbial genera associated with AIEC presence (Figure 2C). Among these genera, *Bacillus*, *Delftia*, and *Roseburia* were also decreased in AIEC-positive CD patients in our previous study.[8] *Finegoldia* and *Rhodococcus* have been reported as pathogens in the periprosthetic joint infection[23] and pneumonia.[24]

We further performed sensitivity analysis using MMUPHin to eliminate potential batch effect and found 16 microbial genera were significantly different in AIEC-positive CD patients (Figure 3A). Fifteen of them were also identified as differentially abundant taxa before batch effect correction, indicating that the selected taxa were relatively robust. A random forest model showed stronger classification performance for AIEC status in urban patients than in rural patients (median AUC from rural: 0.700 vs. median AUC from urban: 0.850, Figure 3B), suggesting that AIEC-associated microbiome signatures were more distinct in urban settings.

3.3. AIEC presence was associated with reduced acetate production capacity

We identified nine functional pathways associated with AIEC presence in rural and urban CD patients using the linear mix model, with most of these pathways being depleted in AIEC-positive groups. Among these pathways, anti-inflammatory pathways such as L-glutamate and L-glutamine biosynthesis (*p*=0.009), chondroitin sulfate degradation I (*p*=0.031), and acetylene degradation (*p*=0.017) were reduced in AIEC-positive groups (Figure 4A and Table S6). Acetylene degradation is a crucial pathway for acetate (one of the short-chain fatty acids [SCFAs]) production.[25] Among 23 microbial genera that were associated with AIEC presence, 21 AIEC were positively associated with these nine functional pathways (Figure 4B), suggesting that the genera depleted in AIEC-positive CD patients may contribute to the reduced functional pathways.

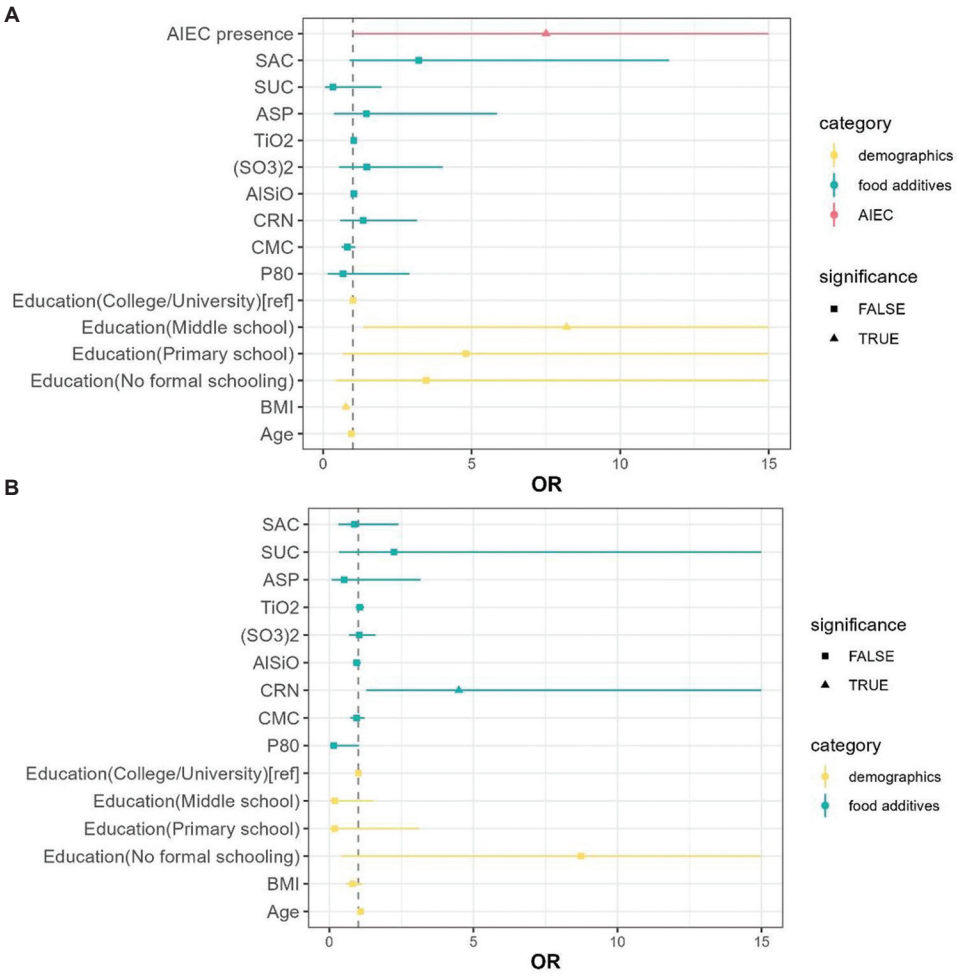

Figure 1. The association between AIEC presence, CD risk, and food additives exposure in the rural cohort. (A) Logistic regression analysis showing factors associated with CD risk. Predictors are grouped into three categories: AIEC presence (pink), demographic variables (yellow), and food additives (blue). Odds ratios with 95% confidence intervals are shown. Triangles indicate statistically significant associations ($p<0.05$), and squares indicate non-significant results. (B) Factors associated with AIEC presence in CD patients from the same rural cohort. Food additives and demographic variables are assessed for their contribution to AIEC presence.

Abbreviations: AIEC: Adherent-invasive *Escherichia coli*; AlSiO: Aluminum silicate; ASP: Aspartame; CD: Crohn's disease; CMC: Carboxymethylcellulose; CRN: Carrageenan; P80: Polysorbate-80; SAC: Saccharine; $(SO_3)_2$: Sulfite; SUC: Sucralose; TiO_2: Titanium dioxide.

4. Discussion

Our study provides the first human population-based evidence linking dietary factors to AIEC prevalence in CD patients. We demonstrate that AIEC is present in CD patients residing in a rural area, with the prevalence lower than that in urban areas. We found that urbanization was associated with increased AIEC prevalence, and that AIEC presence was significantly associated with CD risk and carrageenan intake. These results suggest that dietary emulsifiers may promote AIEC colonization and contribute to CD pathogenesis.

Our study revealed a significant association between AIEC and CD risk after adjusting for education level and food additives. This is consistent with a positive correlation between AIEC and CD reported in a meta-analysis.[26] We found that nearly half of AIEC-positive CD patients had lower education levels compared to AIEC-negative patients. AIEC-positive CD patients with low education levels consumed higher amounts of food additives, specifically carrageenan, than AIEC-negative CD patients. It is highly possible that subjects with low education levels tend to choose foods containing additives than those with high education levels.[27]

Our findings indicate a positive association between urbanization and AIEC prevalence, which may be influenced by accompanying changes in diet and lifestyle. Prior studies from our team reported that the dietary habits differed significantly between people living in Yunnan and

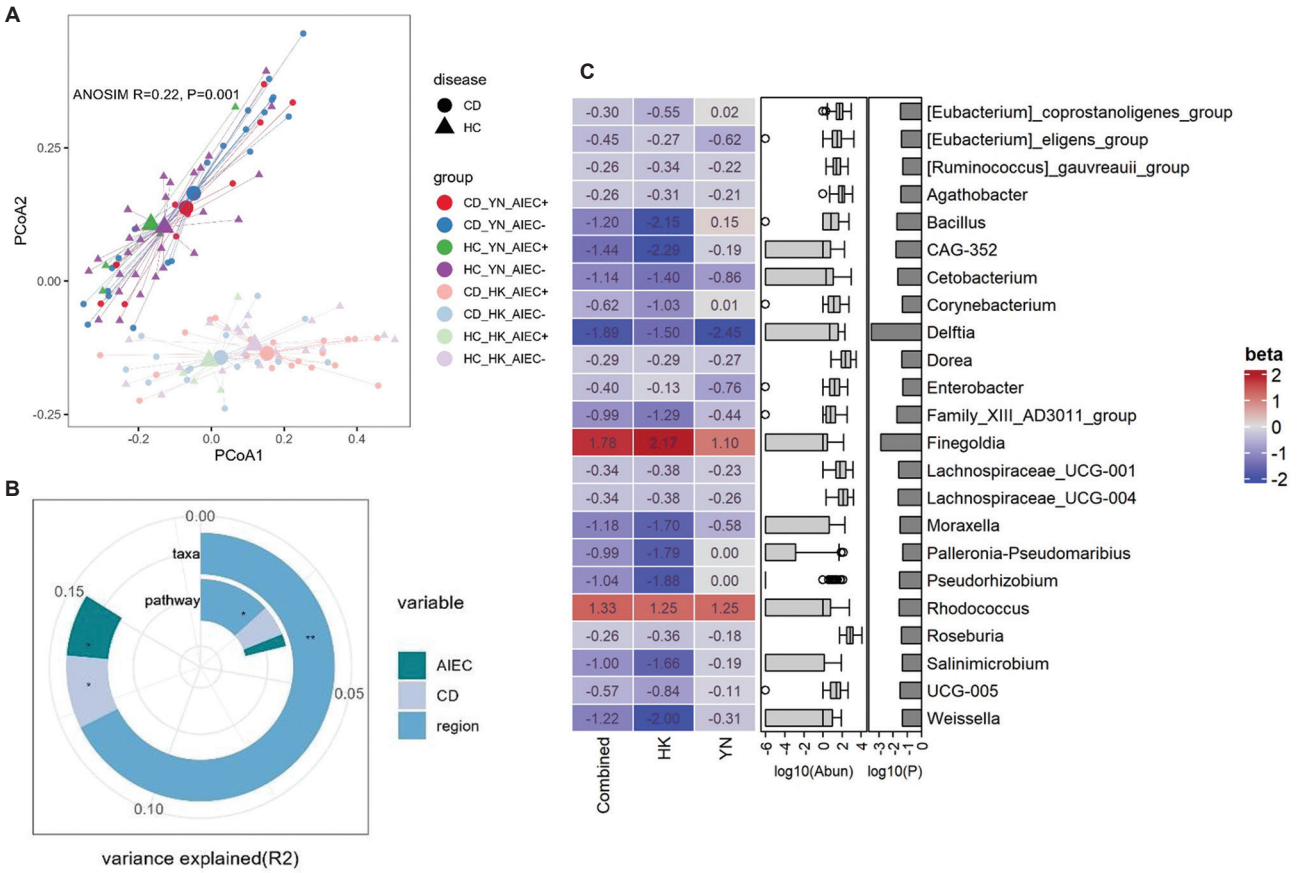

Figure 2. The effect of AIEC on mucosal microbiome composition across rural and urban cohorts. (A) Principal coordinates analysis based on Bray–Curtis distance, comparing microbiome profiles between AIEC-positive and AIEC-negative individuals across CD patients and HCs. The microbiome composition difference between groups was identified by the analysis of similarities test. (B) Permutational analysis of variance (PERMANOVA) showing the proportion of variation in microbiome composition explained by AIEC presence, disease status (CD vs. HC), and geographic region (Hong Kong vs. Yunnan). *p<0.05, **p<0.001. (C) Differentially abundant microbial genera associated with AIEC presence. Heatmap displays the beta coefficients of genera in combined CD patients were calculated using the linear mixed model with the formula ($\log_{10}$(taxa abundance) ~ AIEC presence + (1|region)). The beta coefficients of genera in each cohort were calculated using the linear regression model with the formula ($\log_{10}$(taxa abundance) ~ AIEC presence). Boxplots show the $\log_{10}$-transformed abundance of each genus. Boxplots show p-values with the $\log_{10}$ transformation identified from the linear mixed model.

Abbreviations: AIEC: Adherent-invasive *Escherichia coli*; CD: Crohn's disease; HC: Healthy control; HK: Hong Kong; YN: Yunnan.

Hong Kong, with work stress and dietary habits as the most important factors in explaining the gut mycobiome and virome variation.[28,29] Although Yunnan's vegetable- and mushroom-rich diet likely reduces additive exposure, the persistent association between AIEC and CD, similar to Western populations, suggests that diet and urbanization may not be the only factors affecting the association between AIEC and CD. Absence of dietary fiber was shown to promote AIEC colonization in mice.[30] However, no significant differences in AIEC prevalence and AIEC-associated virulence genes were found between omnivores and vegans consuming high-fiber diets in a human study.[31] Another possible factor was antibiotic usage, as our previous studies reported a higher prevalence of antibiotic-resistant genes in Hong Kong AIEC strains versus France AIEC strains.[8,32] Nevertheless, we did observe lower AIEC prevalence in Yunnan, compared to Hong Kong and France. These findings reinforce that while multiple environmental factors may influence AIEC epidemiology, dietary emulsifiers associated with urbanized lifestyles appear to play a prominent role.

Carrageenan can impact gut health in many ways. The degradation product of carrageenan, poligeenan, can be produced during gastric digestion of carrageenan, triggering the release of inflammatory cytokines.[33] Carrageenan can activate toll-like receptor-4 and stimulate the production of IL-6 in the immune response in mice, further exacerbating gut inflammation.[34] Furthermore, piglets receiving both carrageenan and AIEC infusion exhibited higher fecal

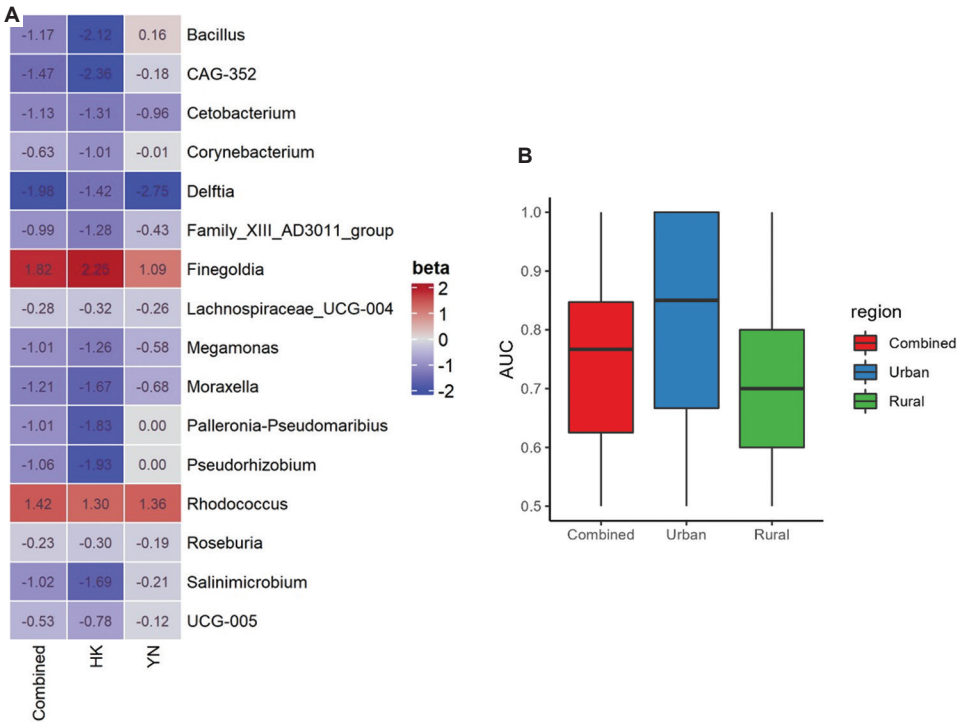

Figure 3. Sensitivity analysis and classification modeling of AIEC presence in CD patients. (A) Heatmap of differentially abundant mucosal microbial genera associated with AIEC presence, after adjusting for batch effect using the MMUPHin approach. For the combined cohort, the beta coefficients of genera based on adjusted data were calculated using a linear mixed model with the formula ($\log_{10}$(taxa abundance) ~ AIEC presence + (1|region)). The beta coefficients of genera in each cohort were calculated using the linear regression model with the formula ($\log_{10}$(taxa abundance) ~ AIEC presence). (B) Performance of a random forest model trained to predict AIEC presence in CD using the selected 23 microbial taxa. Five-fold cross-validation was performed to distinguish AIEC presence in CD patients. The model was validated on the combined CD patients (red), and CD patients from the urban (blue), and rural cohorts (green) in each fold of cross-validation.
Abbreviations: AIEC: Adherent-invasive *Escherichia coli*; CD: Crohn's disease; HK: Hong Kong; YN: Yunnan.

score and IL-6 levels in the ileum compared to the group receiving carrageenan alone.[35] Our findings align with preclinical evidence that: (i) Emulsifiers in mice disrupts the balance between cell proliferation and apoptosis, thereby altering the intestinal microenvironment;[36] (ii) emulsifiers have no significant effect on germ-free mice, indicating that their impact is dependent on the gut microbiome;[16] (iii) emulsifiers can enhance the expression of virulent genes in AIEC, promote its penetration of the mucus layer, and subsequently induce intestinal inflammation and increase disease susceptibility.[17] Taken together, these findings support the hypothesis that emulsifiers influence AIEC pathogenicity through microbiota-mediated mechanisms that compromise intestinal homeostasis. Our study provided the first human evidence that there is an association between dietary emulsifiers and AIEC presence in CD patients. However, given the observational nature of our study, we cannot infer a causal relationship between carrageenan intake and AIEC colonization or inflammation.

We identified several co-differentially abundant microbial genera that were associated with the presence of AIEC. Among them, *Finegoldia* has been associated with CD relapses, inducing gut inflammation through the interaction with human neutrophils,[37,] and *Rhodococcus* was reported to be increased in patients with ulcerative colitis.[38] Several anti-inflammatory SCFAs producers, including *Roseburia*, *Dorea*, *and* Agathobacter, were significantly reduced in AIEC-positive CD patients. One of the SCFAs, butyrate, was known to protect against AIEC-induced mitochondrial dysfunction to reduce gut inflammation.[39] In addition, we found a depletion of *Ruminococcus gauvreauii* group in AIEC-positive CD patients, and the deficiency of *Ruminococcus* was also reported in the recurrent CD patients with AIEC colonization.[40] In the functional analysis, several anti-inflammatory functional pathways were reduced in AIEC-positive CD patients, such as the L-glutamate and L-glutamine biosynthesis, acetylene degradation, and chondroitin sulfate degradation I. Glutamine was shown to alleviate inflammation in CD patients by maintaining the integrity of the intestinal mucosa through increasing the level of heat shock proteins and reducing the expression

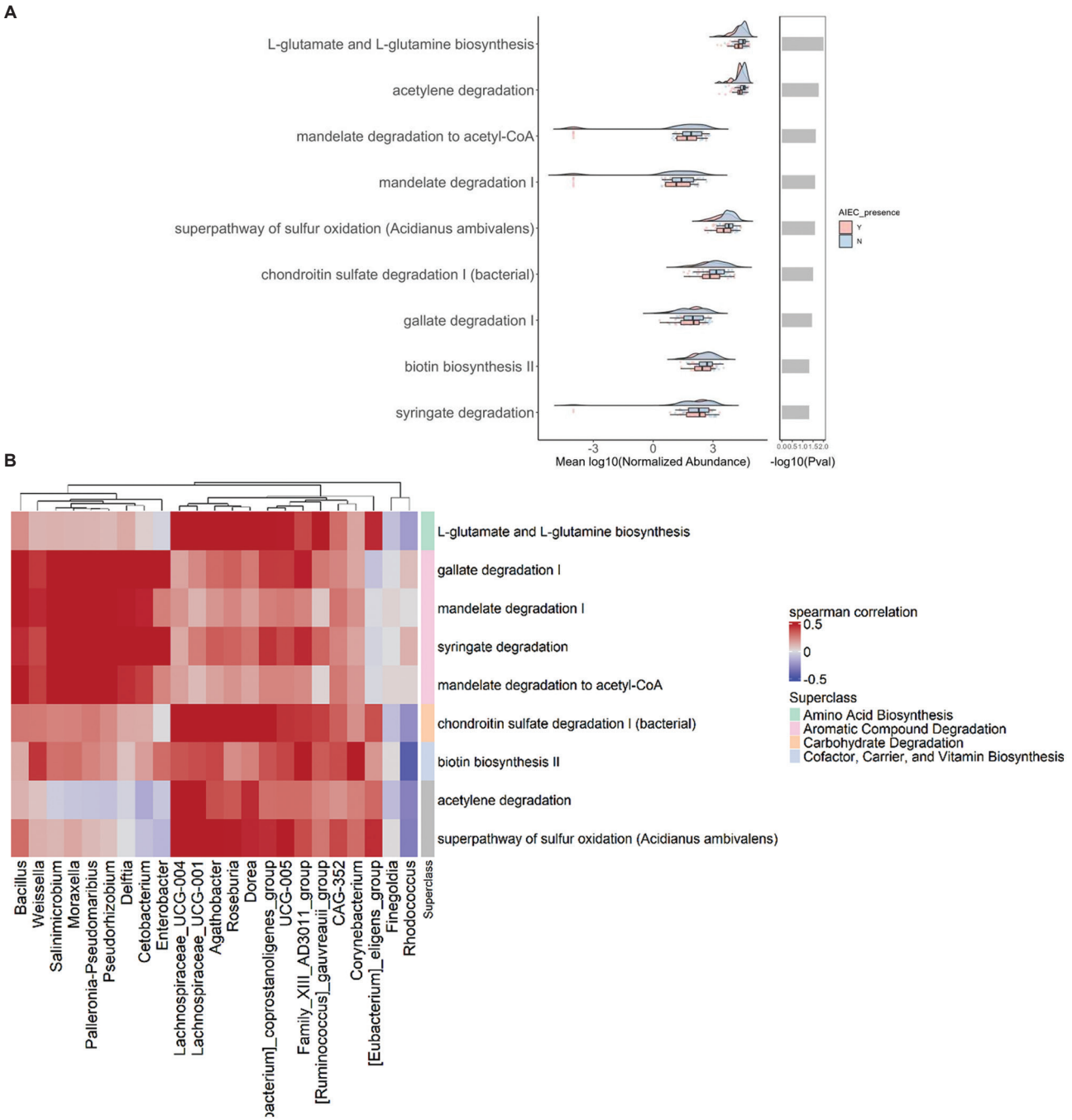

Figure 4. Function analysis of AIEC-related pathways in CD patients. (A) Differential functional pathways associated with AIEC presence in CD patients, as identified by a linear mixed model. The left panel displays boxplots showing $\log_{10}$-transformed normalized abundance of each pathway, stratified by AIEC-positive (pink) and AIEC-negative (blue) CD patients. Overlaid density plots denote the abundance distribution of functional pathways in each group. The right panel shows the corresponding -$\log_{10}$ transformed p-value from the linear mixed model, indicating the statistical significance of group differences. (B) Spearman correlation heatmap showing associations between significantly altered microbial genera and functional pathways for AIEC presence in CD patients. The color-coded bar on the right denotes the Superclass level of each functional pathway, including amino acid biosynthesis, aromatic compound degradation, cofactor/carrier/vitamin biosynthesis, and others.
Abbreviations: AIEC: Adherent-invasive *Escherichia coli*; CD: Crohn's disease.

of inflammation-related transcription factors such as Nuclear factor kappa B.[41] Acetylene degradation is an important pathway for producing acetate—which latter was known to reduce intestinal inflammation.[42] It has

been reported that chondroitin sulfate could reduce IBD relapse in a prospective follow-up study.[43] Altogether, these microbiome alterations may reflect a more dysbiotic gut environment in AIEC-positive individuals.

Given the observed associations among carrageenan intake, AIEC presence, and increased CD risk, our findings suggest that a potential dietary modification strategy, particularly those aimed at reducing the intake of emulsifiers such as carrageenan, may help lower the risk of AIEC colonization and mitigate gut dysbiosis. Promoting nutritional education and reducing the consumption of processed foods, especially among individuals with lower education levels, could be valuable in reducing the risk of CD. Although our study cannot establish causal relationships, these strategies may serve as practical interventions to support the management of high-risk populations. Future interventional studies are warranted to evaluate the effectiveness of these approaches in reducing AIEC colonization and improving clinical outcomes.

This study had several limitations. First of all, dietary and environmental data were only available for the rural subjects. Second, since this is an observational study, the association between carrageenan intake with AIEC presence and the gut microbiome is descriptive, and the causal relationship could not be proven. Further studies using prospective, well-characterized cohorts and mechanistic animal studies or *in vitro* experiments are needed to confirm the findings and make causal inferences.

5. Conclusion

Our findings suggest that reducing dietary emulsifiers, particularly carrageenan, might mitigate AIEC colonization risk—especially among high-risk groups such as less-educated populations who may consume more processed foods in rural areas. This hypothesis is supported by a clinical trial demonstrating that carrageenan exposure increased relapse risk and IL-6 levels in ulcerative colitis patients.[44] Together with our observation of urban-rural differences in AIEC prevalence, these results highlight how both dietary factors and urbanization may shape gut microbiome composition and CD risk. Most importantly, we provide population-based evidence that AIEC should be considered a global risk factor for CD pathogenesis. Future intervention studies should evaluate whether emulsifier-restricted diets can reduce AIEC colonization and improve clinical outcomes in CD patients.

Acknowledgments

We thank Winnie Lin for her assistance in processing the food questionnaire data to estimate food additive content. We would also like to extend our thanks to Alan Chu and Natalie Chen for their guidance during the experimental phase of this project.

Funding

The study was funded by The French National Research Agency (ANR)/Research Grants Council (RGC) Joint Research Scheme (A-CUHK402/17); National Natural Science Foundation of China (82060107, 82160113); "Xingdian Talents" Support Project of Yunnan Province, China; and Applied Basic Research Projects of Yunnan Province, China (202201AW070019, 2019FE001(-039)). Authors affiliated with MagIC are partially supported by InnoHK, the Government of Hong Kong, Special Administrative Region of the People's Republic of China.

Conflict of interest

Francis Ka Leung Chan is a Board Member of CUHK Medical Centre. He is a co-founder, non-executive Board Chairman, non-executive scientific advisor, and shareholder of GenieBiome Ltd. He receives patent royalties through his affiliated institutions. He has received fees as an advisor and honoraria as a speaker for Eisai Co. Ltd., AstraZeneca, Pfizer Inc., Takeda Pharmaceutical Co., and Takeda (China) Holdings Co. Ltd. Siew Chien Ng has served as an advisory board member for Pfizer, Ferring, Janssen, and Abbvie and received honoraria as a speaker for Ferring, Tillotts, Menarini, Janssen, Abbvie, and Takeda. Siew Chien Ng has received research grants through her affiliated institutions from Olympus, Ferring, and AbbVie. Siew Chien Ng is a founder member, non-executive director, non-executive scientific advisor, and shareholder of GenieBiome Ltd. Siew Chien Ng receives patent royalties through her affiliated institutions. RIL is a Medical Affairs Manager of GenieBiome Ltd. Francis Ka Leung Chan, Siew Chien Ng, Zhilu Xu, and RIL are named inventors of patent applications held by the CUHK and MagIC that cover the therapeutic and diagnostic use of microbiome and receive patent royalties through their affiliated institutions.

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Ethical approval and consent to participate

The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Kunming Medical School (reference no. 2017.L.15-1). The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Consent for publication

Participants consented to the publication of their data.

Availability of data

The 16S rRNA sequencing data of the rural and urban cohorts have been deposited in the SRA database under the accession numbers: PRJNA986689 and PRJNA641238.

References

1. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet*. 2017;389(10080):1741-1755.

   doi: 10.1016/S0140-6736(16)31711-1

2. Pascal V, Pozuelo M, Borruel N, *et al*. A microbial signature for Crohn's disease. *Gut*. 2017;66(5):813-822.

   doi: 10.1136/gutjnl-2016-313235

3. Wright EK, Kamm MA, Teo SM, Inouye M, Wagner J, Kirkwood CD. Recent advances in characterizing the gastrointestinal microbiome in Crohn's disease: A systematic review. *Inflamm Bowel Dis*. 2015;21(6):1219-1228.

   doi: 10.1097/MIB.0000000000000382

4. Palmela C, Chevarin C, Xu Z, *et al*. Adherent-invasive *Escherichia coli* in inflammatory bowel disease. *Gut*. 2018;67(3):574-587.

   doi: 10.1136/gutjnl-2017-314903

5. Barnich N, Darfeuille-Michaud A. Abnormal CEACAM6 expression in Crohn disease patients favors gut colonization and inflammation by adherent-invasive *E. coli*. *Virulence*. 2010;1(4):281-282.

   doi: 10.4161/viru.1.4.11510

6. Barnich N, Carvalho FA, Glasser AL, *et al*. CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. *J Clin Invest*. 2007;117(6):1566-1574.

   doi: 10.1172/jci30504

7. Glasser AL, Boudeau J, Barnich N, Perruchot MH, Colombel JF, Darfeuille-Michaud A. Adherent invasive *Escherichia coli* strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. *Infect Immun*. 2001;69(9):5529-5537.

   doi: 10.1128/iai.69.9.5529-5537.2001

8. Zhilu X, Xiangqian D, Keli Y, *et al*. Association of adherent-invasive *Escherichia coli* with severe gut mucosal dysbiosis in Hong Kong Chinese population with Crohn's disease. *Gut Microbes*. 2021;13(1):1994833.

   doi: 10.1080/19490976.2021.1994833

9. Viladomiu M, Metz ML, Lima SF, *et al*. Adherent-invasive *E. coli* metabolism of propanediol in Crohn's disease regulates phagocytes to drive intestinal inflammation. *Cell Host Microbe*. 2021;29(4):607-619.e8.

   doi: 10.1016/j.chom.2021.01.002

10. Kaplan GG, Ng SC. Understanding and preventing the global increase of inflammatory bowel disease. *Gastroenterology*. 2017;152(2):313-321.e2.

    doi: 10.1053/j.gastro.2016.10.020

11. Trakman GL, Lin WYY, Hamilton AL, *et al*. Processed food as a risk factor for the development and perpetuation of crohn's disease-the ENIGMA study. *Nutrients*. 2022;14(17):3627.

    doi: 10.3390/nu14173627

12. Mark-Christensen A, Lange A, Erichsen R, *et al*. Early-life exposure to antibiotics and risk for crohn's disease: A nationwide danish birth cohort study. *Inflamm Bowel Dis*. 2022;28(3):415-422.

    doi: 10.1093/ibd/izab085

13. Anyane-Yeboa A, Quezada S, Rubin DT, Balzora S. The Impact of the social determinants of health on disparities in inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2022;20(11):2427-2434.

    doi: 10.1016/j.cgh.2022.03.011

14. Zuo T, Kamm MA, Colombel JF, Ng SC. Urbanization and the gut microbiota in health and inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol*. 2018;15(7):440-452.

    doi: 10.1038/s41575-018-0003-z

15. Benchimol EI, Mack DR, Guttmann A, *et al*. Inflammatory bowel disease in immigrants to Canada and their children: A population-based cohort study. *Am J Gastroenterol*. 2015;110(4):553-563.

    doi: 10.1038/ajg.2015.52

16. Chassaing B, Koren O, Goodrich JK, *et al*. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature*. 2015;519(7541):92-96.

    doi: 10.1038/nature14232

17. Viennois E, Bretin A, Dube PE, *et al*. Dietary Emulsifiers directly impact adherent-invasive *E. coli* Gene expression to drive chronic intestinal inflammation. *Cell Rep*. 2020;33(1):108229.

    doi: 10.1016/j.celrep.2020.108229

18. Naimi S, Viennois E, Gewirtz AT, Chassaing B. Direct

impact of commonly used dietary emulsifiers on human gut microbiota. *Microbiome*. 2021;9(1):66.

doi: 10.1186/s40168-020-00996-6

19. CEIC. *China Population: Census: Yunnan: Kunming*. CEIC. Available from: https://www.ceicdata.com [Lastb accessed on 2023 May 01].

20. Trakman GL, Lin W, Wilson-O'Brien AL, *et al*. Development and Validation of surveys to estimate food additive intake. *Nutrients*. 2020;12(3):812.

doi: 10.3390/nu12030812

21. CEIC. *Hong Kong SAR, China Population*. CEIC. Available from: https://www.ceicdata.com [Last accessed on 2023 May 01].

22. Ma S, Shungin D, Mallick H, *et al*. Population structure discovery in meta-analyzed microbial communities and inflammatory bowel disease using MMUPHin. *Genome Biol*. 2022;23(1):208.

doi: 10.1186/s13059-022-02753-4

23. Levy PY, Fenollar F, Stein A, Borrione F, Raoult D. Finegoldia magna: A forgotten pathogen in prosthetic joint infection rediscovered by molecular biology. *Clin Infect Dis*. 2009;49(8):1244-1247.

doi: 10.1086/605672

24. Stewart A, Sowden D, Caffery M, Bint M, Broom J. *Rhodococcus equi* infection: A diverse spectrum of disease. *IDCases*. 2019;15:e00487.

doi: 10.1016/j.idcr.2019.e00487

25. Akob DM, Sutton JM, Fierst JL, *et al.* Acetylenotrophy: A hidden but ubiquitous microbial metabolism? *FEMS Microbiol Ecol*. 2018;94(8):fiy103.

doi: 10.1093/femsec/fiy103

26. Nadalian B, Yadegar A, Houri H, *et al.* Prevalence of the pathobiont adherent-invasive *Escherichia coli* and inflammatory bowel disease: A systematic review and meta-analysis. *J Gastroenterol Hepatol*. 2021;36(4):852-863.

doi: 10.1111/jgh.15260

27. Kayışoğlu S, Çoşkun F. Determinatıon of the level of knowledge of consumers about food additives. *IOSR J Environ Sci Toxicol Food Technol*. 2016;10:53-56.

28. Sun Y, Zuo T, Cheung CP, *et al.* Population-Level configurations of gut mycobiome across 6 ethnicities in urban and rural China. *Gastroenterology*. 2021;160(1):272-286.e11.

doi: 10.1053/j.gastro.2020.09.014

29. Zuo T, Sun Y, Wan Y, *et al*. Human-Gut-DNA virome variations across geography, ethnicity, and urbanization. *Cell Host Microbe*. 2020;28(5):741-751.e4.

doi: 10.1016/j.chom.2020.08.005

30. Lau TC, Fiebig-Comyn AA, Shaler CR, McPhee JB, Coombes BK, Schertzer JD. Low dietary fiber promotes enteric expansion of a Crohn's disease-associated pathobiont independent of obesity. *Am J Physiol Endocrinol Metab*. 2021;321(3):E338-E350.

doi: 10.1152/ajpendo.00134.2021

31. Veca R, O'Dea C, Burke J, Hatje E, Kuballa A, Katouli M. A Comparative study of the adherent-invasive *Escherichia coli* Population and gut microbiota of healthy vegans versus omnivores. *Microorganisms*. 2020;8(8):1165.

doi: 10.3390/microorganisms8081165

32. Chevarin C, Xu Z, Martin L, *et al*. Comparison of Crohn's disease-associated adherent-invasive *Escherichia coli* (AIEC) from France and Hong Kong: Results from the Pacific study. *Gut Microbes*. 2024;16(1):2431645.

doi: 10.1080/19490976.2024.2431645

33. Borsani B, De Santis R, Perico V, *et al*. The role of carrageenan in inflammatory bowel diseases and allergic reactions: Where do we stand? *Nutrients*. 2021;13(10):3402.

doi: 10.3390/nu13103402

34. Tsuji RF, Hoshino K, Noro Y, *et al*. Suppression of allergic reaction by lambda-carrageenan: Toll-like receptor 4/MyD88-dependent and -independent modulation of immunity. *Clin Exp Allergy*. 2003;33(2):249-258.

doi: 10.1046/j.1365-2222.2003.01575.x

35. Munyaka PM, Sepehri S, Ghia JE, Khafipour E. Carrageenan gum and adherent invasive *Escherichia coli* in a piglet model of inflammatory bowel disease: Impact on Intestinal mucosa-associated microbiota. *Front Microbiol*. 2016;7:462.

doi: 10.3389/fmicb.2016.00462

36. Viennois E, Merlin D, Gewirtz AT, Chassaing B. Dietary emulsifier-induced low-grade inflammation promotes colon carcinogenesis. *Cancer Res*. 2017;77(1):27-40.

doi: 10.1158/0008-5472.CAN-16-1359

37. Buffet-Bataillon S, Bouguen G, Fleury F, Cattoir V, Le Cunff Y. Gut microbiota analysis for prediction of clinical relapse in Crohn's disease. *Sci Rep.* 2022;12(1):19929.

doi: 10.1038/s41598-022-23757-x

38. Sasaki M, Klapproth JM. The role of bacteria in the pathogenesis of ulcerative colitis. *J Signal Transduct*. 2012;2012:704953.

doi: 10.1155/2012/704953

39. Hamed S. *Butyrate Alleviates Crohn's Diseases' Adherent Invasive E coli-Induced Mitochondrial Dysfunction in Intestinal Epithelium.* (Master's thesis, Calgary, Canada: University of Calgary). 2022.

40. Buisson A, Sokol H, Hammoudi N, *et al*. Role of adherent and invasive *Escherichia coli* in Crohn's disease: Lessons from

the postoperative recurrence model. *Gut*. 2023;72(1):39-48.

doi: 10.1136/gutjnl-2021-325971

41. Deters BJ, Saleem M. The role of glutamine in supporting gut health and neuropsychiatric factors. *Food Sci Hum Wellness*. 2021;10(2):149-154.

doi: 10.1016/j.fshw.2021.02.003

42. Parada Venegas D, De la Fuente MK, Landskron G, *et al*. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol*. 2019;10:277.

doi: 10.3389/fimmu.2019.00277

43. Linares PM, Chaparro M, Algaba A, *et al*. Effect of chondroitin sulphate on pro-inflammatory mediators and disease activity in patients with inflammatory bowel disease. *Digestion*. 2015;92(4):203-210.

doi: 10.1159/000439522

44. Bhattacharyya S, Shumard T, Xie H, *et al.* A randomized trial of the effects of the no-carrageenan diet on ulcerative colitis disease activity. *Nutr Healthy Aging*. 2017;4(2):181-192.

doi: 10.3233/NHA-170023