Composition of the intestinal microbiota in extended-spectrum β-lactamase-producing *Enterobacteriaceae* carriers and non-carriers in Thailand

Pipat Piewngam [a,b], Mariam Quiñones [c], Witoon Thirakittiwatthana [a], Thitiya Yungyuen [a], Michael Otto [b,*], Pattarachai Kiratisin [a,*]

[a] *Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[b] *Pathogen Molecular Genetics Section, Laboratory of Bacteriology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA*
[c] *Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure & Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA*

ARTICLE INFO

ABSTRACT

There is increasing recognition that the intestinal microbiota govern human well-being and prevent diseases. Intestinal colonization by antibiotic-resistant pathogens, however, can lead to the spread of resistance as well as serious infections. Extended-spectrum β-lactamase–producing *Enterobacteriaceae* (ESBL-E) represent particularly dangerous pathogens, which are known to asymptomatically colonize the intestinal tract in the community. Here, we performed a 16S rRNA metagenomics sequence analysis to analyse differences in the microbiota composition between ESBL-E carriers and non-carriers in Thailand, where ESBL-E carriage rates are notoriously high. The most notable difference detected was that the phylum *Bacteroidetes*, and in particular, the species *Bacteroides uniformis,* were significantly more abundant in ESBL-E non-carriers than carriers. The Shannon diversity index in non-carriers (5.10 ± 0.69) was also lower than that in ESBL-E carriers (5.39 ± 0.48) without statistical significance (P=0.13). The overall beta diversity difference of the intestinal microbiota of ESBL-E carriers as compared to non-carriers was statistically significant (Adonis on weighted unifrac: R^2=0.14, P=0.005). Furthermore, ESBL-E carriage was significantly lower in farmers than in those with other occupations. Our findings suggest that a dynamic interaction exists between microbiota diversity and ESBL-E carriage, which is possibly driven by dietary composition and may be exploited using probiotic approaches to control the spread of ESBL-E.

© 2018 Elsevier B.V. and International Society of Chemotherapy. All rights reserved.

1. Introduction

The human gastrointestinal tract is colonized by numerous bacteria. Some of them are opportunistic pathogens and may carry drug resistance genes [1]. This may lead to the rapid spread of drug-resistant pathogenic bacteria in the community. However, the prevalence of this worrisome intestinal carriage of drug-resistant bacteria is not well understood. Extended-spectrum β-lactamase–producing *Enterobacteriaceae* (ESBL-E) represent a particularly dangerous threat to public health, because these bacteria are resistant to most oxyimino-cephalosporins (e.g., ceftriaxone, cefotaxime and ceftazidime). *Escherichia coli* and *Klebsiella pneumoniae* are among the most frequent ESBL-producing isolates and resistance rates in these potentially highly dangerous pathogens are on the rise, in particular in Asia [2,3]. Many recent studies have reported that the healthy population is asymptomatically colonized with ESBL-E, with colonization frequencies ranging from low single-digit percentage rates, for example in the USA and Western Europe [4–6], to much higher frequencies, for example ∼ 34% in Turkey [7], ∼ 30% in Shanghai, China [8], and over 50% in some African and Asian countries including Thailand [9–11]. Due to the high ESBL-E colonization rates in those latter countries, travel to (sub)tropical regions has been recognized as a risk for acquiring ESBL-E colonization [12].

The intestinal microbiota provide an important host defense mechanism by inhibiting the colonization of potentially pathogenic microorganisms either via depletion of nutrients or production of inhibitory substances or conditions that can inhibit the growth of the invading pathogens. For example, Pultz et al. have shown that

* Corresponding authors. Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkok-Noi, Bangkok 10700, Thailand. Tel.: +66 2 419 7058; fax: +66 2 411 3106.

E-mail address: pattarachai.kir@mahidol.ac.th (P. Kiratisin).

colonization with vancomycin-resistant enterococci (VRE) was inhibited by anaerobic microbiota in the colon, which by depleting nutrients within cecal contents limit the association of VRE with the mucus layer [13]. This phenomenon, termed 'colonization resistance,' has the potential to be applied in a therapeutic probiotic manner to the prevention of overgrowth of indigenous pathogens and the inhibition of exogenously introduced foreign organisms [14].

Despite the increasing recognition of the role of the intestinal microbiota in controlling the colonization of specific pathogenic and antibiotic-resistant bacteria, there has been almost no effort yet to investigate this role regarding colonization by ESBL-E. To our knowledge, only one study has addressed this question. That study analysed the population of a remote and isolated village in French Guyana with a very low ESBL-E colonization rate and found increased prevalence in non-ESBL-E carriers of four non-related genera, two of which belong to the Clostridiales [15].

Here, to address ESBL-E colonization as a global public health risk, we analysed the microbiome of ESBL-E carriers versus non-carriers in Thailand, which is known to have high ESBL-E colonization rates. Furthermore, we performed the study on several rural community populations from the entire country to rule out an impact of potentially specific conditions endemic to geographically limited areas.

2. Materials and Methods

2.1. Fecal sample collection

Fecal samples of 200 healthy volunteers living in rural areas in the provinces of Chiang-Mai, Chaiyaphum, Ratchaburi and Surat-Thani, Thailand were collected for ESBL-E screening. The four provinces were selected to represent geographically different regions of the country (Fig. 1). All participants were over 20 years old, gave informed written consent to participate in the study (ethical approval no. Si773/2015, Siriraj Hospital), and had no history of gastrointestinal disease. They had neither received antibacterial treatment nor were hospitalized within the last 3 months prior to participating in this study.

2.2. ESBL–producing Enterobacteriaceae screening

Fecal samples were plated on both MacConkey agar supplemented with 1 mg/L cefotaxime (CTX-MacConkey) and MacConkey agar supplemented with 1 mg/L ceftazidime (CTZ-MacConkey), and then incubated at 37°C for 16–18 h. Bacterial colonies grown from both plates were confirmed for ESBL production by a disk diffusion method using cefotaxime and ceftazidime with and without clavulanic acid according to CLSI guidelines [16]. ESBL-producing isolates were then identified on the species level using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis on a Bruker Microflex LT/SH instrument according to the manufacturer's protocol.

2.3. DNA extraction and 16s rRNA gene sequencing

Genomic DNA was extracted using QIAamp DNA stool Minikits (Qiagen) according to the manufacturer's instructions, amplifying the V4 region of the 16S rRNA genes. The DNA was quantified and processed for 16s rRNA paired-end sequencing, which was performed by Illumina (San Diego, CA, USA) using the Illumina MiSeq system as previously described [17].

2.4. Taxonomic diversity and statistical analysis

All MiSeq sequences were processed using the 16S QIIME Paired-End pipeline implemented in the Nephele platform of the National Institute of Allergy and Infectious Diseases (NIAID) (release 1.6, which uses QIIME 1.9.1) [18]. Operational Taxonomic Units (OTUs) were picked with QIIME's uclust-based [19] open-reference OTU picking protocol [20] and the taxonomic assignment was performed against the Greengenes 13_8 reference sequence set [21] at 99% similarity. Alpha diversity was calculated using Chao1 and Shannon [22] and compared across groups with a nonparametric *t* test with 999 permutations. Beta diversity calculations were performed with QIIME's implementations of weighted and unweighted UniFrac [23,24] using exactly 36,146 randomly selected sequences per sample. In addition, principle coordinate analysis (PCoA) was performed to compare groups of samples based on unifrac distance. We evaluated statistical significance of sample groups by the adonis method. Comparisons of significantly different OTUs across sample groups were performed with negative binomial DESeq2 available via the Calypso web server [25].

Other statistical analysis was performed using GraphPadPrism version 7; all ranges show the standard deviation (SD).

2.6. LDA Effect Size analysis

We performed LDA Effect Size (LEfSe) analysis [26] to identify the biomarker bacterial taxa at different taxonomic levels found in the metagenomes predicted by PICRUSt. The LEfSe analysis was performed using the online Galaxy interface.

3. Results

3.1. Characterization of healthy volunteers

Of 200 Thai healthy volunteers living in four rural regions of Thailand, 108 (54%) were found to be ESBL-E carriers. In order to analyse differences in the microbiota composition between ESBL-E carriers and non-carriers in Thailand, 40 fecal samples were randomly selected from the volunteers and approximately equally represent each sampling region for 16s rRNA metagenomics sequence analysis. The median age of ESBL-E carriers was 45.22 years (range 26–60 years) and that of non-carriers was 59.72 years (range 20–85 years) (Table 1). There were no statistically significant differences in the analysed sociodemographic variables, except that the majority (63.6%) of ESBL-E carriers were general employees (P=0.0002) whereas the majority (72.2%) of non-carriers were farmers (P=0.0003). Furthermore, participants from the non-carrier group reported a significantly higher use of antibiotics without prescription (P=0.0248) in the 9 months preceding the study. However, according to the exclusion criteria of our study, they did not take antibiotics in the 3 months prior to the study.

3.2. Taxonomic composition of intestinal microbiota in ESBL-E carriers and non-carriers

A total of 8,396,814 non-chimeric reads were obtained with an average of 209,920 ± 64,458 per sample. Taxonomic composition of the intestinal microbiota was analysed using QIIME. Fig. 2A shows taxon abundance and sample composition. The phyla *Bacteroidetes* (46.94%) and *Firmicutes* (43.6%) were most abundant, followed by *Proteobacteria* (6.29%), *Actinobacteria* (2.25%), *Tenericutes* (0.28%) and *Fusobacteria* (0.1%). The taxa with a significant difference in abundance between ESBL-E carriers and non-carriers were then identified using the LEfSe algorithm (Fig. 2B), revealing that ESBL-E carriers had a significantly higher abundance of *Firmicutes* (P <0.05), while *Bacteroidetes* were significantly more abundant in non-carriers (P <0.05). The Negative Binomial DESeq2 test was used to identify significant differences between groups on the species level (Table 2). Abundance of *Bacteroides uniformis*
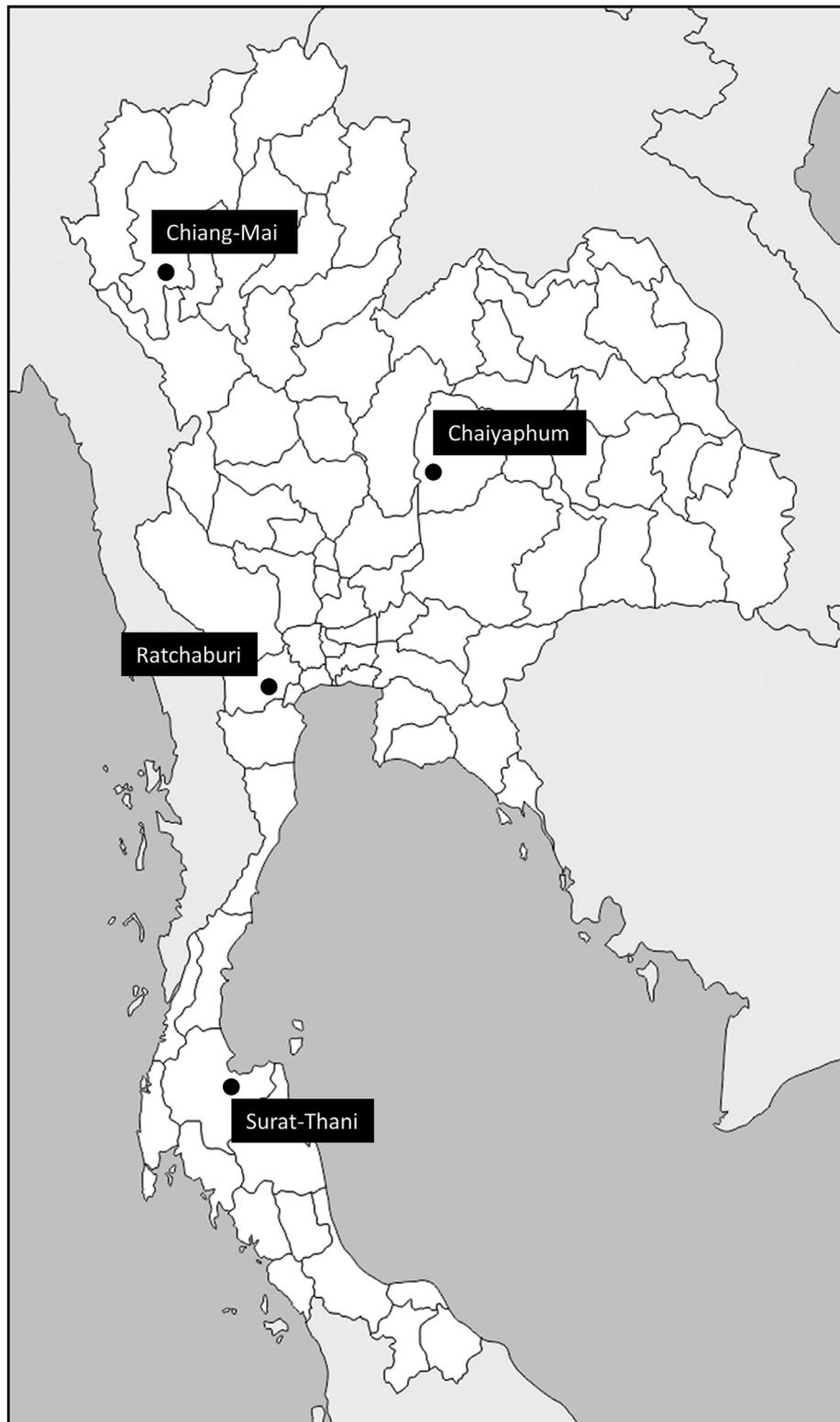
Fig. 1. Geographical zones (shown as provinces) of sample collection in Thailand.

was significantly higher in the non-carrier group, while *Coprococcus eutactus, Collinsella aerofaciens, Akkermansia muciniphila*, and *Pseudomonas fragi* showed higher abundance in the carrier group.

3.3. Comparison of the diversity of the intestinal microbiota between ESBL-E carriers and non-carriers

We performed a comparison of alpha diversity (i.e. the diversity within each group) between ESBL-E carriers and non-carriers.

Chao1 and Shannon diversity index values from all operational taxonomic units (OTU) data were used to quantify the bacterial diversity of each group (Fig. 3). The Chao1 species richness estimator value in non-carriers (2146.47 ± 273.10) was slightly lower than that in ESBL-E carriers (2213.64 ± 232.58), but the difference was not statistically significant (*P*=0.43). The Shannon diversity index in non-carriers (5.10 ± 0.69) was also lower than that in ESBL-E carriers (5.39 ± 0.48) without statistical significance (*P* = 0.13). In
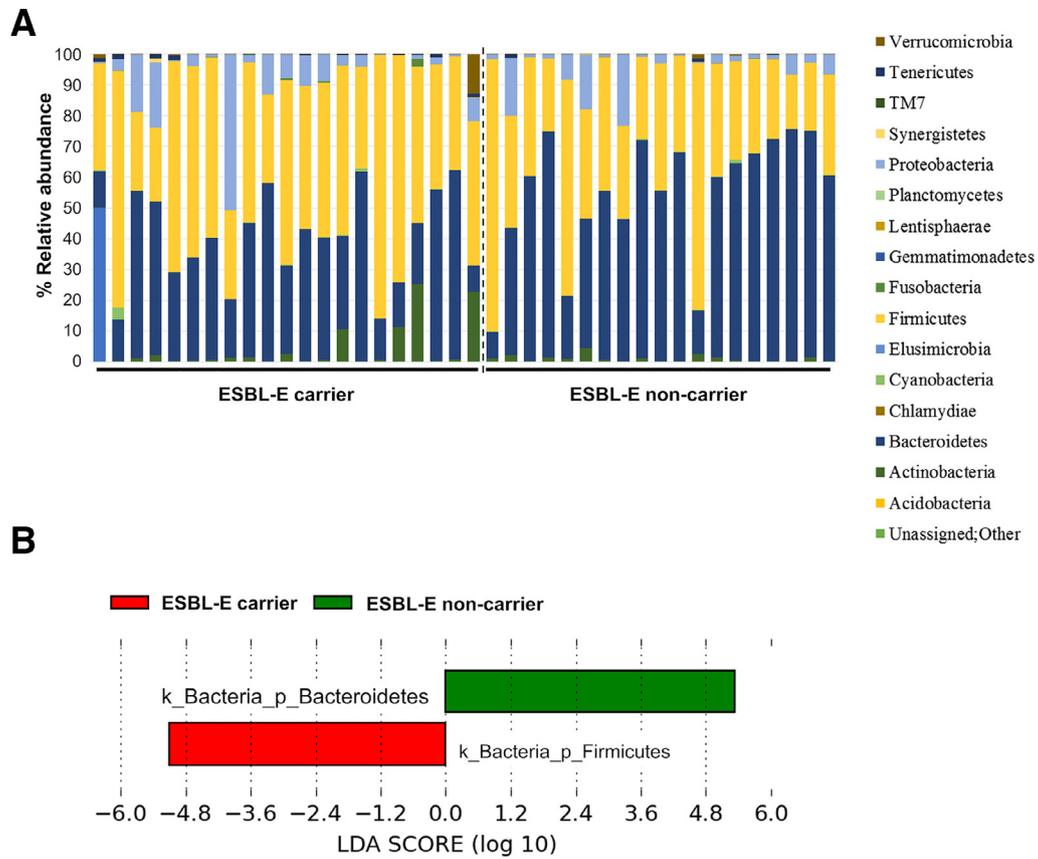
Fig. 2. Relative taxa abundance of the intestinal microbiota in ESBL-E carriers and non-carriers. (A) Taxa abundance was determined by 16S rRNA paired-end sequencing using the Illumina MiSeq system. (B) Taxa with a log LDA (linear discriminant analysis) score above 3.00. as determined using LEfSe.
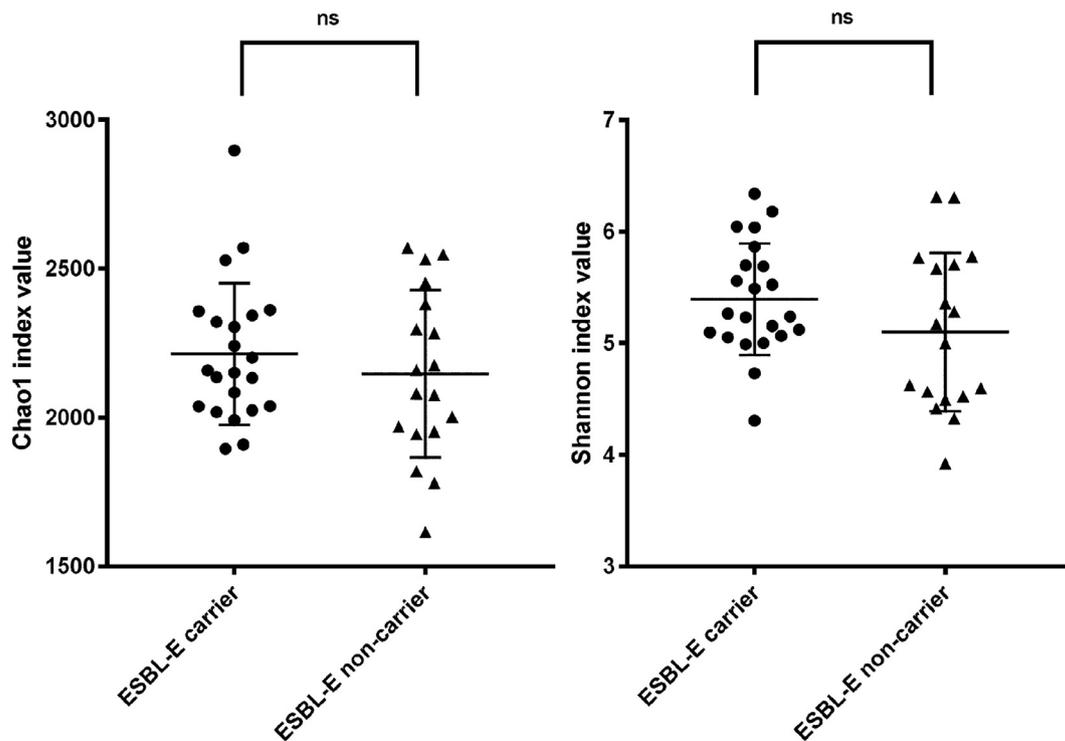
Fig. 3. Microbiome alpha diversity in ESBL-E carrier and non-carriers. Alpha diversity was calculated for species richness by the Chao1 and for both richness and evenness by the Shannon diversity index. Differences in Chao1 richness estimator values and Shannon index values were analysed using a nonparametric *t* test with 999 permutations. Data are presented as a scatter plot with mean and standard deviation (SD).

Table 1

Baseline characteristics between extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriers and non-carriers

Baseline characteristics	ESBL-E carriage status		
	Carrier (n=22)	Non-carrier (n=18)	P[a]
Sociodemographic data			
Age, mean ± SD (years)	45.22 ± 11.12	59.72 ± 20.21	[b]
Age, range (years)	26–60	20–85	[b]
Female gender	15	15	ns
Farmer	3	13	0.0003
General employee	14	1	0.0002
Grocer	2	3	ns
Unemployed	3	1	ns
Medical history[c]			
Used antibiotics without prescription	7	13	0.0248
Admitted to a hospital	4	1	ns
Stopped taking antibiotic early	16	17	ns

ns, not significant; SD, standard deviation.
[a] Results were analysed by Fisher's exact test.
[b] Statistical testing was not performed on matching variables.
[c] Medical history from >3 to 12 months prior to the study.

Table 2

Species with significantly differential abundance between extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriers and non-carriers

Taxa	ESBL-E carriage status (mean)[a]				
	Carrier	Non-carrier	P Bonferroni[b]	FDR[b]	P[c]
Coprococcus eutactus	623.82	277.78	0.044	0.012	0.0017
Collinsella aerofaciens	3454.73	957.33	0.048	0.012	0.0019
Bacteroides uniformis	3504.5	9139.5	0.18	0.035	0.007
Akkermansia muciniphila	975.77	285	1	0.17	0.043
Pseudomonas fragi	297.41	10	1	0.17	0.048

[a] Negative Binomial DESeq2 analysis.
[b] Both the Bonferroni correction (P Bonferroni) and the False-Discovery-Rate (FDR) were applied to reduce the chances of obtaining false-positive results.
[c] After the Negative Binomial DESeq2 analysis, calculated $\underline{P}$-values were adjusted for multiple testing by P Bonferroni and FDR.
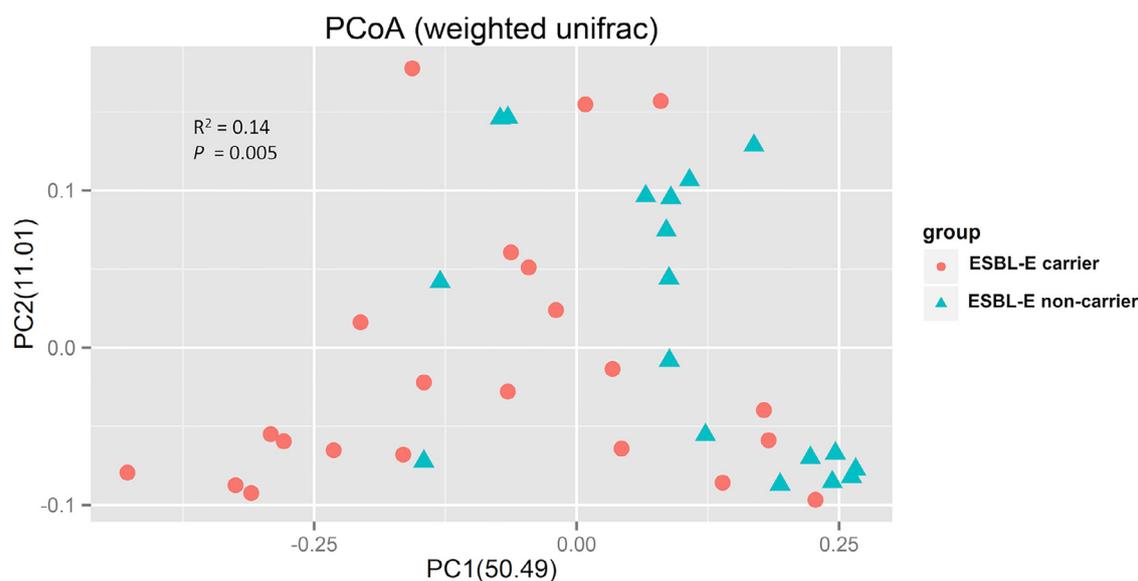
Fig. 4. Principal Coordinate Analysis (PCoA) plot based on weighted UniFrac metrics for samples from extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriers and non-carriers. Each point represents an individual and is plotted in a distance matrix showing the relative diversity. Red circles, ESBL carrier; blue triangle, non-carrier. Statistical analysis was by the Adonis method.

addition, beta diversity analysis (comparing the diversity between the two groups) was performed using the weighted UniFrac analysis. This analysis indicated clear clustering of ESBL carriers along the first principle coordination (PC1), representing 50.49% of intersample variance (Fig. 4). The overall beta diversity difference of the intestinal microbiota of ESBL-E carriers as compared to non-carriers was statistically significant (Adonis on weighted unifrac: R^2 =0.14, P = 0.005). These results indicate that ESBL-E carriage is not associated with altered abundance of taxa, but with the underlying phylogenetic diversity.

4. Discussion

ESBL-E colonization presents a source for the spread of dangerous antibiotic-resistant microorganisms. ESBL-E carriage is on the rise in the community, and especially high in Asia [9–11]. Except for one geographically very limited study in a remote village in South America with low incidence of ESBL-E colonization [15], this is the first study to analyse the role of the intestinal microbiota in determining carriage status of ESBL-E. Our study was performed in subjects from rural areas covering an entire country and ruling out recent antibiotic exposure. Due to the high incidence of ESBL-E carriage in our study (54%), which is in accordance with other reports from Asia [10,11], our results give previously unavailable information on the underpinnings of community ESBL-E antibiotic resistance carriage as a public health risk.

Notably, although participants who used antimicrobial agents and had a history of hospitalization in the previous 3 months were excluded, we still observed a very high incidence of ESBL-E carriers. This suggests that the high ESBL-E colonization rates previously observed in Thai communities [10] may not be due to the recent antibiotic exposure; however, they may be due to complex and multifactorial factors, including the dose, duration, and spectrum of administered antibiotics, the immune status of the host, or the interaction with other components of their microbiota.

The main purpose of our study was, however, to detect a possible association of intestinal carriage of ESBL-E with the composition of the intestinal microbiota. We found that the phyla Bacteroidetes and Firmicutes were generally most dominant in the investigated rural population. This is in accordance with several studies that have also detected Bacteroidetes and Firmicutes as main constituents of the intestinal microbiota, including in Thai populations [27–29]. Notably, we observed a significant difference between the ESBL-E carriers and non-carriers groups in beta but not alpha diversity, implying that the main difference is the relative abundance of some bacterial community members.

The Firmicutes, and on the species level, *C. eutactus, C. aerofaciens, A. muciniphila*, and *P. fragi* showed significantly higher abundance among ESBL-E carriers. While the subjects in our study did not report any signs of acute intestinal disease, *C. eutactus, C. aerofaciens* and *A. muciniphila* have been reported as associated with intestinal inflammation or infection [30–32]. It has been established that intestinal inflammation may change the composition of the microbiota, disrupt colonization resistance, and enhance pathogen growth [33]. Furthermore, it has been reported that host-mediated inflammation altered the colonic microbial community and promoted the overgrowth of either resident or introduced bacteria, particularly of the *Enterobacteriaceae* family [34]. These findings are in accordance with the notion that ESBL-E carriers may suffer from sub-acute inflammation that leads to intestinal dysbiosis [33,34].

Contrastingly, we detected significantly lower abundance of *Bacteroidetes*, and on the species level, *B. uniformis*, in ESBL-E carriers. This association may also be due to intestinal inflammation, as in a murine inflammatory model, an enhanced inflammatory host response was responsible for a dramatic decrease in the *Bacteroides* population [34]. *Bacteroides*, the dominant genus of the intestinal microbiota, can directly inhibit intestinal pathogens by competing for nutrients or producing inhibitory substances [35]. *Bacteroides thetaiotaomicron* can consume carbohydrates needed for *Citrobacter rodentium*, which contributes to the competitive exclusion of this pathogen from the intestinal lumen [36]. Moreover, *Bacteroidetes* are associated with protection against *Salmonella* Typhimurium-induced colitis [37] and *Helicobacter hepaticus*-induced colitis in murine model [38]. Thus, our finding suggesting that *Bacteroidetes* may play a significant role in inhibiting ESBL-E

carriage is in accordance with the discussed reports on probiotic functions of *Bacteroidetes*.

The subjects in our study had not received any antibiotic treatment and did not visit any hospital or healthcare setting within at least the 3 months prior to the study. The participants from the non-carrier group reported significantly more use of antibiotics without prescription within a year but not in the 3 months prior to the study. However, this is not a likely reason for the observed difference in taxa abundance, because generally normalization of the intestinal microbiota is observed within 3 months upon short-term antibiotic exposure [39]. Conversely, any possible long-term antibiotic exposure would not likely lead to the observed differences in the bacterial communities [39–41].

Another notable finding of our study is the observed significantly lower ESBL-E carriage rates in farmers. Dietary conditions impact the composition of the intestinal microbiota, and it is quite likely that these differ in farmers from other occupations. Interestingly, *Bacteroidetes* have been reported to be significantly more abundant in the nasal and oral microbiota of farmers than non-farmers, suggesting that this may also be true for the intestine [42]. Recently, non-toxigenic *Bacteroidetes* was shown to have powerful health benefits to the host, and was recommended as a probiotic candidate [43,44]. These findings may lead to the future identification of specific molecules controlling ESBL-E carriage to potentially be used in probiotic therapeutic approaches to limit ESBL-E colonization. Together, our findings indicate that ESBL-E carriage may be controlled by promoting the abundance of anaerobic bacteria, particularly probiotic intestinal bacteria, possibly by dietary conditions; and may support the notion of probiotic intervention to control the spread of antibiotic resistance [45].

Competing Interests

None.

Ethical Approval

The study was approved by the Siriraj Institutional Review Board (approval No. Si773/2015) at the Faculty of Medicine Siriraj Hospital, Mahidol University.

References

[1] Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol 2004;12:412–16.

[2] Jean SS, Coombs G, Ling T, Balaji V, Rodrigues C, Mikamo H, et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: Results from the Study for Monitoring Antimicrobial Resistance Trends)SMART(, 2010-2013. Int J Antimicrob Agents 2016;47:328–34.

[3] Jean SS, Hsueh PR, Group SA-P. Distribution of ESBLs, AmpC beta-lactamases and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal and urinary tract infections in the Asia-Pacific region during 2008-14: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother 2017;72:166–71.

[4] Islam S, Selvarangan R, Kanwar N, McHenry R, Chappell JD, Halasa N, et al. Intestinal carriage of third-generation cephalosporin-resistant and extended-spectrum beta-lactamase-producing Enterobacteriaceae in healthy US children. J Pediatric Infect Dis Soc 2018;7:234–40.

[5] van Duijkeren E, Wielders CCH, Dierikx CM, van Hoek A, Hengeveld P, Veenman C, et al. Long-term carriage of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in the general population in the Netherlands. Clin Infect Dis 2018;66:1368–76.

[6] Ulstad CR, Solheim M, Berg S, Lindbaek M, Dahle UR, Wester AL. Carriage of ESBL/AmpC-producing or ciprofloxacin non-susceptible Escherichia coli and Klebsiella spp. in healthy people in Norway. Antimicrob Resist Infect Control 2016;5:57.

[7] Hazirolan G, Mumcuoglu I, Altan G, Ozmen BB, Aksu N, Karahan ZC. Fecal carriage of extended-spectrum beta-lactamase and ampc beta-lactamase-producing Enterobacteriaceae in a turkish community. Niger J Clin Pract 2018;21:81–6.

[8] Ni Q, Tian Y, Zhang L, Jiang C, Dong D, Li Z, et al. Prevalence and quinolone resistance of fecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli in 6 communities and 2 physical examination center populations in Shanghai. China. Diagn Microbiol Infect Dis 2016;86:428–33.

[9] Kiratisin P, Chattammanat S, Sa-Nguansai S, Dansubutra B, Nangpatharapornthawee P, Patthamalai P, et al. A 2-year trend of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Thailand: an alert for infection control. Trans R Soc Trop Med Hyg 2008;102:460–4.

[10] Niumsup PR, Tansawai U, Na-Udom A, Jantapalaboon D, Assawatheptawee K, Kiddee A, et al. Prevalence and risk factors for intestinal carriage of CTX-M–type ESBLs in Enterobacteriaceae from a Thai community. Eur J Clin Microbiol Infect Dis 2018;37:69–75.

[11] Kurz MS, Bayingana C, Ndoli JM, Sendegeya A, Durst A, Pfuller R, et al. Intense pre-admission carriage and further acquisition of ESBL-producing Enterobacteriaceae among patients and their caregivers in a tertiary hospital in Rwanda. Trop Med Int Health 2017;22:210–20.

[12] Woerther PL, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. J Travel Med 2017;24:S29–34.

[13] Pultz NJ, Stiefel U, Subramanyan S, Helfand MS, Donskey CJ. Mechanisms by which anaerobic microbiota inhibit the establishment in mice of intestinal colonization by vancomycin-resistant Enterococcus. J Infect Dis 2005;191:949–56.

[14] Lawley TD, Walker AW. Intestinal colonization resistance. Immunology 2013;138:1–11.

[15] Gosalbes MJ, Vazquez-Castellanos JF, Angebault C, Woerther PL, Ruppe E, Ferrus ML, et al. Carriage of enterobacteria producing extended-spectrum beta-lactamases and composition of the gut microbiota in an amerindian community. Antimicrob Agents Chemother 2015;60:507–14.

[16] Performance standards for antimicrobial susceptibility testing. Twenty-First Informational Supplement CLSI document M100-S24. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2014.

[17] Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–4.

[18] Weber N, Liou D, Dommer J, MacMenamin P, Quinones M, Misner I, et al. Nephele: a cloud platform for simplified, standardized and reproducible microbiome data analysis. Bioinformatics 2018;34:1411–13.

[19] Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;26:2460–1.

[20] Rideout JR, He Y, Navas-Molina JA, Walters WA, Ursell LK, Gibbons SM, et al. Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. Peer J 2014;2:e545.

[21] McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J 2012;6:610–18.

[22] Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol 2007;73:1576–85.

[23] Lozupone C, Hamady M, Knight R. UniFrac: an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 2006;7:371.

[24] Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R. Forensic identification using skin bacterial communities. Proc Natl Acad Sci U S A 2010;107:6477–81.

[25] Zakrzewski M, Proietti C, Ellis JJ, Hasan S, Brion MJ, Berger B, et al. Calypso: a user-friendly web-server for mining and visualizing microbiome-environment interactions. Bioinformatics 2017;33:782–3.

[26] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.

[27] Human Microbiome Project C Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–14.

[28] Ruengsomwong S, La-Ongkham O, Jiang J, Wannissorn B, Nakayama J, Nitisinprasert S. Microbial community of healthy Thai vegetarians and non-vegetarians, their core gut microbiota, and pathogen risk. J Microbiol Biotechnol 2016;26:1723–35.

[29] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635–8.

[30] Malinen E, Krogius-Kurikka L, Lyra A, Nikkila J, Jaaskelainen A, Rinttila T, et al. Association of symptoms with gastrointestinal microbiota in irritable bowel syndrome. World J Gastroenterol 2010;16:4532–40.

[31] Koch C, Amati AL, Hecker A, Hoxter M, Hirschburger M, Matejec R, et al. Microbiomic analysis of intra-abdominal infections by using denaturing high-performance liquid chromatography: A prospective observational study. Surg Infect)Larchmt) 2017;18:596–602.

[32] Ganesh BP, Klopfleisch R, Loh G, Blaut M. Commensal Akkermansia muciniphila exacerbates gut inflammation in Salmonella Typhimurium-infected gnotobiotic mice. PLoS One 2013;8:e74963.

[33] Gibson MK, Pesesky MW, Dantas G. The yin and yang of bacterial resilience in the human gut microbiota. J Mol Biol 2014;426:3866–76.

[34] Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007;2:204.

[35] Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013;13:790–801.

[36] Curtis MM, Hu Z, Klimko C, Narayanan S, Deberardinis R, Sperandio V. The gut commensal Bacteroides thetaiotaomicron exacerbates enteric infection through modification of the metabolic landscape. Cell Host Microbe 2014;16:759–69.

[37] Ferreira RB, Gill N, Willing BP, Antunes LC, Russell SL, Croxen MA, et al. The intestinal microbiota plays a role in Salmonella-induced colitis independent of pathogen colonization. PLoS One 2011;6:e20338.

[38] Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008;453:620–5.

[39] Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiome. Microbiology 2010;156:3216–23.

[40] Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012;488:621–6.

[41] Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome Med 2016;8:39.

[42] Shukla SK, Ye Z, Sandberg S, Reyes I, Fritsche TR, Keifer M. The nasal microbiota of dairy farmers is more complex than oral microbiota, reflects occupational exposure, and provides competition for staphylococci. PLoS One 2017;12:e0183898.

[43] Deng H, Li Z, Tan Y, Guo Z, Liu Y, Wang Y, et al. A novel strain of Bacteroides fragilis enhances phagocytosis and polarises M1 macrophages. Sci Rep 2016;6:29401.

[44] Wang Y, Deng H, Li Z, Tan Y, Han Y, Wang X, et al. Safety evaluation of a novel strain of Bacteroides fragilis. Front Microbiol 2017;8:435.

[45] Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B. Probiotic approach to prevent antibiotic resistance. Ann Med 2016;48:246–55.