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Original Article

Stool microbiota analysis for abundance of genus *Klebsiella* among adults and children in endemic area for community *Klebsiella pneumoniae* infection

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Abstract *Background:* Invasive *Klebsiella pneumoniae* syndrome is a significant endemic disease in Taiwan. Intestinal colonization of virulent clones that cause this phenomenon has been demonstrated in asymptomatic adults. Comparisons of healthy adults and children with stool *K. pneumoniae* colonization have rarely been reported. We aimed to evaluate the frequency and abundance of *K. pneumoniae* in the stool of adults and children by stool microbiota analysis.

Methods: Healthy volunteers and their children without antibiotic exposure within 3 months were recruited in a Taiwanese medical center. Stool samples were sent for gut microbiota analysis, using amplification of V3—V4 hypervariable regions of 16sRNA followed by high-throughput sequence. Rectal/stool swabs were sent for *K. pneumoniae* culture and identification by matrix-assisted laser desorption ionization—time-of-flight mass spectrometry (MALDI-TOF MS).

Results: Fifty-five adults with a mean age of 46.9 years (range, 23.1—72.1 years) and 20 children with a mean age of 2.3 years (range, 0.9—5.8) were enrolled, and 29 adults and 6 children had positive *K. pneumoniae* swabs. Children had lower microbiota diversity than adults,

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including higher abundance of phylum *Actinobacteria* and *Proteobacteria*, and lower *Bacteriodetes*. For genus comparison, higher abundance of *Escherichia*, *Streptococcus*, *Enterococcus* and *Bifidobacterium* were found in children, but the composite abundance of *Klebsiella* in adults (median: 0.0156, range: 0—0.031) and in children (median: 0.0067, range: 0—0.043) were similar. *Klebsiella* abundance was significantly higher in participants with positive swabs ($p < 0.0001$). *Klebsiella*-positive swabs were strongly negatively correlated with *Enterobacter* spp. ($p < 0.0001$), but no known demographic factors correlated with *Klebsiella*-positive swabs.

Conclusion: Klebsiella species are present in young children, and the abundance is similar in adults and children. Positive swabs correlate strongly with higher abundance in microbiota analysis.

Introduction

Enterobacteriaceae is a large family of Gram-negative bacteria, and includes the major pathogens *Escherichia coli* and *Klebsiella pneumoniae*, which can cause both community and nosocomial infections. Community-acquired liver abscess is a common presentation for invasive *K. pneumoniae* syndrome (IKS) in Taiwan.[1] The annual incidence of pyogenic liver abscess is 17.59/100,000, and thus is a significant endemic disease in Taiwan.[1,2] Two prospective studies in Taiwan detected monomicrobial *K. pneumoniae* bacteremia (KPB) in about two-thirds of adults in the community setting,[3,4] which is unlike KPB distribution in Western countries. However, the prevalence of community-onset KPB/liver abscess in children is relatively rare compared to that in adults.[3,5—7]

In 15 cases of pediatric liver abscesses identified in southern Taiwan from 1986 to 2001, *K. pneumoniae* was the most common organism isolated, but it was only detected in 40% (6/15) of the cases.[8] Another study from central Taiwan included 15 cases of liver abscess identified from 1995 to 2011, and the 2 most common pathogens were *K. pneumoniae* (6/15, 40.0%) and *Streptococcus* spp. (6/15, 40.0%).[9] A recent study conducted in northern Taiwan identified 38 cases within 20 years (2000—2019), and *K. pneumoniae* was the most common pathogen, but was only detected in 36% of blood cultures (4/11) and 64% of pus cultures (9/14).[10] Overall, the annual incidence of community KPB or liver abscess is rare among children in Taiwan, in spite of the high prevalence in adults.

Intestinal colonization of virulent strains of *K. pneumoniae*, notably K1 and K2, is considered a basis for subsequent infection, and carriers of *K. pneumoniae* are not uncommon in Taiwan.[6,11—14] Long-term follow-up examining the effects of intestinal colonization in the real-world setting is scarce.[6] Methods of analysis of the human intestinal microbiota have advanced rapidly, and the microbiota has been associated with many diseases.[15] However, it is difficult to define a healthy microbiome, since numerous factors affect its composition, especially diet and antibiotics.[16] For decades, the intestinal tract has been considered to be a reservoir and source for the transmission of pathogens.[17,18] For example, intestinal *Proteobacteria* domination (*Proteobacteria* relative abundance over 30%) has been associated with bloodstream infection in the setting of hematopoietic cell transplant.[18] The effects of intestinal domination on other groups or other settings have not been demonstrated.

It is unknown why children have fewer invasive *K. pneumoniae* infections compared to adults. According to the aforementioned studies, the incidence of children with liver abscesses and *K. pneumoniae* as the etiology is much lower than that of adults. No studies have investigated whether healthy children have lower rates of intestinal colonization of *K. pneumoniae* compared to adults. It is also unknown whether traditional culture methods are sensitive enough to determine intestinal colonization status. To answer these questions, we collected anal and stool swabs from healthy volunteers and children to determine the colonization of *K. pneumoniae* and the abundance of *K. pneumoniae* in adults and children in Taiwan using microbiota analysis.

Methods

Study design and sample

In the first part of this 2-part retrospective/prospective observational study, the annual incidence of *K. pneumoniae* bacteremia from 2011 to 2018 was examined in adults and children whose data were retrieved from the microbiology database at Far Eastern Memorial Hospital (FEMH). FEMH is a 1400-bed tertiary hospital in northern Taiwan, that provides services to people of all ages. In the second part of this study, we prospectively enrolled healthy volunteers and children recruited at FEMH.

The study protocol was reviewed and approved by FEMH Internal Review Board (IRB 107027-F). A structured case record form, including underlying illness, medications, recent infection, and probiotic use was used to record data from all included adults and children. Adults and children with antibiotic exposure within 3 months before the initiation of the study were excluded. Participants ≦18 years old were considered children, and those >18 years old were considered adults.

Anal and stool swabs, and *K. pneumoniae* serotypes

Conventional culture swabs (BBL Culture Swab EZ, Becton Dickinson, Sparks, MD, USA) were used for collecting anal

samples. Anal swabs were performed by inserting the swab tube to 1 cm above the rectum. For children, stool swab was applied instead of anal swab. The swabs were collected from the surface of stool samples. The swab specimens were inoculated on MacConkey II agar plates, and subsequent mucoid-like colonies were selected for further identification. The identification of *K. pneumoniae* was carried out by matrix-assisted laser desorption ionization—time-of-flight mass spectrometry (MALDI-TOF). Bacterial isolates were stored for further serotype determination. Capsular type was determined by PCR methods, as previously described.[4]

Stool samples and 16sRNA sequencing

Stool samples were collected and stored in the refrigerator at the homes of the participants if the stool could not be processed by the study team within 3 h. Samples were then stored at −80 °C until tested. Total genomic DNA was extracted using the QIAmp Fast DNA Stool Mini kit (Qiagen, Germany), according to the product instruction manual for frozen samples. After isolation, the DNA yield was approximately 50 μg, and the DNA concentration was 150 ng/μl. The DNA samples were stored at −20 °C until PCR amplification. For PCR amplification, the forward and reverse primers that were complementary upstream and downstream of the V3—V4 region of 16S were designed with Illumina overhang adapters, and used to amplify templates from bacterial genomic DNA. PCR products were purified with a GeneH Low Gel/PCR Purification kit (Geneaid, Taiwan). For the Index PCR and clean-up step, Illumina sequencing adapters and dual indices were attached to the PCR products using the Nextera XT Index kit (Illumina, San Diego, CA, USA). Subsequently, AMPure XP beads were used to clean up the final libraries, and the expected size on a Bioanalyzer trace of the final libraries was approximately 630 bp. For the normalization and sequencing steps, libraries were normalized and pooled and sequenced on the MiSeq system using v3.0 reagents (pair-end 250 bp, Illumina, San Diego, CA, USA).

Sequence quality control

Four major steps were used to analyze the sequence reads in FASTAQ format. 1) The quality value of raw reads was checked using FastQC.[19] 2) Pair-end reads of a sample were merged according to the overlap sequences using PEAR software.[20] Overlapped sequences <10 bp were discarded. 3) Clusters of similar sequences with at least 97% identified were defined as an operational taxonomic unit (OTU) using UCLUST.[21] 4) The QIIME software package (version 1.9.1)[22] with default settings was used to compare sequences to the Green Genes reference database (release 13_8).[23]

Statistical analyses

The alpha diversity, beta diversity (Weighted-UniFrac and Unweighted-UniFrac), microbiome structure, multi-variant statistical analysis, and linear discriminant analysis effect size between adults and children and positivity of swab culture were generated using R software (R Foundation for Statistical Computing, Vienna, Austria). Comparisons between pairs of adults and children were performed according to the abundance of child's genus level. Data were analyzed using SPSS software version 15.0 for Windows (SPSS, Chicago, IL, USA).

Results

In the first part of the study, we reviewed the microbiology database at FEMH for *K. pneumoniae* detected in blood cultures from the years 2011—2018 (Fig. 1). Compared to adults (>200 cases every year), the annual incidence of *K. pneumoniae* bacteremia in children was found to be < 4 cases every year.

The prospective part of the study included 55 adults (15 males and 40 females) with a mean age of 46.0 years (range, 23.1—72.1 years) and a mean body mass index (BMI) of 23.5 ± 3.5 kg/m^2), and 20 children (6 males and 14 females) with a mean age of 2.3 years (range, 0.9—5.8 years). Of the participants, 29 (52.7%) adults and 6 (30%) children had positive anal/stool swabs for *K. pneumoniae* (Table 1). The most common chronic illnesses among adults were diabetes mellitus (n = 7) and hypertension (n = 7); no children had a chronic illness. Children had lower alpha (Fig. 2A) and beta diversity (Unweighted-UniFrac) (Fig. 2B) compared with adults (OTUs 486.5, 181—825 vs. 730, 319—1621, p < 0.001). However, diversity was similar for participants with and without a positive swab for *K. pneumoniae* (OTUs 704, 266—1621 vs. 680.5, 181—1275, p = 0.43) (Fig. 3A and B).

There were differences in phylum distribution between children and adults, as shown in Fig. 4. Children had higher abundance of phylum *Actinobacteria* (0.11, 0.01—0.77 vs. 0.01, 0.00—0.20, p < 0.001) and *Proteobacteria* (0.07, 0.00—0.63 vs. 0.02, 0.00—0.68, p < 0.001), but lower abundance of *Bacteriodetes* (0.15, 0.00—0.71 vs. 0.40, 0.01—0.82, p < 0.001). The distribution of the top 10 OTUs in children is shown in Table 2. OTU 72820 (*Bifidobacterium*) and OTU 1111294 (*Escherichia-Shigella*) were the leading OTUs in children, and were significantly higher than that in adults. Table 3 shows the top 10 OTUs of *Enterobacteriaceae*. OTU 114510 (*Escherichia*) was the second most abundant OTU, and was also significantly higher in children than in adults. However, OTU 813217 (*Klebsiella*) and OTU 411908 (*Klebsiella*), the 2 most common OTUs for genus *Klebsiella*, were not significantly different between children and adults.

Composite genus comparison results are summarized in Fig. 5. Higher abundances of *Escherichia*, *Streptococcus*, *Enterococcus* and *Bifidobacterium* were found in children, but the abundance of *Klebsiella* in adults (median: 0.0156, range: 0 to 0.031) and in children (median: 0.0067, range: 0 to 0.043) were similar. In contrast, *Prevotella*, *Megasphaera* and *Parabacteroides* were higher in adults. Results of the linear discriminant analysis effect size (LEfSe) between adults and children are shown in Table 4; *Bifidobacterium* had the most prominent difference (p = 1.15E-06).

The abundance of genus *Klebsiella* was significantly higher in participants with positive swabs (0.004, 0.00—0.31 vs. 0.00, 0.00—0.07, p < 0.0001) (Fig. 6). Except for *Klebsiella*, genus *Megamonas*, *Prevotella*, and *Veillonella* were

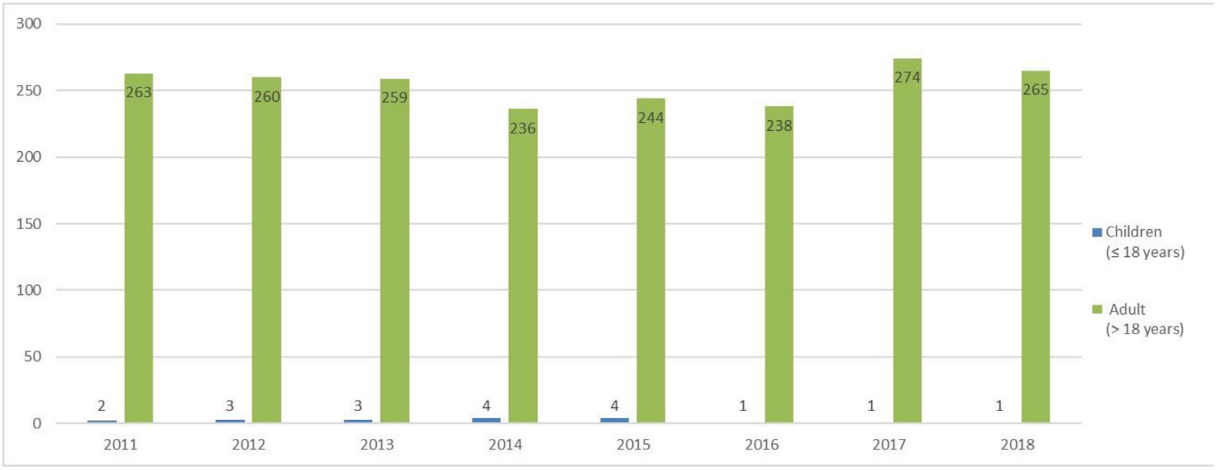

Figure 1. Yearly incidence of *Klebsiella pneumoniae* bacteremia at Far Eastern Memorial Hospital, in children (≤18 years old) versus adults (>18 years old).

Table 1 Participant demographic and clinical characteristics.

	Adult (n = 55)	Children (n = 20)
Age	46.0 ± 13.6	2.3 ± 1.9
Sex (M/F)	15/40	6/14
BMI	23.5 ± 3.5	—
Diabetes mellitus	7 (12.7%)	0
Heart disease	7 (12.7%)	0
Neurological illness	3 (5.4%)	0
Autoimmune disease	2 (3.6%)	0
Liver cirrhosis	1 (1.8%)	0
Chronic renal insufficiency	1 (1.8%)	0
Probiotics	10 (18.2%)	5 (25%)
Vegetarian	3 (5.4%)	0
Daily milk consumption	—	18 (90%)

higher in participants with positive swab cultures; whereas *Bifidobacterium*, *Acidaminococcus*, and *Megasphaera* were higher in participants with negative swabs.

The results of LEfSe between positive and negative swabs is shown in Table 5. Positive swab for *K. pneumoniae* is strongly associated with higher abundance of *Klebsiella* in microbiota analysis (p = 7.28E-05). Interestingly, swab cultures that were positive for *Klebsiella* were negatively correlated with *Enterobacter* spp. (p < 0.0001). No demographic factors examined were correlated with positive swab cultures for *K. pneumoniae*.

Discussion

This is the first study to analyze the microbiota and compare the colonization of *Klebsiella* between adults and children who live in the same community setting, and may have similar diet exposure. The results showed that the

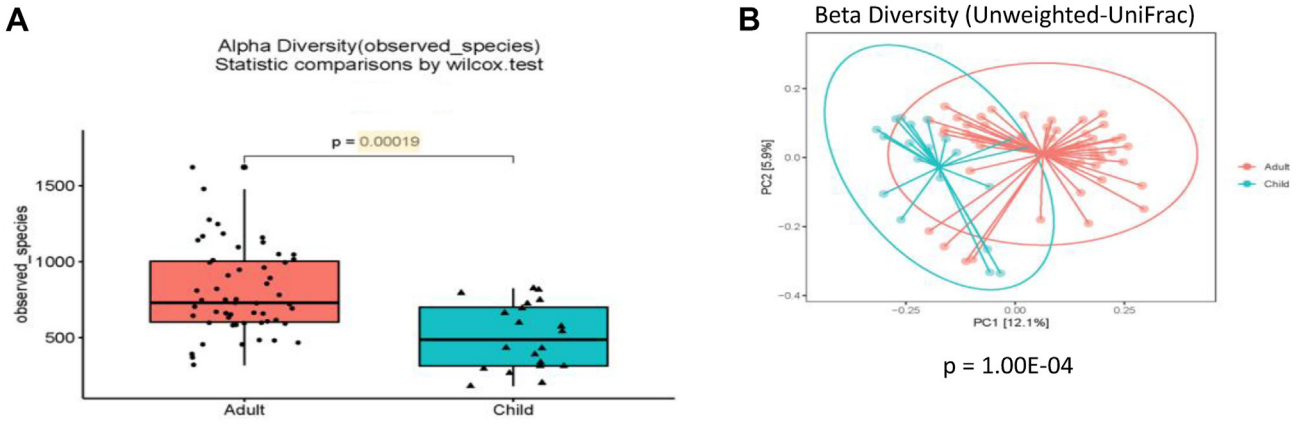

Figure 2. Alpha (2A) and beta (2B) (Unweighted-UniFrac) diversity between adults and children.

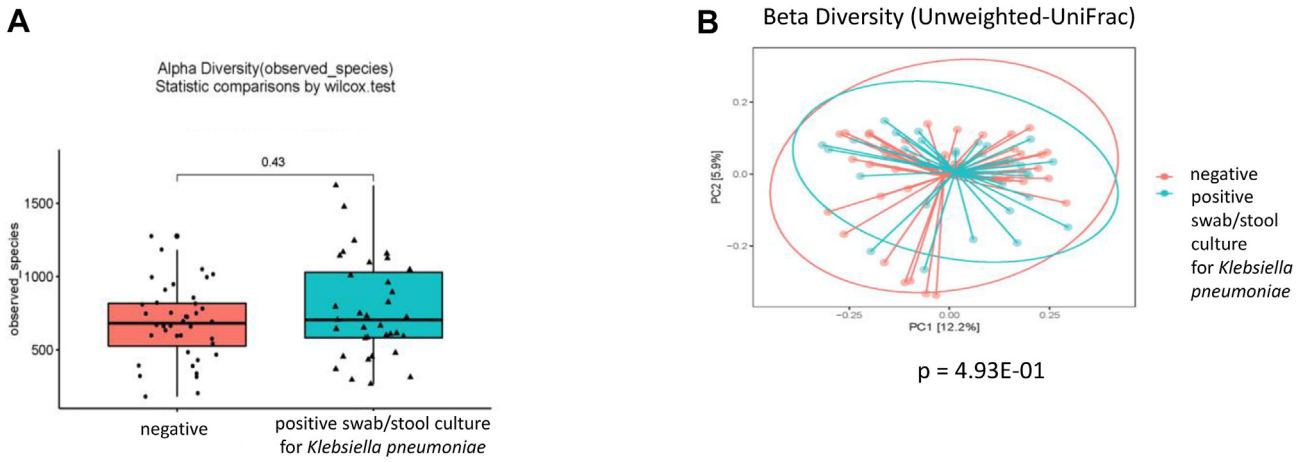

Figure 3. Alpha (2A) and beta (2B) (Unweighted-UniFrac) diversity associated with anal/stool swab for *Klebsiella pneumoniae*.

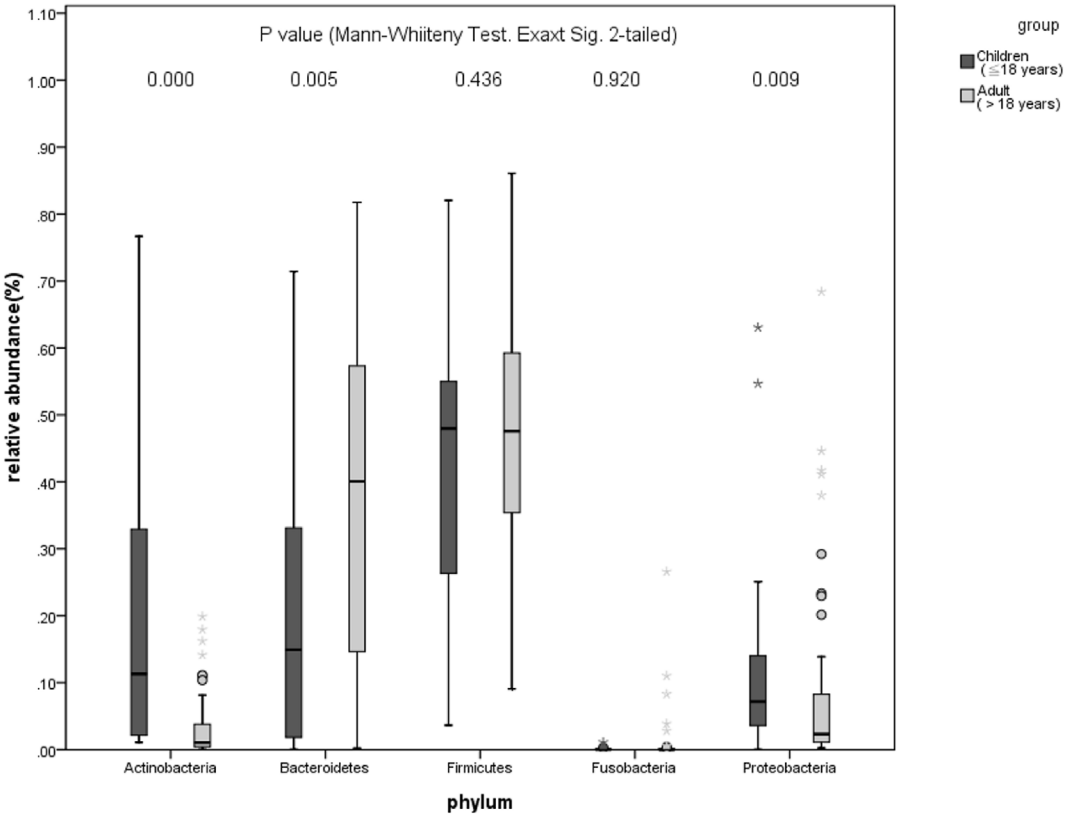

Figure 4. Phylum comparison between adults and children.

application of 16sRNA sequencing reliably analyzes the *Klebsiella* carrier status. Strong correlations were found between the results of traditional culture and 16sRNA analysis. The most notable finding was that adults and children living in a community setting had similar abundance of *Klebsiella*, while children had higher abundance of *Escherichia*, *Streptococcus*, *Enterococcus* and *Bifidobacterium*.

Although the degree of abundance of *Klebsiella* determined by 16sRNA analysis was similar between adults and children, the frequency of *K. pneumoniae* bacteremia or liver abscess are much lower in children than in adults. No prior studies have compared *K. pneumoniae* colonization between children and adults to help explain the discrepancy.

Studies have described risk factors for clinically severe infection caused by *K. pneumoniae* in children, and include premature birth, malignancies, mechanical ventilation, prior antibiotic use, and/or longer hospital stays.[24–29] However, infection caused by *K. pneumoniae* is relatively scarce among healthy children, even in Taiwan, an endemic area for community *K. pneumoniae* infection. It is plausible

Table 2 Top 10 OTU genus according to abundance in children.

#OTU ID	Phylum	Class	Order	Family	Genus	Child (n = 20)				Adult (n = 55)				Mann–Whitney U
						Median	IQR	Mean	SE	Median	IQR	Mean	SE	P value
72820	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	3.11E-02	1.08E-01	1.11E-01	4.22E-02	6.41E-04	2.32E-03	2.81E-03	1.22E-03	**.000**
1111294	*Proteobacteria*	*Gamma proteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	1.31E-02	5.76E-02	5.39E-02	2.29E-02	2.12E-03	5.08E-03	2.06E-02	6.92E-03	**.016**
351231	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	4.30E-03	6.10E-02	5.22E-02	2.96E-02	7.65E-05	6.08E-04	5.33E-03	2.44E-03	**.028**
335550	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	4.28E-03	1.33E-02	1.16E-02	4.01E-03	3.28E-04	1.70E-03	1.57E-03	3.89E-04	**.016**
579608	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	3.02E-03	1.54E-02	1.58E-02	6.59E-03	8.76E-04	2.27E-03	1.93E-03	3.56E-04	**.032**
360015	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*[Ruminococcus]*	2.20E-03	2.71E-02	1.40E-02	4.63E-03	3.25E-04	1.33E-03	1.97E-03	6.79E-04	**.009**
583117	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	2.16E-03	3.50E-02	1.84E-02	6.77E-03	4.88E-03	1.22E-02	8.02E-03	1.24E-03	.534
114510	*Proteobacteria*	*Gamma proteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia*	1.55E-03	5.39E-03	4.51E-03	1.83E-03	1.62E-04	4.63E-04	1.52E-03	4.95E-04	**.003**
589277	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	1.52E-03	8.97E-03	2.42E-02	1.22E-02	2.91E-02	7.15E-02	5.53E-02	8.99E-03	**.000**
132041	*Actinobacteria*	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*	*Bifidobacterium*	1.49E-03	1.71E-02	1.45E-02	7.74E-03	0.00E+00	1.23E-05	1.22E-05	4.14E-06	**.000**

Table 3 Top 10 *Enterobacteriaceae* OTU according to abundance in children.

#OTU ID	Phylum	Class	Order	Family	Genus	Child (n = 20)				Adult (n = 55)				Mann–Whitney U
						Median	IQR	Mean	SE	Median	IQR	Mean	SE	P value
1111294	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	1.31E-02	5.76E-02	5.39E-02	2.29E-02	2.12E-03	5.08E-03	2.06E-02	6.92E-03	**.016**
114510	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia*	1.55E-03	5.39E-03	4.51E-03	1.83E-03	1.62E-04	4.63E-04	1.52E-03	4.95E-04	**.003**
922761	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Erwinia*	8.81E-04	3.69E-03	3.26E-03	1.18E-03	6.53E-05	6.73E-04	1.24E-03	4.29E-04	**.036**
3829957	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	6.57E-04	5.87E-03	4.33E-03	1.58E-03	5.03E-05	4.67E-04	2.61E-03	9.66E-04	**.003**
797229	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	4.45E-04	7.81E-04	1.95E-03	1.15E-03	2.95E-05	1.90E-04	6.27E-04	2.60E-04	**.000**
782953	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	2.41E-04	1.86E-03	3.67E-02	2.62E-02	1.29E-05	9.28E-05	1.59E-02	9.48E-03	**.000**
813217	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Klebsiella*	2.07E-04	6.22E-03	4.44E-03	1.96E-03	2.83E-04	8.11E-03	9.56E-03	3.27E-03	.717
231787	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	9.83E-05	4.69E-04	3.93E-04	1.60E-04	9.45E-06	8.12E-05	9.57E-05	3.00E-05	**.001**
511908	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Klebsiella*	8.98E-05	9.95E-04	2.01E-03	1.03E-03	1.03E-04	1.50E-03	4.78E-03	2.59E-03	.929
581021	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-	8.33E-05	1.98E-03	8.79E-04	2.98E-04	1.97E-05	2.17E-04	4.15E-03	3.58E-03	.295

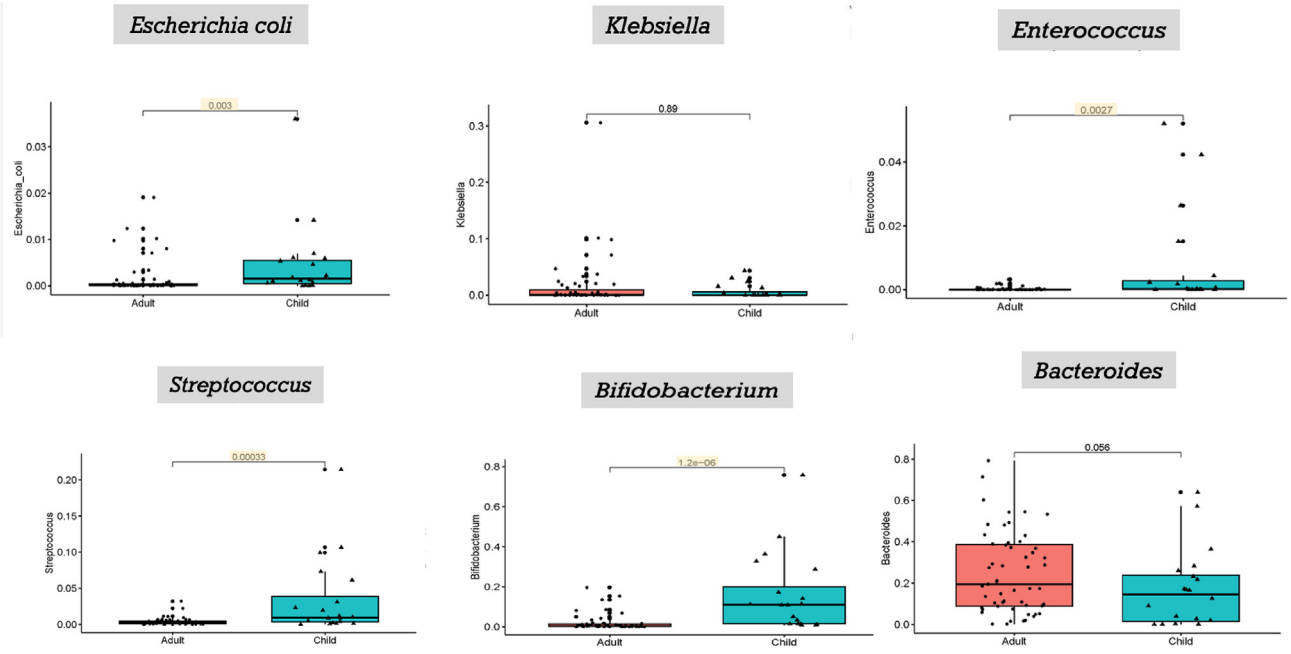

Figure 5. Major genus comparison between adults and children.

that although healthy children may carry a similar abundance of *K. pneumoniae* as adults, adults might have more comorbidities and medications exposure, especially antibiotics and antacid (for example, proton pump inhibitors), which contribute to the higher occurrence of invasive *K. pneumoniae* infection among adults.

In the present study, *K. pneumoniae* was detected in 30% of children by culture. The colonization rate in children was about 10% from fecal samples obtained from healthy and sick preterm neonates reported in another study.[28] A previous study showed an increased incidence of *K. pneumoniae* infection with increased age, despite the absence of correlations with temporal or seasonal changes.[30] Dietary change may be the most important determinants since *Klebsiella* has been recovered from many different food categories.[31] Consumption of dairy food decreases significantly in Taiwanese once children start to eat solid food. Other dietary factors that can affect the gut microbiome are pesticides and preservatives. Further research is needed to understand the factors that contribute to differences in colonization rates.

The results of this study showed that the alpha and beta diversities in children were lower than in adults, which is compatible with previous reports.[15] Alpha diversity refers to the variety and balance of microbial species within an individual microbiota, while beta diversity is a measure of the degree of difference or dissimilarity between groups of samples.[32] Yet, our results showed that the diversities were similar, regardless of positive or negative swabs for *K. pneumoniae*. This may indicate that although diversities are different between children and adults, these factors do not have an impact on *K. pneumoniae* colonization. No previous studies have explored such associations, and the exact mechanism are not fully understood. Our findings indicate that although the microbiota compositions of children were less diverse than adults, *Klebsiella* colonization in the gastrointestinal tract does not significantly impact the overall diversity of microbiota in children. Generally, a children's diet is less diverse compared to that of adults due to food preferences and dietary restrictions, and therefore lack of exposures to certain types of diet that may influence the microbiota composition.[33] Moreover, adults had more chronic illness and exposed to many medications, especially antibiotic exposure may disrupt the balance and diversity of the microbiota.[27,34] Although we excluded participants with antibiotic use in the recent 3 months, it is still not known whether earlier or longer antibiotic or chemicals exposures associated with treatment of infections or illnesses may affect the microbiota diversity in children and adults.

The results of this study showed strong correlations between the abundance of *Klebsiella* by 16sRNA sequencing and the traditional culture method; a finding similar to that of previous reports.[35,36] The traditional culture method is sensitive enough to determine colonization status, but it is still an unresolved question whether a positive *K. pneumoniae* swab in children is associated with clinical confirmed infections. Our results are consistent with those of a prior study that showed rectal colonization with *K. pneumoniae* was detected in nearly half of asymptomatic diabetic adult, but none of these adults eventually developed a liver abscess in 5-year follow up period.[6] Future studies should focus on whether a certain threshold is required for intestinal *K. pneumoniae colonization* to develop into an invasive infection in community-dwelling persons.

Compared to adults, children had a higher abundance of *Actinobacteria* and *Proteobacteria*, but a lower abundance of *Bacteroidetes*. This finding is in line with that of a prior study that reported higher colonization of *Enterobacteriaceae* in children, which may suggest an underdeveloped gut microbiota.[37] The abundance of *Escherichia* is

Table 4 LEfSE analysis for comparison between adults/children.

	Biomarker	Log10	Group	LDA value	p value
1	*Bifidobacteriales*	5.1908	Children	4.890	1.15E-06
2	*Bifidobacterium*	5.1908	Children	4.890	1.15E-06
3	*Actinobacteria* (class)	5.1959	Children	4.893	1.22E-06
4	*Burkholderiales*	3.8975	Adult	3.465	2.64E-06
5	*Betaproteobacteria*	3.8979	Adult	3.464	4.46E-06
6	*Sutterella*	3.8833	Adult	3.458	1.02E-06
7	*Actinobacteria* (phylum)	5.2907	Children	4.951	1.23E-05
8	*Phascolarctobacterium*	4.5597	Adult	4.214	3.05E-05
9	*Odoribacter*	3.1721	Adult	2.817	3.43E-05
10	*Clostridium*	3.9301	Children	3.603	5.55E-05

significantly higher in children compared to adults (notably, *E. coli* is an important pathogen for children), but the abundance of *Klebsiella* is similar. Could the microbiota of children, especially higher *Bifidobacterium*, provide some degree of resistance against invasive infection caused by *Klebsiella*?[38] Moreover, it is interesting to consider whether the abundance of *Klebsiella* and *Enterobacter* is mutually exclusive. *Klebsiella* and *Enterobacter* ranked second and third in the family of *Enterobacteriaceae* in a stool microbiome analysis among hemodialysis patients,[39] which are major Gram-negative pathogens secondary only to *E. coli*.

Strengths and limitations

The major strength of this study is its 2-part retrospective and prospective design, and thus compared microbiota and *K. pneumoniae* colonization between adults and children who might share similar dietary patterns in an endemic area. There are, however, limitations of the study that should be considered. Although all participants indicated

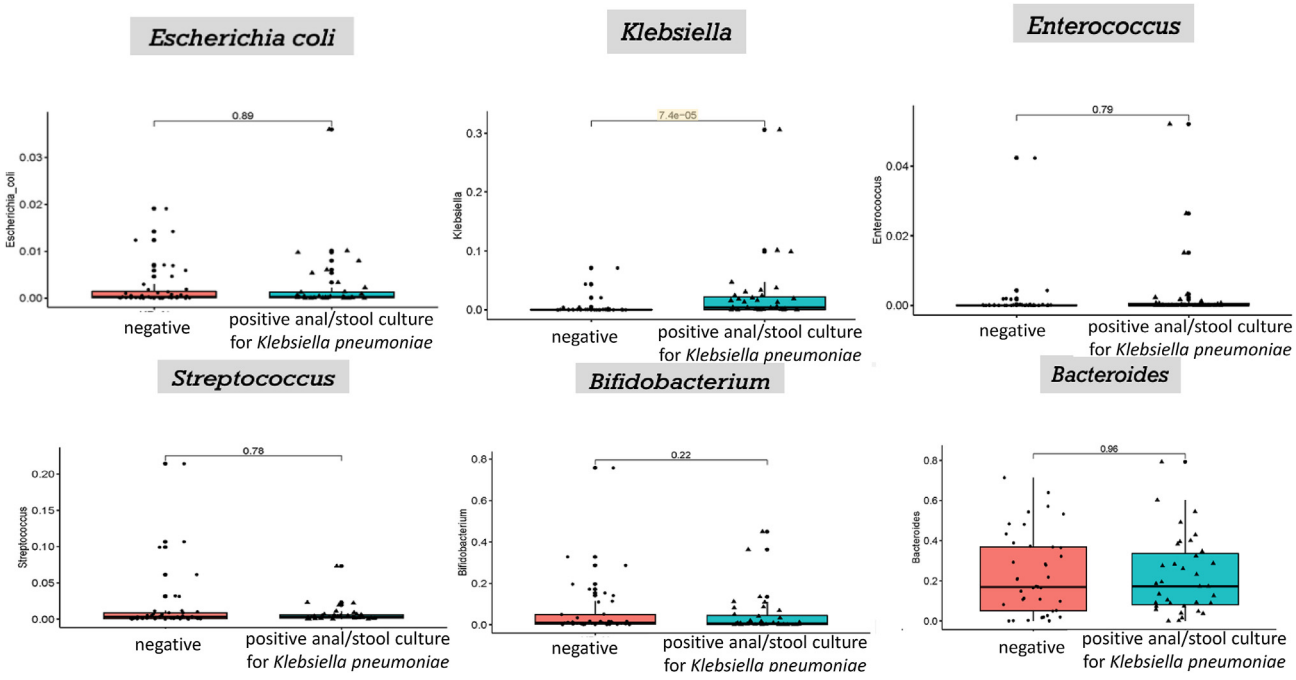

Figure 6. Major genus comparison between positive and negative anal/stool swab culture for *Klebsiella pneumoniae*.

Table 5 LEfSE analysis for positive anal/stool swabs for *Klebsiella pneumoniae*.

	Biomarker	Log10	Group	LDA value	p value
1	Klebsiella	4.3806	Positive culture for *Klebsiella pneumoniae*	3.949	7.26E-06
2	Enterobacter	3.7332	Negative	3.358	0.00076
3	Erwinia	3.4722	Positive culture for *Klebsiella pneumoniae*	2.993	0.00607
4	Salmonella	1.0066	Positive culture for *Klebsiella pneumoniae*	2.307	0.00916
5	Methanobrevibacter	2.7933	Positive culture for *Klebsiella pneumoniae*	2.573	0.01472
6	Morganella	2.2831	Negative	2.038	0.01964
7	Hafnia	2.8376	Negative	2.533	0.02465
8	Catenibacterium	3.4286	Negative	3.139	0.02717
9	Nesterenkonia	0.5378	Positive culture for *Klebsiella pneumoniae*	2.526	0.02912
10	Methanobacteriales	2.9007	Positive culture for *Klebsiella pneumoniae*	2.942	0.04068

no antibiotic use in the prior 3 months, they may have antibiotic exposures that were not detected. This may be a potential confounder that requires attention. Second, our sample size may be too small to reveal the different rates of colonization/diversity of *Klebsiella* between adults and children. Third, 16sRNA V3—V4 hypervariable region sequencing can only confidently identify OTUs to the genus level. It is possible that OTUs belonging to genus *Klebsiella* may include species other than *K. pneumoniae*. There are increasing reports about other species of *Klebsiella* causing human infections.[40]

Conclusions

The present study found that healthy children have less microbiota diversity, but a similar *Klebsiella* abundance as adults. Such findings may imply that the different clinical presentations of *K. pneumoniae* between children and adults may not be due to colonization status. Future research may be designed to investigate whether a certain threshold exists for intestinal *K. pneumoniae* abundance in community-dwelling setting to develop an invasive infection.

Conflicts of interest

None.

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