Cai *et al. BMC Microbiology* (2025) 25:139
https://doi.org/10.1186/s12866-025-03845-4

BMC Microbiology

RESEARCH Open Access

Bacterial translocation and gut microbiome imbalance in an experimental infection model of legionellosis in guinea pigs

Xu Cai[1,2†], Mingtao Xu[1†], Ye Lu[3†], Wei Shen[4], Jian Kang[1], Wei Wang[1] and Yu Chen[3*]

Abstract

Background Recent studies have shown that in critically ill patients such as those with sepsis and shock, the lung and gut microbiomes undergo profound changes. *Legionella pneumophila* (Lp) can cause fatal infection, however, such changes have not been investigated in legionellosis. Here, we evaluated the microbiome of the lungs, blood, liver, and small intestine content in Lp-infected guinea pigs.

Methods We used a culture-independent method by analysing the conserved 16S rDNA sequences of bacteria from the organs of guinea pigs infected with legionellosis. Bacterial DNA was also identified through bacterial probe-fluorescence in situ hybridisation (BP-FISH). Bacterial entry from the intestinal lumen into the submucosa was examined via ultrastructural visualisation.

Results *Anoxybacillus kestanbolensis*, *Geobacillus vulcani*, and other bacteria were identified in the small intestine content of healthy guinea pigs but not in other tissues. However, in Lp-infected guinea pigs, DNA from these bacteria was detected in the small intestine, lungs, blood, and liver tissues at 24 h and 48 h post-infection, indicating the possible translocation of gut bacteria to the remote tissues. This was validated through BP-FISH and ultrastructural visualisation. At 72 h post-infection, *Pseudomonadota* were the dominant gut bacteria, highlighting an imbalance in the gut microbiome.

Conclusion Infection with the Legionella pneumophila serotype 1 disrupted the intestinal microbiota in a subset of guinea pigs during a 72-hour period post-infection, with possible translocation of gut-associated anaerobic bacteria to the lungs and liver based on the presence of genomic DNA detected in tissue from infected guinea pigs.

Keywords Legionellosis, Bacterial translocation, Gut microbiome imbalance

†Xu Cai, Mingtao Xu and Ye Lu contributed equally to this work.

*Correspondence:
Yu Chen
chenyusy@hotmail.com
[1]Department of Pulmonary and Critical Care Medicine, The First Hospital of China Medical University, Heping District, Shenyang 110001, China
[2]Department of Songbei Respiratory Medicine, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150023, P.R. China
[3]Department of Pulmonary and Critical Care Medicine, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Heping District, Shenyang 110004, China
[4]Department of Physical Examination Department, Nangang Branch of Heilongjiang Provincial Hospital, Nangang District, Haerbin 150001, China

Background

Legionella pneumophila (Lp) is an important pathogenic microorganism that causes legionellosis [1]. In various regions across the globe, there has been a notable increase in the incidence of legionellosis [2]. In 2019, the incidence of legionellosis in the European Union/European Economic Area (EU/EEA) was recorded at a rate of 2.2 cases per 100,000 individuals [3]. Legionellosis often progresses to severe pneumonia and may affect multiple organs and systems. A rate ranging from 20 to 40% of patients with legionellosis require admission to the intensive care unit (ICU) for artificial organ support therapy [4]. The severe impact of *Legionella* pneumonia on human health was highlighted by the death toll of 29 out of 182 patients (16%) who suffered from it [5]. In China, *Legionella pneumophila* is recognized as the fourth major causative agent of severe community-acquired pneumonia (CAP) [6]. The mortality rate among patients with *Legionella* pneumonia who were admitted to the ICU was estimated to be around 33% [7].

Legionellosis may lead to extrapulmonary manifestations such as liver dysfunction and diarrhoea [2]. Some patients with legionellosis have poor outcomes even with the administration of effective antibiotics such as levofloxacin [8]. Acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS) are responsible for the high mortality rate in patients with legionellosis [9, 10]. The progression of legionellosis into ARDS or MODS may be associated with the dissemination of Lp, type IV Dot/Icm secretion system, and virulence factors, among others [11, 12]. However, whether there are other mechanisms involved is unclear.

In a previous study using a mouse model of legionellosis, Chen et al. [13] identified the presence of bacteria other than Lp, mainly *Escherichia coli*, *Staphylococcus aureus*, and intestinal *Citrobacter* sp., during the acute phase of the disease. Lieberman et al. [14] reported that 62.5% of the patients with legionellosis were co-infected with at least one more pathogenic microorganism, including *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, viruses, and *Haemophilus influenzae*. In another study, six patients with legionellosis co-infected with *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *and Enterobacter cloacae* developed sepsis [15]. Recent research findings also indicate that in a large number of pneumonia patients, co-infections with *Legionella*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas*, *Enterobacteriaceae*, and other pathogenic bacteria have been detected [16]. The presence of other dominant bacteria in the sputum samples of patients with legionellosis also suggested coinfection [17, 18]. Thus far, superinfection in legionellosis has not been widely investigated, and the source of coinfecting bacteria has not been reported. Given that patients with legionellosis may develop superinfection and diarrhoea, we hypothesised the translocation of gut-associated bacteria to remote organs and an imbalance of gut microbiome in hosts with legionellosis.

To test this possibility, we investigated the microbiomes in different organs in guinea pigs with legionellosis. We used a culture-independent method of bacterial 16S rDNA sequence analysis, which overcomes the limitations of gold-standard bacterial culture methods that cannot detect several gut bacteria [19]. We further conducted a bacterial probe-fluorescence in situ hybridisation (BP-FISH) assay and ultrastructural observation of the small intestines for confirmation.

Methods

Laboratory animals and grouping

The specific pathogen-free Hartley guinea pigs (weight 300–350 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animals were maintained in temperature-controlled conditions (temperature 23–25 ∘C; humidity 50 ± 5%) with 12 h rhythm and fed with sterile food and water. All animal experiments were approved by China Medical University institutional animal care.

The Lp strain used in this study was clinically isolated from the Department of Pulmonary and Critical Care Medicine (Shengjing Hospital of China Medical University) and identified as Lp serotype 1 by slide agglutination test [20], and as sequence type ST2345 by sequence-based typing [21]. The bacterial strain was cultured on *Legionella* charcoal yeast extract agar base containing *Legionella* BCYE growth supplement at 37 °C and 5% CO_2 for four days. The resulting colonies were suspended in sterile 0.9% saline solution and adjusted to a final cell density of 3.33×10^6 colony-forming units (CFU)/mL.

Lp-infected guinea pig model

Hartley guinea pigs were anesthetized and their limbs immobilized by intraperitoneal injection of 40 mg/kg pentobarbital sodium. A midline skin incision was made along the neck of Hartley guinea pigs, and 0.3 mL of bacterial solution containing 1×10^6 CFU Lp serotype 1 was injected through the trachea [20]. Guinea pigs in the control group were injected with 0.3 mL of sterile saline solution only. Intestinal, liver, and lung tissues were collected at 4, 6, 12, 18, 24, 48, and 72 h post-infection from six guinea pigs per group. According to the purpose of the experiment, the whole experiment was divided into control group and Lp-infected group, with 42 Hartley guinea pigs in each group. The weight and temperature of every group at 6, 24, 48, and 72 h observing times were recorded.

DNA extraction, library construction, and sequencing

After the experimental period, all guinea pigs were euthanized by over-anesthesia with pentobarbital sodium (intraperitoneal injection at a dose of 140 mg/kg). Blood samples were taken, and tissue samples were collected from the lungs, liver, and small intestine. Freshly harvested samples were immersed immediately in liquid nitrogen for 2 h and stored at – 80 °C. DNA was extracted from 200 mg samples of the lungs, liver, and contents of the small intestine, and from 2 mL blood using E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The concentration of extracted DNA was measured via Qubit 3.0 (Life Technologies, Carlsbad, CA, USA), and DNA integrity was assessed via 1% agarose gel electrophoresis (150 V, 40 min).

The polymerase chain reaction (PCR) mixture was prepared with 30 ng of DNA sample and 16S V4 (319F-806R) fusion primers. The primers used to amplify the region are: 319F: 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R: 5'-GGACTACHV GGGTWTCTAAT-3'. DNA sequencing was performed on the HiSeq 2500 platform (Illumina) using the HiSeq Rapid SBS Kit v2 Sequencing strategy [22]. Sequencing reactions and execution were performed in accordance with the manufacturer's instructions.

The target DNA fragment was amplified by the following steps: initial denaturation at 98 °C for 3 min, followed by 30 cycles of denaturation at 98 °C for 45 s, annealing at 55 °C for 45 s, extension at 72 °C for 45 s, and final extension at 72 °C for 7 min. The resulting PCR products were purified using the Agencourt AMPure XP beads (Beckman Coulter, Inc., Brea, CA, USA) to remove non-specific amplicons. The average fragment size of DNA libraries was determined using Agilent Technologies 2100 Bioanalyzer (Agilent DNA 1000 Reagents). DNA libraries were quantified using qPCR (EvaGreen) to ensure high accuracy and resolution. EvaGreen dye binds specifically to double-stranded DNA, generating fluorescence signals during the quantitative PCR process, thus providing precise DNA library concentrations. This method not only enhances the assessment of library uniformity but is also highly compatible with downstream sequencing steps.

DNA libraries that met the sample requirements were subjected to paired-end sequencing on the Illumina HiSeq 2500 platform at BGI (Shenzhen, China). Sample Requirements: All DNA libraries used for sequencing must meet certain quality standards. Specific requirements included an average length of DNA fragments between 200 and 500 bp, a concentration of at least 10 ng/μL, and no apparent contamination. Read length information: paired-end sequencing was performed on the Illumina HiSeq 2500 platform used, and the read length was set to 150 bp to ensure high-quality sequencing data and adequate coverage.

Low-quality reads were removed from the raw data, and the paired-end reads were merged into single sequences using Fast Length Adjustment of SHort reads (FLASH v1.2.11) software to generate hypervariable region tags. Low-quality reads were removed from the raw data. We identified low-quality reads using the following criteria: Bases with a Phred Quality Score of less than 30 were considered to be of low quality; Reads with an average quality score of less than 20 for each pair of paired end reads were removed; Reads with a read length of less than 50 bases were considered low quality and removed. The merged tags were clustered using UPARSE software based on the 97% similarity threshold to obtain the representative sequence of each operational taxonomic unit (OTU) [23, 24]. The taxonomy assignment of OTUs was performed by comparing sequences to the Greengene_2013_5_99 by RDP classifierv.2.2, with a 0.6 confidence value as the cutoff.

BP-FISH

The paraffin tissue sections were dewaxed, rehydrated, boiled in the reconstitution solution for 10 min, and left to cool before enzymatic digestion with proteinase K (20 μg/mL) at 37 °C for 30 min. Subsequently, the paraffin sections were incubated in prehybridisation solution at 37 °C for 1 h, followed by overnight incubation with hybridisation solution containing probes (8 ng/μL) at 37 °C. The paraffin sections were then sequentially washed with 2× saline sodium citrate (SCC) solution at 37 °C, 1× SCC at 37 °C, and 0.5× SCC at room temperature for 10 min each, followed by incubation with 4',6-diamidino-2-phenylindole (DAPI) staining solution for 7 min in the dark. Finally, the stained paraffin sections were mounted and observed under a microscope. The probes used to detect Lp and *Anoxybacillus kestanbolensis* were 5′-ATCTGACCGTCCCAGGTT-3′ with fluorescein amidite (FAM) modifications on the 5′-end (488) and 3′-end (488), and 5′-CTCCCCAGCACAACG AGCAGCAAAT-3′ with Cy3 modification on the 5′-end, respectively.

Ultrastructure observations

For ultrastructural observation of the small intestinal mucosa, the ileum tissues were fixed in 2.5% glutaraldehyde, rinsed in 0.1 mol/L phosphate buffer (pH 7.4), and dehydrated in graded ethanol. The tissues were embedded in epoxy resin. The ultrathin tissue sections were stained with uranyl acetate and lead citrate and then examined with a transmission electron microscope (Hitachi HT7700).

Statistical analyses

Statistical analyses were performed using SAS 9.4 software. Each strain's gene expression was statistically represented as mean ± standard deviation, with the measurement unit being percentage.

Results

Weight and temperature of guinea pigs in the control group and Lp-infected group

The control group guinea pigs' weight continued to increase; the Lp-infected group guinea pigs' weight increased until 24 h after infection, then significantly decreased at 48 h, and further decreased at 72 h; the control group showed small fluctuations in body temperature; the Lp-infected group started to show an increase in body temperature 6 h after infection, peaked at 48 h, and significantly decreased at 72 h (Supplement Table 1).

Extraction of 16S rDNA from the lungs, blood, liver, and small intestinal content

At 24, 48, and 72 h post-infection, three randomly selected guinea pigs from each group were tested (a total of 18 guinea pigs and 9 guinea pigs per group). Bacterial 16S rDNA was extracted from the contents of the small intestine of all guinea pigs in the control group (9/9), whereas there was no extractable bacterial 16S rDNA in the lungs, blood, and liver. In contrast, bacterial 16S rDNA was extracted from the lungs (9/9), blood (3/9), liver (3/9), and contents of the small intestine (9/9) of Lp-infected guinea pigs. To confirm negative results, all negative samples were processed using an optimized extraction procedure. Optimization procedures include re-extracting using the same DNA Kits, increasing the temperature and time of sample processing, and introducing additional cleaning steps to improve DNA recovery and reduce the possibility of contamination. The negative results in the first attempt of extraction were further confirmed with optimised extraction procedures.

Bacterial composition in the lungs, blood, and liver at the species level

The lung tissues of two Lp-infected guinea pigs were dominated by *A. kestanbolensis*, *Geobacillus vulcani*, and *Klebsiella oxytoca* at 24 h and 48 h post-infection, whereas Lp became the dominant bacterium at 72 h post-infection (Fig. 1a). *A. kestanbolensis*, *G. vulcani*, and *K. oxytoca* dominated the blood and liver tissues of two Lp-infected guinea pigs at 24 h post-infection and of one Lp-infected guinea pig at 48 h post-infection (Fig. 1a).

Lp infection led to microbiome imbalance in the small intestine

The microbiome of the small intestine of guinea pigs in the control group was dominated by *Bacteroidota* and *Bacillota* at the phylum level (Table 1; Fig. 1b).

At 24 h post-infection, the small intestines of guinea pigs in the Lp-infected were dominated by *Bacteroidota* and *Bacillota* at the phylum level (Table 1; Fig. 1b). However, the proportion of *Pseudomonadota* started to increase gradually at 48 h and at 72 h post-infection, and it became the dominant group compared with the control group at 72 h (Table 1; Fig. 1b). *Escherichia coli* was the dominant bacterium at 72 h post-infection (Supplement Fig. 1).

BP-FISH in the lung liver and small intestine

A. kestanbolensis accounted for the highest proportion of bacterial DNA in the lung and the liver in Lp-infected guinea pigs at 24 h and 48 h post-infection, indicating the possibility of bacterial translocation from gut to remote tissues; this was further identified by BP-FISH assay.

The BP-FISH assays with Lp-specific and *A. kestanbolensis*-specific probes yielded negative results in the lungs, liver, and small intestinal tissues of guinea pigs in the control group at all time points (Figs. 2, 3, 4, 5 and 6; Supplement Fig. 2). The signal intensity of the Lp-specific probe in the lungs (Fig. 2) and small intestines (Fig. 3) of Lp-infected guinea pigs began to increase gradually

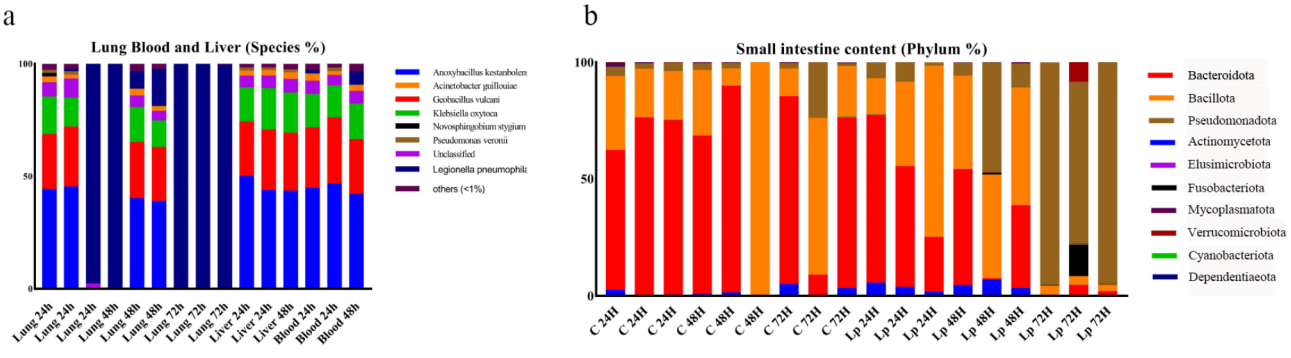

Fig. 1 Legionellosis causes translocation and imbalance of intestinal flora. The taxonomic composition distribution (**a**) in Lung, blood and liver of species-level in Lp- infected group. The taxonomic composition distribution (**b**) in small intestine content of phylum-level. **c** (Control group), and Lp (Lp-infected group)

Table 1 Intestinal flora imbalance caused by Lp infection (Phylum level)

Strain	Cn			Lp		
	24 h	48 h	72 h	24 h	48 h	72 h
Actinomycetota	1.2 ± 1.25	0.87 ± 0.65	2.84 ± 2.48	3.79 ± 1.88	4.93 ± 1.84	0.22 ± 0.26
Bacteroidota	70.15 ± 9.13	52.01 ± 46.24	54.14 ± 39.34	48.92 ± 24.47	28.51 ± 25.15	2.1 ± 2.37
Cyanobacteriota	0.03 ± 0.05	0.02 ± 0.04	0.12 ± 0.21	0.06 ± 0.1	0 ± 0	0 ± 0
Elusimicrobiota	0.03 ± 0.05	0 ± 0	0.3 ± 0.05	0 ± 0	0.03 ± 0.05	0 ± 0
Bacillota	24.52 ± 6.38	45.13 ± 48.34	33.62 ± 29.52	41.77 ± 29.36	44.94 ± 5.2	3.57 ± 0.69
Fusobacteriota	0 ± 0	0 ± 0	0.03 ± 0.05	0 ± 0	0.28 ± 0.49	4.6 ± 7.64
Pseudomonadota	3.28 ± 0.87	1.78 ± 1.51	9.01 ± 12.74	5.44 ± 3.54	21.08 ± 22.85	86.73 ± 14.71
Dependentiaeota	0.12 ± 0.15	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mycoplasmatota	0.61 ± 0.79	0.14 ± 0.13	0.21 ± 0.19	0.03 ± 0.05	0.23 ± 0.18	0 ± 0
Verrucomicrobiota	0.06 ± 0.05	0.03 ± 0.05	0 ± 0	0 ± 0	0 ± 0	2.78 ± 4.81

The proportion of small intestine contents gene expression at the phylum-level. The unit is percentage. Cn (Control group), and Lp (Lp infected group)

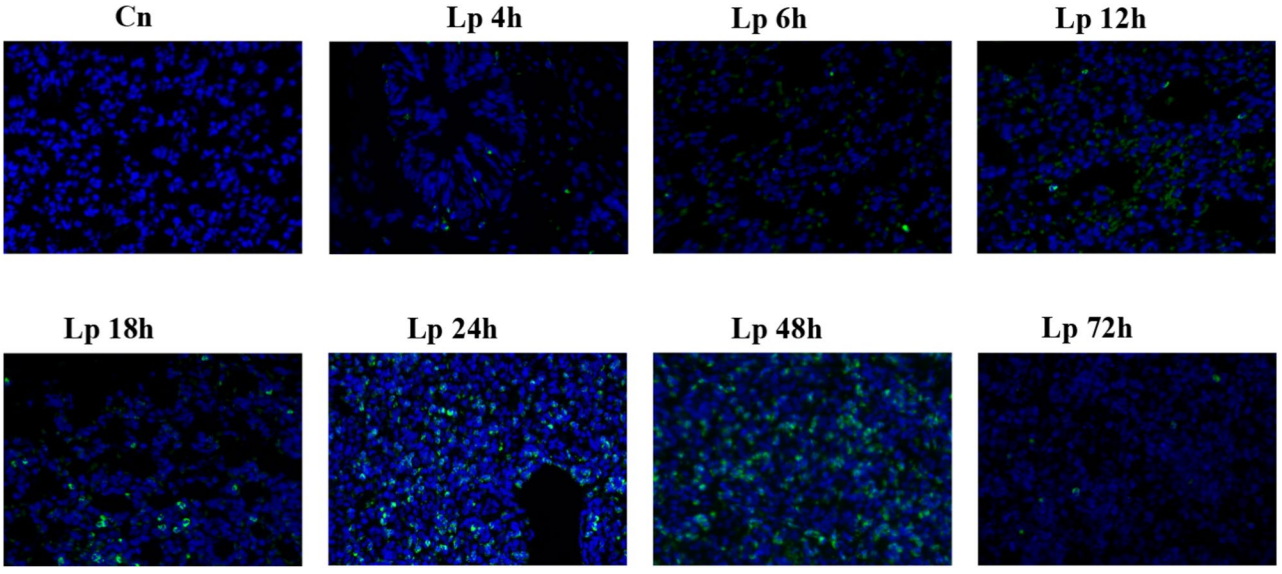

Fig. 2 The presence of Lp in lung by FISH after Lp infected. Cn (Control group), Lp (Lp-infected group)

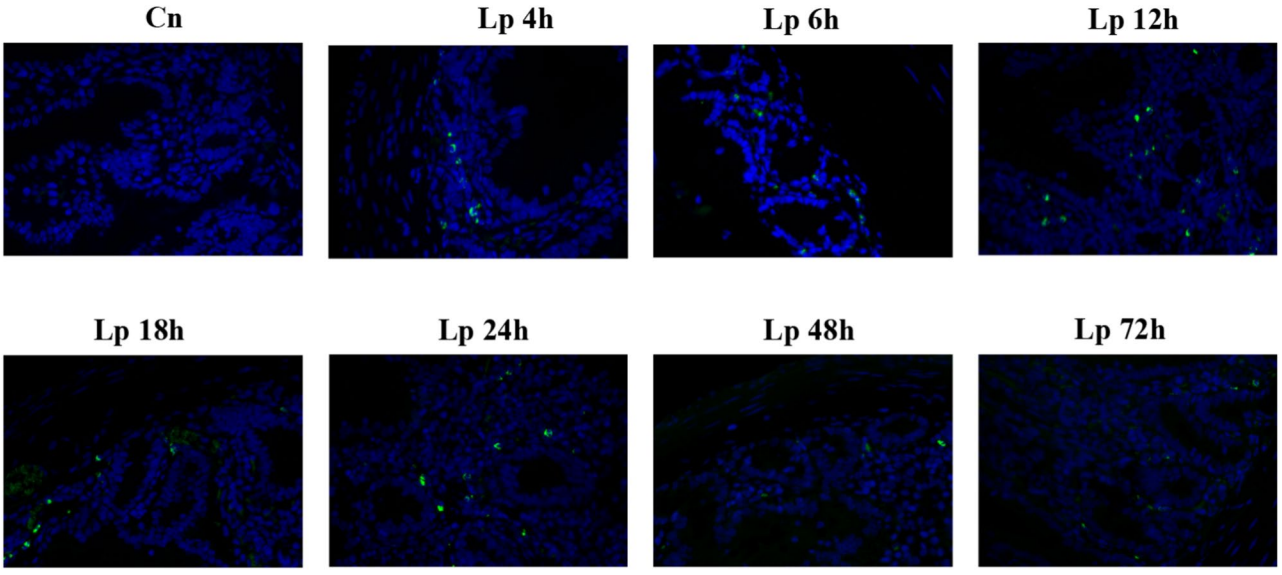

Fig. 3 The presence of Lp in small intestine by FISH after Lp infected. Cn (Control group), Lp (Lp-infected group)

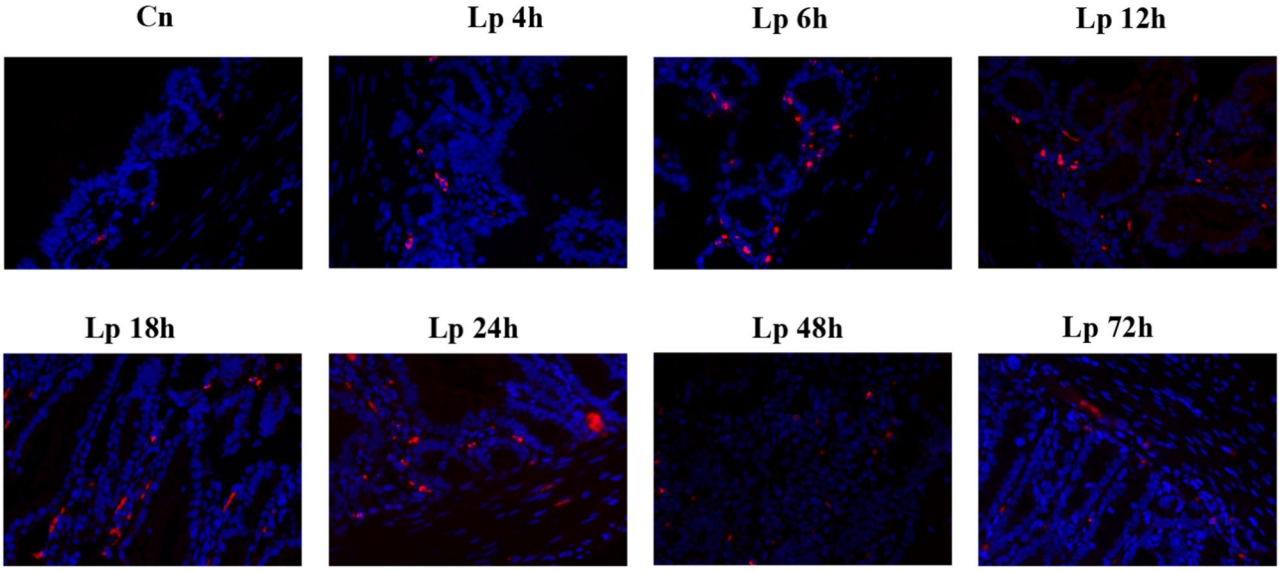

Fig. 4 The presence of *Anoxybacillus kestanbolensis* in small intestine by FISH after Lp infected. Cn (Control group), Lp (Lp-infected group)

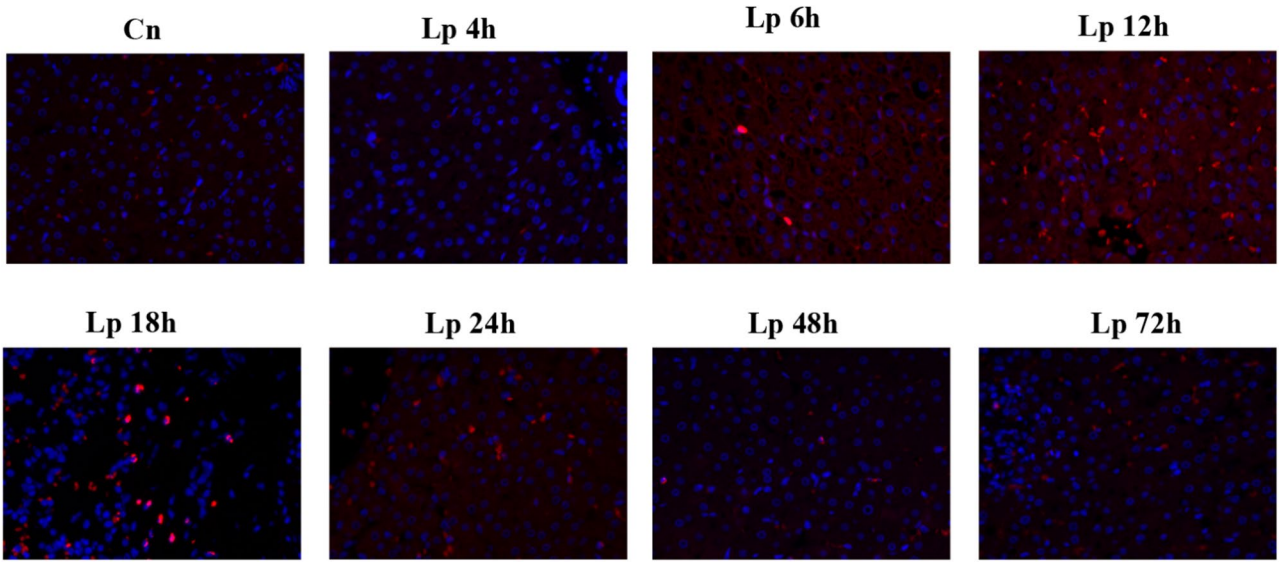

Fig. 5 The presence of *Anoxybacillus kestanbolensis* in Liver by FISH after Lp infected. Cn (Control group), Lp (Lp-infected group)

at 4 h post-infection, peaked at 48 h post-infection, and reduced significantly at 72 h post-infection. The trend in signal intensity of the Lp-specific probe observed in the liver of the Lp-infected group paralleled that observed in the small intestines of the same group. The signal intensity of *A. kestanbolensis*-specific probe in the small intestines of Lp-infected guinea pigs started to increase gradually at 4 h post-infection, peaked at 24 h post-infection, and started to reduce significantly at 48 h post-infection (Fig. 4). In contrast, in the liver of Lp-infected guinea pigs, the signal intensity of *A. kestanbolensis*-specific probe started to increase gradually at 6 h post-infection, peaked at 18 h post-infection, and began to reduce at 24 h post-infection (Fig. 5), whereas in the lungs the signal intensity began to increase gradually at 6 h post-infection, peaked at 24 h post-infection, and reduced significantly at 48 h post-infection (Fig. 6). Extremely weak or no signals were observed in all of the organs at 72 h post-infection.

Ultrastructural observation of the small intestinal mucosa

Electron microscope observations revealed that guinea pigs in the control group had a normal intestinal ultrastructure at all time points. In contrast, the entire process of bacterial entry from the intestinal lumen into the submucosa was visible in Lp-infected guinea pigs at 18 h post-infection (Fig. 7).

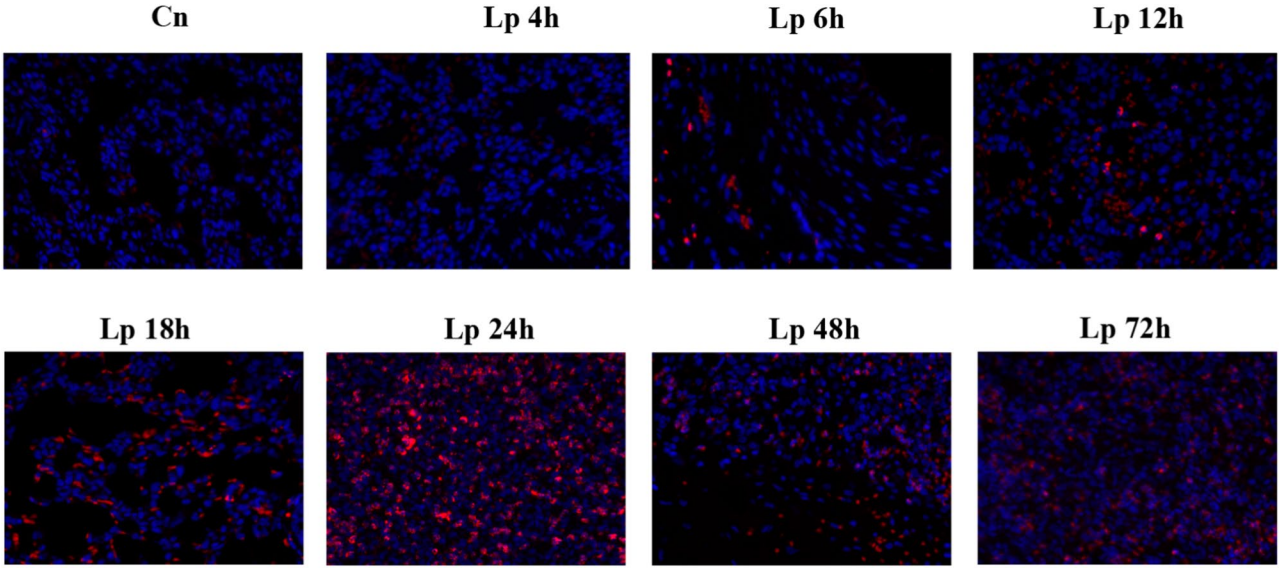

Fig. 6 The presence of *Anoxybacillus kestanbolensis* in lung by FISH after Lp infected. Cn (Control group), Lp (Lp-infected group)

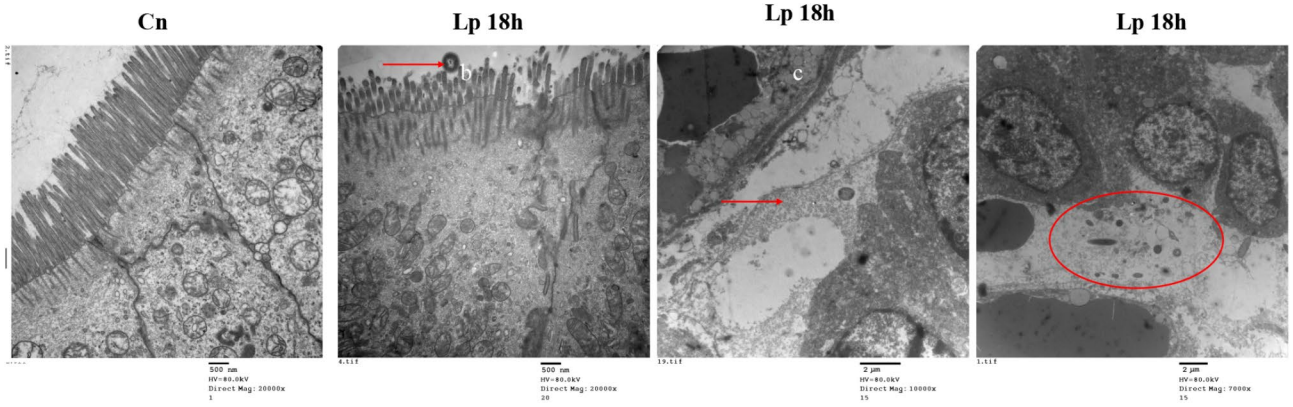

Fig. 7 The entire process of gut bacterial entry from the intestinal lumen into the submucosa at 18 h after Lp infection. (**a**) The structure of the intestinal mucosa in control group; (**b**) The bacteria (red arrow) reached the surface of the intestinal mucosa; (**c**) The bacteria (red arrow) entered the intestinal epithelial cell. (**d**) The intestinal epithelial cells were destroyed and the bacteria enter submucosa. Cn (Control group), Lp (Lp-infected group)

Discussion

Recent studies have shown that in critically ill patients such as those with sepsis, ARDS, and shock, the lung and gut microbiomes undergo profound changes, and gut-associated bacteria translocate to the lung [25, 26]. Lp can cause lethal infection; however, there are no studies so far on the translocation of gut bacteria during legionellosis. The core finding of our study is that genomic DNA from gut-associated anaerobic bacteria is enriched the lung microbiome in experimental Lp-infected model. Previous studies on legionellosis involved the isolation and culture of *Legionella* without performing anaerobic culture, and isolated non-*Legionella* bacteria were considered as contaminants [27, 28]. Specific PCR amplification of conserved regions in the bacterial 16S rDNA sequences allows for more sensitive detection for pathogenic genomic DNA than conventional culture methods [26, 29]. Therefore, we investigated the microbiome in the lungs, blood, liver, and small intestinal contents of guinea pigs infected with legionellosis by culture-independent method. *A. kestanbolensis* and *G. vulcani*, among others, were only detected in the small intestine content in guinea pigs in the control group, whereas no bacterial DNA could be extracted from their lungs, blood, and liver tissues. In contrast, these gut bacterial DNA were also extracted from the lungs, blood, and liver tissues in Lp-infected guinea pigs at 24 h and 48 h post-infection, indicating that the anaerobic bacterial DNA had translocated from the gut to remote organs. Hybridisation with the *A. kestanbolensis*-specific probe yielded positive results. Both of *A. kestanbolensis* and *G. vulcani* are members of *Bacillales* order and *Bacillaceae* family. Recently, a mendelian randomization study found that *Bacillales* order of gut microbiota was causally

associated with lower respiratory tract infections [30]. Another study on respiratory flora in patients with ventilator-related pneumonia revealed a positive correlation between *Bacillales* order and microbial β diversity [31]. However, the mechanisms involved are still unknown, necessitating additional investigation in the future. We also observed the entire process of bacterial entry from the intestinal lumen into the submucosa in Lp-infected guinea pigs by electron microscope. Dickson et al. [26] identified prominent anaerobic gut-associated bacteria in animal model of sepsis and human study of ARDS, which is consistent with our results, although they reported different anaerobic bacteria.

The translocation of gut microflora, also called the "engine" of MODS, has gained substantial attention in cases of ARDS and MODS in recent years [32]. Once the translocated gut bacteria and inflammatory mediators enter the pulmonary microcirculation, they activate the alveolar macrophages and trigger the onset of acute lung injury and ARDS [33]; moreover, they can activate systemic immune responses and promote the cascading effects of inflammatory mediators, leading to the onset of systemic inflammatory response syndrome and MODS [34]. Previous studies about the pathogenesis of legionellosis mostly focused on the direct injury to the lung by Lp per se, while no attention has been paid on the translocated gut-associated bacteria. The translocation of abundant gut anaerobic bacteria (such as *A. kestanbolensis*) and their products (such as catecholase, alpha-L-arabinofuranosidase, and amylase) [35] may further aggravate the lung injury, which may persist even after the discontinuation of bacterial translocation. However, the impact of bacteria that have been translocated to multiple tissues and organs on the body and their "trigger effects" on the progression to ARDS and MODS in legionellosis remain to be further studied.

The gastrointestinal microbiota in adults comprises 1000–1150 bacterial species [36]. The guinea pig is an important animal model for studying the gut microbiome in humans, as the gut of guinea pigs and humans are dominated by *Bacteroidota* and *Bacillota* [37] and our results were in line with these observations. The composition of microbiome in the small intestine of Lp-infected guinea pigs gradually shifted from being dominated by *Bacteroidota* and *Bacillota* to one that was dominated by *Pseudomonadota*, and at 72 h post-infection, *E. coli* became the dominant species, suggesting an imbalance of the gut microflora. *E. coli* is a common bacterium in the gut, accounting for less than 1% of gut microbiota in healthy adults [38]. However, its abnormal increase is associated with various diseases such as acute cholecystitis [39]. *E. coli* is the main gut bacteria involved in colonisation and translocation [40]. Under normal conditions, gut anaerobic bacteria are capable of suppressing the colonisation and translocation of *Enterobacteriaceae* [41]. Our results showed that *E. coli* dominated the imbalanced gut microbiome but did not translocate following Lp infection. However, further studies are needed to determine whether the absence of *E. coli* translocation was attributed to the inadequate observation period (72 h) or whether there were other reasons. Patients with legionellosis may develop diarrhoea [5] but its specific cause has not yet been reported. Based on our results, we hypothesise that the onset of diarrhoea during *Legionella* infections may be associated with the imbalance of gut microflora.

Our results showed that the translocation of gut bacterial DNA occurred in only two out of three Lp-infected guinea pigs at 24 h and 48 h post-infection. Naaber P et al. [42] investigated the translocation of indigenous microflora in an experimental model of sepsis and found the same phenomenon. Cunha et al. reviewed the clinical characteristics of *Legionella* infection and identified that not all patients had extrapulmonary manifestations and only a few patients with legionellosis develop ARDS and MODS [43]. If the translocation of gut-associated bacteria is associated with extrapulmonary manifestations, our findings are consistent with the clinical setting. A recent study revealed that increased lung bacterial DNA burden and enrichment of the lung microbiome with gut-associated bacterial taxa were predictive of poor ICU outcomes and the clinical diagnosis of ARDS [44]. In our study, *A. kestanbolensis* and *G. vulcani* were the main bacterial species that translocated from the gut. As these anaerobic bacteria are sensitive to ampicillin, streptomycin, and gentamicin, among others [35], the use of conventional antibiotics against *Legionella* such as macrolide may result in relatively poor therapeutic outcomes against the translocated bacteria. A multicentre retrospective observational study revealed that patients with severe legionellosis receiving a fluoroquinolone-based antimicrobial regimen in the early course of management had a lower in-ICU mortality [9]. Compared with macrolide, fluoroquinolone is a broad-spectrum antibiotic. Whether the protective effect on severe legionellosis was related to the effective control of the early intestinal translocation bacteria remains to be further studied. Therefore, in the treatment of critically ill patients with legionellosis, it is necessary to pay attention to superinfection and therapeutic strategy to improve the prognosis.

To the best of our knowledge, this is the first study to show Lp-induced dysbiosis in gut and the translocation of gut-associated anaerobic bacterial DNA to remote organs such as the lungs and liver. However, our study has limitations. The sample size included in this study was small. Although Lp1 is the most common type of infection that causes *legionella* pneumonia, only Lp1 strain (ST2345) infection was assessed in the study. Various strains of

Legionella, along with distinct serological and sequenced types of Lp, may exhibit varying levels of pathogenicity. Consequently, further research in the future may be required to investigate the relationship between non-Lp1 (ST2345) *Legionella* types and bacterial translocation or gut microbiome imbalance. Furthermore, only the presence of genomic DNA was confirmed rather than intact, viable translocated cells. This study only "suggests" rather than "confirm" the existence of this phenomenon, and more research is needed to confirm this. In addition, the underlying mechanisms of Lp-induced translocation of gut bacteria and their effects on the hosts were not determined.

Conclusions

Infection with the *Legionella* pneumophila serotype 1 disrupted the intestinal microbiota in a subset of guinea pigs during a 72-hour period post-infection, with possible translocation of gut-associated anaerobic bacteria to the lungs and liver based on the presence of genomic DNA detected in tissue from infected guinea pigs.

Abbreviations

Lp	*Legionella pneumophila*
U/EEA	European Union/European Economic Area
CAP	Community-acquired pneumonia
ICU	Intensive care unit
ARDS	Acute respiratory distress syndrome
MODS	Multiple organ dysfunction syndrome
BP-FISH	acterial probe-fluorescence in situ hybridisation
CFU	Colony-forming units
PCR	Polymerase chain reaction
SCC	Saline sodium citrate
DAPI	4′,6-diamidino-2-phenylindole

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03845-4.

Supplementary Material 1

Acknowledgements

We would like to thank BGI-Shenzhen for conducting the next-generation sequencing and Editage (www.editage.cn) for English language editing.

Author contributions

YC, WW, JK, YL, MX and XC designed the experiments, and XC and MX performed them. WS collected and analysed the data. XC, MX and YL wrote the manuscript. All authors approved the final version of the manuscript.

Funding

The study was supported by grants from the National key R&D Program of China (Grant No. 2017YFC1309702) and the National Natural Science Foundation of China (Grant No. 81170009, 82270107).

Data availability

All sequence reads were deposited into the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA1105769. All the other relevant data of the study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval and consent to participate

The Experimental Animal Welfare and Ethics Committee of the Chinese Medical University (Shenyang, China) approved the experimental protocols.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 8 March 2024 / Accepted: 24 February 2025
Published online: 14 March 2025

References

1. Corre MH, Mercier A, Bouteiller M, Khalil A, Ginevra C, Depayras S, et al. Bacterial Long-Range warfare: aerial killing of Legionella pneumophila by Pseudomonas fluorescens. Microbiol Spectr. 2021;9(1):e0040421.
2. Mondino S, Schmidt S, Rolando M, Escoll P, Gomez-Valero L, Buchrieser C. Legionnaires' disease: state of the Art knowledge of pathogenesis mechanisms of Legionella. Annu Rev Pathol. 2020;15:439–66. https://doi.org/10.1146/annurev-pathmechdis-012419-032742.
3. Iliadi V, Staykova J, Iliadis S, Konstantinidou I, Sivykh P, Romanidou G et al. Legionella Pneumophila: the journey from the environment to the blood. J Clin Med. 2022;11(20).
4. Rello J, Allam C, Ruiz-Spinelli A, Jarraud S. Severe legionnaires' disease. Ann Intensive Care. 2024;14(1):51. https://doi.org/10.1186/s13613-024-01252-y.
5. Cunha BA, Burillo A, Bouza E. Legionnaires' disease. Lancet. 2016;387(10016):376–85. https://doi.org/10.1016/s0140-6736(15)60078-2.
6. Qu J, Zhang J, Chen Y, Huang Y, Xie Y, Zhou M, et al. Aetiology of severe community acquired pneumonia in adults identified by combined detection methods: a multi-centre prospective study in China. Emerg Microbes Infect. 2022;11(1):556–66.
7. Chahin A, Opal SM. Severe pneumonia caused by Legionella Pneumophila: differential diagnosis and therapeutic considerations. Infect Dis Clin North Am. 2017;31(1):111–21.
8. Yang JL, Li D, Zhan XY. Concept about the virulence factor of Legionella. Microorganisms. 2022;11(1).
9. Cecchini J, Tuffet S, Sonneville R, Fartoukh M, Mayaux J, Roux D, et al. Antimicrobial strategy for severe community-acquired legionnaires' disease: a multicentre retrospective observational study. J Antimicrob Chemother. 2017;72(5):1502–9. https://doi.org/10.1093/jac/dkx007.
10. Viasus D, Gaia V, Manzur-Barbur C, Carratalà J. Legionnaires' disease: update on diagnosis and treatment. Infect Dis Ther. 2022;11(3):973–86.
11. Schroeder GN. The toolbox for Uncovering the functions of Legionella Dot/Icm type IVb secretion system effectors: current state and future directions. Front Cell Infect Microbiol. 2017;7:528. https://doi.org/10.3389/fcimb.2017.00528.
12. Liu X, Shin S. Viewing Legionella pneumophila pathogenesis through an immunological Lens. J Mol Biol. 2019;431(21):4321–44.
13. Chen Y, Tateda K, Fujita K, Ishii T, Ishii Y, Kimura S, et al. Sequential changes of Legionella antigens and bacterial load in the lungs and urines of a mouse model of pneumonia. Diagn Microbiol Infect Dis. 2010;66(3):253–60. https://doi.org/10.1016/j.diagmicrobio.2009.11.001.
14. Lieberman D, Porath A, Schlaeffer F, Lieberman D, Boldur I. Legionella species community-acquired pneumonia. A review of 56 hospitalized adult patients. Chest. 1996;109(5):1243–9.
15. Tan MJ, Tan JS, File TM. Jr. Legionnaires disease with bacteremic coinfection. Clin Infect Diseases: Official Publication Infect Dis Soc Am. 2002;35(5):533–9. https://doi.org/10.1086/341771.
16. Gadsby NJ, Musher DM. The microbial etiology of Community-Acquired pneumonia in adults: from classical bacteriology to host transcriptional signatures. Clin Microbiol Rev. 2022;35(4):e0001522. https://doi.org/10.1128/cmr.00015-22.
17. Mizrahi H, Peretz A, Lesnik R, Aizenberg-Gershtein Y, Rodríguez-Martínez S, Sharaby Y, et al. Comparison of sputum Microbiome of legionellosis-associated patients and other pneumonia patients: indications for polybacterial infections. Sci Rep. 2017;7:40114.

18. Pérez-Cobas AE, Ginevra C, Rusniok C, Jarraud S, Buchrieser C. Persistent Legionnaires' Disease and Associated Antibiotic Treatment Engender a Highly Disturbed Pulmonary Microbiome Enriched in Opportunistic Microorganisms. mBio. 2020;11(3).
19. Khan S, Imran A, Malik A, Chaudhary AA, Rub A, Jan AT, et al. Bacterial imbalance and gut pathologies: association and contribution of E. coli in inflammatory bowel disease. Crit Rev Clin Lab Sci. 2019;56(1). https://doi.org/10.1080/10408363.2018.1517144.
20. Cai X, Yu N, Ma J, Li WY, Xu M, Li E, et al. Altered pulmonary capillary permeability in immunosuppressed guinea pigs infected with Legionella pneumophila serogroup 1. Experimental Therapeutic Med. 2019;18(6):4368–78. https://doi.org/10.3892/etm.2019.8102.
21. Jiang L, Tao S, Mu D, Zhang N, Zhao L, Chen Y. Case report: fatal pneumonia caused by new sequence type Legionella Pneumophilia serogroup 1. Med (Baltim). 2020;99(43):e22812. https://doi.org/10.1097/md.0000000000022812.
22. Illumina. HiSeq® 2500 Sequencing System. https://www.illumina.com/documents/products/datasheets/datasheet_hiseq2500.pdf (2015). Accessed 2015-03.
23. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996–8. https://doi.org/10.1038/nmeth.2604.
24. Quince C, Lanzén A, Curtis TP, Davenport RJ, Hall N, Head IM, et al. Accurate determination of microbial diversity from 454 pyrosequencing data. Nat Methods. 2009;6(9):639–41. https://doi.org/10.1038/nmeth.1361.
25. Stanley D, Mason LJ, Mackin KE, Srikhanta YN, Lyras D, Prakash MD, et al. Translocation and dissemination of commensal bacteria in post-stroke infection. Nat Med. 2016;22(11):1277–84. https://doi.org/10.1038/nm.4194.
26. Dickson RP, Singer BH, Newstead MW, Falkowski NR, Erb-Downward JR, Standiford TJ, et al. Enrichment of the lung Microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. Nat Microbiol. 2016;1(10):16113.
27. Kajiwara C, Kusaka Y, Kimura S, Yamaguchi T, Nanjo Y, Ishii Y et al. Metformin Mediates Protection against Legionella Pneumonia through Activation of AMPK and Mitochondrial Reactive Oxygen Species. Journal of immunology (Baltimore, Md: 1950). 2018;200(2):623– 31; https://doi.org/10.4049/jimmunol.1700474
28. Gamradt P, Xu Y, Gratz N, Duncan K, Kobzik L, Hogler S, et al. The influence of programmed cell death in myeloid cells on host resilience to infection with Legionella pneumophila or Streptococcus pyogenes. PLoS Pathog. 2016;12(12):e1006032. https://doi.org/10.1371/journal.ppat.1006032.
29. Xiao L, Sonne SB, Feng Q, Chen N, Xia Z, Li X, et al. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. Microbiome. 2017;5(1):43.
30. Huang S, Li J, Zhu Z, Liu X, Shen T, Wang Y, et al. Gut microbiota and respiratory infections: insights from Mendelian randomization. Microorganisms. 2023;11(8). https://doi.org/10.3390/microorganisms11082108.
31. Zakharkina T, Martin-Loeches I, Matamoros S, Povoa P, Torres A, Kastelijn JB, et al. The dynamics of the pulmonary Microbiome during mechanical ventilation in the intensive care unit and the association with occurrence of pneumonia. Thorax. 2017;72(9):803–10. https://doi.org/10.1136/thoraxjnl-2016-209158.
32. Leaphart CL, Tepas JJ 3. The gut is a motor of organ system dysfunction. Surgery. 2007;141(5):563–9. https://doi.org/10.1016/j.surg.2007.01.021.
33. Deitch EA, Xu D, Kaise VL. Role of the gut in the development of injury- and shock induced SIRS and MODS: the gut-lymph hypothesis, a review. Front Bioscience: J Virtual Libr. 2006;11:520–8.
34. Deitch EA. Bacterial translocation or lymphatic drainage of toxic products from the Gut: what is important in human beings? Surgery. 2002;131(3):241–4.
35. Dulger S, Demirbag Z, Belduz AO. Anoxybacillus ayderensis Sp. Nov. And Anoxybacillus kestanbolensis Sp. nov. Int J Syst Evol MicroBiol. 2004;54(Pt 5):1499–503. https://doi.org/10.1099/ijs.0.02863-0.
36. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59–65. https://doi.org/10.1038/nature08821.
37. Hildebrand F, Ebersbach T, Nielsen HB, Li X, Sonne SB, Bertalan M, et al. A comparative analysis of the intestinal metagenomes present in guinea pigs (Cavia porcellus) and humans (Homo sapiens). BMC Genomics. 2012;13:514. https://doi.org/10.1186/1471-2164-13-514.
38. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635–8.
39. Liu J, Yan Q, Luo F, Shang D, Wu D, Zhang H, et al. Acute cholecystitis associated with infection of Enterobacteriaceae from gut microbiota. Clin Microbiol Infection: Official Publication Eur Soc Clin Microbiol Infect Dis. 2015;21(9):e8511–9. https://doi.org/10.1016/j.cmi.2015.05.017.
40. Zhang W, Gu Y, Chen Y, Deng H, Chen L, Chen S, et al. Intestinal flora imbalance results in altered bacterial translocation and liver function in rats with experimental cirrhosis. Eur J Gastroenterol Hepatol. 2010;22(12):1481–6. https://doi.org/10.1097/MEG.0b013e32833eb8b0.
41. Wells CL, Maddaus MA, Reynolds CM, Jechorek RP, Simmons RL. Role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria. Infect Immun. 1987;55(11):2689–94.
42. Naaber P, Smidt I, Tamme K, Liigant A, Tapfer H, Mikelsaar M, et al. Translocation of Indigenous microflora in an experimental model of sepsis. J Med Microbiol. 2000;49(5):431–9. https://doi.org/10.1099/0022-1317-49-5-431.
43. Cunha BA, Cunha CB. Legionnaire's disease: A clinical diagnostic approach. Infect Dis Clin North Am. 2017;31(1):81–93. https://doi.org/10.1016/j.idc.2016.10.007.
44. Dickson RP, Schultz MJ, van der Poll T, Schouten LR, Falkowski NR, Luth JE, et al. Lung microbiota predict clinical outcomes in critically ill patients. Am J Respir Crit Care Med. 2020;201(5):555–63. https://doi.org/10.1164/rccm.201907-1487OC.

Publisher's note