Comparative genomic analysis of *Proteus* spp. isolated from tree shrews indicated unexpectedly high genetic diversity

Wenpeng Gu¹,², Wenguang Wang¹, Pinfen Tong¹, Chenxiu Liu¹, Jie Jia¹, Caixia Lu¹, Yuanyuan Han¹, Xiaomei Sun¹, Dexuan Kuang¹, Na Li¹, Jiejie Dai¹*

¹ Center of Tree Shrew Germplasm Resources, Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Yunnan Key Laboratory of Vaccine Research and Development on Severe Infectious Diseases, Yunnan Innovation Team of Standardization and Application Research in Tree Shrew, Kunming, China, ² Department of Acute Infectious Diseases Control and Prevention, Yunnan Provincial Center for Disease Control and Prevention, Kunming, China

* djj@imbcams.com.cn

Abstract

*Proteus* spp. are commensal gastrointestinal bacteria in many hosts, but information regarding the mutual relationships between these bacteria and their hosts is limited. The tree shrew is an alternative laboratory animal widely used for human disease research. However, little is known about the relationship between *Proteus* spp. and tree shrews. In this study, the complete genome sequencing method was used to analyse the characteristics of *Proteus* spp. isolated from tree shrews, and comparative genomic analysis was performed to reveal their relationships. The results showed that 36 *Proteus* spp. bacteria were isolated, including 34 *Proteus mirabilis* strains and two *Proteus vulgaris* strains. The effective rate of sequencing was 93.53±2.73%, with an average GC content of 39.94±0.25%. Briefly, 3682.89±90.37, 2771.36±36.01 and 2832.06±42.49 genes were annotated in the NCBI non-redundant nucleotide database (NR), SwissProt database and KEGG database, respectively. The high proportions of macrolide-, vancomycin-, bacitracin-, and tetracycline-resistance profiles of the strains were annotated in the Antibiotic Resistance Genes Database (ARDB). Flagella, lipoooligosaccharides, type 1 fimbriae and P fimbriae were the most abundantly annotated virulence factors in the Virulence Factor Database (VFDB). SNP variants indicated high proportions of base transitions (Ts), homozygous mutations (Hom) and non-synonymous mutations (Non-Syn) in *Proteus* spp. (*P*<0.05). Phylogenetic analysis of *Proteus* spp. and other references revealed high genetic diversity for strains isolated from tree shrews, and host specificity of *Proteus* spp. bacteria was not found. Overall, this study provided important information on characteristics of genome for *Proteus* spp. isolated from tree shrews.

Introduction

*Proteus* spp. are common commensal gastrointestinal bacteria in many hosts and belong to the *Morganellaceae* family [1–2]. Currently, the genus comprises *Proteus mirabilis*, *Proteus
vulgaris, Proteus penneri, Proteus hauseri, and unnamed genomospecies 4, 5, and 6 [3–4]. These bacteria are considered as opportunistic human pathogens and have been isolated from different clinical sources, such as urine and wounds [5]. Many studies have demonstrated their roles in the pathogenesis of infections in human beings, indicating that their virulence factors enable the bacteria to reach and survive different niches of host organisms. However, Proteus spp. is actually a normal, ubiquitous commensal constituent of human flora and it is recognized that the gut is a reservoir for this proteolytic organism. Previous studies have illustrated the Proteus spp. antagonistic or commensal relationships with numerous animals and sometimes found cooperated relations with different animal hosts, acting as animal symbionts [5–7]. However, the complicated interdependence between Proteus spp. and animals coexisting with the bacteria in the environment is still unclear. Information regarding the mutual relationships between Proteus spp. bacteria and their natural habitats is currently limited.

The tree shrew (Tupaia belangeri) is a new laboratory animal belonging to the family Tupaiidae, order Scandentia, widely distributed in South Asia, Southeast Asia and Southwest China [8]. This small mammal is similar in appearance to the squirrel and feeds on insects, fruits and small vertebrates. There are six subspecies of Tupaia belangeri in China, namely, T. belangeri gaoligongensis, T. belangeri modesta, T. belangeri yaoshanensis, T. belangeri tonquinia, T. belangeri yunalis and T. belangeri chinensis [9]. Previous studies have indicated that tree shrews are closely related to humans in genomic signature, biochemical metabolism and physiological function [10–11]. Due to its small body size, life span, short reproductive cycle and low cost of maintenance, tree shrews have been increasingly used as alternative laboratory animals in recent years. Several human diseases have been successfully studied by using this animal, such as hepatitis, influenza, dengue fever, Alzheimer’s disease, social stress and depression [12–15]. Our previous study [16] revealed the gut microbial community between tree shrew and human was different, if we used tree shrew as laboratory animal to study human diseases, especially in gastrointestinal tract, could this animal reflected the pathophysiological features of human infection? Whether there was somewhat similar gut bacterial populations between tree shrew and human, and this might be helpful to know for future clinical research applications. Because Proteus spp. was the most isolated bacteria from feces of tree shrew by using culture method [16], in this study, the complete genome sequencing method was used to analyse the characteristics of Proteus spp. isolated from tree shrews, and comparative genomic analysis was performed to reveal their relationships.

Materials and methods

Sample collections

Thirty-six tree shrew faecal samples were collected at the Center of Tree Shrew Germplasm Resources, Institute of Medical Biology, Chinese Academy of Medical Science and Peking Union Medical College in Kunming, China. The tree shrews were a closed population and healthy, without visible signs of disease or tumours; 20 were male, and 16 were female. The average age was 36.08±23.04 months, ranging from 2 months to 75 months. All tree shrews were divided into four age groups based on the method of a previous study with some modification [17]. The infant group was defined as under 7 months, the young group was between 8 and 18 months, the middle group was 19 to 42 months, and the senile group was over 43 months. Tree shrews were housed in independent sterilized stainless steel cage containing hygienic food and water without any outside contact. The commercial full-price nutritive pellet was used for feeding twice a day, and the clean apple was fed once a week. The details of the tree shrew information were shown in Table 1.
### Table 1. The details of tree shrew information and isolation results.

| Strain ID | Species  | Host sex (tree shrew) | Host age (Months) | Host age group | Host generation |
|-----------|----------|-----------------------|-------------------|----------------|-----------------|
| YNTSP1    | *P. mirabilis* | Male                  | 45                | Senile         | F4              |
| YNTSP12   | *P. mirabilis* | Male                  | 14                | Young          | F4              |
| YNTSP13   | *P. mirabilis* | Male                  | 15                | Young          | F4              |
| YNTSP16   | *P. mirabilis* | Female                | 27                | Middle         | F4              |
| YNTSP17   | *P. mirabilis* | Male                  | 45                | Senile         | F4              |
| YNTSP19   | *P. mirabilis* | Female                | 26                | Middle         | F4              |
| YNTSP21   | *P. mirabilis* | Female                | 26                | Middle         | F4              |
| YNTSP22   | *P. mirabilis* | Male                  | 9                 | Young          | F4              |
| YNTSP24   | *P. mirabilis* | Male                  | 9                 | Young          | F4              |
| YNTSP26   | *P. mirabilis* | Female                | 9                 | Young          | F4              |
| YNTSP3    | *P. mirabilis* | Male                  | 59                | Senile         | F4              |
| YNTSP34   | *P. mirabilis* | Female                | 3                 | Infant         | F4              |
| YNTSP35   | *P. vulgaris*  | Female                | 2                 | Infant         | F4              |
| YNTSP36   | *P. mirabilis* | Female                | 2                 | Infant         | F4              |
| YNTSP40   | *P. mirabilis* | Male                  | 2                 | Infant         | F4              |
| YNTSP42   | *P. mirabilis* | Male                  | 49                | Senile         | F4              |
| YNTSP44   | *P. mirabilis* | Female                | 37                | Middle         | F4              |
| YNTSP48   | *P. mirabilis* | Female                | 62                | Senile         | F4              |
| YNTSP5    | *P. mirabilis* | Male                  | 41                | Middle         | F4              |
| YNTSP53   | *P. mirabilis* | Male                  | 71                | Senile         | F4              |
| YNTSP56   | *P. mirabilis* | Female                | 56                | Senile         | F4              |
| YNTSP57   | *P. mirabilis* | Male                  | 50                | Senile         | F4              |
| YNTSP58   | *P. mirabilis* | Female                | 50                | Senile         | F4              |
| YNTSP59   | *P. mirabilis* | Male                  | 52                | Senile         | F4              |
| YNTSP6    | *P. mirabilis* | Female                | 64                | Senile         | F4              |
| YNTSP62   | *P. mirabilis* | Male                  | 75                | Senile         | F4              |
| YNTSP63   | *P. mirabilis* | Male                  | 58                | Senile         | F4              |
| YNTSP65   | *P. mirabilis* | Male                  | 70                | Senile         | F4              |
| YNTSP66   | *P. mirabilis* | Female                | 37                | Middle         | F4              |
| YNTSP67   | *P. mirabilis* | Female                | 37                | Middle         | F4              |
| YNTSP69   | *P. mirabilis* | Male                  | 34                | Middle         | F4              |
| YNTSP70   | *P. mirabilis* | Male                  | 37                | Middle         | F4              |
| YNTSP72   | *P. vulgaris*  | Female                | 74                | Senile         | F4              |
| YNTSP8    | *P. mirabilis* | Female                | 26                | Middle         | F4              |
| YNTSP9    | *P. mirabilis* | Male                  | 24                | Middle         | F4              |

Bacterial isolation and DNA extraction

The *Proteus* spp. bacteria were isolated according to previous study [18]. All faecal samples were inoculated on MacConkey Agar and Xylose Lysine Deoxycholate (XLD) agar (Luqiao, Beijing) and incubated at 37°C for 24 hours. All suspected *Proteus* spp. colonies were selected, purified by inoculation on Brain Heart Agar (BHI) (Luqiao, Beijing) at 37°C for 24 hours, and finally identified using the Vitek Compact 2 biochemical identification system (bioMérieux). Total genomic DNA of the isolated bacteria was extracted using a bacterial total genomic DNA extraction kit (Tiangen, Beijing) following the manufacturer’s instructions. All DNA samples were stored at -20°C for complete genome analysis.
**Genome sequencing, assembly and annotation**

Bacterial genome sequencing of all isolates was performed by our laboratory on the Illumina MiSeq platform using 2×250 bp paired-end reads. The libraries were built using a Nextera XT DNA Library Prep Kit following the manufacturer’s reference guidelines. Generally, 1 ng genomic DNA of each strain was used. After segment and purification, index PCR was performed to add the Illumina Nextera barcodes using i5 and i7 primers, and then the purification was executed again to remove non-target fragments. Finally, the libraries were normalized, pooled and sequenced using an Illumina MiSeq sequencing system (Illumina, San Diego, USA).

The raw data were trimmed for quality control, and low-quality (<Q40) reads were filtered by Trimmomatic (version 0.38) [19]. Draft genomes were assembled using SOAPdenovo (version 2.04), with k-mer values (25, 31, 37, 47, 59, 71, 83 and 95) optimized to the best assembly results [20–21]. Gapcloser (version 1.12r6) was used to fill the genomic gaps. GenemarkS software (version 4.28) [22] with default parameters was used to predict the open reading frame (ORF) of each genome, and the predicted amino acid sequences were aligned and annotated by DIAMOND (E-value: 1e-5, top 5) [23] to NCBI non-redundant nucleotide database (NR), SwissProt, eggNOG, KEGG, Pfam, Pathogen-Host Interaction database (PHI), Antibiotic Resistance Genes Database (ARDB), and Virulence Factor Database (VFDB). The heatmaps based on PHI, ARDB and VFDB database annotations were drawn using pheatmap in the R package (version 3.2).

**CRISPR, prophage and ICE analyses**

Clustered regularly interspaced short palindromic repeats (CRISPR) locus screening of bacteria was predicted by CRT (version 1.2), with minRL 19, maxRL 38, minSL 19 and maxSL 48 [24]. The repeat and spacer sequences of the CRISPR loci for each strain were counted and extracted. The prophage of isolated *Proteus* spp. was analysed by PhiSpy (version 2.3) with default parameters [25]. The integrative and conjugative elements (ICEs) of bacteria were detected by ICEfinder [26], and the sequences of all predicted ICEs were extracted for further analysis.

**Variant analysis**

For the detection of single nucleotide polymorphism (SNP) variants relative to the reference, we used clean reads mapped to each reference genome (*Proteus mirabilis* HI4320: NC_010554 and *Proteus vulgaris* FDAARGOS_366: NZ_CP023965) with the BWA software package [27–28]. The mapping quality lower than 20 and sequence depths lower than 5 of the variants were filtered for variants calling [29–30]. The bam files were transferred to sam files using SAMtools, and generated the sequence depths (average = 19.47), coverage (10× = 75.38%) and mapping rate (average = 80.14%). Statistical analyses of SNPs, insertions and deletions (InDels) and classification information for each strain were performed by using BCFtools. The variants of bacteria were annotated based on each reference genome using VCFtools (version 4.2) [31].

**Phylogenetic analysis and statistics**

The sequences of the CRISPR repeats were aligned by MEGA 6.0 using the neighbour-joining (NJ) method with 1000 bootstrap replicates to generate a phylogenetic tree. The SNPs of predicted ICEs were called by using the methods mentioned above, and a phylogenetic tree was built by MEGA 6.0 with 18 reference ICE sequences (S1 Table). Phylogenetic analysis based on total SNPs was performed for all isolated *Proteus* spp. in this study with 114 reference *Proteus*
Proteus spp. (102 *P. mirabilis* and 12 *P. vulgaris*) from different sources and countries (S2 Table). A maximum-likelihood (ML) tree was generated by applying PhyML version 3.1 with default parameter (data type: nucleotide, the numbers of bootstrap replicates: 100, model: HKY85, gamma value: e, number of relative substitution rate categories: 4 and transition/tranversion rate: e) to the SNPs differentiating the genomes [32]. The distance-based NJ method was also used to estimate their phylogenetic relations. The phylogenetic tree was visualized and ordered with FigTree (version 1.4.3) and iTol.

Statistical analysis was performed using the SPSS software package (version 16.0, IBM, USA). Kolmogorov-Smirnov Z, T-test or Kruskal-Wallis H test were used if appropriate. A P value of <0.05 was recognized as statistically significant. The Tajima’s D values were calculated by MEGA 6.0 for Proteus spp. in this study.

The sequence data have been deposited into the European Nucleotide Archive, www.ebi.ac.uk/ena with Accession Numbers ERS3013925 to ERS3013960 (PRJEB30582). The sequences of predicted ICEs were deposited into the NCBI database with GenBank Accession Numbers from MK460213 to MK460220.

**Results**

Thirty-four *P. mirabilis* and two *P. vulgaris* strains were isolated in this study, and the details of the strains with host information were shown in Table 1. For the genomic sequencing results, 12,278,826 raw reads were obtained for 36 *Proteus* spp. bacteria, and 11,608,128 clean reads were retained after quality control. The average clean Q20 (%) was 91.25±0.99, clean Q30 (%) was 88.35±1.28, and effective rate (%) was 93.53±2.73, as shown in Table 2.

**Table 2.** The statistics of all sequencing strains for the quality control, assembly, prediction and mapping results.

| Features              | Numbers | Mean       | Std. Deviation | Minimum   | Maximum   |
|-----------------------|---------|------------|----------------|-----------|-----------|
| Quality control       |         |            |                |           |           |
| RawData (reads)       | 36      | 341,000    | 51,965.37      | 241,964   | 487,903   |
| CleanData (reads)     | 36      | 322,000    | 52,110.64      | 224,518   | 472,403   |
| CleanQ20 (%)          | 36      | 91.2533    | 0.99364        | 89.12     | 92.97     |
| CleanQ30 (%)          | 36      | 88.3464    | 1.27638        | 85.6      | 90.62     |
| CleanGC (%)           | 36      | 41.2531    | 0.24703        | 40.67     | 41.86     |
| Effective Rate (%)    | 36      | 93.5264    | 2.72804        | 83.28     | 96.54     |
| Assembly              |         |            |                |           |           |
| Scaffold total_num (>500 bp) | 36  | 1,241.53 | 174.372 | 890       | 1,698     |
| Scaffold total_len (>500 bp) | 36  | 3,620,000 | 104,134.82 | 3,358,482 | 3,878,645 |
| Scaffold N50          | 36      | 4,934.86   | 1,058.41       | 2,783     | 8,196     |
| Scaffold N90          | 36      | 1,277.64   | 210.349        | 895       | 2,022     |
| Max Scaffold (bp)     | 36      | 31,000     | 7,471.51       | 13,766    | 50,230    |
| Min Scaffold (bp)     | 36      | 501.64     | 1.199          | 501       | 506       |
| Scaffold Sequence GC% | 36      | 39.94131973| 0.247879671   | 39.2344   | 40.6334767 |
| Gene predict          |         |            |                |           |           |
| Genome_size (bp)      | 36      | 3,620,000  | 104,134.82     | 3,358,482 | 3,878,645 |
| Gene_total_length (bp)| 36      | 3,040,000  | 104,828.49     | 2,787,918 | 3,291,156 |
| Gene_Number           | 36      | 3,741.39   | 96.938         | 3,467     | 3,947     |
| Gene_average_length (bp)| 36  | 813.36    | 29.04          | 720       | 859       |
| Gene_total_length/Genome (%) | 36  | 84.0486     | 7.7641        | 81.36     | 85.03     |
| Mapping to references |         |            |                |           |           |
| Avg_depth             | 36      | 19.47      | 3.29           | 16.53     | 24.95     |
| Coverage>= 1x (%)     | 36      | 88.83      | 1.27           | 86.82     | 89.93     |
| Coverage>= 4x (%)     | 36      | 84.77      | 1.72           | 81.82     | 86.2      |
| Coverage>= 10x (%)    | 36      | 75.38      | 3.27           | 70.59     | 79.03     |
| Map_rate (%)          | 36      | 80.14      | 7.78           | 67.58     | 88.99     |

https://doi.org/10.1371/journal.pone.0229125.t002
average scaffold number (>500 bp) was 1,241.53±174.37, with a range from 890 to 1,698. The minimum scaffold length was 3,358,482 bp, and 3,878,645 bp was the maximum length. The average scaffold GC% content was 39.94±0.25 (Table 2). The predicted gene results showed that the average genome size was 3.6±0.1 Mbp for 36 isolates, with a range from 3.4 to 3.9 Mbp. The gene numbers were 3,741.39±96.94, with a minimum of 3,467 and a maximum of 3,947. The proportion of total gene length accounted for 84.05±0.78 in the genome, as shown in Table 2. The Kolmogorov-Smirnov Z test showed no statistical significance (P>0.05) for all these features. The details for quality control, assembly and gene prediction results of each strain were shown in S3 Table.

In total, 132,584 NR, 99,769 SwissProt, 159,615 Pfam, 101,954 KEGG, and 126,679 eggNOG numbers were annotated with average numbers 3682.89±90.37, 2771.36±36.01, 4433.75±113.67, 2832.06±42.49, and 3518.86±74.82, respectively (Fig 1A). A total of 4,803 genes were annotated in the ARDB database for all Proteus spp. in this study, with an average number of 133.42±6.31 and a range from 120 to 151 (Fig 1A). We found 24.46% of isolates carried macB and mphA genes, which were associated with resistance to macrolide; 10.04% of isolates carried vanB, vanC, vanE and vanG genes, which were resistant to vancomycin; 8.59% of strains carried lsa, carA and tlrC genes, which were resistant to lincosamide, streptogramin, and

---

**Fig 1.** The annotation results of *Proteus* spp. bacteria in this study. A. The number of annotated genes of each strain based on Pfam, NR, EggNOG, SwissProt, KEGG, PHI, VFDB and ARDB databases. B. The heatmap of annotated ARDB resistance genes for each isolate. C. The heatmap of PHI database annotation results. D. The heatmap of annotated virulence factors based on the VFDB database.

https://doi.org/10.1371/journal.pone.0229125.g001
macrolide; 8.40% of isolates had \( bcrA \) gene with bacitracin resistance profile; 7.70% of strains possessed \( vanA \) and \( vanD \) genes, which were resistant to vancomycin and teicoplanin; 6.25% of isolates carried \( tetB \), \( tetM \), \( tet \) and \( otrA \) genes, which were associated with resistance to tetracycline; 5.77% of strains had \( mdtL \), \( cata \), \( catB \), \( ceo \) and \( cml \) genes, which referred to multi-drug resistant genes; 4.84% of isolates had \( pbp \) gene, which indicated the penicillin resistance. All those ARDB annotated genes and profiles mentioned above were shown in hot spots of Fig 1B. There were 17,185 PHI annotated genes for all strains; the average number was 477.36 ± 12.52, with a range from 449 to 503 (Fig 1A). Reduced virulence (53.28%), unaffected pathogenicity (15.87%), loss of pathogenicity (13.24%), and increased virulence (hypervirulence) (5.43%) were the dominant PHI phenotypes of the mutant deposited in the PHI database, as shown in Fig 1C. The virulence factor investigation showed 15,577 annotated genes obtained from the VFDB database in total, and the average number was 432.69 ± 18.87, with a range from 377 to 470. Flagella (9.24%) was the most annotated virulence factor overall, followed by LOS (lipooligosaccharide) (7.27%), type 1 fimbriae (4.21%), peritrichous flagella (3.96%), type IV pili (3.48%), HitABC (3.28%), capsule (3.07%), FbpABC (2.94%), T6SS (2.89%), pyoverdine (2.28%), alginate (2.02%), and TTSS (2.00%) (Fig 1D). Two isolated \( P. vulgaris \) strains had different virulence factors than \( P. mirabilis \); for example, a high proportion of ompA and RTX toxins were found in \( P. vulgaris \), but a low proportion of T6SS and type 1 fimbriae were found, as shown in Fig 1D. The annotation numbers according to each database had no statistical significance (\( P > 0.05 \)) among the isolates.

CRISPR analysis revealed 245 loci for all strains. Among them, YNTSP16 and YNTSP40 had 12 CRISPR loci, followed by YNTSP22 (11 loci), YNTSP34 (11 loci), YNTSP35 (11 loci), and YNTSP69 (11 loci) (Fig 2A). The details of CRISPR information for each strain were shown in S4 Table. The phylogenetic analysis based on sequences of CRISPR repeats showed five clustering groups for all \( Proteus \) spp. bacteria, presented with red, yellow, green, blue and purple colours, as shown in Fig 2B. The sequences of the CRISPR repeats revealed highly polymorphic characteristics of the isolates in this study, since few identical sequences were found in the phylogenetic tree. However, only eight strains (YNTSP13, YNTSP19, YNTSP34, YNTSP35, YNTSP43, YNTSP44, YNTSP48 and YNTSP56) had prophages, as shown in Fig 2A. The largest numbers of prophage genes were found in YNTSP35 (109), followed by YNTSP48 (89), and YNTSP34 (74); the annotation details of all prophages were shown in S5 Table.

The ICE prediction results showed that eight \( P. mirabilis \) isolates had putative ICEs with T4SS structures and belonged to SXT/R391 family, as shown in Fig 3A. Although all predicted ICE types (SXT/R391) were identical, high diversity was found among them. The locations and insertion sites of ICEs were different. The largest length ICE was 289,114 bp (ICE\(-m\)YNTSP19), while the shortest was 86,907 (ICE\(p\)miYNTSP40). Phylogenetic analysis revealed four clustered groups, indicated with red, yellow, green and blue colours (Fig 3B). Eight ICEs in this study were clustered in green and blue groups, differentiated with other reference ICEs (red and yellow groups). All of the reference ICE strains were isolated from diarrhoea patients or domestic animals (S1 Table), while all of the isolates in this study were from laboratory animals.

For SNP variant investigation, \( P. mirabilis \) HI4320 and \( P. vulgaris \) FDAARGOS_366 were used as reference genomes. Thirty-four \( P. mirabilis \) and two \( P. vulgaris \) strains in this study were mapped to each reference. The depths for all of the sequencing results were >16, with an average depth of 19.47 ± 3.29, and the coverage of sequencing was shown in Table 2. The average mapping rate for all of the \( Proteus \) spp. was 80.14 ± 7.78. In total, 422,163 SNPs were identified for all of the \( Proteus \) spp., with an average of 11,700 ± 2,493.98 SNPs. However, only 3,794 and 2,234 SNPs were found for two \( P. vulgaris \) (YNTSP35 and YNTSP72). A total of
9320.24 ± 2117.44 base transitions (Ts) were obtained for Proteus spp., compared with 2910.35 ± 602.98 base transversions (Tv) (Z = 4.007, P = 0.000), as shown in Fig 4A. The number of homozygous mutations (Hom) was much higher than the number of heterozygous mutations (Het) in this study (Z = 4.243, P = 0.000), and the average number was 12,014.50 ± 2,857.33 and 214.29 ± 262.21, respectively (Fig 4B). Statistical significance was also found between synonymous mutations (Syn) and non-synonymous mutations (Non-Syn) (Z = 3.517, P = 0.000), showing 5,397.29 ± 599.89 and 6,841.97 ± 709.67 mutations, respectively (Fig 4C). There were 695 InDels for all Proteus spp. with an average of 19.31 ± 6.00 InDels. In general, 33.72 ± 9.76 were insertions, and 31.36 ± 10.51 were deletions. No significant difference was found between the insertions and deletions (Z = 0.946, P = 0.336) (Fig 4D). A large number of Hom were identified compared with Het (Z = 4.007, P = 0.000), and the average number was 62.53 ± 18.45 for Hom and 2.56 ± 2.12 for Hem (Fig 4E). The average number for non-frame shift (Non-shift) InDels was 8.42 ± 3.19, compared with 10.89 ± 3.96 for frame shift (Shift), as shown in Fig 4F. Statistical significance was found between non-shift and shift InDels (Z = 1.532, P = 0.018).

Phylogenetic analysis based on ML method of P. mirabilis indicated high genetic diversity for strains isolated from tree shrews because 136 isolates were divided into four clustering groups, indicated with yellow, green, blue and purple colours (Fig 5A), and each group had P. mirabilis bacteria isolated in this study (red font). Twenty-eight strains were clustered into the purple group. Although ten isolates (YNTSP63, 42, 36, 21, 12, 59, 62, 48, 70, and 13) were located at an identical branch, others were clustered into different branches of the tree. Most
**Fig 3. ICE analysis results in this study.** A. The details of eight predicted ICEs in this study. B. Phylogenetic tree based on total SNPs of eight ICEs from Proteus spp. with references.

https://doi.org/10.1371/journal.pone.0229125.g003

| Strain ID | Species       | Location (nt) | Length (bp) | GC content (%) | Insertion site | DRs                                      | Type                      |
|-----------|---------------|---------------|-------------|----------------|---------------|------------------------------------------|---------------------------|
| YNTSP1    | *P. mirabilis*| 689293.800964 | 111672      | 40.76          | Predicted ORF (689272.690130) [-]   | ortl: 689293.689307 (tggtnantaattac) ortF: 800960.800964 (tggtnantaattac) | Putative ICE with T4SS    |
| YNTSP5    | *P. mirabilis*| 2606786.2736886 | 130101      | 41.08          | Predicted ORF (2606622.2607299) [-]   | ortl: 2606786.2606800 (cgtnaacaattgae) ortF: 2736882.2736886 (cgtnaacaattgae) | Putative ICE with T4SS    |
| YNTSP19   | *P. mirabilis*| 478082.767195 | 289114      | 38.41          | Predicted ORF (477268.479226) [-]   | ortl: 478082.478099 (cachaaaagtactacag) ortF: 767178.767195 (cachaaaagtactacag) | Putative ICE with T4SS    |
| YNTSP21   | *P. mirabilis*| 2368194.2500524 | 137331      | 41.68          | Predicted ORF (2367410.2369125) [-]   | ortl: 2368194.2368208 (cttnaagaattgaag) ortF: 2369105.2369124 (cttnaagaattgaag) | Putative ICE with T4SS    |
| YNTSP34   | *P. mirabilis*| 556398.777543 | 221146      | 38.73          | Predicted ORF (555322.556688) [-]   | ortl: 556398.556412 (taaactagacagtatt) ortF: 777529.777543 (taaactagacagtatt) | Putative ICE with T4SS    |
| YNTSP36   | *P. mirabilis*| 3425400.3638974 | 213575      | 41.37          | Predicted ORF (3426079.3427461) [-]   | ortl: 3425400.3425415 (gttgtgagtagctgtag) ortF: 3638959.3638974 (gttgtgagtagctgtag) | Putative ICE with T4SS    |
| YNTSP40   | *P. mirabilis*| 132108.219014 | 86907       | 41.82          | Predicted ORF (128778.220175) [-]   | ortl: 132108.132122 (gtaattaactagttg) ortF: 210000.210014 (gtaattaactagttg) | Putative ICE with T4SS    |
| YNTSP53   | *P. mirabilis*| 1106772.1255395 | 148624      | 40.99          | Predicted ORF (1106574.1106900) [-]   | ortl: 1106772.1106776 (gtgacaattgcgct) ortF: 1255381.1255395 (gtgacaattgcgct) | Putative ICE with T4SS    |
of the reference strains in the purple group were isolated from humans, especially from urinary tract infections, and *P. mirabilis* from tree shrews showed high similarity with these strains, such as 1150_PMIR, 1134_PMIR, Pm_Oxa48, K670, GN2, 51_PMIR, 418_PMIR and 1091.

Fig 4. SNP variants and InDel analysis in this study. A. Comparison of the base transitions (Ts) and base transversions (Tv) for SNPs. B. Comparison of the homozygous mutations (Hom) and heterozygous mutations (Het) for SNPs. C. Comparison of the synonymous mutations (Syn) and non-synonymous mutations (Non-Syn) of SNPs. D. Comparison of the insertions and deletions for InDels. E. Comparison of the homozygous mutations (Hom) and heterozygous mutations (Het) for InDels. F. Comparison of the non-frame shift (Non-shift) and frame shift (Shift) for InDels.

https://doi.org/10.1371/journal.pone.0229125.g004
Furthermore, these reference strains were isolated from different countries without any epidemiological relationship. YNTSP26 was clustered into the blue group, and most strains in this group were also isolated from patients, except *P. mirabilis* Wood, which was isolated from *Lucilia sericata*, BC11-24 from swine, and 25933GTA from beef. There were two strains (YNTSP57 and YNTSP24) divided into the green group, and all of the reference isolates were isolated from patients. The last three *P. mirabilis* bacteria from tree shrews were clustered into the yellow group, and the major sources of strains were from patients as well, except for 11985-2-3 and SAS71, which were environmental strains. We further performed phylogenetic analysis of *P. mirabilis* isolated from tree shrews independently, and 34 strains were clustered into four groups, as shown in Fig 5B. The four groups were presented with red, green, blue and pink coloured branches. Thirteen strains were clustered into the blue-branch group, followed by ten strains in red and nine in pink; only two isolates were in the green group (Fig 5B). There was no obvious correlation between clustering groups and host information, such as sex, age or age groups. The sex and age groups of tree shrews were randomly distributed in each clustering group. Fourteen *P. vulgaris* were separated into three groups, including two strains isolated from tree shrews in this study and 12 references (Fig 5C). YNTSP35 and YNTSP72 were clustered into the green group, showing close similarity with 08MAS1600, CSUR P1867, FDAARGOS_556 and CICC. These reference strains were primarily isolated from the environment or from humans. Other reference isolates were clustered into the yellow and red groups. The alignments and tree files mentioned above were shown as S1 Appendix. The distance-based NJ method for phylogenetic analysis revealed similar results with ML.
method. Generally, three clustering groups were generated by NJ method, shown with purple, yellow and blue colours (Fig 6A). The clustering results of strains for each group showed close similarity with ML method. Most of the \textit{P. mirabilis} from tree shrews were clustered into purple group; YNTSP26 and YNTSP43 were divided into blue group, and the rest of strains were clustered into yellow group (red font). Phylogenetic analysis of 34 \textit{P. mirabilis} isolated from
tree shrews demonstrated four clustering groups were obtained by NJ method, shown with red, green, blue and yellow colours (Fig 6B). In addition, three clustering groups were generated for \( P. \) \textit{vulgaris} by NJ method, and the grouping results were identical with ML method mentioned above (Fig 6C). All the alignments and tree files were shown as S2 Appendix by NJ method. In general, the host specificity of \( P. \) \textit{mirabilis} bacteria was not discovered, especially for \( P. \) \textit{mirabilis}. The Tajima’s D values indicated that no statistical significance were found for 34 \( P. \) \textit{mirabilis} isolated from tree shrews and \( P. \) \textit{vulgaris} \((D = -0.076, P = 0.940; D = 0.742, P = 0.458)\). However, statistical significance \((D = -2.107, P = 0.035)\) was identified for all 136 \( P. \) \textit{mirabilis} in this study.

**Discussion**

A previous study showed that many wild and domestic animals are hosts of \( P. \) \textit{spp.}, including mammals, birds, amphibians, reptiles, and insects [5]. These bacteria may play a fundamental role in animal pathogenic or physiological microbiota, especially in gastrointestinal tracts. For example, Bittar et al. [33] identified both \( P. \) \textit{mirabilis} and \( P. \) \textit{vulgaris} inhabited in the intestines of western lowland gorillas. Gaastra et al. [34] isolated \( P. \) \textit{mirabilis} from the faeces and urine of dogs with urinary tract infections, and Kroemer et al. [35] reported that \( P. \) \textit{mirabilis} bacteria caused urinary tract infections in dogs and cats in European countries. Lowe et al. [36] revealed that \( P. \) \textit{mirabilis} or \( P. \) \textit{vulgaris} strains dominated the pig tonsil, and Kobashi et al. [37] identified \( P. \) \textit{mirabilis} from pig faecal samples that was resistant to tetracycline. Other authors emphasized the role of wild birds in the transmission and spread of \( P. \) \textit{spp.} bacteria to domestic poultry, cattle, or humans [38–39]. However, there is no systemic report about the relations between \( P. \) \textit{spp.} with laboratory animals, specifically for the tree shrew. Our results indicated that \( P. \) \textit{spp.} strains were the most commonly isolated bacteria from the gut of tree shrews, even though all of these laboratory animals were closed populations without any contact from outside environments.

\( P. \) \textit{mirabilis} species possess different antibiotic resistant abilities. Previous studies on clinical patients revealed that polymyxins resistance was mediated via lipid A of bacteria, and they also had intrinsic resistant abilities to tetracycline, tigecycline and colistin [40–41]. Whole genomic sequencing of \( P. \) \textit{mirabilis} HI4320 reference strain demonstrated \textit{tetAJ} encoded tetracycline resistance, \textit{cat} encoded chloramphenicol acetyltransferase, \textit{bcr} encoded sulfonamide resistance, and multidrug efflux genes \textit{mdtG, mdtH, mdtK, mdtL} were all contained in this uropathogenic strain [42]. Recently, \( P. \) \textit{mirabilis} acquired \textit{Salmonella} genomic island 1 (SGI1) was reported, and it was resistant to tetracycline, sulfonamides, fluoroquinolones, streptomycin, trimethoprim, chloramphenicol, and \( \beta \)-lactam antibiotics [43]. Furthermore, \( P. \) \textit{mirabilis} strain PM58, an extensively drug-resistant isolate contained the New Delhi metallo-\( \beta \)-lactamase 1 (NDM-1) gene was also reported. \( P. \) \textit{mirabilis} isolate (NO-051/03), a recent sequenced strain from a patient acquired \( \beta \)-lactam, trimethoprim, sulfonamides, and aminoglycosides resistant genes [44]. Compared the ARDB annotation results of tree shrew \( P. \) \textit{spp.} strains with these clinical ones, we found similar antimicrobial resistant genes acquired by both \( P. \) \textit{spp.} isolates. In our study, ARDB annotations indicated high proportions of macrolide resistance profile. The tetracycline resistance profiles were involved in \textit{tetB, tetM, tetJ} and \textit{otrA} genes, and the descriptions of those genes were tetracycline efflux pump and ribosomal protection protein. \textit{mdtL} and \textit{cat} genes with chloramphenicol resistance profile were annotated, and they mainly referred to multidrug resistance efflux pump and chloramphenicol acetyltransferase. \textit{lsa, carA} and \textit{thrC} genes annotated with lincosamide, streptogramin, macrolide resistance profile. The penicillin resistance profile with \textit{php} gene was also annotated. In addition, our previous study [16] revealed that some of the \( P. \) \textit{spp.} strains possessed NDM-1 gene resistant
to Imipenem. All these pieces of evidences indicated that the antimicrobial resistant profiles or genes were similar between tree shrew Proteus spp. isolates with human clinical ones.

The evolutionary relationships of different niches in humans, animals, and plants using proper techniques could reveal the roles of Proteus spp. bacteria in these hosts. It would be interesting to compare the features of Proteus spp. from clinical isolates and animals, explaining the factors contributing to the different lifestyles of Proteus spp. in various environments [5]. Proteus species are considered potential pathogenic bacteria in human gastrointestinal or urinary tract infections [45–46]. It is postulated that human guts are the reservoir of Proteus spp., especially for the P. mirabilis species. However, the interactions between Proteus spp. and hosts may lead to the pathogenicity of this genus resulting from population expansion. Several virulence factors of this genus have been revealed in previous studies. Proteus spp. possess similar characteristics to other Enterobacteriaceae for lipopolysaccharides (LPS), such as Salmonella spp. and Escherichia coli. The chemical structure of LPS may lead to the adaptation of Proteus spp. bacteria to environmental conditions and enhance their pathogenic abilities [47]. In addition, bacterial flagella are pathogenesis factors of Proteus spp. due to their immunogenic structure. The flagellins activate the inflammatory pathways of hosts and contribute to the stimulation of the host innate immune systems [48–49]. Furthermore, one of the essential pathogenesis factors of Proteus spp. infections is adhesion to epithelial cells or surfaces for urinary or gastrointestinal tracts. A previous study [50] showed 17 fimbrial operons for P. mirabilis HI4320, and six types of fimbriae have been characterized thus far, including MR/P fimbriae (mannose-resistant Proteus-like fimbriae), MR/K fimbriae (mannose-resistant Klebsiella-like fimbriae), NAF (non-agglutinating fimbriae), ATF (ambient-temperature fimbriae), PMP (P. mirabilis P-like pili), and PMF (P. mirabilis fimbriae). All of these fimbriae play an important role in the pathogenesis of P. mirabilis infections. In this study, all of the Proteus spp. strains isolated from tree shrews possessed the virulence factors mentioned above, such as LPS, flagella, type 1 fimbriae and P fimbriae. Therefore, the laboratory animal-isolated Proteus spp. strains showed no significant differences from clinical strains, especially in their pathogenicity.

Furthermore, phylogenetic analysis based on total SNPs of strains demonstrated that P. mirabilis from tree shrew had closer genomic relations with human clinical isolates. There was no host specificity of Proteus spp. isolated from human patients, animals or environments. Close similarity could be found between strains from different epidemiological resources, and even within the same host species, such as the tree shrews in this study, and characteristics of high genetic diversity in the isolates were also identified. This unexpected result contradicted previous studies, and several studies have indicated that microorganism specificity to their hosts supports the co-evolution of the host and its microbes [51–52]. However, comparative genomics of the Proteus spp. strains from our results with other references did not reveal host specificity of isolates.

Based on ARDB, VFDB annotations and phylogenetic relation analysis of tree shrew Proteus spp., we found the antimicrobial resistance, virulence factors and genomic features of these isolates were similar with human clinical Proteus spp. strains. These results indicated some of the gut bacterial population was somewhat similar between tree shrew and human, specifically for Proteus spp. However, the antimicrobial resistant bacteria and related genes, especially for erythromycin, tetracycline and β-lactamase, were commonly existed in this laboratory animal. This should be paid attention in future clinical research applications.

Conclusions

In this study, we firstly analyzed the genomics of Proteus spp. isolated from tree shrews and compared their characteristics with other references. Overall, the host specificity of isolates
was not discovered and *Proteus* spp. strains were somewhat similar between tree shrew and human. This investigation provided important information on characteristics of genome for *Proteus* spp. isolated from tree shrews.

**Supporting information**

S1 Table. The information for reference ICE sequences in this study.
(XLS)

S2 Table. The information for the 114 reference *Proteus* spp. genomes.
(XLS)

S3 Table. The details of the quality control, assembly and gene prediction results in this study.
(XLS)

S4 Table. The details of the CRISPR information for each strain.
(XLS)

S5 Table. The annotation details of all prophages in this study.
(XLS)

S1 Appendix. The alignments and tree files of *Proteus* spp. based on ML method in this study.
(RAR)

S2 Appendix. The alignments and tree files of *Proteus* spp. based on NJ method in this study.
(RAR)

**Acknowledgments**

We sent our manuscript to American Journal Experts (www.aje.com) for English proof reading.

**Author Contributions**

Data curation: Yuanyuan Han.

Formal analysis: Caixia Lu.

Methodology: Wenpeng Gu.

Resources: Wenguang Wang, Pinfen Tong.

Software: Chenxiu Liu, Jie Jia, Dexuan Kuang.

Supervision: Xiaomei Sun, Jiejie Dai.

Visualization: Jiejie Dai.

Writing – original draft: Wenpeng Gu.

Writing – review & editing: Na Li.

**References**

1. Hamilton AL, Kamm MA, Ng SC, Morrison M. *Proteus* spp. as Putative Gastrointestinal Pathogens. Clin Microbiol Rev. 2018; 31(3). Epub 2018/06/15. 31/3/e00085-17 [pii] https://doi.org/10.1128/CMR.00085-17 PMID: 29899011; PubMed Central PMCID: PMC6056842.
2. Adeolu M, Alnajar S, Naushad S, R SG. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacteriales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaeaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. Int J Syst Evol Microbiol. 2016; 66(12):5575–99. Epub 2016/09/14. https://doi.org/10.1099/ijsem.0.02484-M PMID: 27620848.

3. O’Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev. 2000; 13(4):534–46. Epub 2000/10/12. https://doi.org/10.1128/cmr.13.4.534-546.2000 PMID: 11023955; PubMed Central PMCID: PMC888947.

4. O’Hara CM, Brenner FW, Steigerwaldt AG, Hill BC, Holmes B, Grimont PA, et al. Classification of Proteus vulgaris biogroup 3 with recognition of Proteus hauseri sp. nov., nom. rev. and unnamed Proteus genospecies 4, 5 and 6. Int J Syst Evol Microbiol. 2000; 50 Pt 5:1869–86. Epub 2000/10/18. https://doi.org/10.1099/00207713-50-5-1869 PMID: 11034488.

5. Drzewiecka D. Significance and Roles of Proteus spp. Bacteria in Natural Environments. Microb Ecol. 2016; 72(4):741–58. Epub 2016/10/27. https://doi.org/10.1007/s00248-015-0720-6 [pii]. PMID: 26748500; PubMed Central PMCID: PMC5080321.

6. Ma Q, Fonseca A, Liu W, Fields AT, Pimsler ML, Spindola AF, et al. Proteus mirabilis interkingdom swarming signals attract blow flies. ISME J. 2012; 6(7):1356–66. Epub 2012/01/13. ismej20111210 [pii] https://doi.org/10.1038/ismej.2011.121 PMID: 22237540; PubMed Central PMCID: PMC3379643.

7. Morgenstein RM, Szostek B, Rather PN. Regulation of gene expression during swarmer cell differentiation in Proteus mirabilis. FEMS Microbiol Rev. 2010; 34(5):753–63. Epub 2010/05/26. FMR229 [pii] https://doi.org/10.1111/j.1574-6976.2010.00229.x PMID: 20497230.

8. Lu C, Sun X, Li N, Wang W, Kuang D, Tong P, et al. CircRNAs in the tree shrew (Tupaia belangeri chinensis) brain during postnatal development and aging. Aging (Albany NY). 2018; 10(4):833–52. Epub 2018/05/23. https://doi.org/10.1111/aging.13137 [pii] https://doi.org/10.1111/j.1574-6976.2010.00229.x PMID: 20497230.

9. Shen PQ, Zheng H, Liu RW, Chen LL, Li B, He BL, et al. [Progress and prospect in research on laboratory tree shrew in China]. Dongwuxue Yanjiu. 2011; 32(1):109–14. Epub 2011/02/23. https://doi.org/10.24272/j.issn.2095-8137.2011.0322274 [pii]. PMID: 30116606; PubMed Central PMCID: PMC6072924.

10. Yao YG. Creating animal models, why not use the Chinese tree shrew (Tupaia belangeri chinensis)? Zool Res. 2017; 38(3):118–26. Epub 2017/06/07. https://doi.org/10.24427/zool.issn.1995-8137.2017.032 PMID: 28584535; PubMed Central PMCID: PMC5460080.

11. Li R, Zanin M, Xia X, Yang Z. The tree shrew as a model for infectious diseases research. J Thorac Dis. 2018; 10(Suppl 19):S2272–S9. Epub 2018/08/18. https://doi.org/10.21037/jtd.2017.12.121 Jtd-10-S19-S2272 [pii] PMID: 30116606; PubMed Central PMCID: PMC6072924.

12. Feng Y, Feng YM, Lu C, Han Y, Liu L, Sun X, et al. Tree shrew, a potential animal model for hepatitis C, supports the infection and replication of HCV in vitro and in vivo. J Gen Virol. 2017; 98(8):2069–79. Epub 2017/08/02. https://doi.org/10.1099/jgv.0.000869 PMID: 28758632; PubMed Central PMCID: PMC5656785.

13. Yang ZF, Zhao J, Zhu YT, Wang YT, Liu R, Zhao SS, et al. The tree shrew provides a useful alternative model for the study of influenza H1N1 virus. Virol J. 2013; 10:111. Epub 2013/04/12. 1743-422X-10-111 [pii] https://doi.org/10.1186/1743-422X-10-111 PMID: 23575279; PubMed Central PMCID: PMC3639867.

14. Meyer U, van Kampen M, Isioch E, Fluge G, Fuchs E. Chronic psychosocial stress regulates the expression of both GR and MR mRNA in the hippocampal formation of tree shrews. Hippocampus. 2001; 11(3):329–36. Epub 2002/01/05. https://doi.org/10.1002/hipo.1047 PMID: 11769314.

15. Fuchs E. Social stress in tree shrews as an animal model of depression: an example of a behavioral model of a CNS disorder. CNS Spectr. 2005; 10(3):182–90. Epub 2005/03/04. https://doi.org/10.1017/s1092852900010038 PMID: 15744220.

16. Gu W, Tong P, Liu C, Wang W, Lu C, Han Y, et al. The characteristics of gut microbiota and commensal Enterobacteriaceae isolates in tree shrew (Tupaia belangeri). BMC Microbiol. 2019; 19(1):203. Epub 2019/09/04. https://doi.org/10.1186/s12866-019-1581-9 [pii] PMID: 31477004; PubMed Central PMCID: PMC6721287.

17. Han Y, Sun X, Kuang D, Tong P, Lu C, Wang W, et al. Characterization of tree shrew (Tupaia belangeri) interleukin-6 and its expression pattern in response to exogenous challenge. Int J Mol Med. 2017; 40(6):1679–90. Epub 2017/10/19. https://doi.org/10.3892/ijmm.2017.3168 PMID: 29039460; PubMed Central PMCID: PMC5716431.

18. Massad G, Bahrami FK, Mobley HL. Proteus mirabilis fimbriae: identification, isolation, and characterization of a new ambient-temperature fimbria. Infect Immun. 1994; 62(5):1899–94. Epub 1994/05/01. PMID: 7909538; PubMed Central PMCID: PMC186458.
Bittar F, Keita MB, Lagier JC, Peeters M, Delaporte E, Raoult D. Gorilla gorilla gorilla gut: a potential reservoir of pathogenic bacteria as revealed using culturomics and molecular tools. Sci Rep. 2014; 4:7174. doi.org/10.1038/srep07174 PMID: 25417171; PubMed Central PMCID: PMC4241516.

Bolger AM, Lohse M, Usadel B. Trimmmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014; 30(15):2114–20. Epub 2014/04/04. btu170 [pii] https://doi.org/10.1093/bioinformatics/btu170 PMID: 24695404; PubMed Central PMCID: PMC4103590.

Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K, et al. SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics. 2009; 25(15):1966–7. Epub 2009/06/06. btp336 [pii] https://doi.org/10.1093/bioinformatics/btp336 PMID: 19497933.

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience. 2012; 1(1):18. Epub 2012/01/01. 2047-217X-1-18 [pii] https://doi.org/10.1186/2047-217X-1-18 PMID: 23587118; PubMed Central PMCID: PMC3626529.

Besemer J, Lomsadze A, Borodovsky M. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. 2001; 29(12):2607–18. Epub 2001/06/19. https://doi.org/10.1093/nar/29.12.2607 PMID: 11410670; PubMed Central PMCID: PMC55746.

Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015; 12(1):59–60. Epub 2014/11/18. nmeth.3176 [pii] https://doi.org/10.1038/nmeth.3176 PMID: 25402007.

Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyrpides NC, et al. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinformatics. 2007; 8:209. Epub 2007/06/20. 1471-2105-8-209 [pii] https://doi.org/10.1186/1471-2105-8-209 PMID: 17577412; PubMed Central PMCID: PMC1924687.

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPde novo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience. 2014; 3(1):18. Epub 2014/01/01. 2047-217X-1-18 [pii] https://doi.org/10.1186/2047-217X-1-18 PMID: 23587118; PubMed Central PMCID: PMC3626529.

Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 2003; 52(1):103–15. Epub 2002/06/28. 1063-4524-52-1-103 [pii] https://doi.org/10.1080/10634520310001635209 PMID: 12645308; PubMed Central PMCID: PMC12645308.

Handaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009; 25(16):1966–7. Epub 2009/06/06. btp336 [pii] https://doi.org/10.1093/bioinformatics/btp336 PMID: 19451168; PubMed Central PMCID: PMC2703234.

Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25(14):1754–60. Epub 2009/05/20. btp324 [pii] https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168; PubMed Central PMCID: PMC2703234.

Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetic parameter estimation from sequencing data. Bioinformatics. 2011; 27(21):2987–93. Epub 2011/09/10. bt509 [pii] https://doi.org/10.1093/bioinformatics/bt509 PMID: 21903627; PubMed Central PMCID: PMC3198575.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009; 25(16):2078–9. Epub 2009/06/10. btp352 [pii] https://doi.org/10.1093/bioinformatics/btp352 PMID: 19505943; PubMed Central PMCID: PMC2723002.

Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetic parameter estimation from sequencing data. Bioinformatics. 2011; 27(21):2987–93. Epub 2011/09/10. bt509 [pii] https://doi.org/10.1093/bioinformatics/bt509 PMID: 21903627; PubMed Central PMCID: PMC3198575.

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014; 30(15):2114–20. Epub 2014/04/04. btu170 [pii] https://doi.org/10.1093/bioinformatics/btu170 PMID: 24695404; PubMed Central PMCID: PMC4103590.

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience. 2012; 1(1):18. Epub 2012/01/01. 2047-217X-1-18 [pii] https://doi.org/10.1186/2047-217X-1-18 PMID: 23587118; PubMed Central PMCID: PMC3626529.

Besemer J, Lomsadze A, Borodovsky M. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. 2001; 29(12):2607–18. Epub 2001/06/19. https://doi.org/10.1093/nar/29.12.2607 PMID: 11410670; PubMed Central PMCID: PMC55746.

Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015; 12(1):59–60. Epub 2014/11/18. nmeth.3176 [pii] https://doi.org/10.1038/nmeth.3176 PMID: 25402007.

Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyrpides NC, et al. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. BMC Bioinformatics. 2007; 8:209. Epub 2007/06/20. 1471-2105-8-209 [pii] https://doi.org/10.1186/1471-2105-8-209 PMID: 17577412; PubMed Central PMCID: PMC1924687.

Achter S, Aziz RK, Edwards RA. PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity- and composition-based strategies. Nucleic Acids Res. 2012; 40(16):e126. Epub 2012/05/16. gks406 [pii] https://doi.org/10.1093/nar/gks406 PMID: 22584627; PubMed Central PMCID: PMC3419882.

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014; 30(15):2114–20. Epub 2014/04/04. btu170 [pii] https://doi.org/10.1093/bioinformatics/btu170 PMID: 24695404; PubMed Central PMCID: PMC4103590.
36. Lowe BA, Marsh TL, Isaacs-Co sgrove N, Kirkwood RN, Kiupel M, Mulks MH. Microbial commu nities in the tonsils of healthy pigs. Vet Microbiol. 2011; 147(3–4):346–57. Epub 2010/07/29. S0378-1135(10) 00327-5 [pii] https://doi. org/10.1016/j. vetmic.2010.06.025 PMID: 20663617.

37. Akinbowal e OL, Peng H, Barton MD. Diversity of tetracycline resistance genes in bacteria from aquacul -ture sources in Australia. J Appl Microbiol. 2007; 103(5):2016–25. Epub 2007/10/24. JAM3445 [pii] https://doi.or g/10.1111/j.1365-2672.2007.03445.x PMID: 17953612.

38. Winsor DK, Bloebaum AP, Mathewson JJ. Gram-negative, aerobic, enteric pathogens among intestinal microflora of wild turkey vultures (Cathartes aura) in west central Texas. Appl Environ Microbiol. 1981; 42(6):1123–4. Epub 1981/12/01. PMID: 7032423; PubMed Central PMCID: PMC244163.

39. Lee HY, Stephen A, Sushela D, Mala M. Detection of protozoan and bacterial pathogens of public health importance in faeces of Corvus spp. (large-billed crow). Trop Biomed. 2008; 25(2):134–9. Epub 2008/10/25. PMID: 18948884.

40. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resis- tance in bacteria. Front Microbiol. 2014; 5:643. Epub 2014/12/17. https://doi.or g/10.3389/fmicb.2014. 00643 PMID: 25505462; PubMed Central PMCID: PMC4244539.

41. Iredell J, Brown J, Tagg K. Antibiotic resistance in Enterobac teriaceae: mechanis ms and clinical impli- cations. BMJ. 2016; 352:h6420. Epub 2016/02/10. https://doi.org/10.1136/bmj.h6420 PMID: 26858245.

42. Pearson MM, Sebaihia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, et al. Complete genome sequence of uropathogen ic Proteus mirabilis, a master of both adherence and motility. J Bacteriol. 2008; 190(11):4027–37. Epub 2008/04/01. JB.01981-07 [pii] https://doi.org/10.1128/JB.01981-07 PMID: 18375554; PubMed Central PMCID: PMC2395036.

43. Doublet B, Poirel L, Praud K, Nordmann P, Cloeckaert A. European clinical isolate of Proteus mirabilis harbourin g the Salmonella genomic island 1 variant SGI1-O. J Antimicro b Chemother. 2010; 65(10):2260–2. Epub 2010/08/05. dkq283 [pii] https://doi.org/10.1093/jac/dkq283 PMID: 20682564.

44. Qin S, Qi H, Zhang Q, Zhao D, Liu ZZ, Tian H, et al. Emergence of Extensively Drug-Resistant Proteus mirabilis Harboring a Conjugal NDM-1 Plasmid and a Novel Salmonella Genomic Island 1 Variant, SGI1-Z. Antimicro b Agents Chemother. 2015; 59(10):6601–4. Epub 2015/07/22. AAC.00292-15 [pii] https://doi.org/10.1128/AAC.00292-15 PMID: 26195511; PubMed Central PMCID: PMC4576069.

45. Rozalski A, Sidorczyk Z, Kotelko K. Potential virulence factors of Proteus bacilli. Microbiol Mol Biol Rev. 1997; 61(1):65–89. Epub 1997/03/01. PMID: 9106365; PubMed Central PMCID: PMC232601.

46. Schaffer JN, Pearson MM. Proteus mirabilis and Urinary Tract Infections. Microbiol Spectr. 2015; 3(5). Epub 2015/11/07. https://doi.org/10.1128/microbiolspec.UTI-0017-2013 PMID: 26542036; PubMed Central PMCID: PMC4638163.

47. Knirel YA, Perepelov AV, Kondakova AN, Senchenkova SN, Sidorczyk Z, Rozalski A, et al. Structure and serology of O-antigen s as the basis for classif ication of Proteus strains. Innate Immun. 2011; 17(1):70–96. Epub 2010/03/23. 1753425909360668 [pii] https://doi.org/10.1177/1753425909360668 PMID: 20305038.

48. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001; 410(6832):1099–103. Epub 2001/04/27. https://doi.org/10.1038/35074106 [pii]. PMID: 11323673.

49. Lopez-Yglesias AH, Zhao X, Quarles EK, Lai MA, VandenBos T, Strong RK, et al. Flagellin induces anti-body responses through a TLR5- and inflammasome-independ ent pathway. J Immunol. 2014; 192(4):1587–96. Epub 2014/01/21. jimmunol.1301893 [pii] https://doi.org/10.4049/jimmunol.1301893 PMID: 24442437; PubMed Central PMCID: PMC3925749.

50. Kuan L, Schaffer JN, Zouzias CD, Pearson MM. Characterization of 17 chaperone-usher fimbriae encoded by Proteus mirabilis reveals strong conservation. J Med Microbiol. 2014; 63(PT 7):911–22. Epub 2014/05/09. jmm.0.069971-0 [pii] https://doi.org/10.1099/jmm.0.069971-0 PMID: 24809384; PubMed Central PMCID: PMC4064351.

51. Buhnik-Rosenblau K, Matsko-Efimov V, Jung M, Shin H, Danin-Poleg Y, Kaesi Y. Indication for Co-evo-lution of Lactobacillus johnsonii with its hosts. BMC Microbiol. 2012; 12:149. Epub 2012/07/26. 1471-2180-12-149 [pii] https://doi.org/10.1186/1471-2180-12-149 PMID: 22827843; PubMed Central PMCID: PMC3503616.

52. Zhu S, Cao YZ, Jiang C, Tan BY, Wang Z, Feng S, et al. Sequencing the genome of Marssonina brun-nea reveals fungus-poplar co-evolution. BMC Genomics. 2012; 13:382. Epub 2012/08/11. 1471-2164-13-382 [pii] https://doi.org/10.1186/1471-2164-13-382 PMID: 22876864; PubMed Central PMCID: PMC3484023.