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Proceeding Paper

Exploring the Sit-and-Wait Potential of the Bacterial Pathogen *Shigella flexneri*: A Comparative Genomic Study †

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Abstract: The sit-and-wait hypothesis predicts that bacterial durability in the external environment is positively correlated with the evolution of bacterial virulence. Many bacterial pathogens have been recognized as potential sit-and-wait pathogens due to their long-term environmental survival (high durability) and high host mortality (high virulence), such as *Acinetobacter baumannii*, *Burkholderia pseudomallei*, *Mycobacterium tuberculosis*, etc. *Shigella flexneri* is a leading etiologic agent of diarrhea in China with long-term environmental survival capacity, high infection rates, and severe clinical consequences. It has multiple transmission routes like contaminated food (the food-borne route), insanitary water (the water-borne route), and direct person-to-person contacts, etc. These features make *Shigella flexneri* an ideal candidate for sit-and-wait bacterial pathogens. However, there is currently a lack of evidence to support the claim. In this study, we examine the potential of *S. flexneri* as a sit-and-wait pathogen via comparative genomic analysis, which reveals the unique features of *Shigella flexneri* in abiotic stress resistance, energy metabolism, and virulence factors and confirms that *S. flexneri* is a highly potential sit-and-wait bacterial pathogen.

Keywords: *Shigella flexneri*; bacterial transmission; sit-and-wait hypothesis; stress resistance; energy metabolism; virulence factors

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1. Introduction

Shigella spp. belong to the family *Enterobacteriacea*, and a total of four bacterial pathogens are included within the group., that is, *S. boydii*, *S. dysenteriae*, *S. flexneri*, and *S. sonnei*., which originated from *Escherichia coli* via convergent evolution through the acquisition of mobile virulence elements and loss of functional genes [1]. *Shigella* spp. cause the infectious disease termed Shigellosis, which leads to the sickness of thousands of millions of people each year all over the world [2]. Among those infected, there will be more than a million deaths, with a large portion of them under the age of 5 years [3]. Therefore, it is important to prevent and control the infection of *Shigella* spp. in order to save lives and reduce medical expenses. From the perspective of bacterial diagnosis, it is very difficult

and complex to discriminate *Shigella* spp. from *E. coli* due to their similarities, and it is even harder to differentiate the four species within the *Shigella* genus; therefore, novel methods and techniques are needed to improve the efficacy of *Shigella* diagnosis and differentiation, facilitating better control of the pathogen [4,5]. In addition, due to the phenotypical and genotypical similarities in *Shigella* spp., there is currently no availability of a *Shigella* vaccine, while the treatment of *Shigella* infection mainly relies on antibiotics [2,6]. However, due to the increased resistance to antibiotics, *Shigella* infections have gradually become a challenging issue in clinical settings. Epidemiological studies revealed that, among the four *Shigella* species, *S. flexneri* is frequently encountered in developing countries, while *S. sonnei* is more dominant in developed countries [7]. Although, with the increase in China's comprehensive national economic strength year by year, studies have shown that the infection rate of *S. sonnei* in China has an increasing trend, a number of national, provincial and municipal epidemiological surveys have confirmed that *S. flexneri* is still the dominant type of *Shigella* infection in China [8], which will be the focus of the study. The sit-and-wait hypothesis predicts that bacterial durability in the external environment is positively correlated with the evolution of bacterial virulence [9]. Many bacterial pathogens have been recognized as potential sit-and-wait pathogens due to their long-term environmental survival (high durability) and high host mortality (high virulence), such as *Acinetobacter baumannii*, *Burkholderia pseudomallei*, *Mycobacterium tuberculosis*, etc. [10]. *Shigella flexneri* is a leading etiologic agent of diarrhea with high infection rates, severe consequences, and long-term environmental survival. It has multiple transmission routes like contaminated food (the food-borne route), insanitary water (the water-borne route), and direct person-to-person contacts, etc. [11]. These features make *Shigella flexneri* an ideal candidate for sit-and-wait bacterial pathogens. However, there is currently a lack of evidence to support the claim. In this study, we used comparative genomic analysis to investigate the sit-and-wait potential of *Shigella flexneri*, focusing on abiotic resistance, energy metabolism, and virulence factors of the bacterial pathogen. Through our computational analysis, we confirmed that *Shigella flexneri* is a sit-and-wait bacterial pathogen that holds the potential to evolve towards higher virulence. Therefore, more attention should be given to the bacterial pathogen in terms of its transmission control and clinical treatment.

2. Methods

A total of 90 *Shigella* spp. genomes, together with 23 extra bacterial genomes (8 sit-and-wait bacteria, 5 obligate intracellular pathogens, 5 vector-borne bacterial pathogens, and 5 free-living bacteria), were freely downloaded from the public database Bacterial and Viral Bioinformatics Resource Center (BV-BRC), an information system designed to support research on bacterial and viral infectious diseases [12]. For the 90 *Shigella* spp., 34 genomes were *S. sonnei*, 11 genomes were *S. boydii*, 38 genomes were *S. flexneri*, and 7 genomes were *S. dysenteriae*. All the genomes were annotated via the rapid prokaryotic genome annotation tool PROKKA [13]. The protein FASTA file of the translated CDS sequences in the suffix of *faa* for each annotated genome was collected for further analysis. Proteins related to abiotic stress and energy mechanisms were collected from Gene Ontology (GO) via key word searching [14]. Virulence factors were sourced from the Virulence Factor Database (VFDB) [15]. DIAMOND with the blastp command (--query-coverage 90, -e 1e-10) was used to search the homologous protein sequences in each bacterial genome for counting the number of abiotic stress genes, energy mechanism genes, and virulence factors, which were then compared in different categories [16]. Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) was conducted for clustering analysis. All the data were visualized using GraphPad Prism (version 8.0.1). Tukey's Honestly Significant Difference (HSD) test was performed for multi-variant statistical analysis. Means denoted by a different letter indicated significant differences between groups (p-value < 0.05).

3. Results and Discussion

Through the identification of homologous sequences, it was found that the number of genes related to abiotic stress resistance and energy metabolism in *S. flexneri* was significantly higher (p-value < 0.05) than that in the previously defined bacterial groups, that is, sit-and-wait pathogens, obligate-intracellular bacteria, vector-borne pathogens, and free-living bacteria. As for virulence factors, the number was significantly higher (p-value < 0.05) in *S. flexneri* when compared with that in obligate-intracellular bacteria, vector-borne pathogens, and free-living bacteria. However, there was no significance identified between *S. flexneri* and sit-and-wait pathogens (p-value > 0.05), which confirmed that *S. flexneri* had similar distribution patterns as sit-and-wait pathogens in terms of the number of virulence factors. In addition, the OPLS-DA clustering analysis based on the gene distribution patterns of abiotic stress resistance, energy mechanisms, and virulence factors showed that *S. flexneri* could be distinguished from the four bacterial categories, and *S. flexneri* had a much closer relationship with the sit-and-wait pathogen. Therefore, we concluded that *S. flexneri* had high potential to be a sit-and-wait bacterial pathogen.

4. Conclusions

Based on computational analysis of bacterial genomes, this study explored the distribution of genes related to abiotic stress, energy mechanisms, and virulence factors in *Shigella* spp., with a focus on *S. flexneri* due to its predominant prevalence in China. Through the comparison of the number of genes belonging to the three categories with bacteria belonging to previously defined four groups, that is, sit-and-wait bacteria, obligate intracellular bacteria, vector-borne bacteria, and free-living bacteria, it was found that *Shigella flexneri* holds high sit-and-wait potential due to the significant presence of a large number of genes related to abiotic stress, energy mechanisms, and virulence factors. Therefore, more attention should be paid to the control of the bacterial pathogen from an evolutionary perspective so as to restrict its pathogenicity increment and even reduce its pathogenicity in the long term.

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References

1. Lan, R.; Reeves, P.R. Escherichia coli in disguise: Molecular origins of *Shigella*. *Microbes Infect.* **2002**, *4*, 1125–1132. [CrossRef] [PubMed]
2. The, H.C.; Thanh, D.P.; Holt, K.E.; Thomson, N.R.; Baker, S. The genomic signatures of *Shigella* evolution, adaptation and geographical spread. *Nat. Rev. Microbiol.* **2016**, *14*, 235–250. [CrossRef] [PubMed]
3. Fischer Walker, C.L.; Perin, J.; Aryee, M.J.; Boschi-Pinto, C.; Black, R.E. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: A systematic review. *BMC Public Health* **2012**, *12*, 220. [CrossRef]
4. Liu, W.; Tang, J.-W.; Mou, J.-Y.; Lyu, J.-W.; Di, Y.-W.; Liao, Y.-L.; Luo, Y.-F.; Li, Z.-K.; Wu, X.; Wang, L. Rapid discrimination of *Shigella* spp. and *Escherichia coli* via label-free surface enhanced Raman spectroscopy coupled with machine learning algorithms. *Front. Microbiol.* **2023**, *14*, 1101357. [CrossRef] [PubMed]
5. Tang, J.-W.; Lyu, J.-W.; Lai, J.-X.; Zhang, X.-D.; Du, Y.-G.; Zhang, X.-Q.; Zhang, Y.-D.; Gu, B.; Zhang, X.; Gu, B.; et al. Determination of *Shigella* spp. via label-free SERS spectra coupled with deep learning. *Microchem. J.* **2023**, *189*, 108539. [CrossRef]
6. Seferbekova, Z.; Zabelkin, A.; Yakovleva, Y.; Afasizhev, R.; Dranenko, N.O.; Alexeev, N.; Gelfand, M.S.; Bochkareva, O.O. High Rates of Genome Rearrangements and Pathogenicity of *Shigella* spp. *Front. Microbiol.* **2021**, *12*, 628622. [CrossRef] [PubMed]
7. Liang, J.; Zhu, Z.; Lan, R.; Meng, J.; Vrancken, B.; Lu, S.; Jin, D.; Yang, J.; Wang, J.; Qin, T.; et al. Evolutionary and genomic insights into the long-term colonization of *Shigella* flexneri in animals. *Emerg. Microbes Infect.* **2022**, *11*, 2069–2079. [CrossRef]
8. Yang, L.; Lü, B.; Wang, Q.; Wang, K.; Lin, Y.; Yang, C.; Qiu, S.; Li, P.; Song, H.; Gales, A.C. Phylogenetic Characterization Reveals Prevalent *Shigella* flexneri ST100 Clone in Beijing, China, 2005 to 2018. *mSphere* **2020**, *5*. [CrossRef] [PubMed]
9. Walther, B.A.; Ewald, P.W. Pathogen survival in the external environment and the evolution of virulence. *Biol. Rev.* **2004**, *79*, 849–869. [CrossRef] [PubMed]
10. Wang, L.; Liu, Z.; Dai, S.; Yan, J.; Wise, M.J. The Sit-and-Wait Hypothesis in Bacterial Pathogens: A Theoretical Study of Durability and Virulence. *Front. Microbiol.* **2017**, *8*, 02167. [CrossRef] [PubMed]
11. Nisa, I.; Qasim, M.; Yasin, N.; Ullah, R.; Ali, A. *Shigella* flexneri: An emerging pathogen. *Folia Microbiol.* **2020**, *65*, 275–291. [CrossRef] [PubMed]
12. Olson, R.D.; Assaf, R.; Brettin, T.; Conrad, N.; Cucinell, C.; Davis, J.; Dempsey, D.M.; Dickerman, A.; Dietrich, E.M.; Kenyon, R.W.; et al. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): A resource combining PATRIC, IRD and ViPR. *Nucleic Acids Res.* **2023**, *51*, D678–D689. [CrossRef] [PubMed]
13. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef] [PubMed]
14. Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Davis, A.P.; Dolinski, K.; Dwight, S.S.; Eppig, J.T.; et al. Gene Ontology: Tool for the unification of biology. *Nat. Genet.* **2000**, *25*, 25–29. [CrossRef] [PubMed]
15. Liu, B.; Zheng, D.; Zhou, S.; Chen, L.; Yang, J. VFDB 2022: A general classification scheme for bacterial virulence factors. *Nucleic Acids Res.* **2022**, *50*, D912–D917. [CrossRef] [PubMed]
16. Buchfink, B.; Reuter, K.; Drost, H.-G. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat. Methods* **2021**, *18*, 366–368. [CrossRef] [PubMed]