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Extended Abstract

Comparative genomic analysis reveals the basis of phage susceptibility among Salmonella Typhimurium DT104 and DT104b

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Introduction:

Worldwide, infections caused by non-typhoidal Salmonella enterica serovar Typhimurium phage types DT104 and DT104b represent a significant public health concern and an economic burden that is substantially exacerbated by antibiotic resistance. Commonly resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline (ACSSuT resistance profile), S. Typhimurium DT104 and DT104b increasingly become resistant to other antimicrobial agents of clinical importance, such as ciprofloxacin (EFSA and ECDC, 2020). The surge in mortality and morbidity rates, caused by multi-resistant bacteria, prompted a renewal of interest to phages as clinical therapeutics and natural biocontrol agents. Nevertheless, bacteria and phages are continually under the pressure of the evolutionary phage-host arms race for the survival, which is mediated by the co-evolving resistance mechanisms (Sillankorva et al., 2012; Food Standards Agency, 2016; Orzechowska and Mohammed, 2019). Until recently, surveillance and outbreak investigation of S. Typhimurium was performed by phage typing; however, Whole Genome Sequencing (WGS), enabling comprehensive in silico study of microorganisms, began replacing the phenotypic characterisation (Baggesen et al., 2010; Ashton et al., 2015; Kwong et al., 2015). In the Anderson et al. phage typing scheme (1977), the epidemiologically related S. Typhimurium DT104 and DT104b display significantly different phage susceptibility profiles (Table 1). This pilot study aimed to characterise phage resistance mechanisms and genomic differences that may be responsible for the divergent phage reaction patterns in S. Typhimurium DT104 and DT104b. A repertoire of known anti-phage mechanisms was studied, including prophages, restriction-modification (R-M) systems and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) along with CRISPR-associated (Cas) proteins.

Material and Methods:

Bacterial strains: Sequences of eight representative S. Typhimurium DT104b (n = 4) and DT104 (n = 4) were selected for the study. In addition to DT104b and DT104 reference strains, a well-studied S. Typhimurium strain LT2 (DT4 phage type; Gene Bank: AE006468.2) was included in comparative analysis. Details of S. Typhimurium strains, year and country of isolation are presented in Table 2.

Identification of SNPs and Phylogenomics: CSI Phylogeny 1.4 tool at the Center for Genomic Epidemiology (Kaas et al., 2014) was used to call single nucleotide polymorphisms (SNPs) and infer a phylogeny. S. Typhimurium str. LT2 was used as a reference genome. A phylogenetic tree was constructed based on the identified SNPs using Fast Tree. The Newick tree data was visualised on the MEGA X software (Kumar et al., 2018).

Identification of prophages, plasmids, R-M and CRISPR- Cas systems: Lysogenic phages integrated into S. Typhimurium genomes were predicted using web-based tools PHAST (Zhou et al., 2011) and PHASTER (Arndt et al., 2016). Identification of R-M systems was performed using Restriction-Modification Finder 1.1, REBASE (CGE, 2015; Roberts et al., 2015). Detection of CRISPR arrays and subtyping of Cas systems were performed on CRISPRFinder (Grissa et al., 2007) and CRISPRCasFinder (Couvin et al., 2018). In addition, PLSDB (Galata, 2018) was used to identify plasmids present within the S. Typhimurium genomes.

Results and Discussion:

SNPs were used to infer phylogenetic relationships between S. Typhimurium genomes as well as to assess the genetic diversity among the DT104 and DT104b strains. Alas, the phylogenetic analysis could not unambiguously differentiate phage types. Notably, the DT104b reference strain displayed significant divergence among the DT104b studied genomes (Figure 1). This may have been caused by the accumulation of SNPs in prophage regions but also the spacer variation of the CRISPR arrays and acquisition or loss of prophages and plasmids (Rychlik et al., 2006; Lang et al., 2012; Pang et al., 2013; Louwen et al., 2014). Indeed, the comparison of CRISPR-1 arrays of DT104b to DT104b reference implies that DT104b strains lost nine contiguous internal spacers (Figure 2). In contrast, the composition of CRISPR-2 arrays within DT104b genomes suggests greater exposure to multiple phages compared to DT104b reference strain (Figure 3). Besides, unlike the other DT104b pan-genomes, which harbour a low molecular weight (3319 bp) p1PCN033 plasmid, the DT104b reference strain possesses a linear high molecular weight (93862 bp) plasmid that was first identified in S. Typhimurium STMU2UK (NZ_LT855377.1), as illustrated in Table 3. The p1PCN033 plasmid is associated with virulence and resistance traits (Liu et al., 2015), but it is unknown if it has a role in bacterial phage susceptibility or resistance; further studies would be required to determine genetic markers that may be responsible for host-phage interaction. The distribution of intact prophages is shown in Table 4; whereas, Table 5 shows four distinct types of R-M systems (Type I, Type II, Type III, and Type IV) detected in the studied genomes.

Conclusion:

Collectively, the comparison of S. Typhimurium DT104 and DT104b genomes did not reveal unique genetic elements contributing to host adaptation. Perhaps, larger sample size and comprehensive study of p1PCN033 plasmid could uncover the causative phage resistance mechanisms. Besides, this pilot study corroborates the complex dynamics of bacteria-phage interaction that limit conventional phage therapy. It also implies the necessity for further research, such as a study of host receptors involved in recognition and adsorption of phages, as well as phage counterstrategies to circumvent bacterial anti-phage mechanisms. Building up the understanding of host-phage interactions may ultimately lead to the development of phage-based technologies enabling effective infection control.

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