High virulence and stress tolerance of Post-COVID-19 clinical Salmonella Typhimurium strains from Brazil

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Salmonella enterica serovar Typhimurium is a leading cause of foodborne infections worldwide and a frequent agent of human clinical infections. Although, typically associated with self-limiting disease, certain strains exhibit enhanced pathogenic potential, including increased tolerance to host-associated stress conditions and improved ability to invade and persist within host cells. Additionally, the COVID-19 pandemic was associated with changes in antimicrobial use and healthcare dynamics, which may have imposed selective pressures on bacterial populations, potentially influencing the emergence of strains with increased adaptive capacity and pathogenic potential. The characterization of these phenotypic traits in recent clinical isolates is essential to better understand their potential impact on human infection. This study aimed to evaluate the phenotypic pathogenic potential of clinical *S. Typhimurium* strains isolated in the post-COVID-19 period by assessing its stress tolerance, cellular invasion, intracellular survival, and *in vivo* virulence. A total of 28 *S*. Typhimurium clinical strains isolated between 2022 and 2023 were evaluated for survival under acid (pH 4.5) and oxidative stresses (15mM H_2O_2) after 10 minutes and 1 hour of exposure. Additionally, 20 selected strains were assessed for their ability to invade Caco-2 epithelial cells and survive within U937 macrophages. *In vivo* virulence was evaluated using the *Galleria mellonella* infection model. The reference strain *S*. Typhimurium ATCC 14028 was included in all assays for comparison. All strains survived under the two stress conditions performed. In acid and oxidative stress assays, most isolates (>70%) showed survival rates statistically similar to ATCC 14028 at both time points. In U937 macrophages, the majority of strains also exhibited intracellular survival comparable to the reference strain or higher, with a mean intracellular survival of 6.2 log10 CFU/mL. In contrast, in Caco-2 epithelial cells, most isolates demonstrated higher invasion rates than ATCC 14028, with a mean of 6.9 log10 CFU/mL. In the *Galleria mellonella* model, 95% of the strains displayed a highly virulent phenotype, causing 100% larval mortality within 48 hours. The high tolerance to acid and oxidative stresses suggested that these strains are well adapted to overcome key host defense mechanisms. The similarity to the reference strain in stress resistance and intracellular survival indicated preservation of core virulence traits. Importantly, the increased invasion observed in epithelial cells suggested an enhanced capacity for host colonization, which may contribute to the infection establishment. The *in vivo* findings further support the high pathogenic potential of the isolates analysed. Clinical *S. Typhimurium* isolates obtained in the post-COVID-19 period demonstrated high stress tolerance, intracellular survival, and virulence, highlighting their pathogenic potential and reinforcing their clinical relevance.

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