

ABSTRACT

Bacteria, intestinal epithelium, and host innate immune responses are among the most critical interacting factors that determine the fate of bacterial infections and disease outcomes. Recent studies have described multiple infections with evidence of more severe diarrhoea and molecular detection methods, suggesting the association of certain pathogens and commensal bacteria with more aggressive *Shigella* infection. However, the interaction between *Shigella flexneri* and human intestinal epithelial cells co-infected with other intestinal commensal bacteria remains unknown. Therefore, the current study investigated the interaction between *S. flexneri* and human intestinal epithelial cells co-infected with selected intestinal commensal bacteria. This was an experimental study and the bacteria strains tested were selected based on co-infections data obtained from previous studies. The strains were obtained from American Type Culture Collection (ATCC) and routinely grown in Luria Battani agar medium. Human colonic T84 intestinal epithelial cells, which maintain phenotypic characteristics of colonic crypt cells, were maintained in Dulbecco's Modified Eagle Medium-F-12 (DMEM-F12) and polarized in transwell permeable support cell culture inserts. Polarized cells were infected apically with *S. flexneri* 2457T and selected enteric bacteria and assessed for trans-epithelial electric resistance (TEER), invasion, cytotoxicity and cytokine induction. Furthermore, the effects on cellular morphology on non-polarized cells were assessed by scanning electron microscopy. The strains were tested in triplicates and repeated thrice. The current study showed that interaction between *S. flexneri* and *Serratia marcescens* or *Citrobacter freundii* influences invasion, cytotoxicity and Interleukin-8 (IL-8) production by intestinal epithelial cells *in-vitro*. A synergistic invasion effect appeared where the intestinal epithelial cells were co-infected with *S. flexneri* and *S. marcescens*, and *S. flexneri* and *C. freundii* but the difference was not statistically significant between the bacteria strains tested ($p>0.05$). However, TEER dropped significantly in monolayers infected with *S. marcescens* ($p<0.0001$) and in those co-infected with *S. flexneri* and *S. marcescens* ($p<0.001$) accompanied with higher IL-8 response (444pg/mL). A similar IL-8 response appeared in cells co-infected with *S. flexneri* and *C. freundii* (508pg/mL). Lactate Dehydrogenase (LDH) concentration in supernatants from cells co-infected with *S. flexneri* and *S. marcescens* appeared much greater than the concentrations measured from cells co-infected with other enteric bacteria tested ($p<0.001$) suggesting increased cytotoxicity. A similar effect was demonstrated by scanning electron microscope (SEM) which revealed loss of microvilli and vacuolization in the monolayers following exposure to *S. marcescens*. Thus, this study demonstrated that (i) some bacteria previously considered non-pathogenic have the potential to interact with intestinal epithelial cells in culture to induce dramatic alterations similar to those produced by known enteric pathogens (ii) *S. flexneri* and *S. marcescens* or *C. freundii* co-infections can potentially cause severe enteric infection through elevated inflammatory responses and epithelial cell destruction, and (ii) *S. marcescens* manifested significant pathologic effect on epithelial cell morphology thus confirming cytotoxic effect. The finding from this study provides evidence that *S. flexneri* and *S. marcescens* or *C. freundii* can cause deleterious enteric infection in a synergistic manner. Therefore, epidemiologic studies should consider possible association between these microorganisms and diarrhoea in pathogenic states such as necrotizing enterocolitis and severe diarrhoea. The evidence of severe disease in co-infections calls for clinical and laboratory investigations to focus on a wider panel to include *S. marcescens* and *C. freundii* for diagnosis of infectious diarrhoeal diseases.