

Introduction

Gut microbiota constitutes vast and diverse groups of bacteria that mainly occupy the lower gastrointestinal (GI) tract and play a major role in health and diseases of humans (1). *Escherichia coli*, despite making up less than 0.5% of the this microbiota, is the most frequently reported bacterium found in the blood culture of hospitalised patents (2). In the gut these bacteria are located in the mucus layer of large intestine that covers the epithelial cells and are shed into the intestinal lumen. These strains are normally seen in the faeces of the host in much higher numbers than those transiently passing through the intestinal tract.

Although pathogenic strains of intestinal and extra-intestinal *E. coli* have been extensively investigated (3, 4), few studies have focused on population structure of commensal strains of *E. coli* and their possible role in translocation and development of extra-intestinal diseases such as sepsis.

Hypothesis: We hypothesised that *E. coli* strains found at much higher numbers in the faeces of healthy humans have a better ability to adhere the gut epithelium and therefore a much better chance to translocate across the gut epithelium.

Aims: We aimed to characterise a collection of *E. coli* strains isolated from healthy individuals of different gender and age, with respect to their virulence properties and their interaction with a human gut epithelial cell line i.e. Caco-2 cells.

Materials and Methods

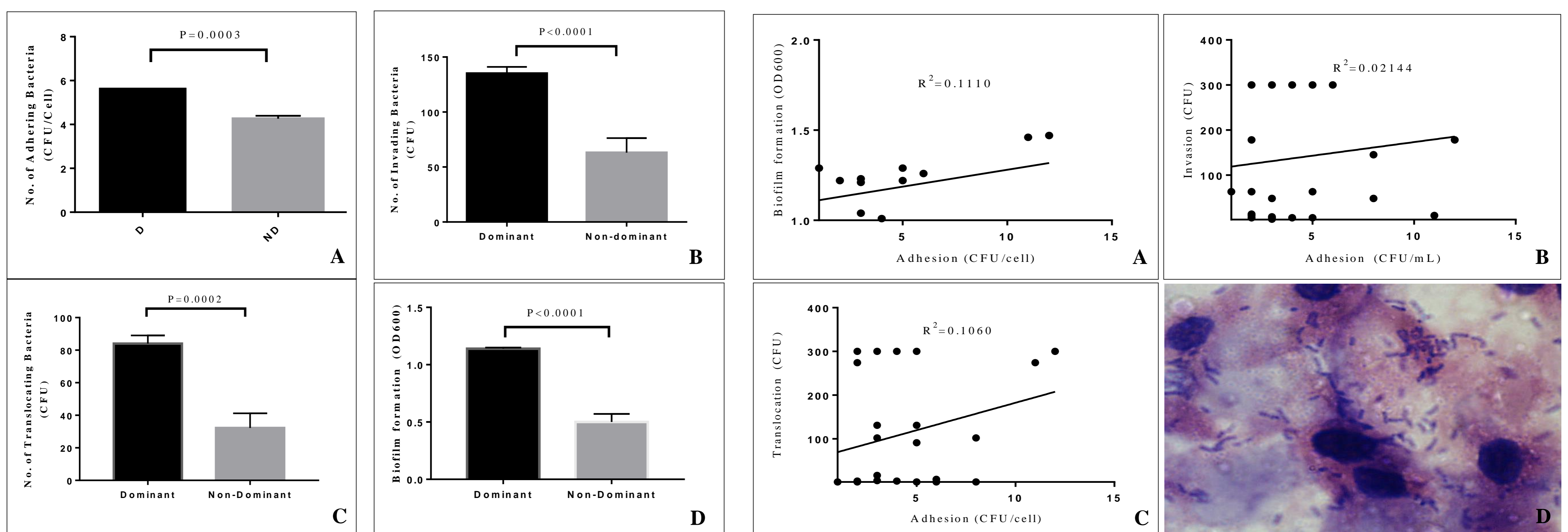
Faecal samples of 46 healthy subjects of different gender and age groups were cultivated and from each faecal sample up to 28 colonies (where possible) were typed using a combination of the PhPlate typing and RAPD-PCR and tested for their phylogenetic groups (5). Strains belonging to the same PhP-RAPD clonal groups were divided into dominant (if they constituted >50% of the population tested) and non-dominant. These strains were then tested for their virulence gene profiles by PCR, the ability to form biofilm measured by crystal violet staining, adherence to, invasion and translocation through a gut epithelial cell line i.e. Caco-2 cells. A comparison was then made between the characteristics of the dominant and non-dominant strains.

Results

The dominant strains adhered to Caco-2 cells significantly more than non-dominant strains (5.7 ± 0.3 versus 4.3 ± 0.1 CFU/cell, $P=0.0003$) (Figure 1A). The dominant strains also invaded (135.1 ± 6 versus 63 ± 13.3 CFU, $P<0.0001$) (Figure 1B) and translocated (84 ± 5 versus 32.2 ± 9 CFU, $P=0.0002$) (Figure 1C) through Caco-2 cells significantly more than non-dominant strains. Moreover, dominant strains showed the ability to form significantly more biofilm (Figure 1D) than non-dominant strains (1.14 ± 0.01 versus 0.5 ± 0.1 OD, $P<0.0001$). We also found high correlations between adhesion of *E. coli* strains to Caco-2 cells and their biofilm formation (Figure 2A), invasion (Figure 2B) and translocation (Figure 2C). Dominant strains carried significantly higher *papG* allele III ($P<0.0001$), *papC* ($P=0.0068$) and *kpsMTII* ($P=0.0166$) than non-dominant strains. Strains belonging to phylogenetic group D constituted 43% and 60% of the dominant and non-dominant groups respectively with B2 being more prevalent among the dominant than non-dominant groups (data not shown).

Figure 1. Comparison between (A) adhesion, (B) invasion, (C) translocation and (D) the degree of biofilm formation of dominant ■ and non-dominant □ strains of *E. coli* isolated from faecal samples of healthy individuals.

Figure 2. Correlations between the adhesion and (A) biofilm formation, (B) invasion and (C) translocation of *E. coli* strains isolated from faecal samples of healthy individuals. (D) A micrograph showing adhesion of *E. coli* to Caco-2 cells.



Conclusion

- *E. coli* strains that are dominantly found in faeces of healthy individuals have a better ability to adhere to, invade and translocated through a human gut epithelial cells model i.e. Caco-2 cells.
- The dominant strains also showed to have a better ability to form biofilm, and carried certain virulence genes significantly higher than non-dominant strains.
- Our data suggest that *E. coli* strains that are dominantly found in faeces of healthy individuals may be well equipped to interact with gut epithelium, translocate to extra-intestinal sites and cause infections such as septicaemia under predisposing conditions in hosts.

References

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