Microbial Pathogenesis 105 (2017) 171–176

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Comparison between virulence characteristics of dominant and non-dominant *Escherichia coli* strains of the gut and their interaction with Caco-2 cells

B. Owrangi, N. Masters, T.L. Vollmerhausen [1], C. O'Dea, A. Kuballa, M. Katouli*

School of Health and Sport Sciences, Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore DC, Queensland 4558, Australia

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form
21 February 2017
Accepted 21 February 2017
Available online 22 February 2017

Keywords:
E. coli
Caco-2 cells
Invasion
Virulence genes
Translocation

ABSTRACT

Escherichia coli strains are normal inhabitants of the gut and are normally found in the faeces of the host at different population sizes. We characterised faecal *E. coli* of 45 healthy male (n = 17) and female (n = 28) volunteers by testing 28 isolates from each individual. These isolates were typed and divided into dominant (if constituted >50% of the population tested) and non-dominant types in each individual. Representative strains of each dominant and non-dominant type were tested for their virulence gene profiles, their ability to form biofilm, adhere to, invade and translocate through a gut epithelial cell line (Caco-2 cells). Strains belonging to dominant types adhered significantly more to Caco-2 cells than non-dominant strains (5.7 ± 0.3 versus 4.3.± 0.13 CFU/cell mean ± SEM, P = 0.0003). They also invaded (135 ± 6 versus 63 ± 13 CFU) and translocated through Caco-2 cells (84 ± 5 versus 32 ± 9 CFU) significantly more than non-dominant strains (P < 0.0001 and P = 0.0002, respectively). Moreover, dominant strains showed the ability to form significantly more biofilm than non-dominant strains (1.1 ± 0.01 versus 0.5 ± 0.1 OD_{600}, P < 0.0001). Majority (51%) of the strains belonged to phylogroup D followed by B2 (23%). Furthermore, out of 25 virulence genes tested, *kps*MTII, *pap*C and *pap*G allele III were found to be significantly higher among dominant than non-dominant strains. Our results suggest that *E. coli* strains dominating the gut may have virulence properties that enable them to efficiently interact with the gut epithelium and translocate under predisposing conditions of the host.

1. Introduction

Gut microbiota constitutes a 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 microbiota, is the most frequently reported Gram-negative bacterium found in blood culture of hospitalised patients [2–4]. In the gut, these bacteria are located in the mucus layer of the large intestine that covers the epithelial cells and are shed into the intestinal lumen [5]. These strains are normally seen in the faeces of the host in much higher population sizes (dominant strains) than those transiently passing through the intestinal tract and are found in low numbers (non-dominant strains). Testing a high number of *E. coli* colonies isolated from the cultures of faecal samples of healthy adults has confirmed that more than half of the isolated colonies are identical in each individual [6] suggesting a strong association between the resident strains and their host [7]. In contrast, other *E. coli* strains are mainly transient and are present in faeces at various levels [8,9]. It has been shown that some *E. coli* strains present at low numbers also have the capacity to colonise the intestinal tract if the host is compromised and increase in number [10,11]. Under these conditions, they translocate through gut epithelium, survive the immune system underlying the epithelium and appear in mesenteric lymph nodes (MLNs) and blood to cause septicaemia [11,12]. Translocation efficiency of these strains however, seems to be host-species specific [13]. Colonization of the gut epithelium by the dominant *E. coli* strains may not always reflect their ability to invade and/or translocate into the MLNs or blood. This is probably because the colonizing strains are normally the ones to which specific immune response has been raised. For instance, development of a sIgA

* Corresponding author.
E-mail address: mkatouli@usc.edu.au (M. Katouli).
[1] Present address: Bacterial Stress Response Group, Microbiology, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland.

http://dx.doi.org/10.1016/j.micpath.2017.02.032
0882-4010/

response can inhibit translocation of a bacterium. On the other hand, transient *E. coli* strains that are normally found in small numbers in the gut may carry properties that under certain conditions allow them to competitively colonise the gut epithelium and translocate.

E. coli strains were initially defined to belong to four phylogroups: A, B1, B2 and D. Whilst some studies suggest that the commensal strains of the human intestinal tract mainly belong to phylogroups A and B1 [14], other studies indicate that phylogroup A and to a lesser extent B2 constitute a majority of the population of commensal *E. coli* of the gut [7] indicating that figures vary among human populations and geographical areas. Although pathogenic strains of intestinal and extra-intestinal *E. coli* have been extensively investigated [15,16], few studies have focused on characterising population structure of *E. coli* strains that are found in high or low numbers in the faeces of healthy individuals and their possible role in translocation and development of extra-intestinal diseases such as septicaemia. In this study, we hypothesised that *E. coli* strains found at much higher numbers than other *E. coli* strains in the faeces of healthy humans have a better ability to adhere to, invade and translocate through human gut epithelial cells.

2. Materials and methods

2.1. E.coli strains

In a previous study, we characterised a collection of 1566 *E. coli* isolates from faecal samples of 59 healthy males and females of different age groups [6]. Details of participants, method of faecal sample collection, isolation and identification of isolates as well as typing of the isolates have been given before [6]. In brief, from each faecal sample grown on MacConkey agar, 28 suspected *E. coli* colonies (where possible) were selected and confirmed as *E. coli* using the universal stress protein (*usp*A) gene [17]. These isolates were typed using a combination of RAPD-PCR and PhPlate typing and their phylogroups were identified as described before [6]. In this study, we randomly selected a subset of the isolates (n = 696) from 45 subjects and grouped them based on their types, into dominant (if they constituted >50% of the isolates tested from each individual) and non-dominant strains in the samples tested. In all, 53 strains representing dominant (n = 23) and non-dominant (n = 30) groups were selected and tested for their interaction with Caco-2 cells including their adhesion, invasion and translocation as well as biofilm formation and virulence gene profiles. On most occasions, both dominant and non-dominant strains came from the same subjects.

2.1.1. Adhesion assay

The adhesion assay was done as previously described [18] using human colorectal cancer (Caco-2) cells (ATCC HTB37A) as an enterocyte *in vitro* model [19]. Cells were grown to 75% confluent [20] in Essential Minimal Eagle Medium (EMEM) (Lonza) supplemented with 10% FBS (Bovogen) and 1% penicillin/streptomycin (Lonza) on sterile round coverslips (13 mm diameter, Knittel-Glass, Germany) in 24-well microplates in an atmosphere of 95% air and 5% CO2 at 37 °C. Bacterial strains were grown in tryptone soy broth (TSB) with agitation (100 rpm) overnight at 37 °C. Bacterial cultures were then centrifuged at 5000 rpm for 10 min, pellets were re-suspended in PBS and the concentration of bacteria was adjusted to 10^9 after measuring the optical density for each strain at 600 nm. Before inoculating bacterial strains, the growth medium was removed, and cells were washed with PBS to remove traces of antibiotics and the medium was replaced with fresh EMEM without antibiotics. Wells were inoculated with 100 μL of bacteria (10^8 CFU/well) and allowed to interact with cells for 60 min, after which bacteria were removed, cells were washed with PBS to remove unattached bacteria. Staining of bacteria and cells was done using Giemsa as described before [17]. Cells were then observed under oil immersion microscope and the number of adhering bacteria was counted on 25 randomly selected cells with adhesion. In all experiments including adhesion, invasion and translocation a positive control i.e. *E. coli* HMLN-1 and a negative control *E. coli* JM109 were included and all experiments were done at least in duplicate.

2.1.2. Invasion assay

The invasion assay was done in duplicate as described by Duncan et al. [21] with some modification including the use of amikacin instead of gentamycin to kill extra-cellular bacteria. In brief, *E. coli* strains were grown in TSB overnight with shaking at 100 RPM at 37 °C prior to the experiment. Bacterial cultures and Caco-2 cells were grown as described above in the adhesion assay section. Bacteria were inoculated to each well at 10^7 CFU/well and the inoculated cells were left at 37 °C under 95% air and 5% CO_2 for 2 h after which wells were inoculated with amikacin (150 μg/mL) for another hour at 37 °C to kill non-invading bacteria. Cells were then lysed with 0.1% Triton-X-100 and the number of invading bacteria were counted after plating serial dilutions of the medium on tryptone soy agar (TSA) followed by an overnight incubation at 37 °C.

2.1.3. Translocation assay

The translocation assay was done according to Vollmerhausen et al. [18] with slight modifications. The Caco-2 cells were grown post-confluence for 15 days as described by Cordeiro et al. [22] in Millicell inserts (Merck Millipore, Ireland) with a permeable base containing 8.0 μm diameter pores. Before inoculation of bacteria, the culture medium was replaced with EMEM without antibiotic. Each well was inoculated with 10^8 CFU of bacteria (see adhesion assay) and allowed to incubate for 60min and the number of translocating bacteria in the outer well was counted after serial dilution on TSA [18,23,24].

2.1.4. Biofilm formation assay

The biofilm formation assay was done using crystal violet staining [25–27] with slight modifications. Briefly, bacterial isolates were grown in 1 mL minimal salt broth (M9) supplemented with 20% glucose and incubated overnight at 37 °C by shaking at 100 rpm. A 1:100 dilution of each bacterial suspension was then prepared in M9 broth (approx. 10^6 CFU/mL) and 100 μL of each bacterial suspension was added to sterile 96-well microtitre plates with flat bottom. The plates were incubated at 37 °C by shaking at 100 rpm overnight. The suspension that contained the unbound bacteria was removed and the adhered cells were washed twice with Dulbecco's phosphate-buffered saline (D-PBS). Each well was stained with 100 μL of prepared 0.1% crystal violet solution for 10 min s. The stain was then removed and the wells were washed with D-PBS. The wells were air dried and the remaining crystal violet was solubilized by 200 μL of ethanol-acetone (80:20) solution. Biofilm formation assay was done in triplicate. The absorbance values of each well were read at 600 nm with a microplate reader.

2.1.5. Virulence gene detection

Genomic DNA of bacterial isolates was extracted using DNeasy Blood &Tissue kit (Qiagen, Australia). Strains were tested for the presence of 25 virulence genes associated with pathogenic *E. coli* strains causing extra-intestinal infections. The functions of virulence factor genes, primer sets, sequences and concentrations have been described in previous studies ([20,28,29]. The virulence genes tested included: Siderophores (*iut*A, *ire*A, *iro*$N_{E.coli}$ and *fyu*A), adhesins (*pap*EF, *fim*H, *pap*AH, *bma*E, *sfa/foc*DE, *pap*G *allele* III, *allele*