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Severity of *Escherichia coli* bacteraemia is independent of the intrinsic virulence of the strains assessed in a mouse model

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Abstract

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains, a major cause of bacteraemia, typically belong to phylogenetic group B2 and express diverse accessory traits that contribute to virulence in mouse infection models. However, their high genomic diversity obscures the relationship between virulence factors and severity of infection in patients. In this study, we analysed concomitantly the strain's expression of virulence in a mouse model, genomic determinants and the clinical severity of infection in 60 bacteraemic patients. We show that bacterial virulence based on an animal model study and virulence factor determination is not correlated with pejorative outcome of *E. coli* human blood infections.

Keywords: Bacteraemia, *Escherichia coli*, ExPEC, mouse model, virulence factor
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Introduction

The *Escherichia coli* species comprise commensal bacteria, which belong to the normal gut microbiota of humans and many animals, as well as highly virulent intestinal (InPEC) and extra-intestinal (ExPEC) pathogenic variants. ExPEC form a heterogeneous group of bacteria responsible for a large spectrum of infectious diseases such as cystitis or pyelonephritis, meningitis, pneumonia, and bone or wound infections [1]. ExPEC are also the leading cause of bacteraemic infections [1].

Phylogenetic analyses have established that *E. coli* can be divided into four main groups (A, B1, B2 and D) and a more recently described group E [2]. Classification of pathogenic *E. coli* is classically based on the presence or absence of specific DNA regions, referred to 'pathogenicity islands' (PAIs), acquired by horizontal gene transfer [3]. These DNA regions encode a large number of virulence factor (VFs) such as adhesins, iron-acquisition systems, capsules and toxins [4]. It is clearly established that these accessory genetic traits contributed to intrinsic virulence of *E. coli* strains in a mouse model of infection [5]. The B2 strains possess the highest level of virulence factors [5].

Epidemiological studies have demonstrated that most clinical strains of ExPEC belong to the phylogenetic group B2, and to a lesser extent to the group D [1]. Also, several VFs are known to be associated with the ExPEC phenotype [6]. However, ExPEC exhibit a considerable genomic diversity [3] and none of the known VFs is constantly expressed in all ExPEC strains [1].

In the present study, we conducted a concomitant analysis of (i) the intrinsic virulence of bacteraemic ExPEC strains based on the presence or absence of specific DNA sequences and virulence in mice and (ii) the severity of the clinical infection caused by these strains to test whether a correlation between these elements exists.

Materials and Methods

Patients, strains, macroarray assay

Sixty strains used for this study were selected among an unbiased collection of 161 ExPEC strains corresponding to all consecutive episodes of *E. coli* bacteraemia in two major university hospitals in Paris [7,8]. This subset of 60 bacteraemic ExPEC was selected as being representative both of the clinical characteristics of the sepsis and the bacterial characteristics of the entire collection [7]. The considered clinical characteristics were the portal of entry of the sepsis, the severity of the disease [9], the immune status (immunodeficiencies included infection with human immunodeficiency virus (HIV) with CD4+ count <200/mm^3, administration of immunosuppressive therapy (corticosteroid therapy >1 mg/kg/day for >30 days, immunomodulating agents and anti-neoplastic chemotherapy) and neutropenia defined as an absolute granulocyte count of <500/μL) and the associated co-morbidities (underlying solid tumour or haematological malignancy, HIV-positive serology, diabetes mellitus (defined as requiring insulin or hypoglycaemiant therapy) and renal failure (defined as a creatinine clearance of <30 mL/min)). The bacterial characteristics encompassed the phylogenetic diversity of the strains based on PCR phylogrouping [10] and MLST analysis [8]. These 60 bacteraemic ExPEC were also characterized for the presence of nine well-documented VFs (*papC, papGIII, papGII, sfa, hlyC, cnf1, iut, iroN, fuyA*) using a multiplex PCR assay [11], and for the 40 most frequently recovered O-antigens by an allele-specific polymerase chain reaction as previously described [12,13]. To evaluate further the accessory genomic content of each strain, a DNA macroarray containing a large *E. coli* flexible gene pool was used [8]. Briefly, DNA probes were selected using the genome sequence of the O6:K15:H31 uropathogenic *E. coli* strain 536, the O6:K2:H1 uropathogenic *E. coli* strain CFT073, and *E. coli* K-12 (MG1655). DNA sequences with more than 90% of homology in the three genomes were excluded. In addition, specific sequences of the meningitis-associated strain RS218, with less than 90% homology in the above three strains, were included. This flexible gene pool DNA macroarray contains a total of 2324 probes that could be classified in functional clusters according to their annotated function (https://www.genoscope.cns.fr/agc/mage); that is, known virulence factors (VF_sequences, n = 208), cell structure membrane proteins (MB_sequences, n = 133), putative functional enzymes (Met_sequences, n = 500), genomic regulator (Transcriptional regulator_sequences, n = 104) and unknown hypothetic protein (ORF_sequences, n = 1033). Genomic DNA preparation and hybridizations were performed as previously described [8]. The macroarray data were analysed using the ArrayVision software (Imaging Reseach, St Catharines, Canada) for signal quantification.

Murine infection

A mouse septicaemia model previously described [5] was used to assess the intrinsic extra-intestinal virulence of all 60 human ExPEC *E. coli*. Between the isolation and the mouse inoculation, these strains were not subcultured more than three times. Briefly, 10 outbred female Swiss mice (6–8 weeks old, 25–30 g) were challenged subcutaneously in the abdomen with 10^9 cfu/mL of log-phase bacteria in 0.2 mL Ringer solution [5]. Mortality was assessed over 7 days post-challenge. It was previously established that when surviving mice were euthanized at 7 days, the organs (blood, kidney, liver and spleen) were sterile. Thus, a longer observation was not contributive [5]. Each experimental series included a positive control (urosepsis strain CFT073) and a negative control (commensal derived strain K-12 MG1655). In this model, lethality is a rather clear-cut parameter and strains were usually classified either as non-killer (MNK, strains killing none or one mouse out of 10) or killer (MK, strains killing nine or ten mice out of 10) [14]. Strains that did not fall into these two categories were considered as being intermediate killer (MIK). No significant difference between the survival curves obtained with MK and MIK strains was observed.

Animal experimentations were carried out according to the authorization no. 6665 given by the Ministère de l'Agriculture, France.

Statistical methods

Comparisons were based on the chi-square test or Mann–Whitney test for categorical variables and the Fisher exact test when numbers were below five. All tests were two-tailed and p <0.05 was considered significant. Factorial analysis of correspondence (FAC), a method suitable for studying a very large amount of data, was used to describe associations among these data [15]. FAC is an eigenvector method of ordination that uses a covariance matrix based on chi-square distances. It describes the dispersion and shape of a cloud of n objects (here, the *E. coli* strains) or p variables (here, the studied variables) in a multidimensional space, by