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Pathophysiology of *Escherichia coli* pneumonia: Respective contribution of pathogenicity islands to virulence

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ABSTRACT

Ventilator-associated pneumonia (VAP) remains the most frequent life-threatening nosocomial infection. *Enterobacteriaceae* including *Escherichia coli* are increasingly involved. If a cumulative effect of pathogenicity islands (PAIs) has been shown for *E. coli* virulence in urinary tract or systemic infections, very little is known regarding pathophysiology of *E. coli* pneumonia. This study aimed to determine the role of each of the 7 PAIs present in pathogenic *E. coli* strain 536 in pneumonia pathophysiology.

We used mutant strains to screen pathophysiological role of PAI in a rat pneumonia model. We also test individual gene mutants within PAI identified to be involved in pneumonia pathogenesis. Finally, we determined the prevalence of these genes of interest in *E. coli* isolates from feces and airways of ventilated patients.

Only PAIs I and III were significantly associated with rat pneumonia pathogenicity. Only the antigen-43 (Ag43) gene in PAI III was significantly associated with bacterial pathogenicity. The prevalence of tested genes in fecal and airway isolates of ventilated patients did not differ between isolates. In contrast, genes encoding Ag43, the F17-fimbriae subunits, HmuR and SepA were more prevalent in VAP isolates with statistical significance for *hmuR* when compared to airway colonizing isolates.

The *E. coli* PAIs involved in lung pathogenicity differed from those involved in urinary tract and bloodstream infections. Overall, extraintestinal *E. coli* virulence seems to rely on a combination of numerous virulence genes that have a cumulative effect depending on the infection site.

1. Introduction

The lung is the first site of infection in intensive care unit (ICU) patients (Vincent et al., 2009). Ventilator-associated pneumonia (VAP) is the most common life-threatening hospital-acquired infection despite considerable efforts to implement guidelines for its prevention (Ricard et al., 2012). Gram-negative bacteria (GNB) predominate in hospital-acquired pneumonias (Chastre and Fagon, 2002; Peleg and Hooper, 2010). In the past, *Pseudomonas aeruginosa* was the most frequent GNB involved in VAP; however, recent data indicate that *Enterobacteriaceae,* and specifically *E. coli*, are more frequently responsible for VAP (Fihman et al., 2015; Hamet et al., 2012; Kollef et al., 2017; Ricard et al., 2012). Surprisingly, little is known about the epidemiology and pathophysiology of *E. coli* pneumonia. We have previously shown that *E. coli* strains isolated in patients under mechanical ventilation with VAP originated predominantly from the B2 phylogenetic group and were highly virulent extraintestinal pathogenic *E. coli* (ExPEC) (Messika et al., 2012). Interestingly, we also found in this study that very few B2 *E. coli* belonged to subgroup II [multilocus sequence type (ST) 73, archetypal strain CFT073] which encompasses strains frequently involved in urinary tract infection (UTI) and urosepsis (Gibreel et al., 2012; Le Gall et al., 2007; Wirth et al., 2006) and that most of them belonged to the subgroup III [ST127, archetypal strain 536 (Hochhut et al., 2006)], more rarely identified in UTI. It thus appears that ExPEC strains responsible for lung infection in ICU patients seem specific and they differ from the ones found in UTI (uropathogenic *E. coli* or UPEC) or bloodstream infections.

A whole repertoire of virulence factors has been identified in ExPEC

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Table 1
Bacterial strains.

E. coli Strains	Characteristics	Origin
K-12 strain MG1655	wild-type commensal *E. coli* lab isolate	
536WT	wild-type uropathogenic *E. coli* isolate (O6:K15:H31), Sm^R	Hacker et al. (1983)
536ΔI	deletion of PAI I, Sm^R	Diard et al. (2010)
536ΔII	deletion of PAI II, Sm^R	Diard et al. (2010)
536ΔIII	spontaneous deletion of PAI III, Sm^R	Middendorf et al. (2004)
536ΔIV	deletion of PAI IV, Sm^R	Diard et al. (2010)
536ΔV	deletion of PAI V, Sm^R	Diard et al. (2010)
536ΔVI	deletion of PAI VI, Sm^R	Diard et al. (2010)
536ΔVII	deletion of PAI VII, Sm^R	Diard et al. (2010)
536ΔI-ΔVII	deletion of all 7 PAIs, Sm^R	Diard et al. (2010)
536ΔF17-fimbriae	deletion of ECP_RS19265 (PAI-I), Sm^R	This study
536ΔCS12-fimbriae	deletion of ECP_RS19345 (PAI-I), Sm^R	This study
536Δ*hlyI*	deletion of ECP_RS19405 to 19420 (PAI-I), Sm^R	Nagy et al. (2006)
536Δ*sfaA*	deletion of ECP_RS01475 (PAIII), Sm^R	This study
536Δ*iroN*	deletion of ECP_RS01520 (PAI-III), Sm^R	This study
536Δ*hmuR*-like	deletion of ECP_RS01575 (PAIII), Sm^R	This study
536Δ*Ag43III*	deletion of ECP_RS01660 (PAIII), Sm^R	Beloin et al. (2006)
536Δ*sepA*	deletion of ECP_RS01730 (PAIII), Sm^R	This study
536Δ*hlyI-II*	deletion of *hlyI* (PAI-I) and *hlyII* (PAI-II), Sm^R	Nagy et al. (2006)

Sm^R: Streptomycin resistant; PAI: Pathogenicity Associated Island.

and more accurately in UPEC strains (Croxen and Finlay, 2010). Virulence genes are mainly organized in large clusters called pathogenicity islands (PAI) on plasmids or integrated in the genome that, by horizontal gene transfer, explain the notable plasticity of *E. coli* genome (Schubert et al., 2009). Several PAIs have been identified in strains 536 and CFT073, and these have been shown to contribute to pathogenicity (Dobrindt et al., 2010). Thus deletion of one or several of these PAI attenuates virulence of the strain in murine models; but the extent to which virulence is affected depends on the strain's genetic background, the nature and the number of PAIs deleted, and the infection site (Brzuszkiewicz et al., 2006; Lloyd et al., 2009; Tourret et al., 2010). A cumulative effect of PAIs in *E. coli* extraintestinal virulence was also observed (Brzuszkiewicz et al., 2006; Tourret et al., 2010).

Because the relative contribution of PAIs to the pathophysiology of *E. coli* pneumonia is unknown, we aimed to test the contribution of each PAI to lung infection in a rat model of *E. coli* pneumonia. After showing that deletion of PAI I and PAI III decreased *E. coli* strain 536 pathogenicity in rat lungs, we tested the principal putative virulence factors carried by those two PAIs to determine their individual roles in lung infection. Finally we evaluated the prevalence of these putative virulence genes in clinical *E. coli* isolates obtained from digestive and respiratory samples from ventilated patients.

2. Material and methods

2.1. Bacterial strains, construction of mutant strains, bioinformatics

2.1.1. Wild-type E. coli strains

E. coli strain 536 is an archetypal ExPEC strain isolated from a urinary tract infection (Hacker et al., 1983). We also used as a non-virulent control *E. coli* K-12 strain MG1655 belonging to the A phylogenetic group.

2.1.2. Single PAI deletion mutant generation

Construction of PAI deletion mutants has been previously described in detail elsewhere (Diard et al., 2010). Briefly, each single PAI deletion mutant except for PAI III was generated using the lambda-Red recombinase gene inactivation method (Datsenko and Wanner, 2000). For each mutant, the antibiotic resistance cassette was removed using flipase (Flp)-encoding helper plasmid pCP20 as described (Datsenko and Wanner, 2000). *E. coli* strain 536ΔIII results from the spontaneous deletion of PAI III of wild-type uropathogenic strain 536 (536WT) (Middendorf et al., 2004). We also tested *E. coli* strain 536 with deletion of the seven known PAIs (*E. coli* 536ΔI-ΔVII).

2.1.3. Bioinformatic analysis of PAI I and PAI III

Based on the published genome of *E. coli* strain 536 (accession number NC_008253), we examined all recognized ORFs and identified putative virulence factors within PAI I and PAI III manually.

2.1.4. Generation of single gene mutant E. coli strains

Putative virulence factors within PAI I and PAI III were individually inactivated using the lambda-Red recombinase gene inactivation method as described previously (17). The antibiotics ampicillin and chloramphenicol were added when needed at final concentrations of 100 µg/ml or 25 µg/ml, respectively.

For PAI I, ECP_RS19265 encoding the F17 fimbrial protein precursor and ECP_RS19345 encoding a putative CS12 major fimbrial protein subunit were inactivated. The *hlyI* mutant (536Δ*hlyI*) was also tested (Nagy et al., 2006).

For PAI III, genes ECP_RS01475 (*sfaA*) encoding the S-fimbrial protein subunit precursor, ECP_RS01520 encoding the salmochelin receptor IroN, ECP_RS01575 encoding a HmuR-like hemin receptor precursor, and ECP_RS01730 encoding the extracellular serine protease SepA, a vacuolating autotransporter toxin, were similarly inactivated. The antigen 43 mutant ($Ag43_{III}$ encoded by gene ECP_RS01660, also known as *flu*, in PAI III) was similarly used (Beloin et al., 2006).

For all isolates, the antibiotic resistance cassette was removed using pCP20 as described previously (Diard et al., 2010). All strains are listed in Table 1. Plasmids and primers used are listed in Tables S1 and S2.

Because hemolytic capacity of *E. coli* strain 536 is based on two functional *hly* operon located on PAI I and PAI II, and because *hly* has been shown to be necessary for full pathogenicity (Nagy et al., 2006; Russo et al., 2005, 2007), we also tested a double *hly* mutant in the rat pneumonia model.

2.1.5. Rat pneumonia model

Animal experiments were performed in compliance with the recommendations of the French Ministry of Agriculture and approved by the French Veterinary Services (accreditation A 75-18-05).

Our *Pseudomonas aeruginosa* unilateral pneumonia model in Wistar male rats (275–300 g) was adapted for *E. coli* (Roux et al., 2009, 2013; Schortgen et al., 2004). With preliminary experiments, we established that an inoculum of 2.25×10^7 CFU of wild type *E. coli* strain 536 caused 80–85% pneumonia (data not shown). This number was chosen in order to be able to detect less-virulent mutant strains.