Bordetella parapertussis isolates not expressing pertactin circulating in France

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Abstract

Surprisingly, most *Bordetella parapertussis* isolates collected in France since 2007 do not express pertactin, owing to mutations in the structural gene encoding this protein. We used a 454 pyrosequencing strategy to study and compare the genetics of two *B. parapertussis* isolates (one expressing pertactin and one not expressing pertactin) and the reference strain. No region of difference was detected between the genomes of the two isolates and the genome of the reference strain. No increase in repeated sequences between both isolates was found, and there were very few sequence differences. Using cellular and animal models, we found no substantial difference between the pathogenicity of these *B. parapertussis* isolates, which is consistent with clinical data. The emergence of these isolates, indicating that pertactin expression is not essential for virulence for *B. parapertussis*, is discussed.

Keywords: *B. parapertussis*, *Bordetella*, France, pathogenicity, pertactin
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Introduction

Bordetella pertussis and *Bordetella parapertussis* are the causative agents of whooping cough, a disease that is still endemic worldwide, despite extensive pertussis vaccination of young children. The reported incidence of *B. pertussis* disease has always been higher than that of *B. parapertussis* all around the world; however, it differs between regions [1] and age groups [2]. The genome sequences of *B. pertussis* and *B. parapertussis* reference strains indicate that these two closely related human-adapted species have evolved independently from a *Bordetella bronchiseptica*-like ancestor [3,4], and that the association of *B. parapertussis* with humans may be more recent than that of *B. pertussis* [3,5].

B. pertussis and *B. parapertussis* share several virulence factors, including the adhesins filamentous haemagglutinin (FHA) and pertactin (PRN), and the toxins adenylate cyclase–haemolysin (AC-Hly) and tracheal cytotoxin. *B. pertussis*, but not *B. parapertussis*, expresses pertussis toxin, tracheal colonization factor and *Bordetella* resistance to killing factor [4]. Like *B. pertussis*, *B. parapertussis* does not express a type - III secretion system (TTSS III). It is generally thought that *B. parapertussis* infection causes a less severe disease than *B. pertussis* infection. However, as observed by Novotny [6], 'Both species can induce mild or severe disease and no clinicians in the world could distinguish between the two infections by clinical symptoms only.' One other important difference between the two species, apart from the expression of pertussis toxin, is the structure of their lipolysaccharides (LPSs) [7]: (i) lipid A does not stimulate toll-like receptor 4 efficiently, enabling the pathogen to avoid host immunity [8]; (ii) the O-antigen, which is not expressed by *B. pertussis* [4], allows *B. parapertussis* to colonize the lower respiratory tract by protecting the pathogen from complement-mediated control [9], and to avoid *B. pertussis*-induced immunity [10]; and (iii) *B. parapertussis* and *B. pertussis* LPSs modulate human dendritic cells differently [11]. Using microarrays, Brinig *et al.* [5] found that the gene contents of genomes of human *B. parapertussis* isolates from patients in nine countries over several decades differed very little. Indeed, the *B. parapertussis* population is highly clonal, as illustrated by the use of different genotyping techniques [12–14].

B. parapertussis was shown to escape *B. pertussis* immunity in animal models [9,10,15] and also in human populations. Despite high homology between the two species, *B. pertussis*

infection or vaccination-induced immunities do not protect against *B. parapertussis* infections [16–18].

Many fewer *B. parapertussis* isolates than *B. pertussis* isolates were sent to the National Centre of Reference (NCR) in France between 1995 and 2006 (0–7%, depending on the year); since 2007, a small increase has nevertheless been noticed (12.6%). Surprisingly, 95% of *B. parapertussis* isolates collected since 2007 do not express PRN. In this study, we report a 454 pyrosequencing analysis of the genetic contents of two isolates and investigations of their pathogenicity in cellular and animal models.

Material and Methods

Clinical isolates, bacterial growth and DNA extraction

The clinical isolates analysed in this study are described in Table 1. They were characterized using classical bacteriological techniques, including growth on Bordet Gengou agar supplemented with 15% sheep blood (BGA), API galleries, oxidase and urease tests, and detection of brownish pigment. They were grown at 36°C for 72 h on BGA, and subcultured for 24 h in the same medium before use. Genomic DNA for 454 pyrosequencing was extracted on Genomic-tip 500/G anion-exchange columns (Qiagen GmbH, Hilden, Germany). DNA for PCR validations was obtained with Dneasy Tissue Kits (Qiagen), according to the manufacturer's recommendations.

Pulsed-field gel electrophoresis (PFGE)

DNA fingerprinting by PFGE was performed as previously described [19].

Genotyping of *prn*

Genotyping of the *prn* gene (regions I and II) was performed as previously described [19].

Western blot analysis

Western blot analysis was performed as described in [15].

Adenylate cyclase activity

Adenylate cyclase activity was measured as previously described [15].

454 Pyrosequencing

A 454 GS-FLX NextGen sequencing platform (Roche Diagnostics GmbH, Beckman Coulter Genomics, Meylan, France) was used for pyrosequencing the two genomes and analysis of the sequences. The two samples were simultaneously sequenced in one GS-FLX run, using one 70 × 75 mm Pico-Titer plate device (Roche Diagnostics GmbH) and one GS LR-70 sequencing kit (Roche Diagnostics GmbH) as previously described [20].

Cell growth conditions

J774-A1 macrophages cells. J774-*A1* cells were cultured in RPMI-1640+ Glutamax (Gibco-BRL, Paisley, UK) supplemented with 10% fetal bovine serum (DAP Vogel Grun, France), 100 U/mL penicillin, 100 mg/L streptomycin and 250 ng/mL amphotericin (Gibco-BRL), 10 mM HEPES (Gibco-BRL) and 1 mM sodium pyruvate (Gibco-BRL) in 75-mL tissue culture flasks (Corning New York, New York, USA). Cells were maintained in a 5% CO_2 atmosphere at 37°C as previously described [21].

Human tracheal epithelial (HTE) cells. Cells were plated in tissue culture trays coated with collagen (Biochrom AG, Berlin, Germany), and cultured in DMEM-Glutamax (Gibco-BRL) supplemented with 2% UltroserG, 100 U/mL penicillin, 100 μg/mL streptomycin and 250 ng/mL amphotericin (Gibco-BRL), in a 5% CO_2 atmosphere at 37°C as previously described [21].

Cytotoxicity assays

Cytotoxicity of bacteria against J774-*A1* cells was measured as previously described [21].

Invasion assays

Invasion assays for HTE cells were performed as previously described [21].

TABLE 1. Characteristics of the isolates used in experiments with cellular and murine models

Identification of the isolate	Expression factor deficiency	Collected in:	Age of the patient	Pertusiss vaccine status of the patient
12822	None	1993	16 months	[26]
BPP1[a]	None	1994	3 months	One vaccination
FR0388	None	1997	5 years	Unknown
FR3286	None	2003	90 years	Non-vaccinated
FR3717	PRN (point deletion in region II of the *prn* gene)	2007	4.5 years	Primary vaccination and one booster
FR3728[a]	PRN (point deletion in region II of the *prn* gene)	2007	7 years	Primary vaccination and one booster
FR3743	PRN (point deletion in region II of the *prn* gene)	2007	2.5 years	Unknown
FR3772	PRN (point deletion in region I of the *prn* gene)	2007	8 years	Unknown

[a]The genomes of these two isolates were subjected to 454 GS-FLX pyrosequencing.