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Distribution of Genes Encoding Virulence Factors *ompB2*, *ompCD*, *ompE,* β-Lactamase and Serotype in Pathogenic and Colonizing Strains of *Moraxella catarrhalis*

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Background and Aims. A total of 115 *Moraxella catarrhalis* isolates from patients with lower respiratory tract and otorhinolaryngeal infections, as well as healthy carriers, were collected to study the prevalence of outer membrane virulence and resistance encoding genes and lipooligosaccharide (LOS) serotypes.

Methods. PCR technique was used to determine the presence of genes *ompB2* (encoding uptake of vital iron), *ompCD* and *ompE* (for adhesion, complement resistance and transporter proteins), *bro1* and *bro2* (for β-lactamases). Serotyping was carried out by monoclonal antibodies (MAb) against LOS serotypes A, B and C.

Results. The frequency of genes determining virulence factors were *ompE* 82.61%, *ompCD* 70.43%, *ompB2* 43.48%, and *bro* 98.26%. Dissemination of virulence genes varied according to the type of infections and carrier state.

Conclusions. The present study revealed the presence of a greater number of virulence factors in isolates from patients compared to that in strains from healthy individuals. In the strains of patients with bronchopulmonary infections, a combination of *ompB2-ompCD-ompE* genes was predominant. In patients suffering from otorhinolaryngeal infections, isolates with combination *ompCD-ompE* prevailed. Isolates from the control group revealed single *ompCD* or *ompE* genes or none of the virulence genes tested. The greatest proportion (91.30%) of β-lactamases was encoded from *bro1* genes. Serotype A was the most frequent (70.43%) serotype. No correlation was found between the strain serotype and presence of the virulence and β-lactamase encoding genes tested.

Key Words: Moraxella catarrhalis, Virulence, Lipooligosaccharide, Serotype, β-lactamase.

Introduction

Moraxella catarrhalis is among the three most frequent causative agents of both otorhinolaryngological (ORL) infections in childhood and chronic obstructive pulmonary disease (COPD) in adults (1–4). Pathogenic characteristics of these bacteria are not yet very clear because for many years they have been considered as commensals of the normal microbial flora of the human upper respiratory tract. As in the case of other opportunistic bacteria during the last decade, *M. catarrhalis* virulence factors differentiating the colonizing from the pathogenic strains were extensively evaluated (5–9). The higher virulence of some *M. catarrhalis* strains that cause serious infections is due mostly to the expression of *uspA2*, *ompB1*, *ompB2, ompCD* genes and intact lipooligosaccharide (LOS), which, giving resistance to serum complement bound bacteriolysis, allows *M. catarrhalis* to surmount a major mechanism of human immune defense (5,10–13). The presence of genes *ompB2* or factors providing the uptake of vital iron from transferrin, lactoferrin and hemoglobin also increases the virulence and protects the development of the *M. catarrhalis* from an unfriendly environment such as blood and other biological fluids (11). OMP E shares borderline homology with *E. coli* FadL, which is involved in uptake of long-chain fatty acids.

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The observation that a mutation in OMP E results in a reduced rate of growth can be explained by the participation of OMP E being in uptake of nutrients (6). The significant etiological role of *M. catarrhalis* is no longer subject to any doubts (14−16).

Infections by *M. catarrhalis* are becoming more and more frequent. This depends directly on the great increase of β-lactamases producing strains in the last years (17,18). The first enzyme producing strains appeared in 1976 (1). Since 1996 >95% of *M. catarrhalis* strains have produced *bro1* and *bro2* coded β-lactamases with gradually extended spectrum due to frequently used antimicrobial agents for treatment of respiratory infections (18−20). Production of extracellular β-lactamases is considered as an indirect virulence factor (21,22).

The aim of this study was to estimate distribution of genes encoding virulence factors *ompB2*, *ompCD*, *ompE,* genes for β-lactamase production in *M. catarrhalis* isolates from patients and healthy individuals and hence to distinguish pathogenic from colonizing strains as well as their association with the known serotypes of this pathogen.

Materials and Methods

Strains and Specimen Collection

Three referent strains of *M. catarrhalis* were used for control of the serotyping and were from the Swedish microbial collection-CCUG 353 (serotype A), CCUG 26400 (serotype B), CCUG 26391 (serotype C). The strains used in the polymerase chain reaction (PCR) were two from the Belgian microbial collection BCCM/LMG 11177, β-lactamase producing, and BCCM/LMG 11178-β-lactamase non-producing; two from the Pasteur Institute collection: CIP 103772, BRO1 producing and CIP 103773, BRO2 producing.

Specimens from different inflammation sites as well as nasal swabs for prophylaxis were taken and delivered in the laboratory obligatorily in transport media as recommended (23,24). Clinical strains of *M. catarrhalis* were isolated from sputum, broncho-alveolar lavage, nasal and pharyngeal swabs, punctures from ears and sinuses of three groups of patients and healthy carriers. Group I was comprised of 52 patients aged from 3−84 years with lower respiratory tract infections (LRTI): pneumonia, chronic exacerbated bronchitis, bronchial asthma and COPD. Group II involved 41 patients aged 1−18 years with the following ORL infections: rhinosinusitis, rhinopharyngitis, adenoiditis, chronically relapsing tonsillopharyngitis, and suppurative otitis media. The third group of the healthy carriers from kindergartens and nurseries included 22 children aged 2−7 years who were prophylactically examined, showing no clinical symptoms of infection and without antimicrobial therapy during the previous 7 days.

Strains were identified by routine criteria using Crystal NH BBL system and stored in skim milk at −70°C. Production of β-lactamases was estimated by Cefinase disc BD BBL.

Serotyping

Monoclonal antibodies against the three LOS chemotypes A, B and C used for serotyping in this study were obtained in our laboratory by hybridoma technology (25).

PCR Analysis

Genomic DNA was extracted by using GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Freiburg, Germany) according to the manufacturer's instructions. For that purpose, *M. catarrhalis* strains were cultured on tryptic-soy agar (BBL) for 24 h at 35°C in 5% CO_2 containing atmosphere to obtain pure cultures from which bacterial lysates were obtained.

PCR was performed in a 25-μl reaction mix using the primers for the genes of outer membrane proteins OMP B2, OMP CD and OMP E (26), as well as for BRO1 and BRO2 β-lactamases (27) as shown in Table 1. The pairs of primers for *bro1* and *bro2* were amplified in 35 cycles using Techne SP Gene 5D thermal cycler with the following parameters: 45 sec at 95°C, 45 sec at 50°C and 45 sec at 72°C. The pairs of primers for OMP were amplified in 25 cycles under the following conditions: 30 sec at 95°C, 1 min at 58°C and 2 min 30 sec at 72°C. PCR products of 235 bp (*bro1*), 214 bp (*bro2*), 2300 bp (*ompB2*), 1200 bp (*ompCD*) and 1300 bp (*ompE*) were separated in 1.5−2% agarose gel for 50−110 min at 130 V, stained with ethidium bromide (0.5 μg/mL) and detected by UV transillumination at wavelength 312 nm. Amplified genes were identified on the basis of fragment size.

Statistical Analysis

Data were compared by alternative statistical analysis; p values <0.05 were considered statistically significant.

Results

Three outer-membrane-associated virulence genes encoding OMPs with different important virulence functions were chosen in the present study. The most frequent genes

Table 1. Primers for PCR detection of virulence factors

Primer	Specific for gene
5′-GTGTGACAGTCAGCCCACTA-3′	*ompCD* (F)
5′-TTGCTACCAGTGATTACTGA-3′	*ompCD* (R)
5′-TTCAACCCTAACCGCAAC-3′	*ompE* (F)
5′-TTTGGCGTGATAAGCAAG-3′	*ompE* (R)
5′-GCCAGCCTAAGGTTGTCT-3′	*ompB2* (2F)
5′-GAAGTTCACGCCAACACG-3′	*ompB2* (2R)
5′-CACCCTGTGGGACAAGC-3′	*bro1,2* sequences
5′-AATGACGGCGTTGCATC-3′	*bro1,2* sequences

PCR, polymerase chain reaction.