BRIEF REPORT

Study of the genetic diversity of Moraxella spp. isolates obtained from corneal abscesses

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KEYWORDS
Moraxella spp.; PFGE; Corneal abscess

Abstract This is the first study of the genetic diversity of Moraxella spp. Isolates were detected in an Eye Hospital in the City of Buenos Aires. Due to the high frequency of Moraxella spp. observed in corneal abscesses, we decided to validate their identification at the species level, determine their drug susceptibility and perform molecular subtyping. Seventeen (17) isolates obtained from corneal abscesses were evaluated. The identification was carried out using a combination of biochemical tests and MALDI-TOF mass spectrometry. Of these isolates, 88.2% were identified as Moraxella lacunata, and 11.8% as Moraxella nonliquefaciens. Molecular subtyping was performed using the pulsed-field gel electrophoresis (PFGE) technique. All isolates were typable and thirteen digestion patterns were identified. Based on the obtained results, the PFGE technique using the Smal enzyme can be used for epidemiological studies of strains of these species.

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PALABRAS CLAVE
Moraxella spp.; PFGE; Absceso corneal

Estudio de la diversidad genética de aislamientos de Moraxella spp. a partir de abscesos corneales

Resumen En este trabajo se presenta el primer estudio de diversidad genética de aislamientos de Moraxella spp. detectados en un hospital de oftalmología de la Ciudad Autónoma de Buenos Aires. Debido a la observación de una elevada frecuencia de Moraxella spp. en abscesos

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corneales, se decidió confirmar su identificación a nivel de especie, conocer su sensibilidad y realizar la subtipificación molecular. Se analizaron 17 aislamientos provenientes de abscesos corneales. La identificación se realizó mediante una combinación de pruebas bioquímicas y espectrometría de masas, MALDI-TOF MS. El 88.2% fueron identificados como Moraxella lacunata y el 11.8% como Moraxella nonliquefaciens. La subtipificación molecular se realizó por la técnica de electroforesis en gel de campo pulsado (PFGE). Todos los aislamientos fueron tipificables y se identificaron 13 patrones de digestión. Nuestros resultados muestran que la técnica de PFGE con la enzima Smal es útil para hacer estudios epidemiológicos en cepas de estas especies.© 2022 Publicado por Elsevier España, S.L.U. en nombre de Asociación Argentina de Microbiología. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (http://creativecommons.org/licenses/by-nc-nd/4.0/).
transfer-formic acid method: MALDI-TOF target plates were inoculated into the spots by picking a freshly grown overnight colony and overlaid with 1 μl of 70% formic acid (Sigma-Aldrich). Each spot was allowed to dry and subsequently overlaid with 1 μl of matrix solution (a cyano-4hydroxycinnamic acid in 50% acetonitrile, 2.5% TFA). The score cut-offs recommended by the manufacturer were used, a score value ≥2.0 indicates species-level identification, a score value between 1.7 and 1.99 indicates genus-level identification, and scores <1.7 indicate no reliable identification. Additionally, the “10% rule” was applied, which states that any species scoring >10% below the top-scoring match may be excluded.

Antibiotic susceptibility testing was performed using the disk-diffusion method (the Kirby–Bauer method). The interpretation criterion used is the one established in the CLSI guideline M45-A2 for *M. catarrhalis*. The culture conditions used were an incubation temperature of 35°C, an atmosphere of 5% CO2 and Mueller Hinton as the culture medium. The ‘susceptible’ category was derived from the extrapolation of the standardized cut-off values for the *M. catarrhalis* species, which is the only species of the genus with standardized cut-off valuesa. The following antimicrobials were tested: amoxicillin-clavulanate, erythromycin, azithromycin, tetracycline, and trimethoprim-sulfamethoxazole.

Patient isolates were analyzed by PFGE to assess genetic relatedness. In brief, chromosomal DNA from the *Moraxella* isolates was digested with restriction endonucleases Smal and Xbal to determine their typeability and discriminatory power. Digestion was performed with 30 units of Xbal (10 U/μl, Fermentas) for 3 h at 37°C and with 50 units of Smal (10 U/μl, Invitrogen) for 3 h at 30°C. Salmonella Braenderup H9812 was used as a molecular weight marker, digested with Xbal.

![Figure 1](image)

Figure 1  Band patterns obtained by PFGE of *M. nonliquefaciens* (lanes 2 and 3) and *M. lacunata* (lanes 4–9) using Smal. Patterns observed for Xbal-PFGE can be observed in lanes 10–14 (lanes 10 and 11 *M. nonliquefaciens*; 12–14 *M. lacunata*). Salmonella Braenderup H9812 was used as a molecular weight marker, digested with Xbal.
Table 1  Traditional biochemical tests that allow the identification of different species of Moraxella.

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<tr>
<th></th>
<th>M. lacunata</th>
<th>M. nonliquefaciens</th>
<th>M. atlantae</th>
<th>M. osloensis</th>
<th>M. lincolnii</th>
<th>P. phenylpiruvicus</th>
<th>M. catarrhalis</th>
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<td>Nutritional requirement</td>
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<td>Colony size</td>
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<td>+ (with Tween80)</td>
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Adapted from Vaneechoutte et al. 8

it is worth mentioning that combining basic biochemical tests and MALDI-TOF MS enabled a correct identification at the species level of the Moraxella spp. isolates that were referred to the RL. It should be noted that retrieving M. catarrhalis from corneal abscesses in the Eye Hospital is about 1 out of every 100 Moraxella isolates, which shows a clear predominance of the M. lacunata species in this type of infection.

With regard to antibiotic susceptibility, only 8 of 17 isolates grew on Mueller Hinton. In 8 strains, antibiotic susceptibility testing was carried out in Mueller Hinton medium with 5% horse blood. One of the isolates did not grow on any media tested, therefore, its susceptibility profile could not be determined. All 16 isolates were susceptible to all the antibiotics tested.

Of the two enzymes that were tested, it was only with Smal that interpretable restriction patterns could be obtained. Therefore, Smal was selected to test all the strains. Fig. 1 shows the resolution of the PFGE patterns produced by Smal and XbaI in a subgroup of the total isolates tested. Of the 15 M. lacunata isolates analyzed, 13 different digestion patterns were obtained. As shown in Fig. 2, the isolates were distributed into eight clonal types (designated with letters A–H) depending on whether there was a difference of four or more bands among the profiles. Clonal type A was divided into subtypes (A1–A6) since there was just one difference of 1 or 3 bands among these isolates. Subtypes A1 and A2 are comprised of two isolates each, which showed the same banding pattern. The two M. nonliquefaciens isolates, identified as 791 and 403, showed a difference of more than 4 bands from one another.

With regard to antibiotic susceptibility, all isolates were susceptible to the five antimicrobials tested. As for the antibiotic treatment administered to the infected patients, after the ocular sample was collected, an intensive medical treatment was recommended, which included fortified antibiotic eye drops of vancomycin (50mg/ml) and cefazidime (50mg/ml), and cycloplegic eye drops for pain management. Once the identification and susceptibility results were obtained, vancomycin was removed and the treatment continued with ceftazidime eye drops and, in some cases, with moxifloxacin eye drops as well.

Comparing the results obtained by PFGE with restriction enzymes XbaI and Smal, it could be observed that the digestion with XbaI produced low molecular weight fragments that could not be adequately resolved in the gel; therefore, this enzyme was considered inappropriate for the analysis of these species by PFGE. The same result was documented for M. catarrhalis when digestion with XbaI was analyzed. Thereby, Smal, another restriction enzyme, was tested. In most of the literature reviewed, SpeI is used for PFGE of M. catarrhalis, being its drawback that it is a very expensive restriction enzyme, which is not available in our laboratory.

SmaI produced restriction patterns ranging from 11 to 15 bands, which enabled sample comparison and typification.
Thirteen (13) different restriction profiles can be observed among the 15 *M. lacunata* isolates. Subtypes A1 and A2 appear to be genetically indistinguishable, since their PFGE patterns have the same number of bands with the same apparent size. The remaining clonal subtypes represent a single isolate. Due to the genetic diversity obtained, an outbreak could be ruled out and they were, evidently, different isolates. Based on the analysis following Tenover’s criterion⁶, those isolates that belong to the same clonal type were considered closely related isolates.

This study demonstrated that digestion with a single enzyme, *SmaI*, can confirm or rule out the clonal relationship of *M. lacunata* and *M. nonliquefaciens* isolates by PFGE. Moreover, it was concluded that the use of MALDI-TOF MS and biochemical tests, alone or in combination, allows the identification of *M. lacunata* and *M. nonliquefaciens*. It is important to mention that 16S rRNA gene sequencing is another useful tool to differentiate *Moraxella* species such as *M. catarrhalis*, *M. nonliquefaciens*, *M. lincolnii* and *M. osloensis*. With reference to antibiotic susceptibility, it should be noted that the report should clarify that the interpretation of the categories “susceptible”, “resistant” or “intermediate” is based on the extrapolation of *M. catarrhalis* cut-off points, and that in the future it should be evaluated whether this extrapolation is valid for all *Moraxella* species. This article highlights the importance of surveillance in the distribution of *Moraxella* species in corneal abscesses to recognize changes over time, to detect the emergence of clones and, if they do emerge, to reveal if they are associated with some antibiotic resistance in particular.

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**Conflicts of interest**

The authors declare that they have no conflicts of interest.

**References**