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Differentiation Between Invasive and Non-Invasive Corynebacterium diphtheriae Isolates

Różnicowanie inwazyjnych i nieinwazyjnych szczepów Corynebacterium diphtheriae

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Abstract

Background. In countries with good diphtheria vaccination coverage, diphtheria cases are very rare. But invasive nontoxigenic C. diphtheriae infections, like bacteriemia, have been emerging during the last decade.

Objectives. The aim of the present investigation was differentiation between C. diphtheriae strains isolated from invasive and non-invasive infections.

Material and Methods. Toxigenic and nontoxigenic C. diphtheriae strains isolated from invasive and local infections and from carriers in the period of the 1960’s to 2008 were analyzed. A PCR MP technique was used for the first time for differentiation between C. diphtheriae isolates. The PCR MP results obtained were compared to PFGE and ERIC-PCR results.

Results. PCR MP differentiates the strains at a similar level as PFGE and ERIC-PCR but, opposite to PFGE and ERIC-PCR, the technique makes it possible to differentiate strains isolated from invasive infections from strains isolated from local infections.

Conclusions. This is the first work revealing genetic differences between nontoxigenic C. diphtheriae strains isolated from invasive and local infections. PCR MP is a useful method for the differentiation of C. diphtheriae. The method gives the possibility for identification of C. diphtheriae strains possessing invasive properties (Adv Clin Exp Med 2011, 20, 6, 717–720).

Key words: Corynebacterium diphtheriae, invasive infections, PCR MP, genetic differentiation.

Streszczenie

Wprowadzenie. W krajach o dużym stopniu zaszczepienia populacji przeciw błonicy przypadki tej choroby zdarzają się niezwykle rzadko. W ciągu ostatniej dekady zaobserwowano jednak pojawianie się inwazyjnych zakażeń, takich jak np. bakteriemia, powodowanych przez nietoksynotwórcze szczepy C. diphtheriae.

Cel pracy. Zróżnicowanie szczepów C. diphtheriae izolowanych z zakażeń inwazyjnych od szczepów izolowanych z zakażeń miejscowych.

Materiał i metody. Badaniami objęto toksynotwórcze i nietoksynotwórcze szczepy C. diphtheriae izolowane z zakażeń inwazyjnych, miejscowych oraz od nosicieli od lat 60. XX w. do 2008 r. W badaniach po raz pierwszy zastosowano technikę PCR MP do różnicowania C. diphtheriae. Uzyskane wyniki typowania techniką PCR MP porównano z wynikami uzyskanymi technikami PFGE i ERIC-PCR.

 Wyniki. Stwierdzono, że technika PCR MP różnicuje badane szczepy na podobnym poziomie jak PFGE i ERIC-PCR, ale w odróżnieniu od PFGE i ERIC-PCR pozwala na odróżnienie izolatów pochodzących z zakażeń inwazyjnych od izolatów z zakażeń miejscowych.


Słowa kluczowe: Corynebacterium diphtheriae, zakażenia inwazyjne, PCR MP, różnicowanie genetyczne.
**Corynebacterium diphtheriae** is a causative agent of diphtheria – an acute disease – due to its exotoxin called diphtheria toxin. Strains that do not produce the diphtheria toxin have been regarded as nonpathogenic. However, more and more invasive infections caused by nontoxigenic *C. diphtheriae* strains have been recorded in many developed countries where a diphtheria vaccine is broadly used. The true number of cases of invasive nontoxigenic *C. diphtheriae* infections is unknown because the reporting obligation only covers cases of classical diphtheria. Nontoxigenic *C. diphtheriae* invasive infections affect intravenous drug users, the alcohol addicted and the homeless but also healthy people [1–6]. There is a question about genome changes which may have given nontoxigenic *C. diphtheriae* invasive properties.

The aim of the presented studies was differentiation between *C. diphtheriae* strains isolated from invasive and non-invasive infections. In these studies we demonstrate, for the first time, the use of a PCR Melting Profile (PCR MP) technique for differentiation between *C. diphtheriae* isolates. PCR MP allows for gradual amplification of the genomic DNA differing in thermal stability, starting from the less stable DNA fragments amplified at lower denaturation temperature values to more stable ones amplified at higher denaturation temperature values. In the present studies, the utility of the PCR MP method was evaluated with data obtained using the pulsed-field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) methods.

### Material and Methods

The group of isolates tested (Table 1) contained isolates from Poland, from diphtheria cases in the 1960’s (1-5/A) and 1990’s (6-9/B), nontoxigenic isolates isolated in 2000 from a diphtheria case and in 2001 from a carrier (10/C and 11/D, respectively) and nontoxigenic isolates from blood and wound infections isolated in 2004–2008 (12-19/E). DNA was extracted with a DNeasy Tissue Kit (Qiagen) according to the manufacturer’s procedure. PCR MP was performed according to Krawczyk et al [7], PCR MP was performed as follows: 7 min at 72°C followed by initial denaturation at 85.5°C for 90 s and 22 cycles of denaturation at 85.5°C for 1 min, and annealing and elongation at 72°C for 2 min. After the last cycle, samples were incubated for 5 min at 72°C. The denaturation temperature was calculated during the optimization experiments using a gradient thermal cycler (Eppendorf) with a gradient range of 82 to 87°C and 86 to 93°C for the denaturation step. The PFGE was performed as described earlier [8]. The ERIC-PCR was performed according to Steinbruechner et al [9]. Data analysis was performed with GelCompar II 5.0 for Windows (AppliedMaths). Dendrograms were constructed by the unweighted pair group method with arithmetic averages (UPGMA), using Dice correlation for PFGE and Pearson correlation for ERIC-PCR and PCR MP. The computer-assisted analysis was performed according to the manufacturer’s instructions.

### Results and Discussion

The PCR MP results obtained were compared to PFGE and ERIC-PCR results (Table 1). The similarity level of all tested isolates was 52.3% for PCR MP and 65% for PFGE and ERIC-PCR. However, the similarity between strains isolated in the period 2004–2008 was very high. Since all of the nontoxigenic *C. diphtheriae* strains in this group were collected within a 5-year period from distinct patients with no epidemiological link (data not shown), we initially expected diverse genotypes. Surprisingly, the resulting PFGE, ERIC-PCR and PCR MP patterns were homogeneous for this group of strains. Only three highly similar PCR MP (genotypes a, a1 and b) and PFGE patterns (genotypes A, A1 and A2) and two ERIC-PCR patterns (V and VI) could be distinguished in the nontoxigenic isolates isolated during last 5 years. This remarkable genetic homogeneity may suggest that Polish nontoxigenic *C. diphtheriae* isolates are clonal currently. But this raises the question whether it is a more virulent genetic clone causing infections or it is the only clone circulating currently in the Polish population. There are no *C. diphtheriae* isolates from carriers in this time frame.

It is worth underlining that all of the compared methods differentiated the studied group of strains at a similar level, but, as opposed to PFGE and ERIC-PCR, PCR MP differentiated strains isolated from invasive infections (from blood samples) from strains isolated from local infections (Fig. 1). The additional DNA band in PCR MP profiles of invasive *C. diphtheriae* isolates may suggest some kind of mutations that give the strains invasive potential, for example the acquisition of new virulence genes located within a pathogenicity island or a profage genome. Only one strain isolated from blood did not reveal the additional band (12/E). Possibly, the strain did not possess all the same virulence genes as the other invasive strains tested, but the pathogenicity level of the strain was enough to cause invasive infection in the patient. The patient could be more sensitive to the infection due to additional diseases or the genetic specificity of the patient’s
body. Unfortunately, the authors were not able to verify this hypothesis because they did not have the necessary information about the patient.

On the basis of the results, the authors cannot say what kind of mutation it is and this needs to be studied further. The additional DNA band detected in PCR MP profiles will be sequenced in the near future in order to clarify the molecular mechanisms of the acquisition of additional genes involved in the invasive properties of some nontoxigenic C. diphtheriae strains. To the best of authors knowledge, this is the first report of the detection of small genotype differences between nontoxigenic C. diphtheriae strains isolated from invasive and non-invasive infections, although the domination of specific clones of toxigenic C. diphtheriae, regarded as more virulent, was reported during diphtheria outbreaks [10–12], as well as the

### Table 1. Characteristics of tested isolates of C. diphtheriae

<table>
<thead>
<tr>
<th>Strain (Szczep)</th>
<th>Toxigenicity (Toksynotwórczość)</th>
<th>Site of isolation (Miejsce izolacji)</th>
<th>PCR MP type</th>
<th>PFGE type</th>
<th>ERIC-PCR type</th>
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</thead>
<tbody>
<tr>
<td>1/A</td>
<td>+</td>
<td>nosopharynx</td>
<td>c1</td>
<td>C</td>
<td>X</td>
</tr>
<tr>
<td>2/A</td>
<td>+</td>
<td>nosopharynx</td>
<td>c</td>
<td>Cl</td>
<td>X</td>
</tr>
<tr>
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<td>C</td>
<td>X</td>
</tr>
<tr>
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<td>+</td>
<td>nosopharynx</td>
<td>f</td>
<td>H</td>
<td>XI</td>
</tr>
<tr>
<td>5/A</td>
<td>+</td>
<td>nosopharynx</td>
<td>d</td>
<td>G</td>
<td>IV</td>
</tr>
<tr>
<td>6/B</td>
<td>–</td>
<td>nosopharynx</td>
<td>i</td>
<td>F</td>
<td>VII</td>
</tr>
<tr>
<td>7/B</td>
<td>+</td>
<td>nosopharynx</td>
<td>e</td>
<td>Bl</td>
<td>I</td>
</tr>
<tr>
<td>8/B</td>
<td>+</td>
<td>nosopharynx</td>
<td>e</td>
<td>B</td>
<td>III</td>
</tr>
<tr>
<td>9/B</td>
<td>+</td>
<td>nosopharynx</td>
<td>e</td>
<td>B</td>
<td>II</td>
</tr>
<tr>
<td>10/C</td>
<td>–</td>
<td>nosopharynx</td>
<td>g</td>
<td>E</td>
<td>VIII</td>
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<tr>
<td>11/D</td>
<td>–</td>
<td>nosopharynx</td>
<td>h</td>
<td>D</td>
<td>IX</td>
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<tr>
<td>12/E</td>
<td>–</td>
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<td>a</td>
<td>A2</td>
<td>V</td>
</tr>
<tr>
<td>13/E</td>
<td>–</td>
<td>blood</td>
<td>a/</td>
<td>A</td>
<td>V</td>
</tr>
<tr>
<td>14/E</td>
<td>–</td>
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<td>a/</td>
<td>A</td>
<td>V</td>
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<tr>
<td>15/E</td>
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<td>wound</td>
<td>a/</td>
<td>A</td>
<td>V</td>
</tr>
<tr>
<td>16/E</td>
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<td>b/</td>
<td>A</td>
<td>V</td>
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<tr>
<td>17/E</td>
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<td>a/</td>
<td>A/</td>
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<tr>
<td>19/E</td>
<td>–</td>
<td>blood</td>
<td>a/</td>
<td>A</td>
<td>V</td>
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**Fig. 1.** The dendrogram illustrating the genetic similarity of PCR MP profiles obtained for nontoxigenic C. diphtheriae strains isolated in Poland in 2004–2008 from infections. Information about the site of the isolation in brackets

**Ryc. 1.** Dendrogram przedstawiający podobieństwo genetyczne profili PCR MP uzyskanych dla nietoksynotwórczych szczepów C. diphtheriae izolowanych w Polsce w latach 2004–2008. W nawiasie podano miejsce izolacji
domination of a single clone of nontoxicogenic *C. diphtheriae* isolated from infections [1, 3, 13, 14].

To summarize, the PCR MP technique differentiates *C. diphtheriae* isolates slightly better than ERIC-PCR and PFGE and might be useful for the differentiation of strains possessing invasive properties from non-invasive strains. Moreover, the presented study showed that nontoxicogenic *C. diphtheriae* strains isolated from invasive infections could possess additional genes or other mutations that result in higher invasiveness and virulence regardless of toxin production. But both these findings must be confirmed using a larger group of strains. The results of the study also point at the necessity of carrying out screening studies of nontoxigenic *C. diphtheriae* carriage in the Polish population to answer the questions about the clonality of strains circulating in Poland.

References


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