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A diagnostic challenge in clinical laboratory: Misidentification of *Neisseria subflava* as *Neisseria meningitidis* by MALDI-TOF MS

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RESEARCH ARTICLE



ABSTRACT

MALDI-TOF MS provides fast, easy to perform and cost-effective diagnosis in clinical microbiology laboratories, however in some cases results of MALDI-TOF MS should be confirmed with additional tests. This confirmation is especially important for causes of life-threatening infections like *Neisseria meningitidis*. In our laboratory, three isolates were identified as *N. meningitidis* by Bruker MALDI Biotyper (BD, USA) between April 2018 and March 2019 from clinical specimens of blood, sputum, and urine. 16S rRNA sequencing was performed for further investigation. Two of the isolates were identified as *Neisseria subflava* and only one was confirmed as *N. meningitidis* by sequencing. These results show that MALDI-TOF MS is not always reliable in the diagnosis of *N. meningitidis* and clinical microbiologists should confirm these results with additional tests. Also, clinical correlations should be determined. Accurate identification of this microorganism is very important because of the necessity of prophylactic antimicrobial usage and biosafety precautions. Enlarged databases of *Neisseria* species are needed to overcome this problem.

KEYWORDS

identification, MALDI-TOF MS, *Neisseria meningitidis*, sequencing

INTRODUCTION

The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) provides a fast, simple and cost-effective diagnosis in clinical microbiology laboratories, which is increasingly found a place in the hospital laboratories. It is simply based on generating a protein mass spectrum of the suspected microorganism and identifying by comparison of its library of spectra. This identification is quantified by the score obtained with the correlation of main spectra profiles in the library. If this score is between 2.0 and 3.0 it is a reliable identification in both genus and species level whereas a score of 1.7–2.0 means a probable identification in the genus level. The scores under 1.7 are considered unreliable [1].

Neisseria meningitidis is an oxidase positive, catalase positive, gram negative cocci causing life threatening infections such as meningitis and meningococemia. Its accurate diagnosis is a significant problem in the clinical microbiology laboratories because phenotypic, biochemical and MALDI-TOF MS testing can lead to misidentification of non-pathogenic *Neisseria* species as *N. meningitidis* [2]. The horizontal DNA transfers in *Neisseria* genus are often accused of this misidentification problem [3].

Apart from the serious clinical manifestation of *N. meningitidis*, the need for heightened biosafety applications and prophylaxis management of healthcare workers and other close contacts point out the requirement for fast and accurate diagnosis of this microorganism [4].

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Table 1. Antimicrobial susceptibility profile of the isolates

Antimicrobials isolates	PEN	AM	CT	TX	MP	CI	TC
<i>N. subflava</i> (sputum)	0.25 (I)	0.5 (I)	0.016 (S)	<0.002 (S)	0.047 (S)	<0.002 (S)	0.38 (S)
<i>N. subflava</i> (urine)	0.75 (R)	0.5 (I)	0.032 (S)	0.008 (S)	0.064 (S)	0.25 (R)	0.19 (S)
<i>N. meningitidis</i> (blood)	0.5 (R)	0.75 (S)	0.032 (S)	<0.002 (S)	0.047 (S)	<0.002 (S)	0.5 (S)

PEN: benzylpenicillin, AM: ampicillin, CT: cefotaxime, TX: ceftriaxone, MP: meropenem, CI: ciprofloxacin, TC: tetracycline.

On the other hand, the misidentification often causes unnecessary employment of patient care and management of healthcare and laboratory workers and other close contacts.

MATERIALS AND METHODS

From April 2018 to March 2019 three isolates were identified as *N. meningitidis* by Bruker MALDI Biotyper (BD, USA) with a score higher than 2.0 in Hacettepe University Hospitals. They were isolated from blood, sputum and urine culture. The blood culture was obtained from a fifty-nine year old patient diagnosed with cervical cancer. She was admitted to the medical oncology department with high fever and sore throat. The sputum culture was obtained from a thirty-nine year old female patient diagnosed with breast cancer, admitted with upper respiratory tract infection. From the molecular investigation, she was diagnosed with Influenza A infection. Lastly, the urine culture was obtained from a twenty-eight year old female patient who was admitted to the emergency department with abnormal vaginal bleeding. She did not have any complaints regarding urinary tract infection.

The samples were inoculated on blood, EMB, and chocolate agar. The blood and chocolate agar revealed grayish mucoid colonies and all isolates were oxidase and catalase positive. The gram stain revealed gram negative cocci. 16S rRNA sequencing was performed for further investigation. DNA of the isolates was extracted using the boiling method. The amplification of the 911 bp location of the 16S rRNA gene was performed using aroE-P1B forward primer (5'-TTTGAAACAGGCGGTTGCGG-3') and aroE-P2B reverse primer (5'-CAGCGTAATCCAGTGCGAC-3') [5]. Sequence reaction was conducted using BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The purification of PCR products were done by an Agencourt CleanSEQ Dye-Terminator removal kit (Beckman Coulter, USA). DNA sequences of the purified products were determined using Beckman Coulter 8000 equipment. The isolates were identified comparing the DNA reference isolates with data stored in the GenBank using the Basic Local Alignment Search Tool (Blast version 2.0) program.

Antimicrobial susceptibility testing was performed according to EUCAST v9.0 guidelines using Mueller Hinton Fastidious Agar and gradient test.

RESULTS

The isolates from sputum and urine culture were indeed identified as *N. subflava* by 16S rRNA sequencing. Only the

isolate from blood culture was confirmed as *N. meningitidis* by sequencing method. The antimicrobial susceptibility testing results were interpreted according to *N. meningitidis* table of EUCAST v9.0 guidelines (Table 1).

DISCUSSION

Our findings correlate with the data presented by Hong et al. in which two isolates of *Neisseria polysaccharaea*, two *Neisseria cinerea* and one *Neisseria bergeri* were misidentified as *N. meningitidis* using Bruker MALDI Biotyper [2]. In a case reported from Japan, a blood isolate of *N. cinerea* was named as *N. meningitidis* by MALDI-TOF MS [6]. Also, Cunningham et al. reports five isolates of *N. polysaccharaea* with an identification problem as meningococci [7]. However, this is the first report of *Neisseria subflava* misidentified as *N. meningitidis*.

We suggest that before reporting the *N. meningitidis* results of MALDI-TOF MS, additional tests should be performed. Also these results should be evaluated with the correspondence of clinical demonstration. The colony morphology is also important since the non-pathogenic species generally diffuses a yellowish pigment. Biochemical tests such as acid production, enzyme substrate test and polysaccharide formation from sucrose can be performed for confirmation. *N. polysaccharaea* also produces acid from glucose and maltose like *N. meningitidis*, so biochemical tests can also be confusing [8]. Alternatively 16s rRNA sequencing or amplification of *N. meningitidis* genes provides accurate identification [9].

Our findings intercalarily to the previous studies introduce the need for enlarged databases for *Neisseria* species. MALDI-TOF MS has extensively become the primary method for identification of bacteria in clinical microbiology laboratories. Accurate identification of this microorganism is very important because of its severe clinical presentation and the necessity of prophylactic antimicrobial usage and biosafety precautions.

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