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Frontline identification of bacterial isolates using the MALDI Biotyper-system during a resistance surveillance study in a central European area, 2010

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Introduction and Purpose

The matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry has become a routine technique for identification of microorganisms. The technique is based on the ionization of the microbial proteome and generation of spectrometric protein peak-pattern that are matched with peak-pattern in the MALDI Biotyper database. Several studies reported that the results of species identification achieved with the MALDI-TOF technique are in agreement with those of conventional identification methods [1–4].

For more than 30 years, the spread of antimicrobial resistance among clinically relevant bacterial species in Germany and a central European area has been surveyed by the Working Party Antimicrobial Resistance of the Paul Ehrlich Society of Chemotherapy. During the resistance surveillance study conducted between October and December 2010, bacterial isolates were identified using the MALDI Biotyper-system for the first time. The manufacturer (Bruker Daltonik GmbH, Bremen, Germany) has recommended three preparation methods for species identification. The aim of the present study was to evaluate how often each method was used to achieve a reliable identification result.

Methods

Bacterial strains

A total of 8,250 non-duplicate Gram-positive and Gram-negative pathogens were prospectively collected from 47 laboratories located in Germany (n=43), Switzerland (n=3) and Austria (n=1). Of these, 2,448 and 5,802 were obtained from outpatients and hospitalized patients, respectively. Centres were requested to collect isolates of the following bacterial groups: *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis, E. faecium, Streptococcus pneumoniae, S. agalactiae, S. pyogenes*, Enterobacteriaceae, *Pseudomonas aeruginosa, Acinetobacter baumannii* group, *Stenotrophomonas maltophilia, Haemophilus influenzae*, and *Moraxella catarrhalis*. At the end of the collection period, isolates were sent to a reference laboratory (Antiinfectives Intelligence) for re-identification and susceptibility testing.

Species identification

Species identification in the reference laboratory was performed using the Microflex-mass spectrometer and the MALDI Biotyper 3.0 Software (Bruker Daltonik GmbH, Bremen). Recommended preparation methods were as follows: The standard method (method 1) comprises the direct transfer of bacteria as a thin film onto a 96-spot steel-plate which after drying is overlaid by 1 µl of a matrix solution. The other two methods each require a pre-treatment of the bacteria, either with applying formic acid alone (method 2) to the smeared sample or using a short extraction protocol by using formic acid and ethanol (method 3). Method 1 was applied to all strains, while method 2 was used for strains that showed inconclusive results with method 1 and method 3 was used for strains that showed inconclusive results with method 2.

Interpretation of results

Species identification of a test strain was regarded as reliable, when the spectrum of proteins matched with protein spectra of the same species included in the Biotyper data base (3,995 cellular organisms) in first and second order, with a log score of ≥2 in first order.

Results

Identification results are summarized in **Table 1**. 8,203 (99.4%) isolates showed reliable results. Of these, 95.5% were identified by method 1, while 221 (2.7%) isolates needed a pre-treatment with formic acid and 99 (1.2%) with formic acid and ethanol. Success rates found for individual bacterial groups with method 1 varied between 93.3% und 97.3%.

A total of 47 (0.6%) isolates provided inconclusive results (**Table 1**). Of these, 15 (31.9%) strains showed a log score of <2.0 in first order and 32 (68.1%) strains a different species identification in first and second order (**Table 2**). 34 of the 47 strains belonged to members of the family Enterobacteriaceae. In 17 cases, inconclusive results were attributed to the low discrimination of the closely related species *Klebsiella oxytoca* and *Raoultella ornithinolytica* or *R. ornithinolytica* and *R. planticola*.

Conclusions

- **For 99.4% of the study isolates, the MALDI Biotyper-system revealed reliable results.**
- **95.5% of isolates were identified by the rapid and easy standard method (direct preparation).**
- **Overall, the MALDI-TOF mass spectrometry is a suitable tool for bacterial identification in high-throughput surveillance studies.**

Table 1: Identification results by preparation method

Bacterial group (no. tested)	Reliable results achieved by method			Inconclusive results
	1	2	3	
Staphylococci (1,818)	96.8% (n=1,759)	2.8% (n=51)	0.4% (n=8)	none
Streptococci (1,339)	97.3% (n=1,303)	1.3% (n=17)	1.0% (n=13)	0.4% (n=6)
Enterococci (730)	93.3% (n=681)	4.9% (n=36)	1.6% (n=12)	0.1% (n=1)
Enterobacteriaceae (2,482)	95.2% (n=2,364)	1.8% (n=47)	1.5% (n=37)	1.4% (n=34)
Non-fermenters (1,413)	93.9% (n=1,327)	3.9% (n=55)	1.8% (n=26)	0.4% (n=5)
Haemophilus spp. (233)	95.3% (n=222)	4.3% (n=10)	0.4% (n=1)	none
Moraxella spp. (234)	97.0% (n=227)	2.1% (n=5)	0.4% (n=1)	0.4% (n=1)
Aeromonas media (1)	none	none	100% (n=1)	none
Total (8,250)	**95.5% (n=7,883)**	**2.7% (n=221)**	**1.2% (n=99)**	**0.6% (n=47)**

Table 2: Inconclusive identification results

Bacterial group	No. of strains
Identical species in 1st und 2nd order, but a log score of <2.0 in 1st order	
Streptococci	5
Enterococci	1
Enterobacteriaceae	4
Non-fermenters	5
Different species in 1st und 2nd order or no result in 2nd order	
Enterobacteriaceae	30
Streptococci	1
Moraxella spp.	1
Total	**47**

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