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Identification of microorganisms grown on chromogenic media by MALDI-TOF MS

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ABSTRACT

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and chromogenic media are widely used in clinical microbiology laboratories to facilitate the rapid selection and identification of pathogens. The aim of this study was to evaluate whether usage of chromogenic media limits the diagnostic performance of MALDI-TOF MS for microbial identification. A total of 386 microorganisms collected and analyzed at five laboratories were included. Isolates were cultured on relevant chromogenic media and non-selective agar plates in parallel and identified using the Bruker MALDI-TOF MS. Among the tested isolates, no misidentification was recorded and there was no medium-related difference in the identification level. However, score values were overall slightly but significantly lower for isolates grown on chromogenic media. In conclusion, the use of chromogenic culture media tested here had no relevant impact on MALDI-TOF MS performance for diagnostic purposes.

1. Introduction

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a widely applied method for identification of bacteria and yeasts in clinical microbiology (Singhal et al., 2015). Species identification is based on peptide mass fingerprints from whole cells which are compared to a database of reference spectra derived from isolates grown on standard media. Clinical specimens are often cultured on various selective agar substrates, including chromogenic media. There are numerous chromogenic media for different purposes on the market. In addition to a selective component, microbial biochemical reactions are visualized by color changes of the medium or the colonies themselves, allowing differentiation and facilitating isolation from polymicrobial samples. Since identification by MALDI-TOF MS is based on unique protein signatures, and microbial protein expression is assumedly influenced by growth conditions, interference of these two methodologies cannot be excluded. In addition to putative direct impact on microbial protein expression, complex components in these media might create medium-associated peaks or interfere with the detection of microbial proteins (Buskirk et al., 2011; Walker et al., 2002).

There is scarce information on the impact of various growth media on MALDI-TOF MS performance for species identification (Anderson et al., 2012; Ford and Burnham, 2013; McElvania Tekippe et al., 2013; Valentine et al., 2005). Moreover, hitherto published studies covered a limited number of chromogenic media. Therefore, comprehensive information on a possible effect of chromogenic media on the clinical performance of MALDI-TOF MS is missing.

The aim of this study was to evaluate the performance of MALDI-TOF MS for identification of bacteria and yeasts grown on chromogenic media. The study was performed at five independent laboratories, to control for systematic factors related to single laboratories, and to cover microbial isolates from different geographical regions.

2. Materials and methods

A total of 386 clinical bacterial and *Candida* isolates were investigated in this study. The isolates were collected at the clinical microbiology laboratories at The Ottawa Hospital, Canada; Barts Health NHS Trust,

Abbreviations: MALDI-TOF MS, Matrix-assisted laser desorption ionization-time of flight mass spectrometry; MRSA, Methicillin-resistant *S. aureus*; VRE, Vancomycin-resistant *Enterococcus*.

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London, United Kingdom; Centre Hospitalier Régional Universitaire de Lille, France; MVZ Dr. Eberhard & Partner Dortmund, Germany; and Karolinska University Hospital, Stockholm, Sweden. Reference strains were included for control purposes. Isolates were grown on relevant CHROMagar media (CHROMagar, Paris, France), including CHROMagar Orientation, CHROMagar MRSA, CHROMagar VRE, CHROMagar Salmonella PLUS, CHROMagar StrepB, CHROMagar Listeria and CHROMagar Candida (Table 1). In parallel, the isolates were cultured on non-selective agar, *i.e.* blood agar for bacteria and Sabouraud dextrose agar for *Candida*. All laboratories used the microflex MS (Bruker Daltonik, Bremen, Germany) with the integrated MALDI Biotyper software. MALDI-TOF MS was performed according to standard procedures using the on-plate extraction method (Ford and Burnham, 2013). In brief, microorganisms were spotted on the MALDI-TOF MS target plate in triplicate and treated with formic acid (70%) before spots were overlaid with α-cyano-4-hydroxycinnamic-acid matrix. Identifications with scores ≥1.7 were accepted, and the highest score from triplicate analysis was used for evaluation. If no identification was achieved, the analysis was repeated. In case of *Candida* species, the second attempt was performed after full extraction with ethanol and formic acid. Score values obtained for identifications from colonies grown on chromogenic media were compared to score values for the same isolate grown on non-selective media by Wilcoxon matched-pairs signed rank test. Fisher's exact test was used to compare the number of identifications with score values <1.7 (no reliable identification), ≥1.7–1.99 (reliable identification on genus level) or ≥2.0 (reliable identification on species level). Differences with *P*-values < 0.05 were considered statistically significant.

3. Results and discussion

The performance of MALDI-TOF MS for microbial identification from non-selective standard media and chromogenic media was compared. Results from different laboratories were overall similar and substantial interlaboratory differences were not observed.

Concerning bacterial identification, the species was correctly identified by MALDI-TOF MS after on-plate extraction for all isolates ($n = 320$), with varying levels of confidence. For 302/320 (94.4%) isolates, identification was reliable to species level (score values ≥ 2.0) from both chromogenic and non-selective media. Among the remaining isolates, 6/320 (1.9%) were reliably identified to genus level (score values ≥ 1.7) from both types of media, 10/320 (3.1%) achieved scores ≥2.0 only from non-selective medium and 2/320 (0.6%) only from chromogenic media. These differences were statistically not significant ($P = 0.1439$). Overall, score values for isolates grown on chromogenic media (2.357, median) were slightly but significantly lower compared to score values obtained from non-selective agar (2.364, median; $P = 0.0102$).

Previous studies have shown that culture media might influence MALDI-TOF MS score values to an extent affecting the level of identification (Anderson et al., 2012; Ford and Burnham, 2013; McElvania Tekippe et al., 2013). Species identification is mainly based on peaks within a mass range of 2–20 kDa, representing ribosomal and few housekeeping proteins (Singhal et al., 2015). Expression of these proteins may be expected to be stable. However, as the identity of peaks used for species specification is hardly known, an impact of growth conditions on microbial identification cannot generally be excluded. Detailed analyses of protein spectra revealed changes in the peptide mass fingerprint throughout mass ranges which might be relevant for microbial identification (Valentine et al., 2005; Walker et al., 2002). In addition, media component could give rise to additional peaks, even though these were mostly located at lower mass ranges (Walker et al., 2002). Moreover, it has been observed that chromogenic substances such as fungal melanin inhibit desorption/ionization of proteins and thus abolish the generation of mass spectra from *Aspergillus niger* (Buskirk et al., 2011). However, the chromogenic media included in this study did not inhibit identification or caused misidentifications among the isolates investigated.

Table 1
Panel of chromogenic media and microorganisms.

Chromogenic medium	Description	Microorganisms (*n*)
CHROMagar orientation	Detection and differentiation of a broad range of bacteria, in particular uropathogens	*Escherichia coli* (24) *Klebsiella pneumoniae* (10) *Klebsiella oxytoca* (10) *Proteus mirabilis* (10) *Proteus vulgaris* (9) *Morganella morganii* (10) *Citrobacter freundii* (9) *Enterobacter cloacae* (10) *Enterobacter aerogenes* (10) *Pseudomonas aeruginosa* (9) *Staphylococcus saprophyticus* (7) *Staphylococcus aureus* (9) *Staphylococcus epidermidis* (9) *Enterococcus faecium* (10) *Enterococcus faecalis* (8)
CHROMagar MRSA	Selection of methicillin-resistant (MR) *S. aureus*	MR-*S. aureus* (24) MR-*S. epidermidis* (10)[a] *Corynebacterium* species (8)[a]
CHROMagar VRE	Selection and differentiation of vancomycin-resistant (VR) *E. faecium* and *E. faecalis*	VR-*E. faecium* (17) VR-*E. faecalis* (9) *Enterococcus casseliflavus* (6)[a] *Enterococcus gallinarum* (9)[a]
CHROMagar Strep B	Selection and differentiation of group B streptococci	Group B streptococci (22) Group A streptococci (8) *Streptococcus dysgalactiae* (8)
CHROMagar *Salmonella* PLUS	Selection and differentiation of *Salmonella*	*Salmonella* species (25) *Pseudomonas aeruginosa* (9)[a] *Aeromonas* species (7)[a]
CHROMagar *Listeria*	Selection and differentiation of *Listeria monocytogenes*	*Listeria monocytogenes* (4)
CHROMagar *Candida*	Selection and differentiation of *Candida* species	*Candida albicans* (19) *Candida dubliniensis* (9) *Candida glabrata* (10) *Candida krusei* (8) *Candida parapsilosis* (10) *Candida tropicalis* (10)

[a] Growth of these species is generally inhibited by agar components, but growth might occur for highly resistant isolates or in case of a heavy inoculum.

One might speculate that highly selective media have a stronger impact on protein expression than less stringent conditions in more universal media and consequently have a more pronounced impact on bacterial identification. We observed subtle differences in the effect of different chromogenic media on MALDI-TOF MS score values (Fig. 1), none significantly affecting the level of identification. No influence on MALDI-TOF MS score values could be established for CHROMagar Orientation (Fig. 1A), but similar results were seen for CHROMagar StrepB (Fig. 1B) and CHROMagar Salmonella Plus (Fig. 1C), which are more selective. The number of tested *Listeria monocytogenes* isolates was low, but all isolates were identified with score values ≥2.0 from both types of media. In contrast, score values for bacterial identification from highly selective media CHROMagar MRSA (Fig. 1D) and CHROMagar VRE (Fig. 1E) were significantly lower compared to results from colonies taken from blood agar plates. Since non-specific growth on selective media might occur, isolates from non-target species were also tested (Table 1). If required, growth of such isolates was facilitated by heavy inocula and longer incubation times. Identification of poorly growing isolates from CHROMagar MRSA and CHROMagar VRE could result in low score values, while all methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) were identified to species level with high confidence (score values ≥ 2.0). However, even for MRSA and VRE isolates alone, score values were significantly lower when grown on CHROMagar MRSA and CHROMagar VRE, respectively, compared to non-selective media. These results suggest that growth conditions selecting for acquired resistance may have a stronger impact on MALDI-TOF MS performance than selective pressure allowing growth of naturally tolerant species.