Diagnostic Microbiology and Infectious Disease 94 (2019) 113–115

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

CHROMagar™ orientation urine culture medium produces matrix-assisted laser desorption ionization–time-of-flight mass spectrometry spectra misidentified as *Mycoplasma arginini and Mycoplasma alkalescens*

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ARTICLE INFO

Article history:
Received 21 August 2018
Received in revised form 16 November 2018
Accepted 24 December 2018
Available online 2 January 2019

Keywords:
MALDI-TOF
Mycoplasma
Chromogenic
Urine
Culture
Identification
Mass spectrometry

ABSTRACT

Matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry is commonly used to identify bacteria and yeasts. Studies indicate that MALDI-TOF is relatively indifferent to the medium used for culture. We report on an investigation into high- and low-confidence MALDI-TOF misidentifications of *Mycoplasma arginini and Mycoplasma alkalescens from* urine specimens plated to CHROMagar™ Orientation medium that appear to be due to the intrinsic mass spectrum of the medium.

The implementation of matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) has largely superseded conventional and time-consuming tests for the identification of bacteria in routine cultures. After MALDI-TOF implementation, we observed urine specimens inoculated to CHOMagar™ Orientation medium showing pinpoint colonies in significant numbers (>1 × 10^5 cfu/mL) that were frequently identified by the Bruker Biotyper MALDI-TOF (Bruker-Daltonics, Boston, MA) as *Mycoplasma* sp., most commonly *Mycoplasma arginini*, a feline commensal and bovine pathogen which has been implicated in human disease (Leach, 1970; Sillo et al., 2012; Tan et al., 1977; Watanabe et al., 2012; Yechouron et al., 1992), and *Mycoplasma alkalescens*, a bovine pathogen with no known human pathogenicity (Kokotovic et al., 2007). In many cases, identification scores were high enough to support genus-level identification (>1.7). Since *M. arginini* as well as human genital mycoplasmas such as *Mycoplasma hominis* can be recovered on routine laboratory media such as sheep blood agar and chocolate agar (McMahon et al., 1990; Watanabe et al., 2012; Yechouron et al., 1992), and mycoplasmas have been implicated in urinary tract infections and urethritis (Taylor-Robinson, 1996), the finding was thought to be potentially relevant. However, in all cases, additional investigations of the isolates revealed either no bacterial organisms on subculture or organisms consistent with typical urogenital flora (e.g., *Corynebacterium* sp., *Lactobacillus* sp., *Gardnerella vaginalis*). Further observation suggested that identification or *Mycoplasma* sp. was only occurring either when no growth was present on the agar or when very small inadequately sampled colonies were present on the media, suggesting something in the media or the residual urine on the agar was responsible for the false identification.

To resolve the discrepancy, a random sample of 10 CHROMagar Orientation™ plates that had been inoculated with urine specimens but showed no visible growth after overnight incubation, 4 uninoculated CHROMagar Orientation™ plates, and 2 uninoculated plates of all other media used in our laboratory was sampled using a sterile plastic loop or bamboo toothpick using standard MALDI-TOF procedure. The other agar media tested included tryptic soy (Becton Dickinson, Mississauga, Canada), sheep blood (Oxoid, Nepean, Canada), blood Mueller–Hinton (Oxoid, Nepean, Canada), brain heart infusion (BHI; Becton Dickinson, Mississauga, Canada), *Brucella* blood (Becton Dickinson, Mississauga, Canada), *Campylobacter* selective (Oxoid, Nepean, Canada), chocolate (Oxoid, Nepean, Canada), colistin-naladixic acid (CNA; Becton Dickinson, Mississauga, Canada), inhibitory mold (Hardy Diagnostics, Santa Maria, CA), MacConkey (Oxoid, Nepean, Canada), modified Thayer–Martin (Oxoid, Nepean, Canada), Mueller–Hinton (Oxoid, Nepean, Canada), phenylethyl alcohol (PEA; Becton Dickinson, Mississauga, Canada), Sabouraud dextrose (Becton Dickinson, Mississauga, Canada), Skirrow's (Becton Dickinson, Mississauga, Canada), sorbitol MacConkey (Oxoid,

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https://doi.org/10.1016/j.diagmicrobio.2018.12.014
0732-8893/

Nepean, Canada), and Xylose-Lactose-Deoxycholate (XLD; Becton Dickinson, Mississauga, Canada) agars as well as chromogenic MRSA Select II (Bio-Rad, Montreal, Canada) and chromogenic VRE Select (Bio-Rad, Montreal, Canada) agars. In brief, a toothpick or plastic loop was used to gently touch the surface of the agar, and then to inoculate a MALDI-TOF target plate, 1 μL of 70% formic acid was added to the inoculum. The spot was allowed to dry and overlaid with 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix and allowed to dry. Target plate controls were also run with formic acid and HCCA matrix alone to rule out exogenous sources for the observed spectra. The target plates were loaded onto the MALDI-TOF MS instrument and spectra analyzed using the MSP 5627 database. Of the inoculated no-growth CHROMagar Orientation™ plates tested, 8/10 (80%) produced spectra, all providing a top identification of *M. arginini*, *M. alkalescens*, or *M. bovis* with variable confidence (Table 1). Of these, 1 had a confidence score high enough to be confident in the genus level. Of the uninoculated CHROMagar Orientation™ plates tested, all 4 produced spectra that matched *M. arginini* with scores ranging from 1.654 to 2.058, with 3 of 4 providing scores sufficiently high to warrant identification to genus (>1.700) or species (>2.000) (Table 1). Spectra were also recovered from blood Mueller–Hinton, BHI, *Brucella* blood, chocolate, CNA, modified Thayer–Martin, PEA, sheep blood, and Skirrow's agars; however, identification scores for these agars were consistently below 1.5 using the BioTyper™ system, and no consistent pattern of identified organisms emerged. Specifically, no identifications of *Mycoplasma* sp. were generated from any of these media. No peaks were generated within the detection range of the instrument for *Campylobacter*, inhibitory mold, MacConkey, Mueller–Hinton, Sabouraud dextrose, sorbitol MacConkey, or XLD agars. Likewise, peaks were not generated by the chromogenic MRSA Select II or the chromogenic VRE Select plates.

MALDI-TOF MS has been shown to be a rapid, accurate, and cost-effective method of identifying pathogens from culture in microbiology laboratories worldwide (Clark et al., 2013; Lagace-Wiens, 2015; Lagace-Wiens et al., 2012). MALDI-TOF MS is used to analyze the mass to charge ratio (*m/z* ratio) of principal components of microbial cells, primarily proteins, to create a mass spectrum. Mass spectra from unknown isolates are compared to a library of spectra to determine the reference organism that most closely matches the generated spectrum (Bizzini et al., 2010; Lagace-Wiens, 2015). Although commonly used for the identification of bacteria and yeasts (Bessede et al., 2011; Bizzini et al., 2010), MALDI-TOF MS has also been successfully used to identify mycobacteria, mycoplasmas, filamentous fungi, and parasites (Bizzini and Greub, 2010; Clark et al., 2013; Seng et al., 2009). MALDI-TOF MS identification has also been shown to be relatively indifferent to the culture medium from which colonies are selected for identification, although inhibitory media may reduce the confidence of the identification without apparently affecting the accuracy (Anderson et al., 2012). Although it remains true that bacterial identification by MALDI-TOF remains relatively unaffected by the media, we have demonstrated that some uninoculated media may produce MALDI-TOF MS spectra that can give low-confidence identifications. The potential for misidentification thus exists when small colonies are sampled and analyzed and the medium may contribute a relatively large proportion of the spectrum, or when specimen on the plate is mistaken for fine growth. We observed that detectable peaks (spectra) are produced from other uninoculated media primarily when blood or tissue addititves are in the media. This is unsurprising given that MALDI-TOF MS is designed to primarily analyze proteins and these media all contain significant protein content by virtue of the blood or tissue added to the medium. However, while it appears that many media with blood or tissue additives produced spectra, these spectra appear to be of minimal consequence to the identification of bacteria or yeast when using the Bruker Biotyper™ as they are not sufficiently similar to reference spectra to produce confident identification scores. However, the spectrum generated by the CHROMagar™ Orientation medium appears sufficiently similar to those of the *M. arginini* and *M. alkalescens* reference spectra that occasional low- and high-confidence misidentification of these organisms may occur when this medium is used and either no microbial biomass is sampled or the relative contribution of the medium to the sample on the target plate is greater than the biomass of bacteria. The components of CHROMagar medium include only the following: agar, peptone and yeast and “chromogenic mix.” As such, 2 hypotheses potentially explain the identification of *Mycoplasma* sp. from the medium. The first is that exogenous mycoplasmas contaminated the components of the agar and were rendered nonviable with subsequent sterilization, and that the mass spectrum represents the detection of residual *Mycoplasma* sp. proteins. *M. arginini* has been frequently isolated from commensal bovine sera (Barile and Kern, 1971), while both *M. arginini* and *M. alkalescens* have been found in cow's milk (due to their role in mastitis) (Gioia et al., 2016). Since milk is one of the primary raw ingredients used in peptone production, mycoplasma proteins could have entered the CHROMagar™ medium through use of milk-derived peptones. The other explanation is that a listed component of the medium is producing a spectrum similar enough to *M. alkalescens* and *M. arginini* that the software cannot reliably distinguish it from the organism's spectrum. In this case, we hypothesize that the spectrum likely originates from either the peptones or the yeast extract due to the fact that chromogens used in microbiology have relatively low molecular weights (200–500 Da) (Perry et al., 2007), which are outside the 2000–20,000 Da range used by MALDI-TOF to identify bacteria.

After recognition of this potential for misidentification, our laboratory implemented practice changes. Primarily, caution was recommended not to sample medium during colony selection. Additionally, use of MALDI-TOF for identification of nonurinary pathogens from urine cultures was discouraged. Due to their frequent poor growth and small colonies on the medium, they were prone to oversampling medium during colony selection. Lastly, where required, subculture of potential pathogens to sheep blood agar prior to reidentification by MALDI-TOF provided an identification of *M. alkalescens* or *M. arginini.*

Table 1
MALDI-TOF MS identification scores and identification results for culture-negative and uninoculated CHROMagar™ Orientation agar

Medium (no. of plates tested)	Identification score	Identification result
Inoculated culture-negative CHROMagar Orientation™ agar (10)[a]	1.618	*M. arginini*
	1.574	*M. arginini*
	1.659	*M. alkalescens*
	1.682	*M. arginini*
	1.090	*M. bovis*
	1.802	*M. arginini*
	1.639	*M. alkalescens*
	1.450	*M. alkalescens*
Uninoculated CHROMagar Orientation™ agar (4)[b]	1.766	*M. arginini*
	1.784	*M. arginini*
	2.058	*M. arginini*
	1.654	*M. arginini*

[a] Eight of 10 (80%) plates tested generated an analyzable spectrum, 1/10 (10%) with a score sufficient for genus-level identification.

[b] Four of 4 (100%) plates tested generated an analyzable spectrum, 2/4 (50%) with a score sufficient for genus-level identification and 1/4 (25%) with a score sufficient for species-level identification.

Conflicts of interest

There are no conflicts of interest for any of the authors.

References

Anderson NW, Buchan BW, Riebe KM, Parsons LN, Gnacinski S, Ledeboer NA. Effects of solid-medium type on routine identification of bacterial isolates by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:1008–13.

Barile MF, Kern J. Isolation of mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proc Soc Exp Biol Med 1971;138:432–7.

Bessede E, Angla-Gre M, Delagarde Y, Sep Hieng S, Menard A, Megraud F. Matrix-assisted laser-desorption/ionization biotyper: experience in the routine of a university hospital. Clin Microbiol Infect 2011;17:533–8.

Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clin Microbiol Infect 2010;16:1614–9.
Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J Clin Microbiol 2010;48:1549–54.
Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 2013;26:547–603.
Gioia G, Werner B, Nydam DV, Moroni P. Validation of a mycoplasma molecular diagnostic test and distribution of mycoplasma species in bovine milk among New York State dairy farms. J Dairy Sci 2016;99:4668–77.
Kokotovic B, Friis NF, Ahrens P. Mycoplasma alkalescens demonstrated in bronchoalveolar lavage of cattle in Denmark. Acta Vet Scand 2007;49:2.
Lagace-Wiens P. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS)-based identification of pathogens from positive blood culture bottles. Methods Mol Biol 2015;1237:47–55.
Lagace-Wiens PR, Adam HJ, Karlowsky JA, Nichol KA, Pang PF, Guenther J, et al. Identification of blood culture isolates directly from positive blood cultures by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry and a commercial extraction system: analysis of performance, cost, and turnaround time. J Clin Microbiol 2012;50:3324–8.
Leach RH. The occurrence of Mycoplasma arginini in several animal hosts. Vet Rec 1970;87:319–20.
McMahon DK, Dummer JS, Pasculle AW, Cassell G. Extragenital Mycoplasma hominis infections in adults. Am J Med 1990;89:275–81.
Perry JD, Morris KA, James AL, Oliver M, Gould FK. Evaluation of novel chromogenic substrates for the detection of bacterial beta-glucosidase. J Appl Microbiol 2007;102:410–5.
Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.
Sillo P, Pinter D, Ostorhazi E, Mazan M, Wikonkal N, Ponyai K, et al. Eosinophilic fasciitis associated with mycoplasma arginini infection. J Clin Microbiol 2012;50:1113–7.
Tan RJ, Lim EW, Ishak B. Significance and pathogenic role of mycoplasma arginini in cat diseases. Can J Comp Med 1977;41:349–54.
Taylor-Robinson D. Infections due to species of Mycoplasma and Ureaplasma: an update. Clin Infect Dis 1996;23:671–82. [quiz 683-674].
Watanabe M, Hitomi S, Goto M, Hasegawa Y. Bloodstream infection due to Mycoplasma arginini in an immunocompromised patient. J Clin Microbiol 2012;50:3133–5.
Yechouron A, Lefebvre J, Robson HG, Rose DL, Tully JG. Fatal septicemia due to Mycoplasma arginini: a new human zoonosis. Clin Infect Dis 1992;15:434–8.