Diagnostic Microbiology and Infectious Disease 82 (2015) 105–108

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Bacteriology

MALDI-TOF identification of Gram-negative bacteria directly from blood culture bottles containing charcoal: Sepsityper® kits versus centrifugation–filtration method☆,☆☆

Kathleen Riederer [a], Kristian Cruz [a], Stephen Shemes [a], Susan Szpunar [a], Joel T. Fishbain [b,*]

[a] Department of Medical Education, St John Hospital and Medical Center, Detroit, MI, USA
[b] Division of Infectious Diseases, St John Hospital and Medical Center, Detroit, MI, USA

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form 10 February 2015
Accepted 4 March 2015
Available online 10 March 2015

Keywords:
MALDI-TOF MS
Direct identification
Gram-negative bacteria
Blood culture
Charcoal bottles
Extraction method

ABSTRACT

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry has dramatically altered the way microbiology laboratories identify clinical isolates. Direct blood culture (BC) detection may be hampered, however, by the presence of charcoal in BC bottles currently in clinical use. This study evaluates an in-house process for extraction and MALDI-TOF identification of Gram-negative bacteria directly from BC bottles containing charcoal. Three hundred BC aliquots were extracted by a centrifugation–filtration method developed in our research laboratory with the first 96 samples processed in parallel using Sepsityper® kits. Controls were colonies from solid media with standard phenotypic and MALDI-TOF identification. The identification of Gram-negative bacteria was successful more often via the in-house method compared to Sepsityper® kits (94.7% versus 78.1%, $P \leq 0.0001$). Our in-house centrifugation–filtration method was further validated for isolation and identification of Gram-negative bacteria (95%; n = 300) directly from BC bottles containing charcoal.

1. Introduction

The isolation and identification of clinically relevant bacterial pathogens have changed very little over the past half century. Phenotypic methods and long incubation periods are required for final species identification and automated systems have not substantially reduced the clinician's waiting times. Recent developments in rapid diagnostic testing capabilities are changing the way clinical microbiology laboratories finalize and report organism identification. The development of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has yielded a product that is easy to use, rapid, cost-effective, and efficient for organism identification to the genus and species levels (Bizzini and Greub, 2010). While this technology has recently been cleared by the US Food and Drug Administration for identification of isolates from solid media, a number of authors have demonstrated the utility of pathogen identification directly from positive blood culture (BC) bottles (Buchan et al., 2012; Clerc et al., 2013; Lagace-Wiens, 2015; Martiny et al., 2012; Prod'hom et al., 2010; Vlek et al., 2012).

The different studies examining the ability and/or impact of direct identification from BC bottles have employed a variety of isolation techniques. Only a few studies have examined these techniques with charcoal-containing bottles, however (Schmidt et al., 2012; Wuppenhorst et al., 2012). One of the MALDI-TOF manufacturers (Bruker Daltonics, Bremen, Germany) makes a kit for processing BC bottles with modified instructions when using charcoal-containing bottles. In order to utilize MALDI-TOF at our institution for direct identification from charcoal BC bottles, we compared the performance of Sepsityper® kits (Bruker Daltonics) to an in-house centrifugation and filtration process. The initial comparative phase was followed by an additional validation phase using only the in-house isolation method.

2. Materials and methods

This study was conducted in the Infectious Disease Research Laboratory at St John Hospital and Medical Center, Detroit, MI. St John Hospital is an 800-bed teaching facility that provides microbiology laboratory services for 2 other hospitals and a long-term acute care facility. Funding was provided by a faculty grant from the Medical Executive Committee of St John Hospital and Medical Center. Bruker Daltonics provided the MALDI-TOF equipment, training, and kits under a no-charge loan agreement.

BCs were performed using BacT/ALERT® FA, FN, and PF (bioMérieux, Durham, NC, USA) charcoal-containing bottles in the BacT/ALERT® 3D system (bioMérieux). The clinical microbiology laboratory monitored and removed positive BCs from the instrument following all standard procedures and policies. In the event the Gram stain demonstrated

☆ The authors have no relevant conflicts of interest.
☆☆ Financial support: Funding provided by a Faculty Grant from the Medical Executive Committee, St John Hospital and Medical Center and Bruker Daltonics, which provided the MALDI-TOF equipment and reagents.

* Corresponding author. Tel.: +1 313-642-9882; fax: +1 313-343-7840.
E-mail address: joel.fishbain@stjohn.org (J.T. Fishbain).

http://dx.doi.org/10.1016/j.diagmicrobio.2015.03.003
0732-8893/

Gram-negative bacilli, the Infectious Disease Research Laboratory staff obtained an aliquot for the study.

2.1. Sepsityper® processing

Sepsityper® kits (Bruker Daltonics) and recommended spin columns (SC1000; Sigma-Aldrich, St Louis, MO, USA) were obtained from the manufacturers. Samples were processed following kit instructions for charcoal bottles. Briefly, 1 mL of BC fluid was added to a reaction tube with 200 μL of lysis buffer, then vortexed for 10 seconds. An 800-μL aliquot was placed into the spin column and centrifuged for 2 minutes at 325×*g*.

2.2. Centrifugation–filtration processing

A 1.5-mL sample of BC fluid was centrifuged for 2 minutes at 850×*g* in a microcentrifuge tube. The supernatant was drawn into a 3-cc syringe with a needle attached. The needle was removed, a Whatman syringe filter (#6888-1327, 13 mm, 2.7 μm pore size) was attached to the syringe, and the fluid completely expressed into a 2-mL tube, using a 1.5-cc air purge.

2.3. Extraction method

Samples from either process were then centrifuged at 14,000×*g* for 2 minutes. The pellet was suspended in 1 mL of wash buffer (Sepsityper® kit) or 1 mL of water (centrifugation–filtration). The tubes were centrifuged for 1 minute at 14,000×*g*, and the pellet suspended in 300 μL of water. Nine hundred microliters of ethanol was added, and another 14,000 × g spin for 2 minutes was performed. The supernatant was removed, and the same spin was repeated on the pellet. All remaining ethanol was removed, and the pellet was allowed to dry. Equal parts of 70% formic acid and acetonitrile (5–50 μL, based on pellet size) were mixed with the pellet. Tubes were centrifuged for 2 minutes at 14,000×*g*, and 1 μL of the supernatant was applied to the MALDI target in duplicate. After drying, 1 μL of α-Cyano-4-hydroxycinnamic acid matrix was applied and allowed to dry.

2.4. MALDI-TOF analysis

MALDI-TOF was performed on the Microflex LT (Bruker Daltonics) instrument using Flex Control 3.4 software and Biotyper Realtime Classification 3.1 with BC specific software. Scores of ≥1.8 were used for identification to species level for direct BC processing as established by the manufacturer's internal software specific for BCs.

2.5. Controls

Controls for organism identification included standard phenotypic results adhering to the Clinical Laboratory Standards Institute guidelines via VITEK 2 (bioMerieux), rapid bench methods, and reference laboratory methods if necessary. MALDI-TOF identification from solid media colonies (score ≥2.0 for species level) following the manufacturer's instructions served as a second control group.

2.6. Data analysis

Data were analyzed using McNemar's test. All data were analyzed using SPSS version 22.0. No statistical test was run when there was a zero cell in any table. A *P*-value of ≤0.05 was considered to indicate statistical significance.

3. Results

Ninety-six positive BCs containing Gram-negative organisms were selected and evaluated in parallel using Sepsityper® kits and centrifugation–filtration processing methods. The centrifugation–filtration method was statistically more likely to provide a result when compared to the Sepsityper® method, 94.7% (91/96) versus 78.1% (75/96), $P \leq 0.0001$. Excluding MALDI-TOF cases of "NO peak" or "NO identification", the MALDI-TOF accurately identified aerobic Gram-negative bacilli to the genus level 100% of the time by both methods.

The centrifugation–filtration method was continued in a validation phase with a total of 300 BC bottles sampled. A MALDI-TOF identification was obtained for 95.3% (n = 286/300) of centrifugation–filtration processed samples. Fourteen samples failed to provide a genus or species level identification (NO peak or NO identification). Species-level identification was obtained for 97.6% (279/286) of organisms identified (Table 1). Only genus-level identification was possible for 7 samples: *Fusobacterium* (n = 2), *Bacteroides* (n = 2), *Capnocytophaga* (n = 1),

Table 1
Identification of Gram-negative organisms by different processing methods.

Phenotypic identification (total = 300)	Sepsityper® kit (total = 96)	Centrifugation–filtration (total = 300)
	% Identified to genus or species level	
Acinetobacter baumannii (16)[a]	66.7% species level (4/6)[b]	100% species level (16/16)[b]
Achromobacter group (1)	0%	0%
Aeromonas hydrophila (1)	ND	100% species level
Bacteroides fragilis (11)	0% (0/2)	100% species level (11/11)
Bacteroides thetaiotaomicron (3)	0% (0/1)	100% species level (3/3)
Bacteroides uniformis (2)	0% (0/1)	100% genus level (2/2)
Bacteroides caccae (1)	ND	100% species level
Bacteroides ovatus (1)	ND	100% species level
Bordetella holmesii[c] (1)	ND	0%
Brevundimonas vesicularis (1)	0%	0%
Capnocytophaga species (1)	ND	100% genus level
Citrobacter amalonaticus (1)	ND	100% species level
Enterobacter aerogenes (7)	100% species level (2/2)	100% species level (7/7)
Enterobacter cloacae complex (8)	50% genus level (1/2)	100% species level (8/8)
Escherichia coli (120)	90.2% species level (37/41)	96.7% species level (116/120)
Eubacterium lentum (1)	ND	100% species level
Fusobacterium species (2)	0% (0/1)	100% genus level (2/2)
Haemophilus influenzae (1)	ND	100% species level
Klebsiella pneumoniae (44)	76.9% species level (10/13)	93.2% species level (41/44)
Klebsiella oxytoca (8)	100% species level (2/2)	100% species level (8/8)
Moraxella group (1)	100% species level	100% species level
Morganella morganii (5)	ND	100% species level (5/5)
Pseudomonas aeruginosa (25)	100% species level (9/9)	100% species level (25/25)
Pseudomonas putida (1)	100% genus level	100% species level
Pseudomonas species (1)	100% genus level	100% genus level
Proteus mirabilis (16)	66.7% species level (4/6)	93.8% species level (15/16)
Providencia stuartii (7)	100% species level	85.7% species level (6/7)
Prevotella melaninogenica (1)	ND	Identified as *B. fragilis*
Salmonella species (1)	ND	100% genus level
Serratia marcescens (6)	100% species level	100% species level (6/6)
Stenotrophomonas maltophilia (4)	50% species level (1/2)	50% species level (2/4)
Yersinia enterocolitica (1)	0%	100% species level

ND = not done, test not performed on these isolates.
[a] Number in parentheses denotes number of isolates for each organism or procedure performed.
[b] Numerator represents the number identified and denominator is the number tested if >1 isolate of that particular species.
[c] Isolate confirmed by 16S ribosomal RNA analysis at the state reference laboratory.