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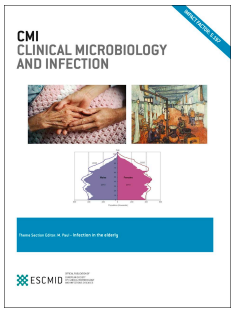

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Direct identification of clinical pathogens from liquid culture media by MALDI-TOF MS analysis

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Running title: Direct MALDI-TOF-based identification of pathogen from liquid cultures

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Abstract

Objectives: We propose using MALDI-TOF MS as a tool for identifying microorganisms directly from liquid cultures after enrichment of the clinical sample in the media, in order to obtain a rapid microbiological diagnosis and an adequate administration of the antibiotic therapy in a clinical setting.

Methods: To evaluate this approach, a series of quality control isolates, were grown in thioglycollate (TG) broth and brain heart infusion (BHI) broth and extracted under 4 different protocols before finally being identified by MALDI-TOF MS. After establishing the best extraction protocol, we validated the method in a total of 300 liquid cultures (150 in TG broth and 150 in BHI broth) of different types of clinical samples obtained from two tertiary Spanish hospitals.

Results: The initial evaluation showed that the extraction protocol including a 5 min sonication step yielded 100% valid identifications, with an average score value of 2.305. In the clinical validation of the procedure, 98 % of the microorganisms identified from the TG broth were correctly identified relative to 97 % of those identified from the BHI broth. In 24 % of the samples analysed, growth by direct sowing was only successful in the liquid medium, and no growth was observed in the direct solid agar cultures.

Conclusions: Use of MALDI-TOF-MS plus the sonication-based extraction method enabled direct and accurate identification of microorganisms in liquid culture media in 15 min, in contrast to the 24 hours of subculture required for conventional identification, allowing the administration of a targeted antimicrobial therapy.

Introduction

Rapid and reliable identification of bacteria is essential for the diagnosis and treatment of patients with infectious diseases. Until recently, biochemical, colorimetric and even antibiotic sensitivity tests were used to identify genera and species. The main limitations of these methods include the time required and the difficulty in distinguishing between poorly reactive, very similar, or difficult-to-culture microorganisms. Many of these problems have been solved by the Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) [1-6]. Cost effectiveness studies have demonstrated that the early diagnosis of bacteraemia and other infectious diseases by MALDI-TOF MS has improved antimicrobial use, allowing a rapid administration of a targeted antimicrobial therapy [7-11]. However, one of the main limitations of MALDI-TOF MS is that more than 10^5 colony forming units (CFU)/ml are required for accurate identification of bacteria [12-13]. Direct identification of bacteria in clinical samples has therefore so far only been possible with urine samples [5-6].

Use of liquid cultures has increased the sensitivity and turnaround time of bacterial culture, especially for samples with low bacterial loads that do not grow in solid culture, e.g. cerebrospinal fluid (CSF), pericardial fluid and joint fluid [14]. However, a period of 24 hours is required to identify the grown up microorganism by subsequent growth on solid culture and final identification. We propose using MALDI-TOF MS as a tool for identifying microorganisms directly from liquid cultures (thioglycollate broth and brain heart infusion broth) after extracting the bacterial protein in a sonication-based procedure.

Material and methods

Clinical setting and sample collection

The study was performed between November 2016 and February 2017 in two tertiary teaching hospitals in Spain, the *Complejo Hospitalario Universitario A Coruña* (CHUAC) and the *Hospital General Universitario Gregorio Marañón* (HGUGM). For the study, each laboratory cultured 150 clinical samples in enriched liquid medium. In the CHUAC, the microorganisms were cultured in thioglycollate (TG) broth supplemented with vitamin K1 and hemin (Becton Dickinson, United States), while in the HGUGM the microorganisms were cultured in brain heart infusion (BHI) broth (Becton Dickinson).

The following different types of samples were cultured in liquid medium: biopsy (n=50), exudate from surgical wounds (n=34), prosthetic material (n=25), cardiac valve (n=10), catheter tip (n=5), pericardial fluid (n=1), pleural fluid (n=10), synovial fluid (n=20), bile fluid (n= 20), peritoneal fluid (n=25) and CSF (n=100).

Optimization of the extraction protocol for direct bacterial identification from liquid media

To evaluate the optimal extraction protocol, various different quality control strains (*Escherichia coli* ATCC 25922, *Haemophilus influenza*e ATCC 49247, *Pseudomonas aeruginosa* ATCC 27083, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619, *Listeria monocytogenes* ATCC 15313 and *Neisseria meningitidis*) were inoculated in parallel in both TG broth and BHI broth with the amount of bacteria filling a 1-µl inoculation loop and incubated for 16-24 hours at 37ºC. After growth was indicated by the turbidity of the media, an aliquot of 1.5 ml of each medium was transferred to an Eppendorf tube. The sample was centrifuged at 14.000 rpm for 2 min and the supernatant was discarded. Five hundred µl of water was added to the sample, and the agar and other debris were removed by pipetting before another 500 µl of water was added. The subsequent steps varied depending on the

protocol (Supplementary material). Each sample was extracted in triplicate and each extract was analysed in duplicate. The following protocol (nº 4) was finally established:

Protocol 4. The sample was sonicated at 200 W (Ultrasons, JP Selecta S. A. Barcelona) for 5 min before being centrifuged at 14.000 rpm for 2 min. The supernatant was discarded and the pellet was washed with 500 µl of water. The sample was vortexed again and centrifuged at 14.000 rpm for 2 min. Finally, the supernatant was discarded to yield the bacterial pellet for MALDI-TOF MS analysis.

Clinical validation

We performed a prospective clinical validation of the assay in 300 liquid cultures of clinical samples. The procedure was applied by researchers who were blinded to the type of samples. First, 139 liquid cultures incubated for 16-24 hours at 37ºC and with no visually detectable turbidity were processed using the extraction protocol selected. Secondly, 161 liquid cultures with visually observed turbidity were processed using the same protocol. All liquid cultures were subcultured, in parallel with the MALDI-TOF MS direct identification, in Trypticase Soy Agar (TSA, Becton Dickinson, EEUU), Chocolate Agar (Becton Dickinson) and Schaedler Agar (Becton Dickinson). TSA and Chocolate agar plates were incubated in 5-10 % CO_2 atmosphere and Schaedler Agar in an anaerobic atmosphere at 37ºC. Colonies grown in the subcultures were identified by MALDI-TOF MS. Cultures were considered negative after 6 days of incubation without growth of microorganisms.

MALDI-TOF MS processing and analysis

The pellet obtained at the end of the extraction procedure was spread with a pipette tip on the MALDI-TOF MS steel plate spots and allowed to dry. One µl of 70% formic acid (Sigma-Aldrich, United States) was added to the sample and allowed to air-dry. The spots were then covered with the MALDI matrix (10mg/mL α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile / 0.1% trifluoroacetic acid; Bruker Daltonik GmbH). Samples were analyzed in duplicates.

Spectra were acquired in a MALDI Microflex LT/SH bench-top mass spectrometer (Bruker Daltonik GmbH) equipped with a 60 Hz nitrogen laser. FlexControl v.3.0 software (Bruker Daltonik GmbH) was used to acquire the spectra and the MALDI Biotyper 3.1 (Bruker Daltonik GmbH) for real time interpretation and identification of the microorganisms. According to the manufacturer, a score > 2.0 indicates species identification, a score between 1.7 and 2.0 indicates genus identification and a score < 1.7 indicates unreliable identification.

Results

Use of a sonication step is key to optimal bacterial extraction

Protocol 1, based on the cell lysis using the lysis buffer from the Sepsityper Kit (Bruker Daltonik GmbH), provided 52.4% (44/84) valid identifications, and *N. meningitidis* and *H. influenzae* isolates were misidentified providing MALDI-TOF MS no peaks in the spectra acquisition (Table 1, supplementary material). The average score value was 1.947 [1.444- 2.397]. Protocol 2, based on cell lysis with lysozyme, yielded 69.0 % (58/84) valid identifications, and *H. influenzae* isolates were misidentified, providing MALDI-TOF MS an unreliable identification. The average score value was 1.905 [1.163- 2.373]. Protocol 3, based on the use of SDS detergent, yielded 63.1% (53/84) valid identifications, and *N. meningitidis* and *H. influenzae* isolates were misidentified. The average score value was 2.048 [1.714-2.383]. Protocol 4, based on a 5 min sonication step, yielded 100% (84/84) valid identifications with an average score value of 2.305 [1.950- 2.525], and no microorganisms were misidentified. The average score value for microorganisms cultured in the BHI broth was 0.084 higher than the score for the microorganisms cultured in the TG broth.

MALDI-TOF-MS direct identification from liquid cultures

In the first part of the study, 139 liquid cultures (61 TG and 78 BHI) with no visually observable turbidity were processed using the previously optimized sonication-based extraction protocol. MALDI-TOF MS did not detect any bacteria and no growth occurred on solid agar plates, so the specificity of the direct identification by MALDI-TOF MS is 100%, for all samples tested.

In the second part of the study, 161 liquid cultures with visually observable turbidity were processed using the previously optimized sonication-based extraction protocol. Of the 89 liquid cultures analyzed in TG broth, 84 were monomicrobial cultures (Table 1). The sensitivity of MALDI-TOF MS for detecting the pathogen in the monomicrobial cultures from the TG broth was 98 % (82/84), with a reliable identification to the species level in 74% (61/82) and an average score of 2.088. The undetected isolates were *Staphylococcus caprae*, isolated from prosthetic material, and *Streptococcus anginosus*, isolated from bile. MALDI-TOF MS successfully detected at least one microorganism in 100% (5/5) of the polymicrobial cultures from TG broth, with an average score of 1.982.

Of the 72 liquid cultures grown in BHI broth (Table 2), 68 were monomicrobial cultures. The sensitivity of MALDI-TOF MS for detecting the pathogen in the monomicrobial cultures grown in BHI broth was 97% (66/68), with a reliable identification to the species level in 77% (51/ 66) with an average score of 2.090. The 2 undetected isolates were *Candida albicans* and *Candida tropicalis.* MALDI-TOF MS enabled identification of at least one microorganism in 100% (4/4) of the polymicrobial cultures grown in BHI broth, with average score of 2.158.

For monomicrobial cultures carrying Gram-negative bacteria, MALDI-TOF MS yielded an average score of 2.172 with a reliable identification to the species level in 85% (33/39) for direct identification from TG broth (n=39) and 2.092 with a reliable identification to the species level in 72% (21/22) from BHI broth (n=22). For Gram-positive bacteria, MALDI-TOF MS yielded an average score of 2.017 with a reliable identification to the species level in 69% (29/42) for direct identification from TG broth (n=42) and 2.071 with a reliable identification to the species

level in 68% (30/44) from BHI broth (n=44). For identification of monomicrobial cultures carrying anaerobic bacteria, MALDI-TOF MS yielded an average score of 2.110 with a reliable identification to the species level in 25% (1/4) for direct identification from TG (n=4), identifying *Bacteroides fragilis* from CSF and peritoneal fluid samples and *Propionibacterium acnes* from two biopsy samples. For monomicrobial cultures carrying anaerobic bacteria, MALDI-TOF MS yielded an average score of 1.809, with no reliable identification to the species level in any of the samples in the direct identification from BHI (n=3), identifying *Clostridium perfringens* in a bile, *Propionibacterium acnes* in a joint prosthesis sample and *C. innocuum* in a surgical wound. For monomicrobial cultures carrying fungus, MALDI-TOF MS yielded an average score of 2.107 for direct identification from TG broth (n=3), identifying *Candida glabrata* in a derivation cardiac valve sample and in a catheter tip sample and *Cryptococcus neoformans* var. *grubii* in a CSF sample. MALDI-TOF MS did not reliably identify any BHI cultures carrying fungus, and subculture revealed *Candida albicans* in a bile and *Candida tropicalis* in a cardiac valve sample.

Unreliable identification by MALDI-TOF MS was not associated with any particular type of clinical sample. The MALDI-TOF MS method for direct identification from liquid cultures did not yield any false positive results. In addition, the overall positive predictive value was 100 % and the negative predictive value, 97 %.

In 24 % (39/ 160) of the samples analysed, growth by direct sowing was only successful in the liquid medium, and no growth was observed in the direct solid agar cultures. These samples comprised CSF (n=13), prosthetic material (n=4), biopsy (n=8), cardiac valve (n=5), catheter tip (n=2), peritoneal fluid (n=4), synovial fluid (n=1) and bile fluid (n=2). Agreement of 100% (39/ 39) was found between the results obtained by direct identification from the liquid medium by MALDI-TOF MS and the results obtained by identification of the corresponding subculture. The average MALDI-TOF MS score was 2.150.

Discussion

In the present study, we demonstrated that MALDI-TOF MS can also provide accurate, reliable and rapid identification of pathogens directly from liquid cultures after enrichment of clinical samples with the sonication-based extraction procedure. This method was able to correctly identify the main species causing meningitis and the most common bacterial species found in clinical microbiology. In the present study, 50 % (13/26) of the positive CSF samples only grew successfully in the liquid culture media, with MALDI-TOF MS providing 100% accurate identification within 16-24 hours of the sample arriving in the laboratory. The method is particularly accurate for pathogens that are scarce and difficult to detect, as was the case for *P. aeruginosa* isolated from a neurosurgery patient and others that were not detected by Gram staining, as was the case for the *Listeria monocytogenes* in an old man and even not suspected (e.g. *C. neoformans*_var_*grubii* isolated from a pulmonary transplant patient). Although the clinical impact is obvious for meningitis, other applications of this novel MALDI-TOF MS identification procedure may be of great value. We have observed that 55 % (5/9) pathogens isolated from cardiac valves were recovered exclusively in the liquid media, having a great impact in the diagnosis of endocarditis and in the management of prosthetic heart valves [15-16]. Furthermore, 33 % (4/12) of the pathogens isolated from prosthetic material were recovered exclusively in the liquid media. This is of great importance for prosthetic joint samples, in which differentiation between infection and aseptic loosening of the replacement joint is difficult to achieve clinically [17-18].

Regarding the extraction procedure from the liquid media, the sonication protocol yielded sensitivities close to 100 % without using as a final step the gold-standard ethanol/formic acid extraction procedure recommended by Bruker Daltonik GmbH. We recommend applying the hole procedure on a second time, only if the identification is not reliable in the first place. Regarding the culture media, we did not observe substantial differences between the TG and

BHI broth. The unidentified microorganisms isolated from the TG media were mainly *Streptococcus anginosus* group. (i.e. *S. anginosus* in a bile and *S. constellatus* and *S. anginosus* in two mixed cultures). The heterogeneity of members of the *Streptococcus anginosus* group has traditionally hampered their correct identification, and although MALDI-TOF MS has helped, identification to the subspecies level has not yet been clearly established [19]. The unidentified microorganisms from the BHI broth were yeasts (i.e. *C. tropicalis* and *C. albicans*), probably because these microorganisms grow less well in the media used [14]. Regarding polymicrobial cultures, at least one microorganism was correctly identified in 100 % of the samples. Use of the MALDI Biotyper MSP identification Mixture Method, relative to the Standard Method used in this study and recommended by the manufacturer for identifying mixed cultures, did not prove useful for the possible identification of mixed cultures in the liquid media (data not shown). Thus, this method must be used with caution in clinical settings. Further improvements in the software should be carried out to validate the possible use of this method in samples of polymicrobial predictable nature. Direct examination on the positive liquid culture could be performed prior extraction to confirm the presence of polymicrobial cultures and reject the direct MALDI-TOF MS identification.

Strengths of our study include the double-center, prospective and blinded sample adjudication of the study. Besides, once the identification is well established, this study opens a way to detect antimicrobial resistance directly from the liquid culture media as previously performed in positive blood cultures and in urine samples [6, 20], being one more step towards the early administration of adequate antimicrobial therapy.

Limitations of the study include the application of the technique exclusively in monomicrobial cultures, the slowness and less sensitivity compared with molecular methods [21-22] and the possibility to bring out contaminants that are further recovered in the liquid cultures, as negative-coagulase Staphylococci. We have informed all isolates recovered exclusively in the liquid media to clinicians responsible of the respective patients, although in case of negative-

coagulase Staphylococci we have warned to evaluate with caution the significance of the microorganism in the clinical setting.

The proposed MALDI-TOF MS method for direct identification from liquid media is able to provide an etiologic diagnosis of the infection only 15 min after observation of the turbidity of the medium, thus saving the 24 hours required for subculture in conventional analysis. Further studies should address the clinical impact of the proposed method by examining its capacity to adapt to different clinical situations and evaluating the yield for the different types of samples and liquid media.

268 **Table 1.** Direct identification of the 89 positive TG broth cultures by MALDI-TOF MS.

Clinical sample (n)[1]	ID solid culture (n)[2]	ID liquid culture (n)[3]	Reliable species identification by MALDI-TOF MS (y)[4]
Biopsies (22)	*E. coli* (2)	*E. coli* (2)	100% (2.109)
	E. cloacae (1)	*E. cloacae* (1) / (1)	100% (2.268)
	M. morganii (1)	*M. morganii* (1)	100% (2.267)
	S. aureus (10)	*S. aureus* (10) / (2)	70% (2.058)
	S. epidermidis (1)	*S. epidermidis* (1)	0% (1.799)
	S. hominis (1)	*S. hominis* (1) / (1)	100% (2.049)
	S. capitis (1)	*S. capitis* (1)	100% (2.070)
	S. oralis (1)	*S. oralis* (1) / (1)	0% (1.538)
	S. pyogenes (1)	*S. pyogenes* (1)	100% (2.303)
	E. faecalis(1)	*E. faecalis*(1) / (1)	100% (2.148)
	P. acnes (2)	*P. acnes* (2) / (1)	0% (1.835)
Surgical wound exudates (14)	*E. coli* (6)	*E. coli* (6)	83% (2.211)
	K. oxytoca (1)	*K. oxytoca* (1)	100% (2.188)
	M. morganii (1)	*M. morganii* (1)	100% (2.369)
	P. mirabilis (1)	*P. mirabilis* (1)	100% (2.111)
	P. aeruginosa (2)	*P. aeruginosa* (2)	50% (2.086)
	S. aureus (3)	*S. aureus* (3)	100% (2.143)
Prosthetic material (4)	*P. agglomerans* (1)	*P. agglomerans* (1) / (1)	0% (1.946)
	K. pneumoniae (1)	*K. pneumoniae* (1)	100% (2.305)
	S. caprae (1)	NRI[5]	0% (<1.6)
	S. agalactiae (1)	*S. agalactiae* (1)	100% (2.013)
Cardiac valves (5)	*K. pneumoniae* (1)	*K. pneumoniae* (1) / (1)	100% (2.305)
	S. hominis (2)	*S. hominis* (2)/ (2)	50% (2.049)
	E. faecium (1)	*E. faecium* (1) / (1)	0% (1.771)
	C. glabrata (1)	*C. glabrata* (1)	100% (2.203)
Catheter tips (5)	*S. aureus* (3)	*S. aureus* (3) / (1)	66% (1.990)
	S. epidermidis (1)	*S. epidermidis* (1) / (1)	100% (2.094)
	C. glabrata (1)	*C. glabrata* (1)	100% (2.159)
Pleural fluids (3)	*P. aeruginosa* (1)	*P. aeruginosa* (1)	0% (1.978)
	S. oralis + S. constallatus (1)	*S. oralis* (1)	100% (2.064)
	C. albicans + G. adiacens (1)	*C. albicans* (1)	0% (1.765)
Syonovial fluid (2)	*S. aureus* (2)	*S. aureus* (2) / (1)	100% (2.324)
Bile fluids (7)	*E. coli* (3)	*E. coli* (3)	100% (2.315)
	E. cloacae (1)	*E. cloacae* (1) / (1)	100% (2.210)
	K. oxytoca (1)	*K. oxytoca* (1)	100% (2.160)
	S. anginosus (1)	NP[6]	0% (<0)
	C. perfringens + S. anginosus (1)	*C. perfringens* (1)	0% (1.750)
Peritoneal fluids (12)	*E. coli* (6)	*E. coli* (6)	100% (2.184)
	B. fragilis (1)	*B. fragilis* (1) / (1)	100% (2.328)
	P. aeruginosa (1)	*P. aeruginosa* (1)	100% (2.001)

	S. epidermidis (2)	*S. epidermidis* (2) / (2)	100% (2.038)
	E. faecium + C. glabrata (1)	*C. glabrata* (1)	100% (2.074)
	K. pneumoniae + E. faecium (1)	*K. pneumoniae* (1)	100% (2.255)
CFS (14)	*E. coli* (2)	*E. coli* (2)	0% (1.759)
	K. pneumoniae (1)	*K. pneumoniae* (1)	100% (2.025)
	P. aeruginosa (2)	*P. aeruginosa* (2) / (1)	100% (2.296)
	N. meningitidis (1)	*N. meningitidis* (1) / (1)	100% (2.304)
	B. fragilis (1)	*B. fragilis* (1)	100% (2.300)
	E. faecium (1)	*E. faecium* (1)	100% (2.337)
	S. epidermidis (1)	*S. epidermidis* (1) / (1)	0% (1.797)
	S. hominis (1)	*S. hominis* (1) / (1)	100% (2.090)
	S. haemolyticus (1)	*S. haemolyticus* (1)	0% (1.870)
	S. capitis (1)	*S. capitis* (1) / (1)	100% (2.165)
	S. pettenkoferi (1)	*S. pettenkoferi* (1) /(1)	100% (2.110)
	L. monocytogenes (1)	*L. monocytogenes* (1) / (1)	100% (2.239)
	C. neoformans var *grubii* (1)	*C. neoformans* var *grubii* (1) / (1)	0% (1.763)

[1] Description of the clinical samples analysed after culture in TG, classified depending on their origin and the number of samples (n).

[2] Identification by MALDI-TOF MS after subculture in solid media with the number of isolates by species (n).

[3] Direct identification by MALDI-TOF MS after sonication-based extraction from the TG broth, with the number of isolates per species (n) and the number of isolates that were exclusively isolated in the TG broth (x) and not recovered in the solid culture by direct seeding.

[4] Percentage of direct reliable identifications (score value >2.0) obtained by MALDI-TOF MS and average scores (y) obtained with the Biotyper MSP identification Standard Method (Bruker Daltonik GmbH). Not reliable identifications to the species level obtained scores among [1.7-2.0], thus accurate to the genus level, excepting the cases further detailed in the table (NRI/NP).

[5] NRI: Not reliable identification (score < 1.6)

[6] NP: No peaks

284 **Table 2.** Direct identification of the 72 positive BHI broth cultures by MALDI-TOF MS.

Clinical sample (n)[1]	ID solid culture (n)[2]	ID liquid culture (n) / (x)[3]	Reliable species identification by MALDI-TOF MS (y)[4]
Biopsies (23)	*E. coli* (5)	*E. coli* (5)	100% (2.481)
	M. morganii (1)	*M. morganii* (1)	100% (2.123)
	E. cloacae (2)	*E. cloacae* (2)	100% (2.209)
	S. aureus (4)	*S. aureus* (4)	100% (2.234)
	S. pyogenes (2)	*S. pyogenes* (2)	0% (1.843)
	E. faecalis (1)	*E. faecalis* (1)	100% (2.330)
	E.faecalis (1)	*E.faecalis* (1)	100% (2.233)
	S.epidermidis (2)	*S.epidermidis* (2)	50% (2.014)
	S. capitis (1)	*S. capitis* (1) / (1)	100% (2.146)
	E. coli + S. agalactiae (1)	*E. coli* (1)	100% (2.071)
	E.coli + C.striatum (1)	*E. coli* (1)	100% (2.237)
Surgical wound exudates (14)	*P. aeruginosa* (1)	*P. aeruginosa* (1)	0% (1.710)
	S. marcescens (1)	*S. marcescens* (1)	100% (2.243)
	K. pneumoniae (3)	*K. pneumoniae* (3)	100% (2.316)
	C. innocuum (1)	*C. innocuum* (1)	0% (1.705)
	S. anginosus (2)	*S. anginosus* (2)	50% (2.094)
	S. aureus (3)	*S. aureus* (3)	100% (2.248)
	S. agalactiae (1)	*S. agalactiae* (1)	0% (1.946)
	S.epidermidis (2)	*S.epidermidis* (2)	100% (2.238)
Prosthetic material (7)	*S. aureus* (2)	*S. aureus* (2) / (2)	100% (2.285)
	S. pyogenes (2)	*S. pyogenes* (2)	0% (1.748)
	E. faecalis(1)	*E. faecalis*(1)	100% (2.491)
	P. acnes (1)	*P. acnes* (1) / (1)	0% (1.823)
	S. aureus + F.magna (1)	*S. aureus* (1)	100% (2.352)
Cardiac valves (4)	*S. mitis* (1)	*S. mitis* (1) / (1)	0% (1.981)
	S. aureus (1)	*S. aureus* (1)	100% (2.822)
	S. epidermidis (1)	*S. epidermidis* (1)	100% (2.305)
	C. tropicalis (1)	NRI[5]	0% (< 1.6)
Bile fluids (6)	*E. coli + E. faecium* (1)	*E. coli* (1)	100% (2.624)
	P. mirabilis (1)	*P. mirabilis* (1)	100% (2.370)
	S. odorifera (1)	*S. odorifera* (1)	100% (2.326)
	C. perfringes (1)	*C. perfringes* (1)	0% (1.898)
	S. constellatus (1)	*S. constellatus* (1) / (1)	100% (2.124)
	C. albicans (1)	NP[6]	0% (<0)
Peritoneal fluids (6)	*K. pneumoniae* (1)	*K. pneumoniae* (1)	100% (2.593)
	P. aeruginosa (1)	*P. aeruginosa* (1)	100% (2.472)
	E. faecalis (2)	*E. faecalis* (2) / (1)	100% (2.231)
	E.faecium (1)	*E.faecium* (1)	100% (2.222)
	E.coli + K.pneumoniae (1)	*E. coli* (1)	100% (2.071)
	E. coli (2)	*E. coli* (1)	100% (2.481)
	S. marcescens (2)	*S. marcescens* (2)	100% (2.254)

CFS (12)	*S. pneumoniae* (2)	*S. pneumoniae* (2) / (1)	100% (2.357)
	S. epidermidis (5)	*S. epidermidis* (5) / (4)	40% (2.001)
	S. simulans (1)	*S. simulans* (1)	100% (2.130)

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287 [1] Description of the clinical samples analysed after being cultured in BHI broth, classified
288 depending on their origin and the number of samples (n).

289 [2] Identification obtained by MALDI-TOF MS after subculture on solid media with the number of
290 isolates per species (n).

291 [3] Direct identification by MALDI-TOF MS after the sonication-based extraction from BHI broth,
292 showing the number of isolates per species (n) and the number of isolates that were
293 exclusively isolated in the BHI broth (x) and not recovered in the solid culture by direct
294 seeding.

295 [4] Percentage of direct reliable identifications (score value >2.0) obtained by MALDI-TOF MS
296 and average scores (y) obtained with the Biotyper MSP identification Standard Method (Bruker
297 Daltonik GmbH). Not reliable identifications to the species level obtained scores among [1.7-
298 2.0], thus accurate to the genus level, excepting the cases further detailed in the table
299 (NRI/NP).

300 [5] NRI: Not reliable identification (score < 1.6)

301 [6] NP: No peaks

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Transparency declarations

None to declare.

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