Title: Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study.
Authors: Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G
Journal: Clinical infectious diseases : an official publication of the Infectious Diseases Society of America
Year: 2013 Apr
Volume: 56
Issue: 8
Pages: 1101-7
DOI: 10.1093/cid/cis1204

Impact of Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF) on the clinical management of patients with Gram-negative bacteremia: a prospective observational study.

Olivier Clerc (1), Guy Prod'hom (2), Christelle Vogne (2), Alain Bizzini (2), Thierry Calandra (1), Gilbert Greub* (1, 2)

(1) Infectious Diseases Service, Lausanne University Hospital Center and University of Lausanne, Switzerland

(2) Institute of Microbiology, Lausanne University Hospital Center and University of Lausanne, Switzerland

Word count: text 2570. Abstract: 250. Summary: 31 words

Figures: 2; Tables: 4

Supplementary table: 1; References: 20

Running title: MALDI-TOF for Gram-negative bacteremia

Key words: MALDI-TOF MS, clinical management, Gram-negative bacteremia

Summary:

We present a prospective observational study of Gram-negative bacteremia. MALDI-TOF allowed an early adaptation of 35% of empirical antibiotic treatments and had a more frequent impact than Gram stain reporting.

Corresponding author:
Gilbert Greub, MD PhD
Institute of Microbiology,
Lausanne University Hospital Center and University of Lausanne,
CH-1011 Lausanne, Switzerland

E-mail: gilbert.greub@chuv.ch. Phone + 41 21 314 49 79. Fax: +41 21 314 40 60.

Alternate corresponding author:
Olivier Clerc, MD
Infectious Diseases Service,
Lausanne University Hospital Center and University of Lausanne,
CH-1011 Lausanne, Switzerland
E-mail: olivier.clerc@chuv.ch. Phone + 41 21 314 10 22. Fax: +41 21 314 10 08.

Abstract

Background:

Early identification of pathogens from blood cultures using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) may optimize the choice of empirical antibiotic therapy in the setting of bloodstream infections. We aimed to assess the impact of this new technology on the use of antibiotic treatment in patients with Gram-negative bacteremia.

Methods:

We conducted a prospective observational study from January to December 2010 to evaluate the sequential and separate impacts of Gram stain reporting and MALDI-TOF bacterial identification performed on blood culture pellets in patients with Gram-negative bacteremia. The primary outcome was the impact of MALDI-TOF on empirical antibiotic choice.

Results:

Among 202 episodes of Gram-negative bacteremia, Gram stain reporting had an impact in 42 cases (20.8%). MALDI-TOF identification led to a modification of empirical therapy in 71 of all 202 cases (35.1%), and in 16 of 27 cases (59.3%) of monomicrobial bacteremia caused by AmpC-producing *Enterobacteriaceae*. The most frequently observed impact was an early appropriate broadening of the antibiotic spectrum in 31 of 71 cases (43.7%). In total, 143 of 165 episodes (86.7%) of monomicrobial bacteremia were correctly identified at genus level by MALDI-TOF.

Conclusions:

In a low prevalence area for extended spectrum betalactamases (ESBL) and multi-resistant Gram-negative bacteria, MALDI-TOF performed on blood culture pellets had an impact on the clinical management of 35.1% of all Gram-negative bacteremia cases, demonstrating a greater impact than Gram stain reporting. Thus, MALDI-TOF could become a vital second step beside Gram stain in guiding the empirical treatment of patients with bloodstream infection.

Introduction

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF), formerly confined to research laboratories, has emerged as a recent revolution in clinical microbiology.[1] For bacterial identification, this technology compared favourably with the classical phenotypical methods in bacteriology in terms of time to result, accuracy, ease of use and cost-effectiveness.[2,3] Due to the speed of this technology, MALDI-TOF was very soon applied to identify microorganisms detected in blood cultures,[4-6] since an appropriate antibiotic therapy covering the etiological agent is of paramount importance in decreasing mortality of bacteremic patients with sepsis.[7,8] In the setting of bloodstream infections, MALDI-TOF applied to bacterial pellet obtained from positive blood culture is allowing a rapid identification of around 80% of pathogens. This identification is accurate in ≥ 99% of cases.[4-6] Identification of Gram-negative pathogens has been shown to be very reliable, although the success rate is lower for Gram-positive bacteria (mainly streptococci) and for encapsulated bacteria such as *Klebsiella pneumoniae* and *Haemophilus influenzae*.[4-6]

In clinical practice, the microbiology laboratory has an impact on the management of bloodstream infections at first by reporting the results of positive blood cultures. Munson *et al.* demonstrated that the Gram stain result reported by telephone to the clinician had an even greater influence on the antibiotic regimen than antimicrobial susceptibility testing.[9] A same-day transmission of MALDI-TOF-based identification of the pathogen isolated from blood cultures would obviously represent another opportunity to increase the appropriateness of empirical antibiotic therapy. Although this strategy is theoretically promising, very few data are available analysing the routine use of MALDI-TOF in this setting.[10, 11]

The aim of our study was thus to assess the impact of MALDI-TOF on the clinical management of Gram-negative bacteremia. We prospectively performed MALDI-TOF MS-based bacterial identification on blood culture pellets immediately after Gram stain reporting[5] and assessed the impact of this procedure on antimicrobial treatment.

Methods

Design and case definition:

This prospective observational study was conducted between January and December 2010 in the University Hospital of Lausanne, an 850-bed primary and tertiary care hospital in Western Switzerland. Patients with a first episode of Gram-negative bacteremia (including polymicrobial infections) for whom an infectious diseases (ID) consultation was performed were included (figure 1). Data on successive antibiotic therapies were prospectively assessed by the clinical microbiologist when reporting the results to the clinicians using a standardized case report form (CRF). The sequence of antibiotic prescription was checked using the ID consultation report and a final CRF was filled in to include standardized clinical and epidemiological data. The impact of the Gram stain (first step) and the MALDI-TOF reporting (second step) on the antimicrobial management were assessed by two ID specialists (O.C and G.G) as described below. The primary outcomes were the respective and separate impacts of each step on the empirical antibiotic therapy. The design of this study was in accordance with the ethical standards of our local ethics committee.

Routine procedures

Positive blood cultures were detected by the BACTEC 9240 automated blood culture system (Becton Dickinson, Sparks, MD). The microbiology laboratory ran daily from 8 AM to 17 PM. Gram stain was immediately performed on all positive blood cultures during this period, and early the next morning when the blood cultures became positive overnight. Direct MALDI-TOF has been routinely performed in our center since September 2009 on all positive blood cultures immediately after the Gram staining. According to local procedure, pellets from positive blood cultures were prepared as described by Prod'hom *et al.*[5] during the same day when bacterial growth was detected before 17 PM. Under these circumstances, Gram stain reporting was followed about one hour later by a second call to the clinician in charge of the patient in order to transmit the MALDI-TOF-based identification.

Mass spectra were acquired on a Microflex LT MALDI-TOF (Bruker Daltonics, Bremen, Germany). Spectral analysis and comparison with the database were performed using MALDI BioTyper 2.0 software. According to the criteria proposed by the manufacturer, an identification was considered reliable at the species level when the score value was $x \geq 2$ and at the genus level when the score was $1.7 \leq x < 2$. Blood culture results were transmitted by clinical microbiologists directly to the clinicians and during a daily meeting to ID consultants. ID consultations were requested by clinicians in charge of the patients.

Hospital setting

The incidence of Gram-negative bacteria producing extended spectrum betalactamases (ESBL) was low according to the hospital microbiology database (< 5% of all Gram-negative bacilli). Similarly, community-associated ESBL-producing *Escherichia coli* remained rare in 2010 (3.4%, source: www.anresis.ch). Carbapenemase-producing organisms were not documented during the study period. As a consequence, third generation cephalosporins were the empirical first choice for non-AmpC-producing *Enterobacteriaceae* except in previously known ESBL carriers and in patients with severe sepsis or neutropenia, where broad spectrum antibiotic treatments were considered adequate. Fourth generation cephalosporins or carbapenems were chosen when AmpC-producing *Enterobacteriaceae* were detected. Our local antibiotic policy was summarized in local consensus guidelines developed by ID specialists and based on local epidemiology. These guidelines did not support the use of quinolones as empirical therapy.

Study definitions

Polymicrobial bacteremia was defined as the isolation of > 1 microorganism during the same bacteremic episode, except when the second microorganism was a coagulase-negative staphylococci (generally considered as a contaminant). A *previous ESBL carriage* was recorded according to the hospital infection control database. *Immunosuppression* included HIV infection and specific medications (prednisone

equivalent ≥ 5 mg/d, cancer chemotherapy, TNFα inhibitors and other immunosuppressive drugs). *Penicillin allergy* was included as stated in the medical record regardless of stage/gravity of the presumed reaction. A bacteremia was considered *hospital-acquired* if blood cultures were taken ≥ 48h after hospitalization. *Neutropenia* was defined as an absolute neutrophil cell count < 0.5 x 10^9 neutrophils/L. *Severe sepsis* and *septic shock* were defined according to standard definitions.[12]

Streamlining was defined as the reduction of the antibiotic spectrum, either after Gram staining or after MALDI-TOF reporting, as for instance, the interruption of a specific anti-Gram-positive coverage of vancomycin when only Gram-negative pathogens were seen on the blood culture Gram stain. Similarly, *spectrum broadening* was defined after one of the two steps when the antibiotic coverage changed toward a broader spectrum. For instance, spectrum broadening occurred following MALDI-TOF reporting of *Enterobacter cloacae,* changing empirical therapy from ceftriaxone to cefepime. Gram stain could lead to the *introduction of an empirical antibiotic therapy*. MALDI-TOF reporting allowed the *introduction of a focused empirical antibiotic therapy* as for instance, the introduction of an initial treatment with cefepime in case of the early detection of *E. cloacae*.

Statistics

Categorical variables were compared using the chi-square or Fisher's exact tests when appropriate; continuous variables were compared using the Mann-Whitney test. Analyses were conducted using the GraphPad Prism software version 5.03 (GraphPad software, San Diego, California).

Results

General

There were 202 first episodes of Gram-negative bacteremia leading to an ID consultation during the study period (figure 1), among which 37 (18.3%) were polymicrobial and 120 (59.4%) were *hospital-acquired*. Main sources of infection were

the digestive tract in 69 cases (34.2%), the urinary tract in 65 cases (32.2%) and intravascular catheters in 26 cases (12.9%) (Table 1).

Among the 242 episodes of Gram-negative bacteremia that were excluded as they did not lead to an ID consultation (figure 1 and supplementary table), 100 (41.3%) were diagnosed in the emergency department. *Enterobacteriaceae* were the more frequently identified class, with 207 episodes (85.5% of all), among which were 146 cases of *E. coli* bacteremia. AmpC-producing bacteria were found in 23 cases (9.5% of all) and non-fermentative bacteria in 15 cases (6.2%).

Pathogens and identification scores (Table 2)

Among the 202 episodes of Gram-negative bacteremia, 37 (18.3%) were polymicrobial and 165 (81.7%) were monomicrobial. Altogether, 143 of 165 episodes of monomicrobial bacteremia (86.7%) were correctly identified with a score ≥ 1.7. Among the 37 episodes of polymicrobial bacteremia, at least one pathogen could be reliably identified at the genus level in 28 cases (75.7%). Nevertheless, a correct identification at the genus level of all pathogens in a given polymicrobial blood culture was only possible in 11/37 cases (29.7%).

Among the 165 episodes of monomicrobial bacteremia, *Enterobacteriaceae* were documented in 120 cases (59.4%). MALDI-TOF identification performed on the blood culture pellet confirmed a high reliability for this bacterial family since 117 of all *Enterobacteriaceae* (97.5%) were correctly identified with a score ≥ 1.7. All 27 AmpC-producing *Enterobacteriaceae* species (13.4%) were correctly identified at the genus level.

Non-fermentative bacteria were documented in 25/202 cases, representing 12.4% of all cases. *Pseudomonas* spp. were identified in 9 out of 22 cases (40.9%), whereas the other three non-fermentative bacteria were all correctly identified at species level.

Impact of Gram stain and MALDI-TOF reporting

The sequential impact of Gram stain and MALDI-TOF identification reporting on empirical antibiotic therapy are summarized in figure 2. Overall, Gram stain reporting had an impact in 42 cases (20.8% of all 202 Gram-negative bacteremia). Despite a positive impact of Gram stain, MALDI-TOF reporting further influenced the treatment in 8/42 cases (19%). When Gram stain had no impact, MALDI-TOF reporting led to a modification of empirical antibiotic therapy in 63/160 cases (39.4%). Altogether, MALDI-TOF had an impact on empirical therapy in 71 cases (35.1% of all 202 bacteremia cases). The most frequent impact of MALDI-TOF was an early appropriate broadening of the antimicrobial spectrum (table 3). MALDI-TOF had an impact on 16/27 episodes (59.3%) of monomicrobial bacteremia caused by AmpC-producing *Enterobacteriaceae*, whereas an impact was documented in only 39/93 cases (41.9%) of other *Enterobacteriaceae*. MALDI-TOF had an impact in only 8/37 episodes (21.6%) of polymicrobial bacteremias, in only 6/25 episodes (24%) of monomicrobial bacteremia with non-fermentative bacteria and had no impact in the four previously known ESBL carriers. Impact of Gram stain reporting in cases of polymicrobial bacteremias (12/37, 32.4%) was greater than that of MALDI-TOF MS.

Among the 131 cases where the MALDI-TOF did not lead to treatment modification, an impact would have been possible in 31 cases but no modification occurred. Factors associated with lack of modification of empirical antibiotic therapy are shown in table 4. Among these, intensive care unit (ICU) acquisition of bacteremia was associated with the absence of consideration of MALDI-TOF result, as were the male sex and younger age. Adaptation of antibiotic treatment was more likely for patients presenting with urosepsis ($P = 0.06$).

Discussion

In this prospective, single-arm observational study including 202 episodes of Gram-negative bloodstream infections, we observed that MALDI-TOF reporting had an impact on antibiotic therapy in 35.1% of bacteremia, whereas Gram stain had an impact in 20.8% of cases. In addition, we confirmed the excellent reliability of MALDI-TOF for bacterial identification on blood culture pellets outside of validation studies,[4-6] in the real-

life setting of routine microbiology practice. As previously reported, the reliability of this new technology was especially high for *Enterobacteriaceae.*[4-6] A maximal impact was observed when AmpC-producing *Enterobacteriaceae* were documented, given their particular pattern of antibiotic resistance. As already published,[4,13] we documented a lower reliability of MALDI-TOF in cases of polymicrobial bacteremia, a situation where the Gram stain result is more informative. Thus, this justifies always performing direct Gram staining examination of any positive blood cultures even in the MALDI-TOF era.

Reducing the time to result in clinical microbiology has been achieved using various approaches and aims (i) to impact on the clinical outcome of patients, (ii) to optimize the use of antibiotics and (iii) to reduce costs.[14-17] Gram stain reporting is part of routine management of bloodstream infections, as it has been shown to have the greatest impact on antimicrobial empirical therapy.[9] Improvement in Gram stain turnaround time has even been associated with a decrease in patient mortality.[18] Indeed, as early appropriate antibiotic therapy has been shown to reduce the mortality of bloodstream infections,[19] there is an obvious need for techniques which could increase initial antibiotic appropriateness. Although there has been numerous publications in the recent years on the additional value of bacterial identification with MALDI-TOF in blood cultures,[4-6,13,20] there are few data on the clinical impact of this new strategy to justify its implementation in routine practice. Vlek *et al.* compared two sequential periods in a prospective study: one standard period during which only Gram staining and results of susceptibility testing could influence the antibiotic therapy, and one intervention period in which MALDI-TOF was added to study its possible impact on empirical treatment. Among 253 episodes of bacteremia, 89 led to MALDI-TOF identification with an early correct identification in 56.2% of episodes. The application of MALDI-TOF increased the proportion of appropriate treatments within 24 hours of blood cultures positivity by 11.3%.[10] Hodiamont *et al*. recently presented unpublished data showing that the addition of MALDI-TOF identification to Gram stain reporting led to an early adaptation of antibiotic therapy in up to 29% of 73 cases of bloodstream infection.[11] Both studies came from the Netherlands where the incidence of antimicrobial resistance remains low.

Our data from a country with low but increasing ESBL prevalence are in line with these two studies.

Our study has limitations. Its single arm, observational design precluded us from evaluating the impact of MALDI-TOF on the clinical outcome of patients as, in our hospital, MALDI-TOF has been included in the routine management of blood cultures since we initially validated this approach[5]. We included only cases managed with the help of an ID consultation, as these were clinically the most relevant and as the resulting report allowed us to obtain precise information regarding the sequence of antibiotic therapy. The overrepresentation of non-AmpC-producing *Enterobacteriaceae* (mostly *E. coli*) in the 242 cases that were excluded and the predominance of ambulatory cases in that group, suggest that among these 242 cases, there were more common infections such as urosepsis, for which the impact of MALDI-TOF identification might be less important and might be managed without the need of an ID consultation. However, this study design corresponds to the routine clinical management of Gram-negative bacteremia in our hospital and probably in many others. In this sense, we think that our results are useful to estimate the true benefits of this new technology in a real-life setting. Our study was conducted in a low prevalence area for ESBL and for multi-resistant Gram-negative bacteria, which could possibly lead to an overestimation of the impact of MALDI-TOF MS. Finally, prescription of an empirical antibiotic therapy is a complex decision involving epidemiological considerations, severity of disease and experience or education of the clinician in charge, as suggested by the determinants of the effective impact of MALDI-TOF MS. The impact of MALDI-TOF may thus vary according to the epidemiological setting, the site where bacteremia was managed (particularly ICU) or the presumed site of infection. Indeed, clinicians might feel more comfortable with the early identification of bacteria using an unfamiliar technology if the urinary tract was the suspected source of bacteremia, as uropathogens might be more predictable than pathogens from a suspected catheter infection. Further studies including educational approaches might help to target these hypotheses.

The main strength of our study is that we document the clinical application of MALDI-TOF performed directly on blood culture pellets as well as its successful implementation in routine clinical microbiology. Indeed, we observed that this new technology had an impact in more than one third of all episodes of Gram-negative bacteremia. Although these findings should be confirmed by larger, randomized studies including clinical outcomes, our data suggest that rapid MALDI-TOF MS-based identification of bacteria grown from blood cultures could become a second critical step in the management of patients with positive blood cultures besides Gram stain reporting.

Figures legends:

Figure 1: Selection of patients (ID = infectious diseases)

Figure 2: Sequential impact of Gram stain and MALDI-TOF identification on empirical antibiotic therapy. Cases that benefited from MALDI-TOF are highlighted in dark grey.

Table 1: Characteristics of the 202 cases of Gram-negative bacteremia included in the study.

Characteristics	**N = 202**
Male sex	122 (60.3)
Age (mean, years)	62
Previous ESBL carriage	4 (2.0)
Immunosuppression	62 (30.7)
Agranulocytosis	9 (4.5)
Severe sepsis/septic shock	28 (13.9)
History of penicillin allergy	11 (5.4)
Hospital-acquired bacteremia	120 (59.4)
ICU acquisition	23 (11.4)
Polymicrobial bacteremia	37 (18.3)
Sources of bacteremia	
Digestive tract	69 (34.2)
Urinary tract	65 (32.2)
Catheter infection	26 (12.9)
Respiratory tract	10 (5.0)
Other source	25 (12.4)
Unknown	7 (3.5)

Data are presented as n (%), unless stated otherwise.
Abbreviation: ICU = intensive care unit.

Table 2: Types of pathogen and identification scores

Pathogens	**N = 202**	**Score > 2**	**1.7<= score < 2**	**Score < 1.7**
Enterobacteriaceae	**120 (59.4)**			
Escherichia coli	56 (27.7)	53	3	-
Klebsiella spp.	25 (12.4)	18	4	3
Enterobacter spp.	18 (8.9)	15	3	-
Citrobacter freundii	1 (0.5)	1	-	-
Citrobacter koseri	4 (2.0)	4	-	-
Serratia spp.	5 (2.5)	5	-	-
Proteus spp.	8 (4.0)	8	-	-
Morganella spp.	2 (1.0)	2	-	-
Hafnia alvei	1 (0.5)	-	1	-
Non-fermentative	**25 (12.4)**			
Pseudomonas spp	22 (10.9)	5	4	13
Acinetobacter spp	1 (0.5)	1	-	-
Stenotrophomonas spp	2 (1.0)	2	-	-
Other aerobic	**8 (4.0)**			
Salmonella spp	4 (2.0)	2	1	1
Haemophilus spp	3 (1.5)	1	-	2
Campylobacter spp	1 (0.5)	1	-	-
Anaerobes	**12 (5.9)**			
Bacteroides spp	7 (3.5)	3	3	1
Other	5 (2.5)	-	1	4
Polymicrobial (best identification)	**37 (18.3)**	26	2	9

Data are presented as n (%)

Table 3: Impact of sequential Gram stain and MALDI-TOF reporting

Impact of the sequential reporting	N = 202
Gram stain	**42 (20.8)**
Streamlining	16 (7.9)
Spectrum broadening	16 (7.9)
Introduction of empirical antibiotic therapy	10 (5.0)
MALDI-TOF MS	**71 (35.1)**
Streamlining	22 (10.9)
Spectrum broadening	31 (15.3)
Introduction of focused empirical antibiotic therapy	18 (8.9)

Data are presented as n (%)

Table 4: Determinants of the impact of MALDI-TOF on empirical therapy

Characteristics	**Impact N = 71**	**Possible impact N = 31**	**P value**
Male sex	45 (63.4)	6 (19.4)	< 0.0001
Age (mean, years)	65.8	61.2	< 0.0001
Previous ESBL carriage	0	0	n.a.
Immunosuppression	17 (23.9)	9 (29.0)	0.63
Agranulocytosis	0	1 (3.2)	0.30
Severe sepsis/septic shock	9 (12.7)	3 (9.7)	1.00
History of penicillin allergy	3 (4.2)	2 (6.5)	0.64
Hospital-acquired bacteremia	43 (60.6)	22 (71.0)	0.37
ICU acquisition	1 (1.4)	9 (29.0)	< 0.0001
Polymicrobial bacteremia	8 (11.3)	1 (3.2)	0.27
Source of bacteremia			
Urinary tract	26 (36.6)	5 (16.1)	0.06
Digestive tract	19 (26.8)	14 (45.2)	0.11
Catheter infection	12 (16.9)	3 (9.7)	0.54
Unknown	3 (4.2)	0	0.55

Data are n (%), unless stated otherwise.
Abbreviations: ICU = intensive care unit, n.a = not applicable

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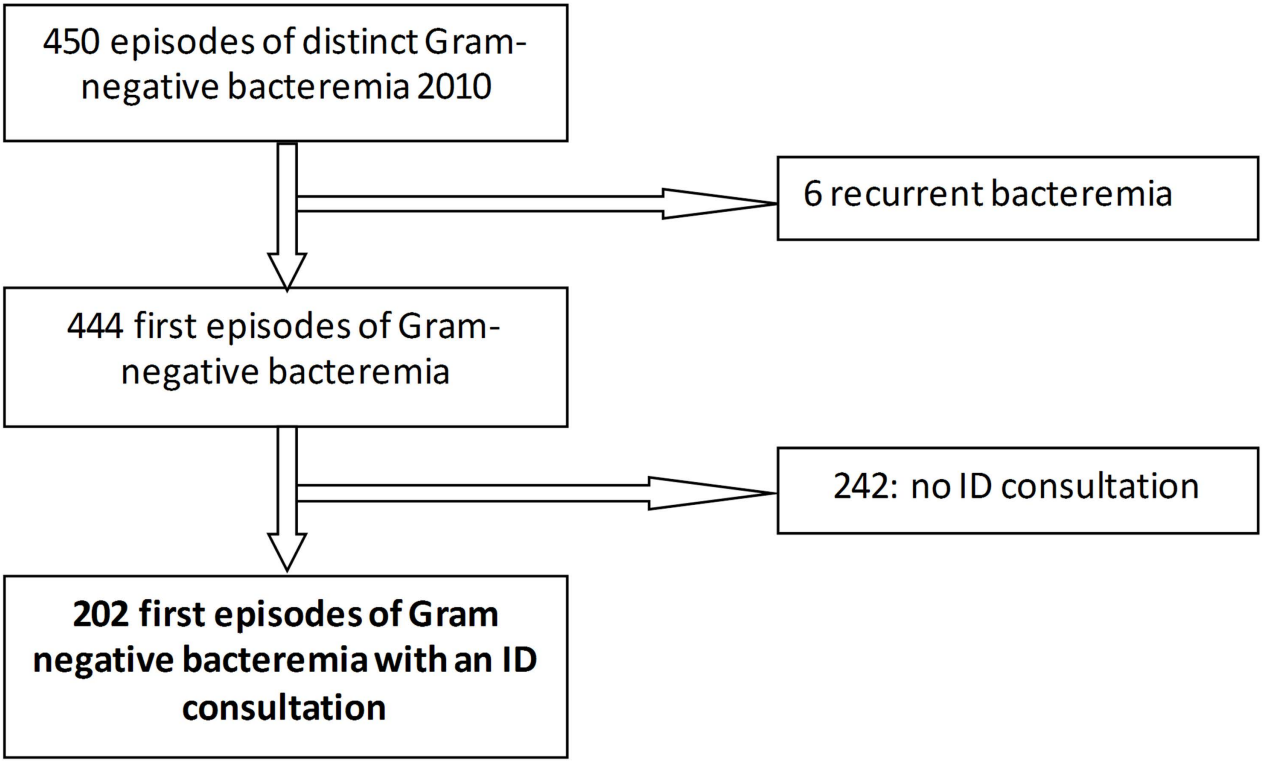
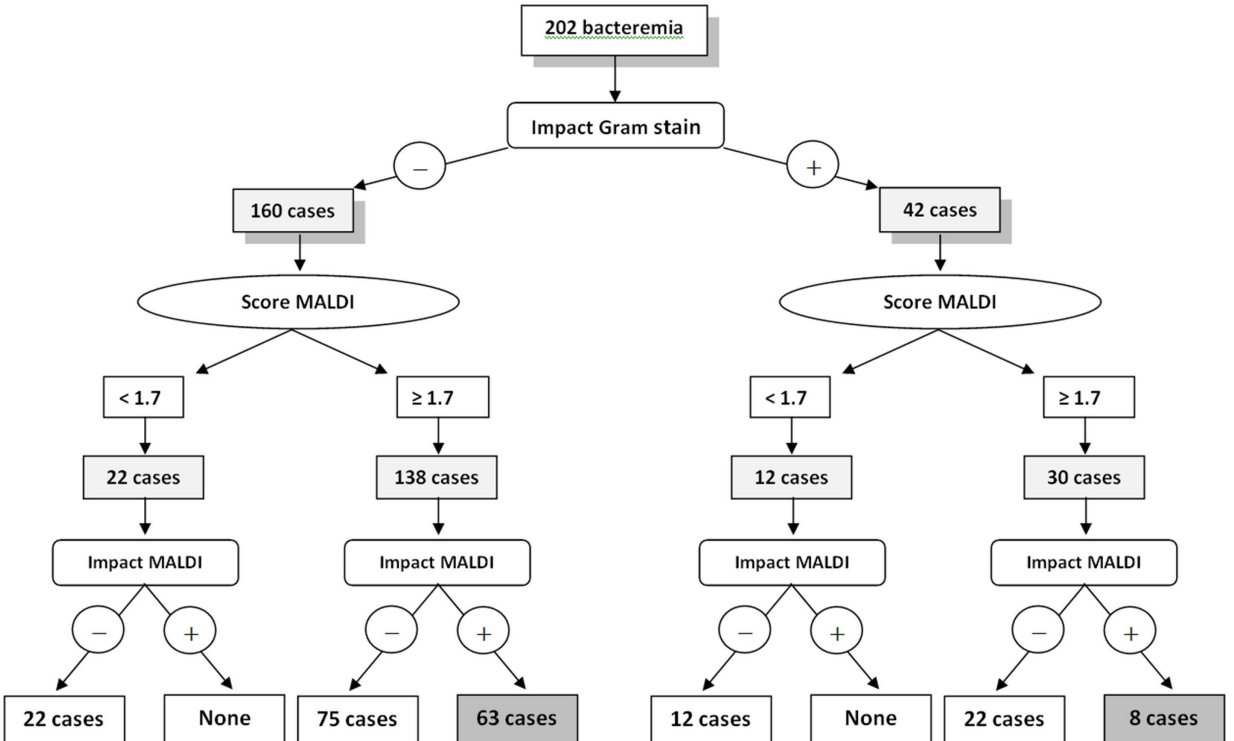
Acknowledgment

We thank Dr Katharine Darling for her critical review of the manuscript. **Source of funding**: none. **Conflits of interest**: none, for all authors.


450 episodes of distinct Gram-negative bacteremia 2010
6 recurrent bacteremia
444 first episodes of Gram-negative bacteremia
242: no ID consultation
202 first episodes of Gram negative bacteremia with an ID consultation


202 bacteremia
Impact Gram stain
−
+
160 cases
42 cases
Score MALDI
Score MALDI
< 1.7
≥ 1.7
< 1.7
≥ 1.7
22 cases
138 cases
12 cases
30 cases
Impact MALDI
Impact MALDI
Impact MALDI
Impact MALDI
22 cases
None
75 cases
63 cases
12 cases
None
22 cases
8 cases

Supplementary table: Characteristics of the 242 cases of Gram-negative bacteremia that were excluded.

Characteristics	**N = 242**
Male sex	126 (52.1)
Age (mean, years)	52*
Diagnosed in emergency ward	100 (41.3)*
Enterobacteriaceae	**207 (85.5)***
Escherichia coli	146 (60.3)*
Klebsiella spp.	28 (11.6)
Proteus spp.	7 (2.9)
AmpC-producers	23 (9.5)
Non-fermentative	**15 (6.2)***
Pseudomonas spp.	12 (5.0)
Other aerobic bacteria	**8 (3.3)**
Anaerobes	**11 (4.5)**
Polymicrobial bacteremia	**0 (0)***

Data are presented as n (%), unless stated otherwise.
* indicates $p < 0.05$ vs. included cases.