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# Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Detection of β-Lactam Resistance in *Enterobacteriaceae* Derived from Blood Cultures

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**The identification of pathogens directly from blood cultures by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) can be a valuable tool for improving the treatment of patients with sepsis and bacteremia. However, the increasing incidence of multidrug-resistant Gram-negative bacteria makes it difficult to predict resistance patterns based only on pathogen identification. Most therapy regimens for sepsis caused by Gram-negative rods consist of at least one β-lactam antibiotic. Thus, it would be of great benefit to have an early marker of resistance against these drugs. In the current study, we tested 100 consecutive blood cultures containing *Enterobacteriaceae* for resistance against 3rd-generation cephalosporins in a MALDI-TOF MS β-lactamase assay. *Escherichia coli* was also tested for resistance against aminopenicillins. The results of the β-lactamase assay were compared with those of conventional methods. The assay permitted discrimination between *E. coli* strains that were resistant or susceptible to aminopenicillins with a sensitivity and a specificity of 100%. The same was true for resistance to 3rd-generation cephalosporins in *Enterobacteriaceae* that constitutively produced class C β-lactamases. Discrimination was more difficult in species expressing class A β-lactamases, as these enzymes can generate false-positive results. Thus, the sensitivity and specificity for this group were 100% and 91.5%, respectively. The test permitted the prediction of resistance within 2.5 h after the blood culture was flagged as positive.**

Sepsis, severe sepsis, and septic shock are among the most common conditions resulting in admission to intensive care units, and mortality rates of up to 54% for septic shock have been described (1). To improve the outcomes of these patients, early initiation of appropriate antibiotic therapy is the single most important parameter (2–4). Empirical treatment must be administered at the time sepsis is recognized and should be adjusted if necessary based on the antibiogram upon its availability (5–8). β-Lactam antibiotics are among the most potent compounds for the treatment of sepsis, and the majority of therapy regimens consist either of a β-lactam antibiotic alone or a β-lactam antibiotic in combination with other antimicrobials. The application of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) technology for the identification of microorganisms in clinical diagnostics has significantly improved the time it takes to obtain results. This is especially true for the use of MALDI-TOF MS in identifying pathogens in blood cultures (BCs), a procedure that has been well evaluated (9–11). However, with the emergence of multiresistant Gram-negative bacteria, valid prediction of susceptibility based on the species alone is no longer possible (12, 13). Thus, methods that allow early prediction of antibiotic susceptibility are of great interest. Recently, it has been shown that MALDI-TOF MS can be used to detect resistance against β-lactam antibiotics (14–17). The mass spectrometric β-lactamase assay is based on a short coincubation of the Gram-negative bacterium with the antibiotic in question. If the bacterium produces a β-lactamase that is effective against the drug, hydrolysis of the β-lactam ring occurs. Hydrolyzed and nonhydrolyzed substances differ in their molecular weights, and these differences can be detected by MALDI-TOF MS.

In the present study, we evaluated this technique in patients with Gram-negative bacteremia. One hundred consecutive BCs containing *Enterobacteriaceae* were tested. Immediately after the results of the Gram staining were available, bacterial pellets were extracted from the blood culture flasks for identification and for detection of resistance against aminopenicillins and 3rd-generation cephalosporins.

## MATERIALS AND METHODS

**Sample preparation.** BD Bactec Plus Aerobic/F and Anaerobic flasks were used to process all BCs. BCs were incubated in a Bactec FX blood culture system (Becton, Dickinson, Heidelberg, Germany). Incubation started within 6 h after blood was taken from the patient. Gram staining was performed for all positive BCs. If Gram-negative rods were found, MALDI-TOF MS identification was performed using the MALDI Sepsityper kit (Bruker Daltonik, Bremen, Germany), according to the manufacturer's instructions. In parallel, the same kit was used to isolate an additional bacterial pellet for each β-lactamase assay. Briefly, 1 ml of BC fluid was mixed with 200 μl of lysis buffer. After vortexing, the sample was centrifuged at 14,000 rpm for 2 min, the supernatant was discarded, and the pellet was washed with 1 ml of deionized water. The water was thoroughly removed, and the bacteria (pellets contained $10^7$ to $10^9$ CFU) were resuspended in 10 μl of antibiotic solution (0.5 mg/ml cefotaxime [CTX]) (Serva Electrophoresis GmbH, Heidelberg, Germany) in water supple-

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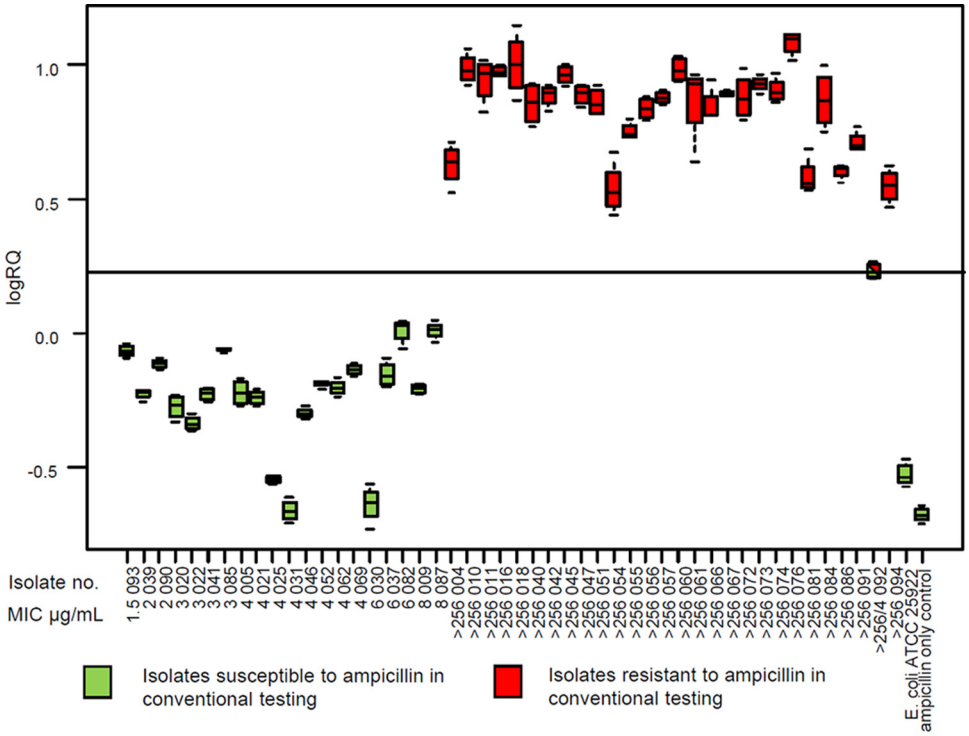


**FIG 1** Results of the ampicillin hydrolysis assay. The logRQ values for ampicillin are displayed in a box plot diagram. For comparison with conventional susceptibility testing, boxes that represent *E. coli* strains with MIC values of >8 μg/ml are colored in red. Boxes representing *E. coli* strains with MIC values of ≤8 μg/ml (susceptible breakpoint according to CLSI and EUCAST) are colored in green. Blood culture flask 92 contained one susceptible and one resistant *E. coli* strain.

mented with 0.01% sodium dodecyl sulfate (SDS) (Sigma-Aldrich, Taufkirchen, Germany) or 10 mg/ml ampicillin (Sigma-Aldrich) in water without SDS (14, 15). The suspensions were incubated in an Eppendorf Thermomixer comfort (Eppendorf, Hamburg, Germany) at 37°C with agitation (950 rpm) for 90 min. After centrifugation at 14,000 rpm for 2 min, the cell-free supernatants were analyzed by MALDI-TOF MS.

For the β-lactamase assays from subcultures, strains were grown on Columbia blood agar, MacConkey agar, and Mueller-Hinton agar (bioMérieux, Nürtingen, Germany) for 24 h. The bacterial colonies were dissolved in water, and the suspension was adjusted to an optical density at 600 nm ($OD_{600}$) of 0.75. A pellet derived from 1 ml of this suspension was resuspended in 10 μl of the antibiotic solution and processed as described above. For the negative controls and the comparison of the different types of media, BD Bactec Plus Aerobic blood culture flasks were inoculated with 8 ml of fresh human blood spiked with $10^4$ CFU of the respective bacterium. The flasks were incubated as described above. The reference strain, *Escherichia coli* ATCC 92112 (TEM and SHV negative), was used as a negative control and was included in every run (18). For quality control of the antibiotic solution and for detecting spontaneous hydrolysis, a control without bacteria (β-lactam only) was processed in the same manner as the samples.

**MALDI-TOF MS analysis.** The samples were measured in quadruplicate. One microliter of supernatant was applied to each spot. The dried spots were overlaid with 1 μl of matrix (10 mg/ml α-cyano-4-hydroxycinnamic acid [HCAA] in 50% acetonitrile-2.5% trifluoroacetic acid) (Bruker Daltonik, Bremen, Germany). The dried spots were measured using a microflex LT benchtop mass spectrometer (Bruker Daltonik). The spectra were acquired in the linear positive mode at a laser frequency of 60 Hz. A method optimized for the low mass range was set up using the following parameters: acquisition range, 100 to 1,000 Da with maximum laser frequency; acceleration voltage, 18.98 kV; IS2 voltage, 17.09 kV; and extraction delay time, 30 ns. For instrument calibration, an external standard consisting of bradykinin 1-5 ($M + H^+$, 573.31 Da) (Sigma-Aldrich, Germany), bradykinin 1-7 ($M + H^+$, 757.40 Da) (Sigma-Aldrich), and 2 HCCA peaks ($M + H^+$, 190.05 Da; and $2M + H^+$, 379.02 Da) was used.

**Data analysis.** For automated data analysis, an in-house program was written in R, a freely available software tool (19). Automated peak picking and calculation of the intensities of the hydrolyzed and nonhydrolyzed peaks were performed using the MALDIquant package with the parameters chosen as described before (14, 20). For calibration, the 2HCCA peak at 379.02 Da was used. Peaks were automatically picked with a tolerance of 0.5 Da as follows: ampicillin nonhydrolyzed, 350.4, 372.4, and 394.4 Da; ampicillin hydrolyzed, 368.4, 390.4, 412.4, and 324.4 Da; cefotaxime nonhydrolyzed, 456.5, 478.5, 500, and 396 Da; and cefotaxime hydrolyzed, 370 and 414 Da (14). Peak intensities were further processed using the formula logRQ = log(sum[hydrolyzed peak intensities])/(sum[nonhydrolyzed peak intensities]).

The results of the four measurements are displayed with a box plot diagram with the median indicated by a bold line, the 25th and 75th percentiles indicated by a box, and the minimum and maximum indicated by whiskers (for a sample calculation, see Fig. S1 in the supplemental material).

**Strain characterization.** For all isolates, a complete antibiogram was created using the BD Phoenix automated system (Becton, Dickinson, Heidelberg, Germany). For more detailed information on the MIC, Epsilometer tests (Etests) were used. In brief, Mueller-Hinton agar plates (bioMérieux, Nürtingen, Germany) were inoculated with 0.5 McFarland standard suspensions of the bacteria. Etest strips (Liofilchem, Roseto degli Abruzzi, Italy) were placed within 15 min after inoculation. Plates were incubated at 35°C and 5% $CO_2$ for 18 h. The MIC values were determined at the intersection of the elliptical growth margin with the Etest strip and interpreted according to CLSI regulations (21). For phenotypic extended-spectrum β-lactamase (ESBL) determination, double-disk synergy testing with cefotaxime, ceftazidime, and cefpodoxime with or without clavu-

lanate was performed. Genotypic characterization of isolates suspicious for AmpC production was performed using Check-MDR CT 101 and Check-MDR CT 102 microarray kits (Check-Points, Wageningen, Netherlands) according to the manufacturer's protocol. These tests detect the following genes and mutations: CTX-M ESBLs (CTX-M-1, -2, -3, -8, -25, -9, -15, and -32), TEM ESBLs (E104K, R164S, R164H, and G238S), SHV ESBLs (SHV G238S, SHV G238A, SHV G238S, and SHV E240K), AmpCs (CMY I/MOX, ACC, DHA, ACT/MIR, and CMY II), and carbapenemases (KPC, NDM, VIM, IMP, and OXA-48). For genotypic characterization in all *E. coli* strains, PCRs for the detection of SHV and TEM genes were performed using the following primers: blaSHV-F, ATCGGCCCTCACTCAAGG; blaSHV-R, TTAGCGTTGCCAGTGCTC; TEM-F, TCGGGGAAATGTGCCG; and TEM-R, TGCTTAATCAGTGAGGCACC. The isolates with an ESBL phenotype were also checked for CTX-M positivity by PCR using the following primers: CTX-M-F, TTTGCGATGTGCAGTACCAGTAA; and CTX-M-R, CGATATCGTTGGTGGTGCCATA. Amplification products were sequenced for further subgrouping.

## RESULTS

**Reproducibility of the β-lactamase assay performed on blood cultures.** To assess the reproducibility of the quantitative MALDI-TOF MS measurement itself, each setup was measured in quadruplicate, and the results are displayed in box plot diagrams. Comparisons of the box plots from blood cultures and subcultured bacteria showed similar spreads of the logRQ values in both systems (Fig. 1 to 4). For evaluation of the variability of the complete test procedure, when performed directly on blood culture fluid, we tested 10 BC flasks for cefotaxime resistance in four independent sets side by side. Measurements from the blood culture flasks showed slightly higher variations compared with measurements from subcultured bacteria, but the results were still robust (see Fig. S2 in the supplemental material).

**Ampicillin susceptibility of *E. coli* from blood cultures.** Susceptibility testing for ampicillin as a surrogate marker for aminopenicillins was performed on all blood culture flasks containing *E. coli*. The isolates other than *E. coli* were not tested for ampicillin resistance, as this antibiotic is not a therapeutic option for most Gram-negative rods. *E. coli* was the most common pathogen among those found in the blood cultures. In total, 48 *E. coli* isolates were identified, and the hydrolysis of ampicillin was detected by MALDI-TOF MS. Spectra were analyzed according to the algorithm described above and are displayed in a box plot diagram (Fig. 1). With the exception of one sample (BC 92), clear separation of the *E. coli* isolates into 2 groups could be observed. A comparison of these results with the MIC values obtained from the Etest showed that all susceptible isolates had a mean logRQ value of $<0$, whereas all resistant isolates had logRQ values of $>0.5$. A cutoff value for logRQ of 0.25 was set to distinguish between susceptible and nonsusceptible strains. The β-lactamase assay performed with BC flask 92 showed an ambiguous result. Subcultivation of this flask revealed that it contained two *E. coli* biotypes. The more prominent biotype had an MIC of 4 μg/ml, whereas the other had an MIC of $>256$ μg/ml.

**Cefotaxime resistance of *Enterobacteriaceae* from blood cultures.** Next, we explored the value of the β-lactamase assay for detecting resistance against 3rd-generation cephalosporins directly from blood cultures. One hundred consecutive BCs that were identified by MALDI-TOF MS to contain *Enterobacteriaceae* were tested for cefotaxime resistance with the β-lactamase assay (Table 1). The results of the test were compared with those of the conventional antibiogram, which was available the next day. To

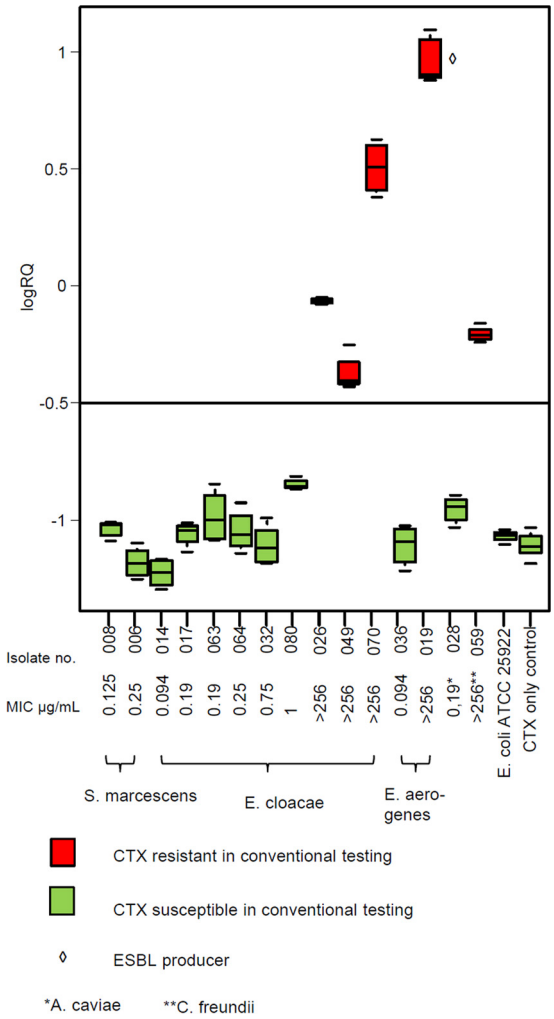


**FIG 2** β-Lactamase assay with cefotaxime. Results for isolates that constitutively produce AmpC enzymes. All isolates with logRQ values of less than −0.5 were susceptible to 3rd-generation cephalosporins. Isolates with stable hyperproduction of AmpC β-lactamase had a moderately elevated hydrolysis rate. One isolate with an additional plasmid-encoded CTX-M-type β-lactamase exhibited logRQ values comparable to those of the other ESBL producers.

achieve clarity, the different resistance mechanisms are discussed separately as follows. First, the results from BCs containing bacteria that constitutively express AmpC β-lactamase genes (e.g., *Enterobacter cloacae* and *Citrobacter freundii*) are described. Strains with phenotypic susceptibility to cefotaxime showed almost no hydrolysis of cefotaxime. The logRQ values were within the same range as those observed in the *E. coli* ATCC 92112 reference strain (β-lactamase negative) and in the β-lactam only control, in which only spontaneous hydrolysis occurs (Fig. 2). In contrast, in isolates resistant to cefotaxime due to overexpression of the chromosomally encoded AmpC enzyme, logRQ values were more than −0.5 after 90 min of incubation (Fig. 2). One isolate in this group of AmpC producers (isolate no. 19) also produced a CTX-M ESBL enzyme; this isolate exhibited the highest logRQ values (Table 2). Based on these observations, we set the cutoff value at a logRQ value of −0.5. All strains with logRQ values greater than this cutoff were defined as resistant to 3rd-generation cephalosporins. Isolates with logRQ values of less than −0.5 were

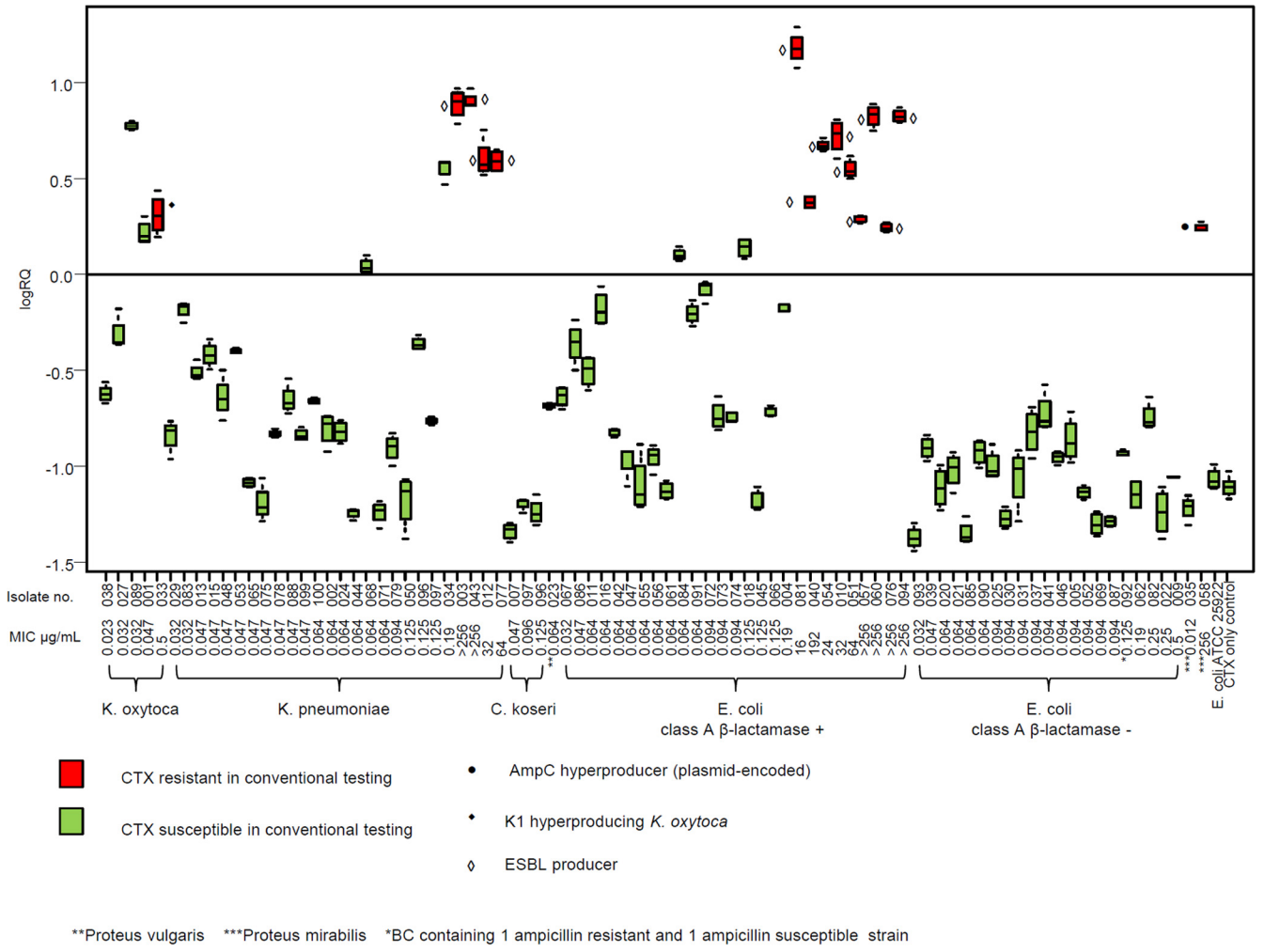


**FIG 3** Eighty-five blood culture flasks contained *Enterobacteriaceae* that typically express class A β-lactamase genes but do not have chromosomally encoded AmpC activity. The logRQ values for cefotaxime are displayed in a box plot diagram. In SHV- and TEM-negative isolates, the logRQ values were comparable to those for isolates with low AmpC expression. Cefotaxime-susceptible isolates with class A β-lactamase activity showed a wider dispersion of the logRQ values. All of the isolates that were phenotypically resistant to cefotaxime due to either ESBL or AmpC enzyme activity showed logRQ values of >0. An arbitrary logRQ cutoff value of 0 was set.

defined as susceptible to cefotaxime. By applying these criteria, complete accordance with phenotypic susceptibility testing could be achieved.

Second, blood cultures containing bacteria that typically exhibit either chromosomally- encoded or plasmid-encoded class A β-lactamases (primarily *Klebsiella pneumoniae* and *E. coli*) are described. Strains that were negative for SHV and TEM in the genotypic testing exhibited consistently low logRQ values that were comparable to those of isolates with low-level AmpC production (Fig. 3). The results were more heterogeneous in cefotaxime-susceptible but TEM- or SHV-positive isolates, as these strains showed a wider range in their logRQ values, which did not correlate with higher MIC values in the phenotypic testing (Fig. 3). Isolates that were phenotypically resistant to cefotaxime due to ESBL production or a plasmid-encoded AmpC enzyme exhibited logRQ values of >0. Therefore, for data interpretation, we set a cutoff value at a logRQ value of 0 for these species. Following this cutoff value for class A β-lactamase producers, the β-lactamase assay had a sensitivity of 100% and a specificity of 91.5% compared with those of conventional methods. Most discrepancies with the phenotypic testing occurred for *Klebsiella oxytoca* strains. Of the 5 isolates tested, 3 showed logRQ values greater than the cutoff, but only 1 isolate exhibited hyperproduction of the OXY enzyme (resistance to aztreonam and tazobactam in the conventional antibiogram), which could potentially be identified as a reason for this phenomenon. Overall sensitivity and specificity of the β-lactamase assay from all tested culture flasks were 100% and 92%, respectively, when cutoff values of logRQ of 0 and −0.5 were applied. Of note, testing of the patient-derived blood cultures was performed from the first blood culture flask that was flagged as positive. This led to testing of the aerobic flask in 72 patients and to testing of the anaerobic flask in 18 patients. We did not observe a negative impact on the test results when anaerobic flasks were used for the β-lactamase assays.

For comparison between the β-lactamase assay when performed on bacteria grown on solid media and on bacteria extracted from BC fluid, all isolates were subcultured on Columbia blood agar, and the β-lactamase assay was repeated (Fig. 4). Under

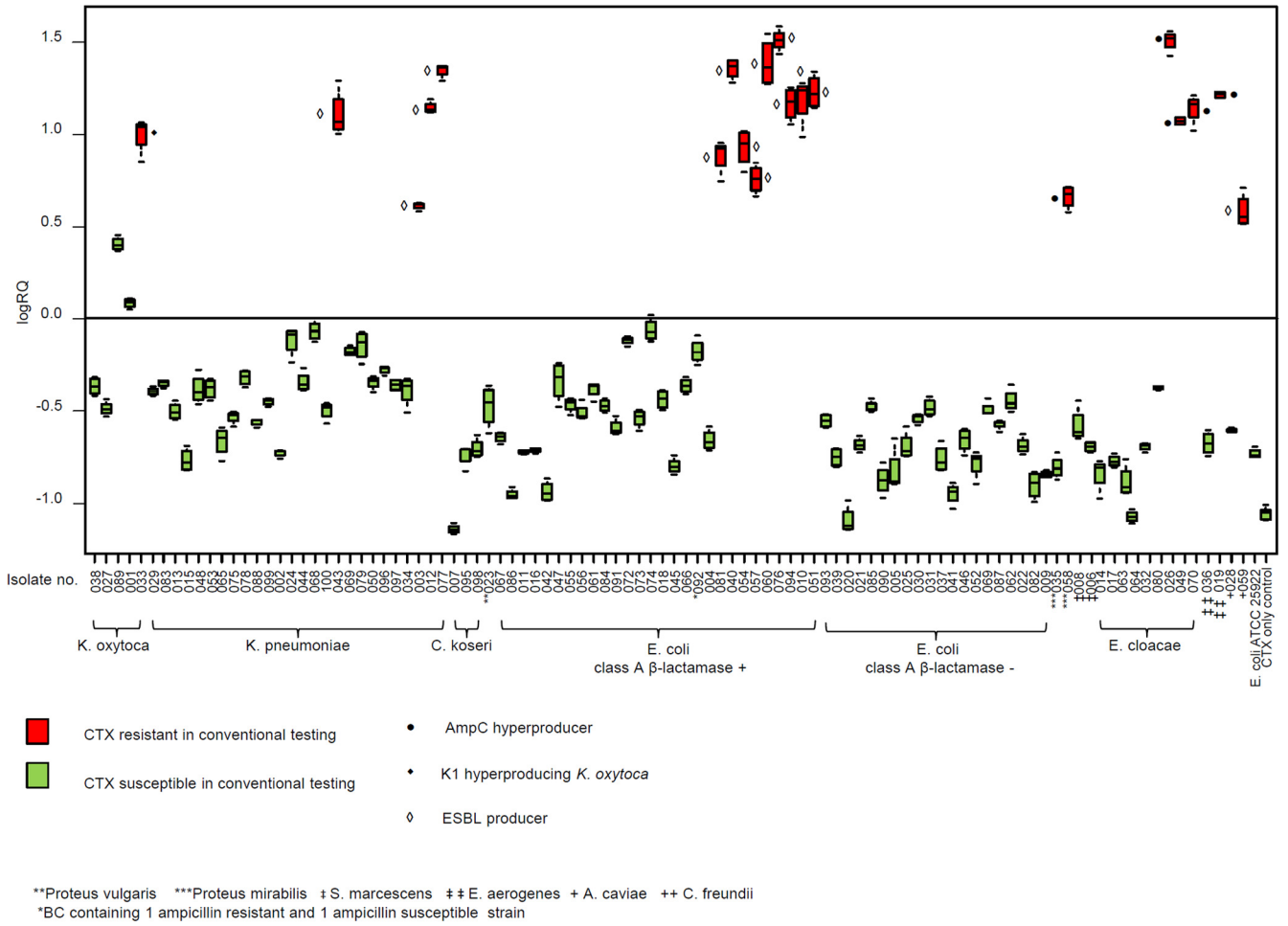


**FIG 4** Cefotaxime logRQ values from subcultures on Columbia blood agar. LogRQ values were elevated compared to those from blood culture fluid. No differences in the logRQ values between AmpC hyperproducers and ESBL positive strains were observed. The cutoff was set at a logRQ value of 0.

these conditions, two notable effects occurred. First, more hydrolyzed drug was detected in both the susceptible and resistant isolates. This effect was enhanced in the AmpC hyperproducers. As a consequence, a universal cutoff value at a logRQ of 0 for all resis-

**TABLE 1** Distribution of species and β-lactamase enzymes among 100 BC flasks tested

| Isolate | Total no. | No. of BCs containing: TEM wt[a] | SHV wt | CTX-M | AmpC | Increase in AmpC |
|---|---|---|---|---|---|---|
| *Aeromonas caviae* | 1 | | | | 1 | |
| *Citrobacter freundii* | 1 | | | | | 1 |
| *Citrobacter koseri* | 3 | | | | | |
| *Enterobacter aerogenes* | 2 | | | 1 | | |
| *Enterobacter cloacae* | 9 | | | | 6 | 3 |
| *Escherichia coli* | 48 | 27 | 1 | 9 | | |
| *Klebsiella oxytoca* | 5 | | | | | |
| *Klebsiella pneumoniae* | 26 | | 26 | 4 | | |
| *Proteus mirabilis* | 2 | | | | | 1 |
| *Proteus vulgaris* | 1 | | | | | |
| *Serratia marcescens* | 2 | | | | | |

[a] wt, wild type.

**TABLE 2** MICs and underlying resistance mechanism of isolates resistant to cefotaxime

| Patient no. | Species | MIC (μ/ml) | Mechanism(s) of resistance |
|---|---|---|---|
| 3 | *K. pneumoniae* | >256 | ESBL, CTX-M 15 |
| 10 | *E. coli* | 32 | ESBL, CTX-M 1 |
| 12 | *K. pneumoniae* | 32 | ESBL, CTX-M 1 |
| 19 | *E. aerogenes* | >256 | ESBL, CTX-M 15 |
| 26 | *E. cloacae* | >256 | AmpC, ACT/MIR |
| 33 | *K. oxytoca* | 0.5 | K1 hyperproducer |
| 43 | *K. pneumoniae* | >256 | ESBL, CTX-M 15 |
| 49 | *E. cloacae* | >256 | AmpC, ACT/MIR |
| 51 | *E. coli* | 64 | ESBL, CTX-M 1 |
| 52 | *E. coli* | 192 | ESBL, CTX-M 1 |
| 54 | *E. coli* | 24 | ESBL, CTX-M 15 |
| 57 | *E. coli* | >256 | ESBL, CTX-M 15 |
| 58 | *P. mirabilis* | >256 | AmpC, CMY II |
| 59 | *C. freundii* | >256 | AmpC, CMY II |
| 60 | *E. coli* | >256 | ESBL, CTX-M 15 |
| 70 | *E. cloacae* | >256 | AmpC, ACT/MIR |
| 76 | *E. coli* | >256 | ESBL, CTX-M 15 |
| 77 | *K. pneumoniae* | 64 | ESBL, CTX-M 15 |
| 81 | *E. coli* | 16 | ESBL, CTX-M 1 |
| 94 | *E. coli* | >256 | ESBL, CTX-M 15 |

tance mechanisms was defined. The second effect was that the logRQ values were more homogeneous, particularly among the cefotaxime-susceptible isolates with class A β-lactamase activity. False-positive results were obtained only in the 2 *K. oxytoca* isolates that also exhibited elevated logRQ values in the blood culture assay. Thus, sensitivities were 100% for both subcultures on Columbia blood agar and blood cultures. Specificities were 97% for subcultures versus 92% for blood cultures. Furthermore, to investigate the influence of growth conditions on our results, a subgroup of isolates was used to compare logRQ values obtained from Columbia blood agar with either Mueller-Hinton agar, MacConkey agar, or blood culture fluid supplemented with human blood. The lowest logRQ values were observed in bacteria grown on MacConkey agar or in blood culture flasks. The highest logRQ values were detected in bacteria grown on Columbia blood agar and Mueller-Hinton agar (see Fig. S3 and S4 in the supplemental material).

## DISCUSSION

In the present study, we evaluated the applicability of the mass spectrometric β-lactamase assay performed directly on blood culture flasks. A total of 100 *Enterobacteriaceae* cultures were analyzed following direct identification by MALDI-TOF MS, which is routinely performed on Gram-negative rods in our laboratory. Samples in which MALDI-TOF MS identified nonfermentative bacteria were excluded *a priori* from our study, as permeability- or efflux-based resistance mechanisms play an important role in the drug resistance of nonfermentative Gram-negative bacteria (22, 23). Consequently, testing for β-lactamase activity would not lead to valid therapeutic information. Gram-positive bacteria were not included in this study, as the main focus was to detect β-lactam resistance in patients with Gram-negative sepsis.

Testing for resistance against 3rd-generation cephalosporins was performed with cefotaxime as the indicator drug. Our preliminary studies with ceftazidime and cefpodoxime, which are both widely recommended for phenotypic ESBL screening, indicated that these drugs gave false-negative results with ceftazidime in ESBL producers and AmpC hyperproducers and cefpodoxime mainly in AmpC hyperproducers (21, 24, 25). The β-lactamase assay was able to distinguish between resistant and susceptible strains. However, the method did not permit good correlation between the detected β-lactamase activity and MIC values. One reason for this may be the inability of the β-lactamase assay to detect resistance mechanisms, such as alterations in outer membrane proteins or efflux pumps. Nevertheless, in our study of *Enterobacteriaceae*, these effects were not strong enough to generate false susceptibility results either with ampicillin or cefotaxime (26–28).

For all of the blood culture flasks, the β-lactamase assay was repeated on subcultures on Columbia blood agar. In general, resistant strains showed higher logRQ values when they were tested from subcultures. This effect was much more prominent in strains with derepressed AmpC enzymes, which, under these conditions, gave logRQ values within the same range as those for the ESBL strains. Hence, a species-independent cutoff could be applied here. The lower logRQ values obtained from the direct testing may be due, at least in part, to the extraction process, which is needed for isolation of bacteria from the blood. However, lower logRQ values were also observed in AmpC-producing bacteria when these bacteria were grown on MacConkey agar instead of Columbia blood agar. These findings suggest that the growth medium has a significant impact on the activity of the AmpC enzyme.

For the 100 BCs tested, no false-negative results were obtained. This is relevant, as the aim of the test is to provide rapid information that leads to an effective antibiotic treatment. For patient safety, the sensitivity of the test is more important than the specificity. Moreover, high sensitivity can also help in terms of antibiotic stewardship, as better sensitivity can permit a higher level of confidence in the administration of narrow-spectrum antibiotics in cases of susceptible results. Of the six false-positive results that were obtained, two were given by *K. oxytoca*. These two false-positive results were reproducible with the subcultured isolates, but they did not correlate with elevated Etest MICs for cefotaxime, tazobactam, or aztreonam. Whether these findings reflect reduced effectiveness of cefotaxime *in vivo* might be an area of further investigation. Of the four false-positive results that were not reproducible in subcultures, two patients had received piperacillin-tazobactam before the blood sample was taken. Overall, the rate of patients who had been treated with antibiotics prior to the acquisition of the blood samples was approximately 30% (for some patients, this information was not available). These data are not sufficient to make any assumptions about whether substances present in the patient's blood (e.g., empirical antibiotics) influence the outcome of the test.

With the protocol presently used, a prediction regarding the effectiveness of β-lactam therapy is available within 2 to 2.5 h after the blood culture is flagged positive. Elevated logRQ values indicate that ESBL or AmpC overproduction is likely, and therapy should be switched to a carbapenem or, if already given, should be continued. For laboratories performing MALDI-TOF MS identification from blood cultures, the additional hands-on time is moderate because the extraction processes from the BC flask are largely the same for identification and resistance testing and thus can be done in parallel. In this study, measurements were performed in quadruplicate to assess whether potential residues of the blood culture fluid interfere with the reproducibility of the quantitative measurement. Such interference was not observed. Thus, in routine clinical practice, we consider a measurement in triplicate to be sufficient. In terms of the measurement, it is possible to set up a method specific for the β-lactamase assay. This enables the user to switch between the optimized methods for identification and β-lactamase detection in one click.

In conclusion, our study is the first to evaluate the use of the β-lactamase assay on blood cultures derived directly from patients. The method was able to detect resistance against β-lactam antibiotics with a high sensitivity 24 to 48 h earlier than conventional methods. This can make the test a valuable tool for early therapeutic guidance in critically ill patients. However, prospective studies are needed to test a larger variety of ESBL- and AmpC-producing isolates and to validate our *post hoc*-defined cutoffs. Furthermore, in a patient collective with a higher prevalence of infections with carbapenemase-producing bacteria, it would be of interested to test also for carbapenem resistance.

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