The use of MALDI-TOF MS for the detection of β-lactamase activity in Gram-negative anaerobic bacteria

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Abstract

Because of the increasing prevalence of β-lactam resistant Gram-negative anaerobic strains and the lack of a rapid and reliable method for β-lactamase detection, there is an urgent need to develop alternative rapid and reliable detection methods. Recent studies have focused a general interest in using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a tool for the detection of β-lactamase activity. Moreover, this mass-spectrometric β-lactamase assay is already reported successful in protocols requiring only small amounts of bacteria. Literature search showed that the most relevant β-lactamases for a diagnostic purpose produced by anaerobic bacteria belong to the functional subgroups 2e cephalosporinases and, more rare but emergent, 3a carbapenemases. Activity of β-lactamases belonging to the subgroup 3a carbapenemases has already been detected using small amounts of bacteria. However, the specific 3a carbapenemase produced by the strictly anaerobic *Bacteroides fragilis* is only examined using a relatively high inoculum. Additionally, in contrast to the activity of 3a carbapenemases, activity of 2e cephalosporinases is neither examined using high inoculums nor using small amounts of bacteria. The results represented here show that the activity of the most common β-lactamases in Gram-negative anaerobes, the 2e cephalosporinases, has not been examined yet using the mass spectrometric β-lactamase assay. Therefore, future research should determine if it is possible to detect activity of 2e cephalosporinases using this upcoming tool. Additionally, it remains to be seen if Gram-negative anaerobic bacteria secrete sufficient β-lactamases for a rapid detection using amounts of bacteria suitable for diagnostic use, namely, most β-lactamases of Gram-negative bacteria are located intracellular. Otherwise, increasing cell wall permeability or performing lysis could probably provide the answer. Including a lysis-step in the protocol is presumably the most beneficial.

Keywords: MALDI-TOF MS, Beta-lactamases, Beta-lactam antibiotics, Gram-negative anaerobic bacteria.

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1 Introduction

Gram-negative anaerobes are a common cause of endogenous infections and the prevalence of antibiotic resistance in those species is rising. Especially resistance to β-lactam antibiotics in *Bacteroides* and *Prevotella* species constitute an emerging problem among clinical isolates (4, 20). Beta-lactam antibiotics include a large group of different antibiotics that block the biosynthesis of the peptidoglycan layer by interacting with peptidoglycan transpeptidases (24). They comprise different subtypes, including penicillin derivatives, cephalosporins, cephamycins, monobactams and carbapenems and are frequently administered because of their minimal side effects (35).

Antibiotic resistance developed by microorganisms is based on different mechanisms, including increased efflux or decreased influx of the antibiotic agent, altering of the target binding site of the antibiotic agent and enzymatic modification or degradation of the antibiotic agent (6, 35). These different mechanisms are all observed in β-lactam resistance (29). However, the most common resistance mechanism developed against β-lactam antibiotics is based on an enzymatic modification of the antibiotic agent due to the (over)expression of β-lactamase enzymes (4, 35). Beta-lactamases act by hydrolyzing the central β-lactam ring, shared among all β-lactam antibiotics (24). The resulting hydrolyzed product is thereby incapable to interact with the transpeptidases and will no longer block the biosynthesis of peptidoglycan (35).

Nowadays, susceptibility of bacteria to β-lactam antibiotics is analyzed on the phenotypic

level by determination of the minimum inhibitory concentration (MIC). In diagnostic laboratories, MICs for both aerobes and anaerobes are most commonly determined using the Epsilometer test (Etest), which yield results after 48 hours (4). In 1991, after its commercial emergence, it was seen as a rapid and reliable test for anaerobic bacteria (9). However, concerning the increased prevalence of resistant anaerobes and their less predictable susceptibility, results after 48 hours are not considered sufficient anymore (4, 20). Another phenotypic based test for the detection of β-lactam antibiotics resistance is the Cefinase disk test. This test detects specific β-lactamase production and reveals results within 30 minutes. However, no endpoint based on the interaction of the bacteria and the β-lactams has been defined, which make the results based on subjective observations (37). Therefore, recent studies have focused interest on the possibility of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) in the detection of β-lactamase activity (5, 16, 18, 19, 20, 21, 23, 35). Sparbier et al. (35) showed that even the amount of bacteria filling a 1-µl inoculation loop is sufficient for a rapid β-lactamase detection of various β-lactam antibiotics by various aerobic strains. The mass spectrometric β-lactamase (MSBL) assay reveals information by direct monitoring enzymatic activity of the secreted β-lactamases. MALDI-TOF MS is designed to determine small mass differences and can therefore easily distinguish between an intact β-lactam antibiotic and its hydrolyzed form. Hydrolysis of the β-lactam ring results in a molecular mass shift of +18 Da and is usually followed by a decarboxylation, resulting in a mass shift of -44 Da from the hydrolyzed product. Concerning these features, sensitivity and resistance patterns for different antibiotics can be determined, which allow a reliable detection of β-lactamase activity after 1-4 hours (25, 35). Furthermore, during the MSBL assay, resistance testing and identification can be done parallel (21). However, recent studies are particularly focused using this method for detection of the β-lactamase activity in aerobic bacteria. Until now, there is only one study that examined the MSBL assay on a strictly Gram-negative anaerobic bacterium. Johannson et al. (20) wanted to develop a protocol for detection of β-lactamase activity of the most commonly isolated anaerobic pathogen, *Bacteroides fragilis*, by determining hydrolysis of the β-lactam antibiotic ertapenem. This study yielded promising results, the strains that were expected to produce a β-lactamase showed all clear hydrolysis of ertapenem after 2,5 hours of incubation (20). However, in their protocol an inoculum of McFarland 4 was used, which is not ideal for the purpose of a rapid diagnostic identification of anaerobic isolates.

In this report, the β-lactamases specific to anaerobes are determined and compared with the β-lactamases of the aerobic bacteria used in previous MSBL assays. Furthermore, by means of this comparison, suggestions for future research were made. Hence, the aim of this report was to speculate in what extent the MSBL assay used for the detection of β-lactam resistant aerobic bacteria is suitable for anaerobic bacteria as well, with emphasis on the Gram-negative *Bacteroides* and *Prevotella* species.

2 Methods

Relevant information was obtained through keyword searches on PubMed and various internet search engines (e.g. via Google) to April 2014. The PubMed and internet keyword searches included the following terms or combinations of terms: 'beta-lactamases', 'MALDI-TOF MS', 'beta-lactam resistance' and 'anaerobic bacteria'. The electronic database of the University of Groningen provided access to many free full texts of articles. The cited references of the retrieved papers were reviewed to obtain information as well. In this way, 38 papers relevant to the subject were collected.

3 Results

3.1 The diversity of β-lactamases

Beta-lactamases are very efficient enzymes in protecting the organisms against β-lactam antibiotics. Hundreds of β-lactamase enzymes have been described, encoded either by chromosomal genes or by genes located on transferable elements such as plasmids and transposons (22). They can be produced in a constitutive or inducible manner and are very precisely located at optimal positions (33). Namely, β-lactamases produced by Gram-positive bacteria are usually extracellular, whereas those produced by Gram-negative bacteria are particularly located in the periplasmic space (31). The most commonly used classifications of β-lactamases is based on molecular similarities (Ambler classification) (1) or functional similarities (Bush-Jacoby-Medeiros classification) (8). The first distinction in both molecular and functional similarities can be made on the fact that β-lactamases belong to two different superfamilies (11). Because of this, the enzymatic activity of β-lactamases is based on either the utilization of one or two zinc ion(s) or the serine ester mechanism, represented by metallo-β-lactamases (MBLs) or serine-β-lactamases respectively (7). Besides the nature of active site, other properties included in the classification are: isoelectric point, molecular mass, relative activity towards different β-lactam antibiotics, interaction with inhibitors and inactivators, amino acid sequence and three dimensional structures (33). This resulted in the differentiation of four classes (A, B, C and D) according to their molecular structure and differentiation of three groups (1, 2 and 3) based on functional similarities. In 2009 the functional classification scheme was updated by Bush et al. (7), which led to the subgroups 1e, 2b, 2be, 2br, 2c, 2e 2f, 3a and 3b (Table 1).

Table 1. Classification of β-lactamase enzymes (7)

Bush-Jacoby group (2009)	Molecular class (subclass)	Distinctive substrate(s)	Inhibited by		Defining characteristic(s)
			CA or TZB[a]	EDTA	
1	C	Cephalosporins	No	No	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins
1e	C	Cephalosporins	No	No	Increased hydrolysis of ceftazidime and often other oxyimino-β-lactams
2a	A	Penicillins	Yes	No	Greater hydrolysis of benzylpenicillin than cephalosporins
2b	A	Penicillins, early cephalosporins	Yes	No	Similar hydrolysis of benzylpenicillin and cephalosporins
2be	A	Extended-spectrum cephalosporins, monobactams	Yes	No	Increased hydrolysis of oxyimino-β-lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam)
2br	A	Penicillins	No	No	Resistance to clavulanic acid, sulbactam, and tazobactam
2ber	A	Extended-spectrum cephalosporins, monobactams	No	No	Increased hydrolysis of oxyimino-β-lactams combined with resistance to clavulanic acid, sulbactam, and tazobactam
2c	A	Carbenicillin	Yes	No	Increased hydrolysis of carbenicillin
2ce	A	Carbenicillin, cefepime	Yes	No	Increased hydrolysis of carbenicillin, cefepime, and cefpirome
2d	D	Cloxacillin	Variable	No	Increased hydrolysis of cloxacillin or oxacillin

Bush-Jacoby group (2009)	Molecular class (subclass)	Distinctive substrate(s)	Inhibited by		Defining characteristic(s)
			CA or TZB[a]	EDTA	
2de	D	Extended-spectrum cephalosporins	Variable	No	Hydrolyzes cloxacillin or oxacillin and oxyimino-β-lactams
2df	D	Carbapenems	Variable	No	Hydrolyzes cloxacillin or oxacillin and carbapenems
2e	A	Extended-spectrum cephalosporins	Yes	No	Hydrolyzes cephalosporins. Inhibited by clavulanic acid but not aztreonam
2f	A	Carbapenems	Variable	No	Increased hydrolysis of carbapenems, oxyimino-β-lactams, cephamycins
3a	B (B1)	Carbapenems	No	Yes	Broad-spectrum hydrolysis including carbapenems but not monobactams
	B (B3)				
3b	B (B2)	Carbapenems	No	Yes	Preferential hydrolysis of carbapenems

a: CA, clavulanic acid; TZB, tazobactam,
β-lactams: β-lactam antibiotics

3.2 The β-lactamases of anaerobes

As mentioned above, among all Gram-negative anaerobic bacteria, the prevalence of resistant *Bacteroides* and *Prevotella* species is most concerning. The most common β-lactamases found among these species belong to the functional subgroup 2e cephalosporinases (15). Only one strain of *Prevotella intermedia* (formerly *Bacteroides intermedius*) is known to produce a group 1 cephalosporinase (3, 32). In *Prevotella* species the production of 2e chephalosporinases is associated with the expression of the cfxA and cfxA2 genes (13). Chephalosporin resistant *Bacteroides* species due to expression of the cfxA gene are found as well, but expression of the cepA gene, also referred to as the ccrA gene, is more prevalent (10). Furthermore, the *Bacteroides* species *Bacteroides uniformis* produces a species-specific cephalosporinase, encoded by the cblA gene (27, 34). The functional subgroup 2e cephalosporinases include the ability to hydrolyze penicillin derivatives and extended-spectrum cephalosporins, such as cefotaxime and ceftazidime (7) (Table 1). The sensitivity and resistance patterns of these two cephalosporins and two penicillins derivatives, ampicillin and piperacillin, determined by Sparbier et al., (35) are listed in table 2 and 3 respectively.

The biggest concerns are anaerobes that are able to produce the MBLs (15). These β-lactamases comprise activity towards the most powerful β-lactam antibiotics, the carbapenems, and are not inhibited by the classical β-lactamase inhibitors, such as clavulanic acid and tazobactam (26). In contrast, they are inhibited by ion chelators, such as EDTA (6) (Table 1). Furthermore, in clinical isolates, this unique group of β-lactamases is usually produced in combination with a second or third β-lactamase (7). However, until now, *B. fragilis* is the only MBL producing anaerobic bacteria (10). MBLs differ structurally and functionally with the other β-lactamases and are assigned to the molecular class B and the structural group 3. The MBL produced by *B. fragilis* belongs to the functional subgroup 3a carbapenemases. It is encoded by the chromosomal cfiA gene and could rapidly inactivate penicillin derivatives, cephalosporins and the carbapenems imipenem, meropenem and ertapenem (7). The sensitivity and resistance patterns of the carbapenems, determined by Sparbier et al., (35) are listed in table 2 and 3 respectively. As much as 5-7% of *B. fragilis* strains actually carry the cfiA gene (10). However, most of those strains produce insufficient carbapenemase levels in order to become resistant to carbapenems (15). Currently, the prevalence of carbapenem resistant *B. fragilis* strains is only

0,8% (10). However, the expression levels in most strains are dependent on promotor containing insertion sequence (IS) elements inserted upstream of the cfiA gene, whereby insertion of such IS element will cause an increased expression. Because of the fact that these IS elements are mobile genetic elements; they could play a critical role in the spread of *B. fragilis* strains that are able to produce high MBL levels (10, 15, 20).

Table 2. Sensitivity patterns for different antibiotics defined by calculated masses and corresponding molecular forms (35)

Antibiotic	Mol wt (g/mol)	Sensitivity pattern (Da)						
		$[M + H]^+$	$[M + Na]^+$	$[M + K]^+$	$[M + 2 Na]^+$	$[M + Na + K]^+$	$[M + 3 Na]^+$	$[M-X^a+ H]^+$
Ampicillin	349.4	350.4	372.4		394.4			
Piperacillin	517.5	518.5	540.5		562.5			
Cefotaxime	455.5	456.5	478.5					396.5
Ceftazidime	546.6	547.6						468.6
Ertapenem	475.5	476.5	498.5	514.5	520.5	536.5	542.5	
Imipenem	299.4	300.4						
Meropenem	383.4	384.5	406.5		428.5			

[a] X, acetyl for cefotaxime and pyridine for ceftazidime.

Table 3. Resistance patterns for different antibiotics defined by calculated masses and corresponding molecular forms (35)

Antibiotic	Mol wt (g/mol)	Resistance pattern (Da)								
		$[M_{hydr.} + H]^+$	$[M_{hydr.} + Na]^+$	$[M_{hydr.}+ 2 Na]^+$	$[M_{hydr.}+ Na + K]^+$	$[M_{hydr./decarb.}+ H]^+$	$[M_{hydr.}-X + H]^+$	$[M_{hydr./decarb.}-X + H]^+$	$[M_{hydr./decarb.}+ Na]^+$	[Mhydr./decarb. + K]+
Ampicillin	349.4	368.4	390.4	412.4		324.4				
Piperacillin	517.5	536.5	558.5	580.5		(492.5)				
Cefotaxime	455.5						414.5	370.5		
Ceftazidime	546.6						486.6	442.6		
Ertapenem	475.5	494.5	516.5	538.5	554.5	450.5			472.5	488.5
Imipenem[a]	299.4									
Meropenemen[a]	383.4									

a: No hydrolysis products could be detected. (35)

Other anaerobes capable of producing β-lactamases include *Clostridium*, *Fusobacterium* and *Porphyromonas* species and two specific anaerobic strains including an *Acidaminococcus fermentan* and a *Brachyspira pilosicoli* strain (3, 12, 15, 28). However, except from *Bacteroides* and *Prevotella* species, little research has been done on β-lactamase production of anaerobic species. Despite a prevalence of 30% of β-lactamases producing strains of *Porphyromonas* species, no β-lactamase of these species has been classified (3). More is known about β-lactamases produced by *Clostridium* species and *Fusobacterium* species. These β-lactamase enzymes are most commonly penicillinases (3, 15) falling within the functional group 2a. However, exceptions among strains have been reported. For example, the β-lactamases of a strain of *Clostridium clostridioforme* and a strain of *Fusobacterium* species were characterized belonging to the functional group 2d (2, 36). The β-lactamases produced by the *A. fermentan* strain and the *B. pilosicoli* strain were assigned to

the functional group 2be and 2d respectively (12, 28).

However, besides *Bacteroides* and *Prevotella* species, resistant clinical isolates of other anaerobic bacteria are rare. Therefore, the most relevant β-lactamases produced by anaerobes concerning diagnostic identification are the β-lactamases belonging to the functional subgroups 2e cephalosporinases and, less frequently, 3a carbapenemases.

3.3 Detection of β-lactamase activity using the MSBL assay

In September 2011, the first two studies on direct β-lactamase detection by MALDI-TOF MS were reported (5, 17, 19). Nowadays, several studies have followed (5, 16, 18, 19, 20, 21, 23, 35). However, most of the used protocols are unsuitable for diagnostic identifications, because relatively high inoculums were used throughout their experiments. Only two studies reported a MALDI-TOF MS based assay with amounts of bacteria suitable for diagnostic use, including the studies of Sparbier et al. (35) and Burckhardt et al. (5). Additionally, the study of Sparbier et al. (35) detected besides carbapenemase activity, penicillinase and cephalosporinase activity as well, which is currently done by only two studies (16, 35).

Sparbier et al (35) showed that the amount of bacteria filling a 1-µl inoculation loop was sufficient for the detection of various β-lactamases by using the protocol described by a manufacturer of MALDI-TOF MS systems (Bruker Daltonics, GmbH, Bremen, Germany) (35). They used five different β-lactamase producing strains of *Escherichia coli* and two different carbapenemase producing *Klebsiella pneumoniae* strains. The Bruker protocol comprises seven different antibiotics, including two penicillin derivatives, ampicillin and piperacillin, two cephalosporins, cefotaxime and ceftazidime, and three carbapenems, ertapenem, imipenem and meropenem. Of these β-lactam antibiotics, the *E. coli* strains provided resistance patterns of the penicillins derivatives and the cephalosporins (Table 2 and 3). Genotyping of these strains revealed that the strains contained the genes ampC, TEM-1 and CTX-M, which encode for β-lactamases belonging to the functional groups 1, 2b and 2be respectively (7, 35). Three of the five strains produced both 2b and 2be β-lactamases, one strain produced both 1 and 2b β-lactamases and one strain produced only a group 1 β-lactamase. The other study that reported detection of cephalosporinase activity used an *E. coli* strain expressing the gene ampC as well (16).

Group 1 and group 2 cephalosporinases are assigned to different molecular classes. Subgroup 1 enzymes are cephalosporinases belonging to the molecular class C, while group 2e and 2be cephalosporinases belong to the molecular class A. In contrast to subgroup 2e enzymes, group 1 enzymes are usually resistant to inhibition of clavulanic acid and tazobactam. Furthermore, group 1 enzymes can be differentiated from subgroup 2e enzymes by their high affinity for the monobactam aztreonam, in contrast to the poor affinity towards aztreonam by subgroup 2e enzymes (7). The differences between the subgroups 2e, 2b and 2be are found in their rates of hydrolyses and their substrates. The β-lactamases assigned to subgroup 2b can, besides penicillins, only hydrolyse the early cephalosporins, while 2e and 2be cephalosporinases hydrolyze all cephalosporins (Table 1). However, the E. coli strains that contained the TEM-1 gene, encoding for a 2b β-lactamase, were containing group 1 or subgroup 2be β-lactamase genes as well. Compared to 2e cephalosporinases, the subgroup 2be β-lactamases contains higher hydrolytic activities towards certain cephalosporinases, including cefotaxime and ceftazidime (Table 1).

For the detection of carbapenemase activity using the Bruker protocol, the carbapenemases ertapenem, imipenen and meropenem were used. However, the MSBL assay only revealed a clear sensitivity pattern and resistance pattern of ertapenem (Table 2 and 3). The

K. pneumoniae strains used for the detection of carbapenemase activity contained the plasmid-encoded genes kpc2 and kpc3 (35). KPC enzymes belong to the functional subgroup 2f β-lactamases. This subgroup consists of serine carbapenemases from molecular class A (7) (Table 1). Burckhardt et al. (5) revealed clear resistance patterns of ertapenem as well. Their method worked for strains carrying the KPC-2 enzymes and worked for strains carrying the subgroup 3a β-lactamases NDM-1, VIM-1, VIM-2 and IMPs (5, 7).

The greatest difference between 2f and 3a carbapenemases is the fact that 3a carbapenemases require two bound zinc ions for maximal enzymatic activity, while serine carbapenamases do not need to utilize zinc ions. Furthermore, in contrast to serine carbapenemases, 3a carbapenemases are unable to hydrolyze monobactams and, as mentioned above, are not inhibited by clavulanic acid or tazobactam.

In conclusion, while 3a carbapenemases have already been detected successfully in MSBL protocols using small amounts of bacteria, the hydrolase-activity of 2e cephalosporinases has not yet been examined using the MSBL assay, neither with the use of a high inoculum, nor with an amount of bacteria suitable for diagnostic use.

4 Discussion

The MALDI-TOF MS-based detection of β-lactamase production with amounts of bacteria suitable for diagnostic use has been successful for several aerobic species producing various β-lactamases, including the subgroups 1, 2b, 2be, 2f and 3a β-lactamases, according to the updated functional β-lactamase classification of Bush et al. (7). The most relevant β-lactamases of anaerobes in clinical microbiology belong to the functional subgroup 2e cephalosporinases. However, β-lactamases belonging to this subgroup have not yet been tested.

The subgroups whose activity has been demonstrated which are most similar to 2e cephalosporinases are the subgroups 1 and 2be. These subgroups consist of cephalosporinases as well and the hydrolytic activities of subgroups 1 and 2e are quite similar. However, there are some clear differences between these subgroups. With respect to 2e and 2be cephalosporinases, subgroup 1 cephalosporinases belong to a different molecular class and contain therefore some different characteristics. Furthermore, 2be cephalosporinases can be distinguished from 1 and 2e cephalosporinases due to their higher hydrolytic activity against certain cephalosporinases. For this reason, for detection of activity against those cephalosporinases, the incubation time of 1 or 2e cephalosporinases-producing strains with the antibiotic solution should probably be longer than the incubation time for 2be cephalosporinases-producing strains. However, in the Bruker protocol no distinction is made between the different subgroups of cephalosporinases. In contrast, the Bruker protocol does distinguish between different carbapenemase genes. While for bacteria containing kpc genes, encoding for 2f carbapenemases, the given incubation time with ertapenem is set on 1 hour, the incubation time for the detection of OXA genes, encoding for 2de and 2df carbapenemases, and for MBL genes, encoding for 3a and 3b carbapenamases, is set on at least 4 hours.

Noteworthy, although activities of the unique 3a carbapenemases have been demonstrated using small amounts of bacteria, the study on carbapenemase production by the anaerobic *B. fragilis* did use a relatively high inoculum. This approach was probably used because they wanted to make sure that there was sufficient release of β-lactamases in the antibiotic solution for a rapid detection. Namely, the MSBL assay is only depending on the extracellular β-lactamases, while in Gram-negative bacteria, most of the β-lactamases are located in the periplasmic space. In addition, it is unclear which percentage of the total of β-lactamase contains

extracellular activity and this fraction differs greatly among Gram-negative strains (38). Of importance, the extracellular fraction of β-lactamase could be increased by permeabilize the cells by Osmotic Shock (30) or by adding SDS at a concentration of 0,1% to the incubation set up (18). However, the level of resistance of strains is determined by the level of their expression and not by the level of secretion. Therefore, in order to achieve a good determination of the specific level of resistance, either the extracellular fraction of β-lactamase should be determined or, more easily, the intracellular β-lactamases should be freed. Intracellular enzymes can be released by causing lysis of the bacterial cells. Lysis can be achieved enzymatically by lytic enzymes or mechanically by several methods including sonication, homogenization, bead beating and freezing and grinding. Additionally, if a lysis-step gets included in the protocol, the incubation times will decrease, which is, in turn, beneficial for a rapid antibiotic therapy. Hooff et al. (16) have already established a protocol for detection of β-lactamase activity based on cell lysis. They achieved lysates by using the lytic enzyme lysozyme suspended in Tris-HCl lytic buffer in combination with an EDTA solution. This lysis-step will take approximately 75 minutes prior to the current protocol and validation studies have to show whether the resulting decrease in the incubation times will sufficiently compensate these 75 minutes in order to retain quick results. However, because of the fact that MBLs are inhibited by EDTA, this lysis protocol will be unsuitable for the detection of 3a and 3b carbapenemases.

In conclusion, recent studies have shown the ability of MALDI-TOF MS for a rapid and reliable detection of various β-lactamases of aerobic bacteria in protocols using amounts of bacteria suitable for diagnostic use. Since the increased prevalence of resistant anaerobes and their less predictable susceptibility, a MALDI-TOF MS based resistance assay suitable for anaerobic bacteria is desirable as well. However, activity of the most common β-lactamases in anaerobes, the 2e cephalosporinases, has not been examined yet using the MSBL assay. Therefore, future studies should explore the possibility of detecting 2e cephalosporinases using this upcoming tool. Additionally, it remains to be seen if Gram-negative anaerobic bacteria secrete sufficient β-lactamases for a rapid detection using small amounts of bacteria. Otherwise, performing an Osmotic Shock, adding SDS to the incubation set up or performing lysis could probably provide the answer. Moreover, performing lysis prior to the MSBL assay for all Gram-negative bacteria could provide more insight in the specific level of their resistances and will possibly provide an even faster detection. Studies on the above points would contribute to the development and improvement of MALDI-TOF MS as a routine diagnostic tool in β-lactamase detection.

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