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REVIEW

Progress on the development of rapid methods for antimicrobial susceptibility testing

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Synopsis

Antimicrobial susceptibility testing is essential for guiding the treatment of many types of bacterial infections, especially in the current context of rising rates of antibiotic resistance. The most commonly employed methods rely on the detection of phenotypic resistance by measuring bacterial growth in the presence of the antibiotic being tested. Although these methods are highly sensitive for the detection of resistance, they require that the bacterial pathogen is isolated from the clinical sample before testing and must employ incubation times that are sufficient for differentiating resistant from susceptible isolates. Knowledge regarding the molecular determinants of antibiotic resistance has facilitated the development of novel approaches with potential for rapidly detecting resistance in bacterial pathogens. PCR-based techniques, mass spectrometry, microarrays, microfluidics, cell lysis-based approaches and whole genome sequencing have all demonstrated the ability to detect resistance in various bacterial species. However, it remains to be determined if these methods can achieve sufficient sensitivity and specificity compared to standard phenotypic resistance testing that would justify their use in routine clinical practice. In the present review, we discuss recent progress in the development of methods for rapid antimicrobial susceptibility testing, and highlight the limitations of each approach that still remain be addressed.

Introduction

The administration of appropriate antibiotic therapy for many types of bacterial infections requires that a microbiologic diagnosis, consisting of identification of the causative agent of infection and its resistance profile, is obtained. In routine clinical practice, this process can require between 24 and 72 hours, during which empiric therapy is selected based on the suspected causative organism and local epidemiology. The use of methods that are able to rapidly detect antibiotic resistance in bacterial isolates therefore has the potential to reduce the duration of empiric therapy and facilitate early initiation of targeted treatment with proven antibiotic activity against the causative agent of infection. This is of importance in light of studies that have demonstrated that the rapid availability of antimicrobial susceptibility testing results can improve patient outcomes,[1-3] and that a delay in the initiation of appropriate antibiotic therapy is associated with higher patient mortality in certain bacterial infections.[4, 5] In addition, rapid antimicrobial resistance testing may contribute to reducing healthcare costs given studies showing that the early availability of antibiotic susceptibility information can result in the ordering of fewer laboratory tests, a decrease in the number of invasive procedures performed, and reduced hospital stay.[1, 2] An additional foreseeable benefit of the rapid availability of antibiotic susceptibility testing results is its potential to contribute to antimicrobial stewardship efforts, which include the administration of appropriate antibiotic therapy once susceptibility testing results are available.[6] In this context, the early initiation of adequate therapy with the narrowest spectrum needed for appropriate treatment of the infecting bacteria could play a role in reducing the emergence and transmission of resistant strains. In the present review, we give an overview of the techniques that have been developed for rapidly detecting antibiotic resistance in bacterial pathogens (excluding *Mycobacteria*, a topic which has

recently been reviewed[7, 8]) and provide examples of how these techniques have been employed for detecting resistance in a variety of bacterial pathogens. In addition, we discuss the questions that still remain to be addressed regarding these approaches.

Currently-used methods

The most widely used methods for characterizing antibiotic resistance in clinical isolates detect phenotypic resistance by measuring bacterial growth in the presence of the antibiotic being tested. These techniques include broth microdilution, antimicrobial gradient methods (e.g. Etest strips), disk diffusion, and various commercially available automated systems (e.g. the MicroScan WalkAway system from Siemens, the Phoenix Automated Microbiology System from BD Diagnostics and the Vitek systems from bioMerieux). In addition to their high sensitivity for detecting antibiotic resistance, a major advantage of these techniques is that they have been highly standardized, a process that has been facilitated by the elaboration of international guidelines for antimicrobial susceptibility testing such as those published by the CLSI and the EUCAST.[9, 10] For each of these methods, a variety of commercial suppliers provides reagents in ready-to-use formats that facilitate their use in clinical microbiology laboratories with high work loads. In the cases of broth microdilution and antimicrobial gradient methods, an MIC value is obtained, which provides information on the concentration of antibiotic necessary for inhibiting bacterial growth. However, despite their broad use and high sensitivity, there are some limitations associated with these approaches. These methods generally require purity culture before susceptibility testing can be performed, although some protocols may allow for testing directly with clinical samples. In addition, because these techniques measure bacterial growth in order to detect resistance, incubation times that are sufficient for differentiating susceptible from

resistant strains must be employed. In the following sections, we comment on the advantages and disadvantages of techniques and protocols that are being developed for rapidly identifying antibiotic resistance with respect to these commonly-used methods.

PCR-based techniques

PCR-based techniques (both conventional and real-time) rely on the sequence specific amplification of nucleic acids. For this reason, PCR was initially used in microbiologic testing for the rapid identification and quantification of causative agents of infections through the amplification of sequences specific to a particular pathogen.[11] With increased knowledge of the genetic bases of antibiotic resistance that has been acquired in recent years, PCR-based approaches have been developed for detecting the presence of genetic determinants of resistance to a variety of antibiotics for a number of different bacterial species. One salient example has been the use of PCR to identify methicillin resistant *Staphylococcus aureus* (MRSA) through the detection of the *mecA* gene, which encodes a modified penicillin-binding protein with reduced affinity for β-lactam antibiotics. Many PCR assays (both conventional and real-time) have been described for detecting *mecA* and/or associated sequences,[12-16] including commercial systems that in some cases have the ability to detect MRSA directly from clinical samples in less than two hours.[17-19] Examples of commercialized systems that employ real-time PCR for detection are the BD GeneOhm MRSA assay from Becton-Dickinson and the GeneXpert system from Cepheid, the latter of which showed high sensitivity (>93%) compared to routine methods for detecting MRSA in a multicenter study.[20] A recent study demonstrating that the clinical implementation of a test for detection of the *mecA* gene reduced the time to receiving optimal antibiotic therapy by an average of 25.4 hours in patients with *S. aureus* bacteremia illustrates the potential utility of this

122 method.[21] PCR-based approaches have also been developed for detecting vancomycin
123 resistance associated with the *vanA* and *vanB* genes, and have primarily been used for
124 detecting resistance in *Enterococcus* species. The sensitivity and specificity of these
125 assays varies depending on the study, however a number of these studies have reported
126 high false positive rates for the detection of the *vanB* gene, resulting in reduced
127 specificity.[22-25] Assays for detecting the presence of resistance genes in Gram negative
128 bacteria have also been developed. Most notably, this has included assays for
129 identifying numerous carbapenemase-encoding genes including, but not limited to,
130 KPC, NDM, IMP, VIM, AmpC, TEM, SHV, and the OXA carbapenemases in
131 *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae* and
132 *Escherichia coli*.[26-32]
133 The major advantage of these PCR-based approaches is that they can be carried
134 out in a relatively short period of time, in some cases using clinical samples without the
135 need for purity culture. PCR thus clearly has the potential to significantly reduce turn-
136 around times and rapidly provide information on antibiotic resistance. The major
137 limitation of this approach however, is that the presence of resistance genes may not
138 always correlate with phenotypic resistance. This is less worrisome in cases were the
139 presence of a genotype is highly associated with phenotypic resistance, such as the
140 presence of the *mecA*, *vanA* and *vanB* genes. However, other cases are not so clear cut,
141 such as the presence of carbapenemases in Gram negative bacteria. There are dozens of
142 distinct carbapenemases for which phenotypic resistance may depend not only on the
143 presence of the gene, but on its level of expression. One example is the OXA-51 gene
144 of *A. baumannii* which only produces phenotypic resistance if it is highly expressed, for
145 example due to the integration of mobile insertion sequences in the gene's promoter
146 region that increase expression.[33, 34] The development of techniques that rely on the

measurement of gene transcripts (RNA levels) instead of the presence of a gene may provide a potential solution to this problem. PCR-based techniques that detect the presence of resistance genes are also unable to detect novel or uncharacterized mechanisms of resistance for which the genetic determinant is unknown. This may be especially important in the case of carbapenemases in Gram negative bacteria given the continuous emergence of new variants. This is a critical point given that the inability of a technique to detect resistance would lead to the inappropriate classification of a resistant isolate as susceptible, an error that could lead to the administration of ineffective therapy. A final consideration is that these methods do not provide MIC values, which can be useful for guiding clinical decisions regarding therapy.

In addition to using PCR for detecting the presence of genetic determinants of resistance, the ability of real-time PCR to accurately quantify the number of copies of a specific nucleic acid in a sample has led to the development of approaches that employ this method for measuring bacterial growth. This approach monitors the number of bacterial genome copies present during growth of the isolated bacteria in the presence of the antibiotic being tested. Since quantitative real-time PCR can provide precise information regarding genome copy numbers, very short incubation times can be used for differentiating susceptible from resistant strains. One recent example is the detection of resistance to imipenem, ciprofloxacin and colistin in clinical isolates of *A. baumannii* using a real-time PCR assay targeting highly conserved sequences of the *ompA* gene.[35] As shown in Figure 1, growth of a resistant strain in the presence of antibiotics could be detected with relatively short incubation times. This approach has been applied to a number of bacterial species with various antibiotics.[36, 37] One advantage of this approach with respect to the PCR-based approaches described above is that it does not depend on the mechanism of resistance and that the assay is indirectly measuring

phenotypic resistance by detecting growth in the presence of antibiotic. The major disadvantage is that, unlike PCR-based approaches that detect the presence of resistance determinants, this approach requires previous culture and cannot be used directly with clinical samples.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS identifies molecules based on their time of flight through a vacuum tube after laser irradiation of a matrix which is co-crystallized with the sample. The time of flight allows for determination of the mass/charge ratio (*m/z*) of the ions present, and a spectrum of the sample is generated. The spectrum is then compared with a reference database in order to identify the analyte. Similar to PCR, MALDI-TOF was originally introduced into clinical microbiology laboratories for the identification of pathogens, and has only recently been applied to the detection of resistance. The use of MALDI-TOF to detect resistance most commonly aims to differentiate spectra from resistant and susceptible isolates using whole cells or crude extracts. Comparison of fingerprints from *E. coli* ATCC 700926 and the same strain carrying the β-lactamase-producing plasmid pUC19 determined just one discriminatory peak corresponding to a β-lactamase.[38] However, no pattern was found that could reliably identify β-lactamase resistance in clinical isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa.*[39] The detection of vancomycin-resistant enterococci has been recently demonstrated by identifying discriminatory peaks between *vanB*-positive *Enterococcus faecium* isolates from those lacking *vanB.*[40] In the case of MRSA, different results have been obtained in individual studies. Some studies have reported measureable differences in spectra when comparing to methicillin-susceptible *S. aureus* isolates,[41-44] whereas other authors

affirm that these differences are due to differences in clonality between the resistant and susceptible strains.[45, 46] The major advantages of the use of MALDI-TOF for identification of resistant strains based on differences in spectra are that it is extremely rapid and highly automated. However, similar to PCR-based techniques, the results obtained using this approach may not always directly correlate with phenotypic resistance, and differences between strains that are not related to resistance can complicate the interpretation of results.

MALDI-TOF MS has also been employed to detect the hydrolysis of antibiotics during incubation with a bacterial isolate in order to detect degradation products. Two reports describing the detection of β-lactamase activity using this approach were published in 2011.[47, 48] Hrabak *et al.* validated this method in Enterobacteriaceae and *P. aeruginosa* using 124 strains by detecting carbapenemase activity through meropenem degradation, reporting a sensitivity and specificity of 96.67% and 97.87%, respectively.[48] Moreover, the same authors reported an improvement in the technique by adding SDS to the reaction in order to decrease the incubation time and the number of bacteria necessary for the assay.[49] Carbapenem resistance has also been analyzed in *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *C. freundii* using ertapenem.[47] In separate studies, the presence of β-lactamases was detected by hydrolysis of various antibiotics including penicillin G, ampicillin, imipenem and cefoxitin in lysates of *E. coli* and ampicillin, piperacillin, cefotaxime, ceftazidime, ertapenem, imipenem, and meropenem in *E. coli* and *K. pneumoniae* from supernatants, and ertapenem directly in *K. pneumoniae* positive blood cultures.[50, 51] Recently published results have determined that the detection of class D β-lactamase activity requires more incubation time compared to class A and B in *Acinetobacter* spp.[52] and a study with 106 *A. baumannii* strains determined that the use of imipenem to identify carbapenem resistance showed a

sensitivity and specificity of 100%.[53] The main advantage of this method is the detection of activity without considering the type of enzyme involved.

Microarrays

Microarrays identify the presence of specific nucleic acid sequences using complementary oligonucleotides. Due to the fact that these oligonucleotides can be assembled onto solid supports in very close proximity, this method has the ability to detect numerous (i.e. thousands) of sequences in a single assay. For this reason, this method offers the possibility of creating arrays with the ability to detect a broad range of resistance genes present in bacterial isolates. One example is the use of microarrays for the detection of β-lactamase genes in Gram negative bacteria. Numerous studies have employed microarrays for the detection of β-lactamases, some of which can provide results in one working day.[54-60] In a recently published study, real-time PCR was combined with a microarray in order to identify respiratory pathogens that produce ventilator-associated pneumonia and detect the presence of 24 genes associated with resistance to β-lactam antibiotics directly from clinical samples.[32] This technique demonstrated high sensitivity and specificity for the detection of resistance genes, with a limit of detection of 10-100 DNA copies.

Microarray technology offers the ability to detect vast numbers of different resistance genes in a single assay, a clear distinction between PCR-based approaches which by comparison can only identify a handful of genes. For this reason, microarrays are ideally suited to bacteria in which there are numerous distinct mechanisms of resistance or variants of a single mechanism, such as the case of β-lactamases in Gram negative bacteria. However, similar to the approaches described above, data obtained from microarrays may not always correlate with phenotypic resistance and this

247 approach does not provide data on MIC values. In addition, this method may have
248 limited ability to detect resistance in isolates harboring novel or uncharacterized
249 mechanisms of resistance.
250
251 **Microfluidics**
252 Advances in bioengineering and nanotechnology have facilitated the
253 miniaturization of molecular assays that can be used for the detection of antibiotic
254 resistance. These so-called "lab on a chip" platforms can be carried out using extremely
255 small volumes of reagent and analyte (e.g. picoliters). These devices can incorporate
256 multiple functionalities onto a single chip including, bacterial culture, nucleic acid
257 hybridization and amplification, and cell lysis. The detection methods vary widely
258 depending on the device employed, and can be based on electrochemical, magnetic, and
259 optical detection, among others. A handful of recent studies have illustrated the
260 potential of microfluidic devices for rapidly providing information regarding antibiotic
261 resistance. Choi *et al.* demonstrated that a device consisting of microfluidic agarose
262 channels could track the growth of single cells using microscopy in the presence of
263 antibiotics.[61] Interestingly, approximate MIC values could be obtained using this
264 approach in only 3-4 hours. A second study, the electrochemical quantification of 16S
265 rRNA levels was used to measure bacterial growth in the presence of antibiotic.[62] This
266 method was validated directly with patient urine samples and was able to provide results
267 in 3.5 hours with 94% agreement with standard antibiotic susceptibility testing methods.
268 In a separate study, a microfluidic pH sensor was developed that could be used to detect
269 pH changes that occur during bacterial growth in the presence of antibiotics due to the
270 accumulation of metabolic products.[63] With this approach, bacterial growth curves
271 could be generated in as few as two hours in nanoliter scale cultures.

In addition to the very small volumes of analyte that are needed for these assays, this approach has the advantage of being highly automated with the potential for providing results extremely rapidly. Due to their small size, the chips used in these assays can be incorporated into portable devices, which may facilitate antimicrobial susceptibility testing at the point of care. In many cases, microfluidic devices indirectly measure bacterial growth in the presence of antibiotic, making it likely that the results obtained will correlate well with phenotypic resistance. This aspect also makes this approach amenable for use in detecting resistance in bacteria for which the mechanisms of resistance are not well characterized.

Cell lysis-based approaches

Recently, an approach based on detecting bacterial cell lysis after incubation with the antibiotic being tested has been described. The bacterial isolate is first incubated with the desired concentrations of the antibiotic being tested, and then immobilized in an agarose microgel. The immobilized bacteria are subsequently immersed in a lysing solution which results in disruption of the nucleoid in bacteria that have been affected during incubation with the antibiotic in the previous step. The preparation is incubated with a DNA-specific fluorescent stain and nucleoid integrity is visualized by microscopy. As can be seen in Figure 2, which shows ampicillin susceptible and resistant strains of *Enterococcus faecalis* processed as described above, nucleoid fragmentation is evident in the susceptible strain whereas the resistant strain maintains an intact nucleoid. This approach has been validated for the detection of quinolone and ampicillin resistance in *E. coli*,[64, 65] and recently for detecting carbapenem resistance *A. baumannii*.[66] The procedure could be carried out in 100 minutes and showed good correlation with microdilution and Etest data.[66] Interestingly,

297 although it has not been rigorously determined experimentally, this approach may have
298 the ability to provide approximate MIC values since nucleoid fragmentation is
299 visualized after incubation with different concentrations of the antibiotics being tested.
300 The studies described to date have only assessed this technique using culture purified
301 bacteria, and it remains to be determined if this approach can be used directly with
302 clinical samples. An advantage of this approach is that a result is obtained regardless of
303 the mechanism that is producing resistance.
304
305 **Whole genome sequencing**
306 Advances in DNA sequencing technology have made it possible to sequence
307 entire bacterial genomes extremely rapidly. These methods, coupled with
308 bioinformatic tools that can quickly assemble and analyze the massive amount of data
309 obtained from these sequencing runs, open the possibility of using these techniques
310 for detecting antibiotic resistance. A number of studies describing whole genome
311 sequencing of small numbers of clinical isolates in order to characterize the genetic
312 determinants of antibiotic resistance have been described.[67-70] The objective of these
313 studies has primarily been to characterize strains with interesting phenotypic
314 resistance profiles. In a recent study, whole genome sequencing was used to
315 characterize resistance profiles of 200 bacterial isolates from four bacterial species to
316 a variety of antibiotics, and the results were compared to those obtained using
317 phenotypic susceptibility testing.[71] High concordance (99.74%) was observed
318 between the two techniques, demonstrating that data obtained from genome
319 sequences can correlate well with phenotypic resistance in some cases. For these
320 studies, the speed with which the sequencing runs and subsequent analyses are carried
321 out are of less importance than would be the case if this technology were to be

applied in the clinical setting for susceptibility testing. Taking into account the turn-around times necessary for whole genome sequencing, and the elevated cost compared to traditional and other emerging techniques, the use of whole genome sequencing for routine susceptibility testing may not yet be practical.[72] Although in its current form it may not be suited for routine testing, whole genome sequencing has demonstrated its utility in tracking outbreaks of clinically-important strains, as was demonstrated during a hospital outbreak of MRSA in the United Kingdom,[73] and during an outbreak of *E. coli* O104:H4 in Germany.[74-76] Undoubtedly, genome sequencing will increasingly be employed for a variety of applications in the clinical microbiology laboratory as the cost of sequencing decreases and the speed of sequencing and analysis increases. However, it should be noted that similar to the microarrays and PCR-based approaches described above, DNA sequencing relies on the identification of the genetic determinants of resistance, raising the possibility that a limitation of this approach may be the detection of novel or uncharacterized mechanisms of resistance.

Conclusions

The methods described here all aim to shorten the time necessary for detecting resistance in bacterial pathogens, however in many cases it remains to be determined if these approaches provide sufficient sensitivity and specificity. In addition, although the methods discussed here have undergone microbiological testing to determine if they give similar results to those obtained using standard methods, studies providing information on the clinical validation of these approaches have yet to be performed in many cases. For a method to be approved by the U.S. Food and Drug Administration the very major error rate (the classification of a resistant isolate as susceptible) must be

below 1.5% and the major error rate (the classification of a susceptible isolate as resistant) must be below 3.0%.[77] One of the key limitations that must be addressed regarding many of these methods in order to ensure that they meet these criteria is how often there is discordance between the presence of a resistant determinant and phenotypic resistance. This is of particular importance with the use of techniques that rely solely on the detection of resistance determinants, such as PCR for gene detection or MALDI-TOF. How these tests will deal with novel or uncharacterized resistance mechanisms must also be considered, since the inability of a test to identify resistance will lead to very major errors. Additional aspects that will need to be addressed on a case-by-case basis are whether or not the tests being developed require highly-qualified personal, the added expense associated with the test, and the acceptance of test results by clinicians. However, given the potential benefits in terms of improving patient outcomes by reducing the period of time during which empiric therapy is administered and in terms of reducing healthcare costs, the continued development of these approaches is warranted.

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Transparency Declaration

All authors declare no potential conflicts of interest.

References

1. Barenfanger J, Drake C, Kacich G. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J Clin Microbiol* 1999; **37**: 1415-8.

2. Doern GV, Vautour R, Gaudet M et al. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. *J Clin Microbiol* 1994; **32**: 1757-62.

3. Trenholme GM, Kaplan RL, Karakusis PH et al. Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. *J Clin Microbiol* 1989; **27**: 1342-5.

4. Iregui M, Ward S, Sherman G et al. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. *Chest* 2002; **122**: 262-8.

5. Luna CM, Aruj P, Niederman MS et al. Appropriateness and delay to initiate therapy in ventilator-associated pneumonia. *Eur Respir J* 2006; **27**: 158-64.

6. Dryden M, Johnson AP, Ashiru-Oredope D et al. Using antibiotics responsibly: right drug, right time, right dose, right duration. *J Antimicrob Chemother*; 2011; **66**: 2441-3.

7. Moore DA, Shah NS. Alternative methods of diagnosing drug resistance--what can they do for me? *J Infect Dis*; 2011; **204 Suppl 4**: S1110-9.

8. Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clin Infect Dis*; 2011; **52**: 1350-5.

9. Clinical Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement M100-S15*. CLSI, Wayne, PA, USA, 2013.

10. Leclercq R, Canton R, Brown DF et al. EUCAST expert rules in antimicrobial susceptibility testing. *Clin Microbiol Infect*; 2013; **19**: 141-60.

11. Espy MJ, Uhl JR, Sloan LM et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol Rev* 2006; **19**: 165-256.

12. Cuny C, Witte W. PCR for the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains using a single primer pair specific for SCC*mec* elements and the neighbouring chromosome-borne orfX. *Clin Microbiol Infect* 2005; **11**: 834-7.

13. Huletsky A, Giroux R, Rossbach V et al. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004; **42**: 1875-84.

14. Jones CH, Tuckman M, Howe AY et al. Diagnostic PCR analysis of the occurrence of methicillin and tetracycline resistance genes among *Staphylococcus aureus* isolates from phase 3 clinical trials of tigecycline for complicated skin and skin structure infections. *Antimicrob Agents Chemother* 2006; **50**: 505-10.

15. Sinsimer D, Leekha S, Park S et al. Use of a multiplex molecular beacon platform for rapid detection of methicillin and vancomycin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 2005; **43**: 4585-91.

16. Zhang K, McClure JA, Elsayed S et al. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005; **43**: 5026-33.

17. Grobner S, Dion M, Plante M et al. Evaluation of the BD GeneOhm StaphSR assay for detection of methicillin-resistant and methicillin-susceptible *Staphylococcus*

428 *aureus* isolates from spiked positive blood culture bottles. *J Clin Microbiol* 2009; **47**:
429 1689-94.
430 18. Paule SM, Hacek DM, Kufner B et al. Performance of the BD GeneOhm
431 methicillin-resistant *Staphylococcus aureus* test before and during high-volume clinical
432 use. *J Clin Microbiol* 2007; **45**: 2993-8.
433 19. Wang XP, Ginocchio CC. Automation of the BD GeneOhm methicillin-resistant
434 *Staphylococcus aureus* assay for high-throughput screening of nasal swab specimens. *J*
435 *Clin Microbiol* 2009; **47**: 1546-8.
436 20. Wolk DM, Picton E, Johnson D et al. Multicenter evaluation of the Cepheid
437 Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening
438 method for detection of MRSA in nares. *J Clin Microbiol* 2009; **47**: 758-64.
439 21. Carver PL, Lin SW, DePestel DD et al. Impact of *mecA* gene testing and
440 intervention by infectious disease clinical pharmacists on time to optimal antimicrobial
441 therapy for *Staphylococcus aureus* bacteremia at a University Hospital. *J Clin*
442 *Microbiol* 2008; **46**: 2381-3.
443 22. Gazin M, Lammens C, Goossens H et al. Evaluation of GeneOhm VanR and
444 Xpert *vanA/vanB* molecular assays for the rapid detection of vancomycin-resistant
445 enterococci. *Eur J Clin Microbiol Infect Dis*; 2012; **31**: 273-6.
446 23. Mak A, Miller MA, Chong G et al. Comparison of PCR and culture for
447 screening of vancomycin-resistant *Enterococci*: highly disparate results for *vanA* and
448 *vanB*. *J Clin Microbiol* 2009; **47**: 4136-7.
449 24. Stamper PD, Cai M, Lema C et al. Comparison of the BD GeneOhm VanR assay
450 to culture for identification of vancomycin-resistant enterococci in rectal and stool
451 specimens. *J Clin Microbiol* 2007; **45**: 3360-5.

452 25. Usacheva EA, Ginocchio CC, Morgan M et al. Prospective, multicenter
453 evaluation of the BD GeneOhm VanR assay for direct, rapid detection of vancomycin-
454 resistant *Enterococcus* species in perianal and rectal specimens. *Am J Clin Pathol*;
455 2010; **134**: 219-26.
456 26. Monteiro J, Widen RH, Pignatari AC et al. Rapid detection of carbapenemase
457 genes by multiplex real-time PCR. *J Antimicrob Chemother* 2012; **67**: 906-9.
458 27. Chroma M, Hricova K, Kolar M et al. Using newly developed multiplex
459 polymerase chain reaction and melting curve analysis for detection and discrimination
460 of beta-lactamases in *Escherichia coli* isolates from intensive care patients. *Diagn*
461 *Microbiol Infect Dis* 2011; **71**: 181-91.
462 28. Mendes RE, Kiyota KA, Monteiro J et al. Rapid detection and identification of
463 metallo-beta-lactamase-encoding genes by multiplex real-time PCR assay and melt
464 curve analysis. *J Clin Microbiol* 2007; **45**: 544-7.
465 29. Brolund A, Wisell KT, Edquist PJ et al. Development of a real-time SYBRGreen
466 PCR assay for rapid detection of acquired *AmpC* in Enterobacteriaceae. *J Microbiol*
467 *Methods* 2010; **82**: 229-33.
468 30. Geyer CN, Reisbig MD, Hanson ND. Development of a TaqMan multiplex PCR
469 assay for detection of plasmid-mediated *ampC* beta-lactamase genes. *J Clin Microbiol*
470 2012; **50**: 3722-5.
471 31. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated *AmpC* beta-
472 lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002; **40**:
473 2153-62.
474 32. Bogaerts P, Hamels S, de Mendonca R et al. Analytical validation of a novel
475 high multiplexing real-time PCR array for the identification of key pathogens causative

476 of bacterial ventilator-associated pneumonia and their associated resistance genes. *J*
477 *Antimicrob Chemother* 2012; **68**: 340-7.
478 33. Figueiredo S, Bonnin RA, Poirel L et al. Identification of the naturally occurring
479 genes encoding carbapenem-hydrolysing oxacillinases from *Acinetobacter*
480 *haemolyticus*, *Acinetobacter johnsonii*, and *Acinetobacter calcoaceticus*. *Clin Microbiol*
481 *Infect* 2011; **18**: 907-13.
482 34. Turton JF, Ward ME, Woodford N et al. The role of *ISAba1* in expression of
483 OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006;
484 **258**: 72-7.
485 35. Martín-Peña R, Domínguez-Herrera J, Pachón J et al. Rapid detection of
486 antibiotic resistance in *Acinetobacter baumannii* using quantitative real-time PCR. *J*
487 *Antimicrob Chemother* 2013; doi:10.1093/jac/dkt057
488 36. Beuving J, Verbon A, Gronthoud FA et al. Antibiotic susceptibility testing of
489 grown blood cultures by combining culture and real-time polymerase chain reaction is
490 rapid and effective. *PLoS One* 2011; **6**: e27689.
491 37. Rolain JM, Mallet MN, Fournier PE et al. Real-time PCR for universal antibiotic
492 susceptibility testing. *J Antimicrob Chemother* 2004; **54**: 538-41.
493 38. Camara JE, Hays FA. Discrimination between wild-type and ampicillin-resistant
494 *Escherichia coli* by matrix-assisted laser desorption/ionization time-of-flight mass
495 spectrometry. *Anal Bioanal Chem* 2007; **389**: 1633-8.
496 39. Schaumann R, Knoop N, Genzel GH et al. A step towards the discrimination of
497 beta-lactamase-producing clinical isolates of Enterobacteriaceae and *Pseudomonas*
498 *aeruginosa* by MALDI-TOF mass spectrometry. *Med Sci Monit* 2012; **18**: MT71-7.
499 40. Griffin PM, Price GR, Schooneveldt JM et al. Use of matrix-assisted laser
500 desorption ionization-time of flight mass spectrometry to identify vancomycin-resistant

501 enterococci and investigate the epidemiology of an outbreak. *J Clin Microbiol* 2012; **50**:
502 2918-31.
503 41. Du Z, Yang R, Guo Z et al. Identification of *Staphylococcus aureus* and
504 determination of its methicillin resistance by matrix-assisted laser desorption/ionization
505 time-of-flight mass spectrometry. *Anal Chem* 2002; **74**: 5487-91.
506 42. Edwards-Jones V, Claydon MA, Evason DJ et al. Rapid discrimination between
507 methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* by intact cell mass
508 spectrometry. *J Med Microbiol* 2000; **49**: 295-300.
509 43. Jackson KA, Edwards-Jones V, Sutton CW et al. Optimisation of intact cell
510 MALDI method for fingerprinting of methicillin-resistant *Staphylococcus aureus*. *J*
511 *Microbiol Methods* 2005; **62**: 273-84.
512 44. Majcherczyk PA, McKenna T, Moreillon P et al. The discriminatory power of
513 MALDI-TOF mass spectrometry to differentiate between isogenic teicoplanin-
514 susceptible and teicoplanin-resistant strains of methicillin-resistant *Staphylococcus*
515 *aureus*. *FEMS Microbiol Lett* 2006; **255**: 233-9.
516 45. Bernardo K, Pakulat N, Macht M et al. Identification and discrimination of
517 *Staphylococcus aureus* strains using matrix-assisted laser desorption/ionization-time of
518 flight mass spectrometry. *Proteomics* 2002; **2**: 747-53.
519 46. Wolters M, Rohde H, Maier T et al. MALDI-TOF MS fingerprinting allows for
520 discrimination of major methicillin-resistant *Staphylococcus aureus* lineages. *Int J Med*
521 *Microbiol* 2011; **301**: 64-8.
522 47. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-
523 time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours.
524 *J Clin Microbiol* 2011; **49**: 3321-4.

525 48. Hrabak J, Walkova R, Studentova V et al. Carbapenemase activity detection by
526 matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin*
527 *Microbiol* 2011; **49**: 3222-7.
528 49. Hrabak J, Studentova V, Walkova R et al. Detection of NDM-1, VIM-1, KPC,
529 OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-
530 time of flight mass spectrometry. *J Clin Microbiol* 2012; **50**: 2441-3.
531 50. Hooff GP, van Kampen JJ, Meesters RJ et al. Characterization of beta-lactamase
532 enzyme activity in bacterial lysates using MALDI-mass spectrometry. *J Proteome Res*
533 2012; **11**: 79-84.
534 51. Sparbier K, Schubert S, Weller U et al. Matrix-assisted laser desorption
535 ionization-time of flight mass spectrometry-based functional assay for rapid detection of
536 resistance against beta-lactam antibiotics. *J Clin Microbiol* 2012; **50**: 927-37.
537 52. Carvalhaes CG, Cayo R, Assis DM et al. Detection of SPM-1-producing
538 *Pseudomonas aeruginosa* and class D beta-lactamase-producing *Acinetobacter*
539 *baumannii* isolates by use of liquid chromatography-mass spectrometry and matrix-
540 assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol*
541 2013; **51**: 287-90.
542 53. Kempf M, Bakour S, Flaudrops C et al. Rapid detection of carbapenem
543 resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption ionization-
544 time of flight mass spectrometry. *PLoS One* 2012; **7**: e31676.
545 54. Cohen Stuart J, Dierikx C, Al Naiemi N et al. Rapid detection of TEM, SHV and
546 CTX-M extended-spectrum beta-lactamases in Enterobacteriaceae using ligation-
547 mediated amplification with microarray analysis. *J Antimicrob Chemother* 2010; **65**:
548 1377-81.

549 55. Grimm V, Ezaki S, Susa M et al. Use of DNA microarrays for rapid genotyping
550 of TEM beta-lactamases that confer resistance. *J Clin Microbiol* 2004; **42**: 3766-74.
551 56. Lee Y, Lee CS, Kim YJ et al. Development of DNA chip for the simultaneous
552 detection of various beta-lactam antibiotic-resistant genes. *Mol Cells* 2002; **14**: 192-7.
553 57. Leinberger DM, Grimm V, Rubtsova M et al. Integrated detection of extended-
554 spectrum-beta-lactam resistance by DNA microarray-based genotyping of TEM, SHV,
555 and CTX-M genes. *J Clin Microbiol* 2010; **48**: 460-71.
556 58. Naas T, Cuzon G, Bogaerts P et al. Evaluation of a DNA microarray (Check-
557 MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-
558 lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. *J Clin*
559 *Microbiol* 2011; **49**: 1608-13.
560 59. Naas T, Cuzon G, Truong H et al. Evaluation of a DNA microarray, the check-
561 points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-
562 spectrum beta-lactamases and KPC carbapenemases. *Antimicrob Agents Chemother*
563 2010; **54**: 3086-92.
564 60. Peter H, Berggrav K, Thomas P et al. Direct detection and genotyping of
565 *Klebsiella pneumoniae* carbapenemases from urine by use of a new DNA microarray
566 test. *J Clin Microbiol* 2012; **50**: 3990-8.
567 61. Choi J, Jung YG, Kim J et al. Rapid antibiotic susceptibility testing by tracking
568 single cell growth in a microfluidic agarose channel system. *Lab Chip*; 2013; **13**: 280-7.
569 62. Mach KE, Mohan R, Baron EJ et al. A biosensor platform for rapid
570 antimicrobial susceptibility testing directly from clinical samples. *J Urol*; **185**: 148-53.
571 63. Tang Y, Zhen L, Liu J et al. Rapid antibiotic susceptibility testing in a
572 microfluidic pH sensor. *Anal Chem*; 2013; **85**: 2787-94.

573 64. Santiso R, Tamayo M, Gosalvez J et al. A rapid in situ procedure for
574 determination of bacterial susceptibility or resistance to antibiotics that inhibit
575 peptidoglycan biosynthesis. *BMC Microbiol*; 2011; **11**: 191.
576 65. Tamayo M, Santiso R, Gosalvez J et al. Rapid assessment of the effect of
577 ciprofloxacin on chromosomal DNA from *Escherichia coli* using an in situ DNA
578 fragmentation assay. *BMC Microbiol* 2009; **9**: 69.
579 66. Bou G, Otero FM, Santiso R et al. Fast assessment of resistance to carbapenems
580 and ciprofloxacin of clinical strains of *Acinetobacter baumannii*. *J Clin Microbiol*;
581 2012; **50**: 3609-13.
582 67. Huang H, Yang ZL, Wu XM et al. Complete genome sequence of *Acinetobacter*
583 *baumannii* MDR-TJ and insights into its mechanism of antibiotic resistance. *J*
584 *Antimicrob Chemother* 2012; **67**: 2825-32.
585 68. O'Neill CE, Seth-Smith HM, Van Der Pol B et al. *Chlamydia trachomatis*
586 clinical isolates identified as tetracycline resistant do not exhibit resistance in vitro:
587 whole-genome sequencing reveals a mutation in *porB* but no evidence for tetracycline
588 resistance genes. *Microbiology*; 2013; **159**: 748-56.
589 69. Snitkin ES, Zelazny A, Gupta J et al. Genomic insights into the fate of colistin
590 resistance and *Acinetobacter baumannii* during patient treatment. *Genome Res*; 2013;
591 doi:10.1101/gr.154328.112
592 70. Tan SY, Chua SL, Liu Y et al. Comparative Genomic Analysis of Rapid
593 Evolution of an Extreme-Drug-Resistant *Acinetobacter baumannii* Clone. *Genome Biol*
594 *Evol*; 2013; **5**: 807-18.
595 71. Zankari E, Hasman H, Kaas RS et al. Genotyping using whole-genome
596 sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial
597 susceptibility testing. *J Antimicrob Chemother*; 2013; **68**: 771-7.

598 72. Torok ME, Peacock SJ. Rapid whole-genome sequencing of bacterial pathogens
599 in the clinical microbiology laboratory--pipe dream or reality? *J Antimicrob Chemother*;
600 2012; **67**: 2307-8.
601 73. Koser CU, Holden MT, Ellington MJ et al. Rapid whole-genome sequencing for
602 investigation of a neonatal MRSA outbreak. *N Engl J Med*; 2012; **366**: 2267-75.
603 74. Mellmann A, Harmsen D, Cummings CA et al. Prospective genomic
604 characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak
605 by rapid next generation sequencing technology. *PLoS One*; 2011; **6**: e22751.
606 75. Rasko DA, Webster DR, Sahl JW et al. Origins of the *E. coli* strain causing an
607 outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med*; 2011; **365**: 709-17.
608 76. Rohde H, Qin J, Cui Y et al. Open-source genomic analysis of Shiga-toxin-
609 producing *E. coli* O104:H4. *N Engl J Med*; 2011; **365**: 718-24.
610 77. U.S. Food and Drug Administration. Class II special controls guidance
611 document: antimicrobial susceptibility test (AST) systems; guidance
612 for industry and FDA. Rockville, MD: US Food and Drug Administration. 2003.
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Figure Legends

Figure 1. Use of real-time PCR to measure bacterial growth in the presence of antibiotics. The figure shows the fold-change in genome copy numbers as determined by real-time PCR (compared to time zero) during the growth of two strains of *A. baumannii* in the presence of the indicated antibiotic. Strain 1 is susceptible to colistin, imipenem, and ciprofloxacin whereas Strain 2 is resistant to the three antibiotics.

Figure 2. Detection of antibiotic resistance by characterizing cell lysis. The panels show fluorescent microscopy images of strains of *Enterococcus faecalis* assessed for resistance to ampicillin using the procedure described in the text. The photographs show a resistant strain processed after incubation with 8 mg/L of ampicillin (a), a susceptible strain processed after incubation with 8 mg/l of ampicillin (b), the same resistant and susceptible strains processed after incubation without antibiotic (c and d, respectively). As can be seen, nucleoid fragmentation is seen in the susceptible strain after incubation with ampicillin. This figure appears in color in the online version of *JAC* and in black and white in the printed version of *JAC*.