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Rapid Detection of Antibiotic-Resistant Organism Carriage for Infection Prevention

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Rapid detection of multidrug-resistant organism (MDRO) carriers could help reduce MDRO infections by allowing for faster institution of prevention measures. However, improving the turnaround time (TAT) of a test requires attention to more than the analytic TAT, and will only occur if postanalytic processes (test reporting and care interventions) are also rapid and efficient. Obstacles to rapid MDRO test development include complex evolving resistance mechanisms, performance directly on mixed samples (eg, nares, stool), and adaptation of new methods for routine clinical diagnostic use. Existing data to support the clinical utility of rapid detection (vs standard culture methods) are scant. For these reasons, rapid detection of MDRO carriers remains a work in progress. Future efforts should be on developing rapid tests to detect multidrug-resistant gram-negative rods, particularly those harboring β-lactamases, and on performing clinical trials to determine how best to incorporate rapid detection of MDRO carriage into healthcare-associated infection prevention efforts.

Keywords. antimicrobial resistance; surveillance; infection prevention.

As the emergence and spread of antibiotic resistance continues, the dearth of new agents for treatment of multidrug-resistant organisms (MDROs) has become an urgent public health crisis [1]. Among the MDROs, the most prevalent as causes of healthcare-associated infection are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and a wide variety of multidrug-resistant gram-negative rods (MDR-GNRs: organisms resistant to most or all available antibiotic classes through a myriad of mechanisms, including production of β-lactamases with increasingly broad activity) [2–4].

In the face of rising resistance rates and limited treatment options, prevention of MDRO infections is paramount. MDRO prevention approaches can be broadly categorized into (1) measures to improve antimicrobial use, (2) measures to prevent transmission of MDROs, and (3) measures to prevent infection among patients who are uninfected carriers of an MDRO. For example, the Centers for Disease Control and Prevention (CDC) recommends use of contact precautions for all MDRO carriers to prevent the spread of MDROs in healthcare settings [5], and some hospitals practice focused decolonization of MRSA carriers to prevent subsequent infection [6]. Given the fact that routine clinical cultures identify only a minority of those who carry an MDRO [7, 8], the use of active screening for MDRO carriage is becoming more common. Current CDC guidance recognizes active MDRO surveillance as an important "tier 2" measure, to be applied during outbreaks or when rates of a targeted MDRO are not decreasing [5].

One of the major obstacles to active MDRO surveillance has been the turnaround time of culture-based screening methods. Depending upon the MDRO, culture assays can take between 24 and 72 hours to complete, during which time any intervention must either be delayed or must be applied to all screened patients. For this reason, in 2004 we called rapid

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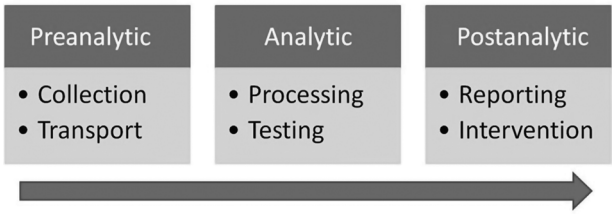

Figure 1. Components of the actual turnaround time of a laboratory test.

detection of MDRO carriage an "unmet clinical need" [9]. The purpose of this review is to update progress in the rapid detection of MDRO carriage for prevention of healthcare-associated infections. We report advances in availability of rapid diagnostics for MDROs, emphasizing lessons learned from this early experience. This is not a comprehensive review of available technologies, but rather a practical guide to both the promise and the limitations of rapid (mostly molecular) detection of MDRO carriage.

HOW RAPID IS RAPID? ANALYTIC VERSUS ACTUAL TURNAROUND TIME

For the purposes of this discussion, rapid tests will be defined as those providing a same-day turnaround time (TAT). It is important to distinguish the *analytic* TAT from the *actual* TAT of a test—the time from when a test is ordered to when the result is translated into a change in patient care (eg, institution or removal of contact precautions, decolonization) (Figure 1). A rapid test with analytic TAT of 2 hours that is batched and performed once daily represents little improvement in TAT over some agar-based methods. Similarly, tests that require an isolated bacterial colony to perform are not really "rapid," given that it often takes 24 hours or more to grow the organism. Thus, the most useful rapid tests are those that can be applied directly to patient samples (or to broth cultures after an abbreviated incubation).

If rapid testing is to provide any benefit, there must be a postanalytic system in place for the results to be translated into action (eg, institution of isolation, discontinuation of isolation, decolonization). Unless the performance characteristics are much better, there is no reason to convert to a more expensive test with shorter analytic TAT if the actual TAT of the test will not be meaningfully reduced.

METHICILLIN-RESISTANT *S. AUREUS*

Among MDROs, MRSA is the organism for which there is the most experience with rapid detection of carriage. There has now been wide adoption of US Food and Drug Administration (FDA)–approved molecular (real-time polymerase chain reaction [PCR]) tests for detection of MRSA directly from patient samples (eg, nares) [10]. However, despite the fact that the presence of a single gene (*mec*A) is the gold standard for MRSA detection, the development and optimization of molecular assays applied directly to patient samples has been surprisingly complicated. The *mec*A gene found in *S. aureus* is highly homologous to that carried by coagulase-negative staphylococci (CoNS), and most CoNS are methicillin resistant [11, 12]. Thus, the major obstacle to early assays utilizing *mec*A primers was the false-positive test resulting from the simultaneous presence of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant CoNS (MR-CoNS) in mixed samples. To overcome this limitation, the widely used commercial assays target a region on the *S. aureus* genome that links the staphylococcal cassette chromosome mec (SCCmec) where it inserts, the SCC*mec-orfX* junction. This target does not itself contain *mec*A [13]. This assay demonstrated good performance characteristics, even though some strains of MSSA contain this target as a result of having "lost" the *mec*A gene through excision or mutation (the so-called *mec*A dropout strains or empty cassette variants) [14]. Although initially thought to be rare, these strains were soon recognized to be common causes of false-positive tests. In our institution, almost 8% of all positive tests using the Xpert MRSA assay (Cepheid, Sunnyvale, California) were falsely positive due to MSSA containing SCC*mec* elements without the *mec*A gene [15]. Modification of this test (inclusion of *mec*A primers) has largely addressed this problem, although the assay may still be vulnerable to false-positives from simultaneous presence of an empty cassette variant of MSSA and an MR-CoNS. Recent data from the MOSAR group also suggests that certain MR-CoNS may alone give false-positive results owing to homogeneity of the SCC*mec-orf*X region [16]. These false-positivity issues can result in low positive predictive values (<70%) in low-prevalence populations [17, 18].

False-positive tests are not the only issue for current molecular MRSA assays. As has been widely reported, strains of MRSA with divergent SCC*mec* elements and *mec*A genes can result in false-negative tests [19, 20]. The International Working Group on the Classification of Staphylococcal Cassette Chromosomal Elements recently published a guideline for reporting novel *mec*A gene homologues [21]. The frequency and range of such strains is not yet known, but ongoing surveillance and cataloguing of different *mec*A types will be necessary, in order for molecular detection assays to keep up with the evolution of MRSA.

These issues in molecular screening for MRSA carriage highlight the importance of ongoing use of culture in parallel, to help monitor for emerging strains that present problems for the performance of the tests. Additional advantages of continuing to perform culture are that it provides an organism for molecular typing and susceptibility testing (including testing

Table 1. Selected Challenges to Rapid Multidrug-Resistant Organism Detection Directly From Patient Samples

Challenge	Example(s)
Resistance gene shared by commensals	• *mec*A in CoNS • *van*B in intestinal anaerobes
Resistance gene not expressed or epidemiologically important	• Chromosomal AmpC cephalosporinase in *Escherichia coli*
Resistance phenotype multifactorial	• Carbapenem resistance associated with porin protein mutation + AmpC overexpression
Natural evolution and mutation of resistance genes	• Empty cassette variants of MSSA • Novel *mec*A homologues • Emergence of new β-lactamases
No organism available for molecular typing, additional susceptibility testing, or prospective validation of assay	• Broadly applicable to molecular tests, requires running culture in parallel
Approved/validated only for 1 sample type	• MRSA nares-only testing misses carriers at other body sites (eg, throat, skin)

Abbreviations: CoNS,coagulase-negative staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

against agents used for topical decolonization, such as mupirocin and chlorhexidine [22]), and that it provides considerably more flexibility for testing body sites that fall outside of FDA-approved indications for the commercial molecular assays, and that must also be sampled to improve sensitivity for detection of MRSA carriage [23]. Thus, the molecular test is used to reduce TAT but may not replace culture (Table 1).

Other rapid methods for detection of MRSA carriage are being developed, but these tests are neither widely adopted or yet FDA-approved for direct testing on mixed (eg, nares) samples.

VANCOMYCIN-RESISTANT *ENTEROCOCCUS*

In US hospitals, VRE is the next most common target of tests for detection of MDRO carriage. Vancomycin resistance in enterococci is mediated by several genes designated *van*A, *van*B, *van*C, *van*D, etc. Among these, *van*A and *van*B predominate among clinically significant enterococci and are the only 2 that have major epidemiologic significance owing to the transmissibility of the resistance genes and outbreak potential [24]. Culture-based assays for detection of VRE carriage have traditionally taken 48–72 hours or longer, so VRE seems a good target for rapid direct detection from complex samples such as perirectal swabs or stool.

The challenges to rapid detection of VRE carriage arise from the fact that the reservoir for VRE is within the rich microbiota of the intestine, a microbial community that is profoundly impacted during hospitalization and by receipt of antimicrobial therapy [25]. All *van*A and/or *van*B detection assays have lower limits of detection ranging from 10 to 100 colony-forming units per milliliter [9, 26, 27], so it is possible that VRE may be present at levels below detection upon hospital admission and become detectable after antimicrobial receipt causes expansion of the VRE population [25]. This sequence of events could be misinterpreted as VRE acquisition (which could in turn focus prevention efforts solely on transmission prevention activities rather than on antimicrobial stewardship). An additional problem with direct detection of VRE from fecal flora is that intestinal anaerobes may also carry the *van*B resistance determinant [28]. Because *van*B is also less prevalent among VRE than is *van*A, published evaluations of the performance of rapid *van*B detection assays reveal positive predictive values as low as 5%–10% [27]. Given this, rapid molecular VRE screening tests are useful only for detection of *van*A-bearing enterococci.

In contrast to screening for MRSA carriage (which is now mandated in some states), little enthusiasm has developed for routine screening for the VRE carrier state. There are several reasons for this. First, VRE are much less common as invasive pathogens than MRSA and tend to impact a smaller subset of highly immunocompromised patients [3]. Second, there are no effective decolonization options for VRE carriers, as there are for MRSA carriers. Third, the available screening tests do not perform as well as do those for MRSA, and the test results are more difficult to interpret, owing to some of the considerations we have outlined above.

MULTIDRUG-RESISTANT GRAM-NEGATIVE RODS

Compared to MRSA and VRE, for which a single gene (*mec*A, *van*A) provides a gold standard for MDRO detection, MDR-GNRs present a much greater challenge. Resistance mechanisms among MDR-GNRs are multiple and highly complex [29], and

Table 2. Major β-Lactamases of Epidemiologic Importance

Bush-Jacoby Classification	Molecular Class	Common Name	Example β-Lactamases (No. of Types)
1	C	Cephalosporinase	OXA (18), AmpC types (many)
2be	A	Extended-spectrum β-lactamase	SHV (168), TEM (204), CTX-M (134)
2df	D	Carbapenemase	OXA-48–like
2f	A	Carbapenemase	KPC (12), SME
3	B	Metallo-β-lactamase	NDM (7), IMP (39), VIM (37)

there is no general agreement around the definition of MDR-GNR across species. In addition to the multiplicity of potential targets for rapid testing, the mere presence of a resistance gene does not mean that the gene is being expressed or is of epidemiological concern as a risk for rapid spread (eg, chromosomal AmpC cephalosporinases in *Escherichia coli*) [30]. Moreover, some important resistance phenotypes involve multiple mechanisms (eg, carbapenem resistance from high levels of cephalosporinase production in combination with porin protein mutations). All of these factors have delayed the development and availability of rapid MDR-GNR tests for application directly to patient samples. Most labs that perform screening for MDR-GNR carriage use one of a variety of culture-based methods with analytic TATs of 24 hours or longer.

As it has become clear that most clinical laboratories do not have the capacity to characterize the molecular basis of resistance among the GNRs, the Clinical and Laboratory Standards Institute has lowered minimum inhibitory concentration (MIC) breakpoints for Enterobacteriaceae against cephalosporins and carbapenems, suggesting that therapeutic decisions should be based on MICs rather than on knowledge of the resistance mechanism [31]. The new (lower) breakpoints are intended to obviate the need for extended-spectrum β-lactamase confirmatory testing or modified Hodge testing (confirmation of carbapenemase) for clinical use. The result of this change in testing and interpretive criteria has been the loss of epidemiologic data for some infection prevention personnel who have come to rely on these confirmatory tests to guide prevention activities [32], and has also led to an increase in the number of isolates characterized as resistant to cephalosporins and carbapenems, with major implications for infection prevention [32, 33]. One institution reported that this change resulted in a 35% increase in the number of MDR-GNRs identified and a concomitant increase in the hospitals' use of contact precautions [34]. Thus the question arises: Which MDR-GNRs require additional or extraordinary infection prevention interventions, and which do not?

The MDR-GNRs that have the greatest potential for rapid spread and devastating outbreaks are those that harbor β-lactamases (including and especially carbapenemases) [35]. There are hundreds of β-lactamases, some of which are already endemic in many US hospitals, and a few of which are global public health problems causing deadly outbreaks as they spread widely [36]. Table 2 lists many of the most important β-lactamases spreading currently. Most tests in development or in use in reference laboratories focus on 1 or more of these β-lactamases, with a recent focus on the *Klebsiella pneumoniae* carbapenemases (KPCs). Many of these are culture-based methods with molecular detection of the resistance determinant from isolated colonies, and have prolonged TATs (48–72 hours). Recently, investigators have reported more-rapid detection of KPC-producing organisms using either an abbreviated broth enrichment (reducing TAT to 24 hours from 64–72 hours [37], with 97% sensitivity) or direct testing from nasal and rectal swabs (with TAT of only 2 hours [38]).

Even more challenging will be to develop rapid screening approaches (applied directly to samples) for multiple β-lactamase targets. The most promising available method for detection of multiple targets simultaneously relies upon microarray technology (Check-Points, Wageningen, the Netherlands), which has demonstrated the ability to accurately detect multiple important β-lactamases when applied to isolated bacterial colonies [39]. It remains to be seen if this technology, or the multiplex real-time PCR methods that are in development by several companies, will perform well when applied directly to complex patient samples (eg, rectal swabs) to reduce TAT. Such molecular assays will also require continuous updating, to incorporate detection of new β-lactamase genes that can arise via minor mutations.

Although truly rapid detection of MDR-GNR carriage is not yet widely available, any method that could accurately detect the most important MDR-GNR resistance mechanisms would be a tremendous advance upon the ability of most clinical laboratories to characterize MDR-GNRs and for infection prevention programs and public health authorities to better track emerging resistances.

WILL ADOPTING RAPID DETECTION ASSAYS REDUCE MDRO TRANSMISSION AND INFECTION RATES?

As the technology for rapid detection of MDRO carriers advances, the most important question is whether such

technology will be helpful in reducing MDRO transmission and infection rates. As discussed previously, active MDRO surveillance rests on the assumption that detecting uninfected MDRO carriers guides interventions that will either prevent MDRO transmission (eg, contact precautions) or prevent infection among carriers (eg, decolonization). Published data to support this assumption are weak and conflicting; however, some of the best-designed trials demonstrate no impact of active MRSA surveillance [40–42]. Notably, proponents of active MDRO surveillance sometimes blame the slow TAT of results for the failure of some studies to demonstrate effectiveness [42, 43].

To determine if this is the case, we need studies that compare rapid (<24-hour TAT) MDRO testing to slower (culture-based) testing, using concurrent control groups and measuring meaningful outcomes (MDRO transmission/acquisition and infection events). Such studies have been performed only for MRSA testing, and are summarized in 2 recent systematic reviews [44, 45]. Both reviews found the existing literature to be scant and the individual studies to have important limitations; as a result, rapid MRSA detection tests have not been convincingly demonstrated to reduce MRSA transmission or infection rates when compared with culture-based screening. As summarized in Table 3, however, some of the best-designed studies were only able to reduce the overall TAT of the rapid (PCR) tests to 19–22 hours [46–48]. This reiterates the challenge described earlier, of setting up a system to expedite all aspects of testing, including the extremely important postanalytic arm of notification and intervention.

In addition to comparative clinical studies, several groups have performed modeling studies to determine the likely impact of rapid MRSA testing on outcomes [49–51], and found that the improvement in TAT compared with culture methods provided little benefit at substantially increased cost, particularly if applied universally (ie, to all admitted patients).

Thus, currently, there is not sufficient evidence to conclude that investments in routine rapid MDRO testing will reduce MDRO transmission or infection rates. However, there is reason to believe that moving to rapid testing can reduce the number of contact precaution (isolation) days under policies that involve preemptive isolation either of all admitted patients or of those with prior history of MDRO carriage [52]. Given the negative consequences that contact precautions can have on patient satisfaction, patient safety, and cost of care [53], this impact of rapid MDRO detection should be considered in those hospitals that employ preemptive contact precautions while awaiting MDRO surveillance culture results.

The existing literature on rapid MRSA testing does not address the potential importance of rapid results reporting when using active surveillance during an MDRO outbreak. During outbreaks, the prompt detection and isolation of carriers can be important steps in containing spread [7]. Because certain MDR-GNRs, such as KPC-producing *K. pneumoniae*, can cause explosive, deadly, and difficult-to-control outbreaks [36], any test that could more rapidly detect carriers would be important in outbreak response.

In addition, as MRSA is endemic in healthcare settings worldwide, the MRSA testing literature also does not address the potential impact of early detection of an MDRO that has not yet been detected in a given locale. The challenges of detecting epidemiologically important MDR-GNRs with available culture-based phenotypic methods make it likely that a newly emerging MDR-GNR could spread within a facility long before it is discovered. Thus, a widely available molecular method that rapidly and accurately detects epidemiologically important MDR-GNRs will be an important step forward for clinical laboratories.

Table 3. Summary of Studies Assessing Impact of Rapid Versus Culture-Based Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Carriage, Limited to Those Using Concurrent Control Groups and Reporting MRSA Infection or Colonization Outcomes

Study	Design	TAT Difference	MRSA Outcome	Major Limitations
Aldeyab et al [46]	Nonrandomized cluster crossover trial	PCR: 19 h Culture: 52 h	No difference in event rates (acquisition + infection)	• Long TATs • Not randomized
Hardy et al [47]	Nonrandomized cluster crossover trial	PCR: 22 h Culture: 79 h	Reduced acquisition rate in PCR group (0.29 vs 0.41 per 100 bed-days)	• Long TATs • Not randomized • More unscreened in culture arm • 71% decolonized in PCR arm vs 41% in culture arm • Only 17% of MRSA carriers placed in isolation rooms
Jeyaratnam et al [48]	Cluster-randomized crossover trial	PCR: 22 h Culture: 46 h	No difference in acquisition or infection	• Long PCR TAT

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction; TAT, turnaround time.

FUTURE DIRECTIONS

Because of the many challenges discussed here, rapid detection of MDRO carriage remains in its infancy, with assays only widely available for MRSA. Future work will bring improved rapid testing options for other emerging MDROs, including new MDR-GNRs that are increasingly important public health threats. Those tests that have good performance characteristics will be useful adjuncts during management of MDRO outbreaks or when rates of a specific MDRO are elevated and not responding to standard ("tier 1") MDRO prevention approaches [5].

At the same time, well-designed clinical trials should be performed to help determine how these tests should be incorporated, if at all, into routine MDRO prevention efforts (ie, in nonoutbreak settings). Endemic MDRO infection and colonization rates are associated with so many factors that detecting any incremental benefit of rapid detection of MDRO carriers will require large multicenter cluster-randomized trials. These trials will be difficult to perform and resource intensive. However, funding such studies is an essential step in determining how best to approach MDRO prevention well into the future, and thus will be more than worth the investment.

Note

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