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# MINIREVIEW

# Current Trends in Rapid Diagnostics for Methicillin-Resistant *Staphylococcus aureus* and Glycopeptide-Resistant *Enterococcus* Species▽†

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Hospital-acquired (HA) infections are an increasing global problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) and glycopeptide-resistant *Enterococcus* (GRE) are multidrug-resistant organisms and are particularly frequent causes of HA infections that often prove difficult and expensive to treat. Major sources of MRSA and GRE causing infections are either the patient's own floras (endogenous infection), those acquired from another person (cross-infection), or those from substances recently contaminated by a human source (environmental infection). Active surveillance cultures from patients for carriage of MRSA and GRE facilitate an early contact isolation (and even treatment), thus preventing spread in the hospital and reducing costs (42). However, the time to result with conventional cultures is 2 to 3 days, which allows these organisms a sizeable time window for potential spread prior to the institution of contact precautions. Recently, several "rapid" diagnostic tests have been introduced that would be very beneficial in decreasing the time to detection, therefore reducing the risk of nosocomial transmission and infections, especially in high-risk patients. This review discusses the current state of the art on rapid and direct detection methods for MRSA and GRE from patient material and hopes to facilitate the infectious disease specialist or microbiologist in choosing an appropriate diagnostic test.

## MRSA

Shortly after the introduction of methicillin into clinical use, the occurrence of MRSA began increasing steadily in hospitals and in nursing homes. MRSA strains now constitute 20% to 55% of clinical isolates in Europe and the United States (1). Moreover, MRSA strains expressing heterogeneous resistance (a predominantly low-level-resistance population coexisting with a small proportion of highly resistant cells) are often mistaken for methicillin-sensitive *S. aureus* (MSSA) by conventional culture and represent a hidden reservoir in hospitals (reviewed in reference 3). Methicillin resistance in *S. aureus* is primarily mediated by the *mecA* gene, which codes for the modified penicillin-binding protein 2a (PBP 2a). *mecA* expression can be constitutive or inducible. *mecA* is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (*SCCmec*), and at least five types of *SCCmec* elements have been reported (reviewed in reference 21). Characteristics shared by all *SCCmec* elements are carriage of the *mec* and *ccr* (cassette chromosome recombinases) gene complexes and integration in the *S. aureus* genome at the 3′ end of an open reading frame (*orfX*) with an unknown function. Four and five types of *mec* and *ccr* genes have been identified, respectively, and *SCCmec* elements can be differentiated based on various combinations of these alleles.

The recent emergence of community-associated MRSA (CA-MRSA) within the hospital setting poses another significant public health threat (reviewed in reference 14). CA-MRSA can be distinguished from HA-MRSA by the presence of *SCCmec* types IV and V and the Panton-Valentine leukocidin exotoxin, the latter often associated with severe skin infections and necrotizing pneumonia. On the other hand, HA-MRSA strains mainly harbor *SCCmec* types I, II, and III and in contrast to CA-MRSA strains tend to be multidrug resistant with hallmark resistance to fluoroquinolones (reviewed in reference 14).

## GRE

While MRSA strains emerged shortly after methicillin began to be clinically used, GRE strains were observed only after almost 25 years of vancomycin use in clinical practice in the 1980s (9). The prevalence of GRE increased dramatically in the 1990s, and currently more than 20% of enterococcal isolates in U.S. hospitals are vancomycin resistant. The incidence of GRE infections in European hospitals is currently low. However, asymptomatic carriage in healthy individuals is common (reviewed in reference 28), and a recent report of an in vivo transmission of vancomycin resistance from GRE to MRSA underscores the potential danger of a coexisting reservoir of both pathogens (5).

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TABLE 1. Overview of currently available chromogenic media for MRSA

| Chromogenic medium | Gold standard or comparators | Reference | Total assay time (h) | Sampling site(s) | Direct inoculation or OE, h of incubation | % Sensitivity | % Specificity |
|---|---|---|---|---|---|---|---|
| ChromID | CHROMagar MRSA and ORSAB | Compernolle et al., 2007 (7) | 22–24 | Perineum, throat, nose, mouth, wounds and others | Direct, 22–24 h | 60.0 | 98.0 |
| | | | 48 | | Direct, 48 h | 77.0 | 94.0 |
| | IDI-MRSA, GenoType MRSA Direct, MRSA Select and CHROMagar MRSA | van Hal et al., 2007 (39) | 24–48 | Nose | Direct, 24 h | 72.0 | 95.0 |
| | | | | | Direct, 48 h | 81.0 | 65.0 |
| | | | | Groin and axilla | Direct, 24 h | 68.0 | 98.0 |
| | | | | | Direct, 48 h | 84.0 | 47.0 |
| | CHROMagar MRSA, ORSAB and selective mannitol broth | Perry et al., 2004 (32) | 22–24 | Nose, throat, axilla, perineum and wounds | Direct, 22–24 h | 80.0 | 99.5 |
| | | | 48 | | Direct, 48 h | 89.0 | 85.6 |
| | CHROMagar MRSA, MRSA Select and ORSAB | Nahimana et al., 2006 (30) | 16–18 | Nose, throat, perineum and infected sites | Direct, 16–18 h | 51.0 | 100.0 |
| | | | 42 | | Direct, 42 h | 82.0 | 98.0 |
| | | | | | OE, 24 h | 93.0 | 100.0 |
| | Conventional culture | Cherkaoui et al., 2007 (6) | 24 | Pooled nose and groin | Direct, 24 h | 76.0 | 97.0 |
| | | | 48 | | Direct, 48 h | 90.0 | 95.0 |
| | | | | | OE, 24 h | 94.0 | |
| MSRA Select | IDI-MRSA, GenoType MRSA Direct, MRSA Select and CHROMagar MRSA | van Hal et al., 2007 (39) | 24–48 | Nose | Direct, 24 h | 68.0 | 98.0 |
| | | | | | Direct, 48 h | 77.0 | 69.0 |
| | | | | Groin and axilla | Direct, 24 h | 52.0 | 98.0 |
| | | | | | Direct, 48 h | 60.0 | 66.0 |
| | Mannitol salt agar with 8 μg/ml cefoxitin | Louie et al., 2006 (26) | 18–24 | Nose | Direct, 18–24 h | 90.0 | 99.6 |
| | | | 48 | | Direct, 48 h | 97.0 | 93.0 |
| | | | 18–24 | Perianal and rectal | Direct, 18–24 h | 97.0 | 99.3 |
| | | | 48 | | Direct, 48 h | 100.0 | 90.0 |
| | ORSAB, mannitol salt agar and "in-house" selective medium | Ben Nsira et al., 2006 (2) | 24 | Nose, ulcers and suppurations | Direct, 24 h | 99.0 | 99.8 |
| | | | 48 | | Direct, 48 h | 99.0 | 99.3 |
| | CHROMagar MRSA and mannitol salt agar with oxacillin or cefoxitin | Stoakes et al., 2006 (38) | 18 | Nose and perineal | Direct, 18 h | 97.3 | 99.8 |
| | CHROMagar MRSA, ChromID and ORSAB | Nahimana et al., 2006 (30) | 16–18 | Nose, throat, perineum and infected sites | Direct, 16–18 h | 65.0 | 100.0 |
| | | | 42 | | Direct, 42 h | 80.0 | 98.0 |
| | Conventional culture | Cherkaoui et al., 2007 (6) | 24 | Pooled nose and groin | Direct, 24 h | 77.0 | 96.0 |
| | | | 48 | | Direct, 48 h | 91.0 | 79.0 |
| | | | | | OE, 24 h | 96.0 | |
| CHROMagar MRSA | IDI-MRSA, GenoType MRSA Direct, MRSA Select and CHROMagar MRSA | van Hal et al., 2007 (39) | 24–48 | Nose | Direct, 24 h | 75.0 | 88.0 |
| | | | | | Direct, 48 h | 79.0 | 79.0 |
| | | | | Groin and axilla | Direct, 24 h | 40.0 | 100.0 |
| | | | | | Direct, 48 h | 48.0 | 70.0 |
| | ChromID, MRSA Select and ORSAB | Nahimana et al., 2006 (30) | 16–18 | Nose, throat, perineum and infected site | Direct, 16–18 h | 59.0 | 99.0 |
| | | | 42 | | Direct, 42 h | 75.0 | 97.0 |
| | | | | | OE, 24 h | 95.0 | 99.0 |
| | MRSA ID and ORSAB | Compernolle et al., 2007 (7) | 22–24 | Perineum, throat, nose, mouth, wounds and others | Direct, 22–24 h | 67.0 | 98.0 |
| | | | 48 | | Direct, 48 h | 73.0 | 90.0 |
| | MRSA Select and mannitol salt agar with oxacillin or cefoxitin | Stoakes et al., 2006 (38) | 18–48 | Nose and perineal | Direct, 18–48 h | 82.9 | 99.1 |
| | ChromID, ORSAB and selective mannitol broth | Perry et al., 2004 (32) | 22–24 | Nose, throat, axilla, perineum and wounds | Direct, 22–24 h | 59.0 | 99.3 |
| | | | 48 | | Direct, 48 h | 72.0 | 92.1 |
| ORSAB | ChromID and CHROMagar | Compernolle et al., 2007 (7) | 22–24 | Perineum, throat, nose, mouth, wounds and others | Direct, 22–24 h | 57.0 | 92.0 |
| | | | 48 | | Direct, 48 h | 77.0 | 83.0 |
| | CHROMagar MRSA, MRSA Select and ChromID | Nahimana et al., 2006 (30) | 16–18 | Nose, throat, perineum and infected site | Direct, 16–18 h | 47.0 | 99.0 |
| | | | 42 | | Direct, 42 h | 67.0 | 98.0 |
| | | | | | OE, 24 h | 79.0 | 100.0 |
| | ChromID, CHROMagar and selective mannitol broth | Perry et al., 2004 (32) | 22–24 | Nose, throat, axilla, perineum and wounds | Direct, 22–24 h | 62.0 | 97.9 |
| | | | 48 | | Direct, 48 h | 78.0 | 93.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MRSA Select, mannitol salt agar and "in-house" selective medium | Ben Nsira et al., 2006 (2) | 24 | Nose, ulcers and suppurations | Direct, 24 h | 91.9 | 92.6 |
| | | 48 | | Direct, 48 h | 96.0 | 92.6 |
| Conventional culture | Cherkaoui et al., 2007 (6) | 24 | Pooled nose/groin | Direct, 24 h | 76.0 | 67.0 |
| | | 48 | | Direct, 48 h | 87.0 | 68.0 |
| | | | | OE, 24 h | 91.0 | |
| In-house PCR | Velasco et al., 2005 (40) | 24 | Clinical isolates | Direct, 24 h | 100.0 | 92.1 |
| | | 48 | | Direct, 48 h | 100.0 | 66.6 |
| MRSA Ident Agar | ChromID, CHROMagar, and Denim Blue agar | Hansen et al., 2006 (18) | 24 | Nose | Direct, 24 h | 50.0 |
| | | 48 | | Direct, 48 h | 50.0 | 84.1 |
| Chromogen Oxacillin *S. aureus* medium | Conventional culture | Cherkaoui et al., 2007 (6) | 24 | Pooled nose and groin | Direct, 24 h | 1.0 |
| | | | 48 | | Direct, 48 h | 53.0 |
| | | | | | OE, 24 h | 53.0 |
| Chromogenic MRSA/Denim Blue agar | ChromID, CHROMagar, and MRSA Ident agar | Hansen et al., 2006 (18) | 24 | Nose | Direct, 24 h | 90.0 |
| | | | 48 | | Direct, 48 h | 90.0 |

Resistance to glycopeptides is mediated by the *van* gene clusters, which produce resistance by altering the drug target from D-alanine-D-alanine to D-alanine-D-lactate (reviewed in reference 9). So far, eight genotypes of glycopeptide resistance, which differ in the level and range of resistance to glycopeptides and in transferability, have been described for enterococci. Five of the *van* genes are acquired (*vanA*, *B*, *D*, *E*, *G*) and three (*vanC1*, *C2*, *C3*) are intrinsic. Of these, *vanA* is the most prevalent and is predominantly found in *E. faecium* and *E. faecalis*, the enterococcal species responsible for most infections in humans. Both *vanA* and *vanB* are inducible; *vanA* codes for high-level resistance to both vancomycin and teicoplanin, and *vanB* for variable levels of resistance only to vancomycin. *vanC*-harboring enterococci have low-level resistance to vancomycin and rarely cause human infections. The *vanD*, *E*, and *G* gene clusters are relatively uncommon; *vanD* is associated with variable levels of resistance to both vancomycin and teicoplanin, while *vanE* and *vanG* are characterized by low-level resistance to vancomycin (9).

## "RAPID" CULTURE-BASED DETECTION METHODS

The general perception of culture-based methods as being slow and laborious is rapidly undergoing a change with the introduction of the chromogenic agars, some of which can efficiently produce results within 24 h and without the added setup, expertise, and costs required for molecular tests. An ideal chromogenic medium allows a definitive identification of microorganisms directly from the primary isolation plate, eliminating further subcultures and biochemical confirmatory tests. Detection depends on the incorporated colorless chromogenic substrate, which is designed to mimic a metabolic substrate and is cleaved by the targeted bacterial enzyme (selectivity also depends largely on the incorporated antibiotics). Once cleaved, the chromogen becomes both insoluble and colored and builds up within the bacterial cell, allowing an easy differentiation of the organism possessing the enzyme based on colony color. Other factors influencing the medium's performance include the ability to support the growth of bacterium (especially with small inocula), the effective inhibition of the competing bacterial flora (dependent on the type and concentration of antimicrobial compounds incorporated), user friendliness (ease of colored colony differentiation), and optimal culture sites.

**Chromogenic media for MRSA.** Chromogenic media for MRSA are shown in Table 1 (also see the supplemental material). Currently available chromogenic media for MRSA detection include ChromID (bioMérieux, Marcy l'Etoile, France), MRSA Select (Bio-Rad Laboratories, Belgium), CHROMagar MRSA (CHROMagar Microbiology, France; BD Diagnostics, Belgium), Chromogenic MRSA/Denim Blue agar (Oxoid, Basingstoke, United Kingdom), oxacillin resistance screening agar base (ORSAB; Oxoid), MRSA Ident agar (Heipha GmbH, Eppelheim, Germany), and Chromogen oxacillin *S. aureus* medium (Axon Labs AG, Stuttgart, Germany).

The chromogen in ChromID targets the α-glucosidase enzyme of *S. aureus*, and the inhibition of competing flora is brought about by the incorporation of cefoxitin (4 mg/liter), resulting in green-colored colonies of MRSA. MRSA Select incorporates a cephamycin derivative (proprietary formula-

tion, concentration unknown) and characterizes MRSA colonies by a pink color. CHROMagar contains cefoxitin (6 mg/liter) and a chromogen that also results in rose to mauve MRSA colonies. The CHROMagar formulation is available as dehydrated medium from CHROMagar Microbiology and as prepoured plates from BD Diagnostics. The chromogen in Chromogenic MRSA or Denim Blue agar detects phosphatase activity in *S. aureus* strains and, coupled with a selection with cefoxitin, produces denim blue colonies of MRSA. ORSAB, a modified version of mannitol salt agar, is made selective by the addition of oxacillin (2 mg/liter) to inhibit MSSA and polymyxin to suppress gram-negative bacteria. This medium incorporates aniline blue as a pH indicator, giving MRSA colonies a characteristic blue color. A latex agglutination test is recommended to confirm MRSA on ORSAB after 24 h of incubation. Colonies of MRSA on MRSA Ident agar are dusky pink- or ruby-colored due to a chromogenic phosphatase substrate and an antibiotic supplement including cefoxitin. Chromogen oxacillin *S. aureus* medium characterizes MRSA colonies by a pink-mauve color.

**(i) Antibiotics used as selective agents.** The penicillinase-resistant penicillins methicillin and oxacillin have been traditionally used in selective media for MRSA; however, cefoxitin, a cephamycin, shows better selectivity and is currently preferred for MRSA selection. MRSA strains exhibiting inducible resistance to methicillin grow much more readily in the presence of cefoxitin than oxacillin, possibly due to an enhanced induction of PBP 2a by cefoxitin (34). Moreover, induction with oxacillin requires an extended period for full expression. Hence, oxacillin-containing media achieve sufficiently high sensitivities only after 48 h of incubation (2, 32). Finally, inhibition of chromogen activity is also lower with cefoxitin than with oxacillin (32).

**(ii) Analytical sensitivity, specificity, and influence of prolonged incubation time and enrichment.** The currently available chromogenic media for MRSA detection show almost uniformly high specificities after 24 h of incubation, although sensitivities tend to vary widely both between media and between studies (2, 7, 26, 30, 38, 39). Prolonging incubation time to 48 h can improve sensitivities; however, specificities are adversely affected, necessitating confirmatory tests before reporting MRSA.

Studies investigating ChromID and MRSA Select generally corroborate the manufacturers' claims that after 24 h of incubation, characteristically colored colonies can be reported as MRSA without any need for confirmatory tests (2, 26, 30, 39). However, a few studies report problems with false-positive colonies on these media and recommend a quick confirmatory test at 24 h (Gram stain, latex agglutination) (7, 26, 38). False-positive colonies on ChromID are mainly coagulase-negative staphylococci (CoNS), *Enterobacter*, and *Stenotrophomonas maltophilia* (also an α-glucosidase producer) (7). Gram-negative bacteria are also not completely inhibited on ChromID due to an insufficient cefoxitin concentration (4 mg/liter), although these appear as colorless colonies and do not hamper MRSA detection (7). On MRSA Select, enterococci grow as colorless colonies and also do not hinder MRSA detection, although false-positive pink-mauve colonies of enterococci or diphtheroids and rarely *Enterobacteriaceae* or CoNS have also been observed (26, 38). On CHROMagar, however, enterococci appear as large, dark blue colonies due to which the smaller, pink MRSA colonies, especially when present in smaller numbers, might be obscured or completely missed (38). The frequency of false-positive colonies with ORSAB is high (31.6%) and is probably related to the low oxacillin concentrations in this medium (2 mg/liter) (40). Coupled with a long latency period for oxacillin induction, this medium requires 48 h of incubation and a coagulase test to achieve higher specificity and sensitivity (2).

Compernolle et al. compared ChromID, CHROMagar, and ORSAB and found CHROMagar to be the most sensitive (67%) after 24 h of incubation, although at 48 h, ChromID and ORSAB showed higher sensitivities (77%) (7). Specificities of both ChromID (98%, 24 h; 94%, 48 h) and CHROMagar (98%, 24 h; 90%, 48 h) were high and superior to those of ORSAB (92%, 24 h; 83%, 48 h) (7). Positive results on ChromID and CHROMagar after 24 h could be reported without additional testing. However, after 48 h, confirmatory tests are necessary and ChromID's superior specificity and sensitivity resulted in a workload lesser than that seen for the other two media (7). Also, in another study, not only did ChromID perform better than CHROMagar and ORSAB, but the former medium's sensitivity (80%) after 22 to 24 h of incubation was superior to those of both CHROMagar (72%) and ORSAB (78%) incubated for 48 h (32). van Hal et al. also found sensitivities of ChromID to be higher than those of CHROMagar and MRSA Select. However, the 72% and 81% sensitivities of ChromID at 24 and 48 h, respectively, meant that approximately 18% to 29% of MRSA-colonized patients would have been missed if this medium was utilized as a MRSA screening assay (39). The high specificities of all three media (95% to 99%) reiterated that confirmatory testing would not be necessary at 24 h (39). Cherkaoui et al. showed comparable sensitivities (76% to 77%) and specificities (96% to 97%) and a good colorimetric discrimination at 24 h on ChromID and MRSA Select, and both outperformed ORSAB, partly due to the substitution of oxacillin with a cephamycin in the first two media (6). At 48 h, sensitivities for both media increased to 90% to 91% and the specificity of ChromID remained high at 95%, while that of MRSA Select decreased to 79% (6). Another study compared ChromID and CHROMagar to the newly introduced Denim Blue agar (18). The latter medium showed sensitivities higher than and specificities comparable to (90%; 96%) those of CHROMagar (70%; 99.1%) and ChromID (60%; 99.3%) at 24 h. However, at 48 h, there was a large increase in false-positive colonies on Denim Blue agar, and the specificity of this medium decreased to 44.2% (18). Nahimana et al. found differences in sensitivities of ChromID, ORSAB, CHROMagar, and MRSA Select to be especially significant after 16 to 18 h of incubation. MRSA Select was the most sensitive (65%), followed by CHROMagar (59%), ChromID (51%), and ORSAB (47%), while at 42 h, the sensitivity of ChromID was maximum (82%) (30). Specificities for all four media were close to 100% at 24 h, and in contrast to most studies, which observe a decrease in specificity on prolonged incubation, specificities remained at 97% to 98% at 42 h (30). Prolonging incubation times to 42 to 48 h undoubtedly provides an improved sensitivity and yield; however, its overall utility remains debatable. Studies with MRSA Select show that the majority of the MRSA isolates (96% to 99.8%)

can be correctly detected after 18 to 24 h of incubation without any requirement for confirmatory testing (2, 26). Furthermore, sensitivities achieved in real practice might be even higher than those observed by these studies. Unlike analytical studies that inoculate test media using either a swab suspension in sterile saline or randomly inoculate a single swab on several media plates, in real practice, a single swab is inoculated onto one medium plate, delivering a much larger inoculum. The effect of inoculum size on the performance of CHROMagar, ChromID, and ORSAB was studied by Perry et al. by use of pure MRSA strains (32). With a large inoculum (10,000 CFU) and 24 h of incubation, CHROMagar could support the growth of all 37 MRSA strains, and ChromID and ORSAB could support the growth of 35 strains. With a smaller inoculum (100 CFU), 35, 34, and 30 strains could be identified as MRSA on CHROMagar, ChromID, and ORSAB, respectively (32).

The advantages of sample preenrichment versus direct plating were studied by Nahimana et al., who showed a substantial increase in sensitivity of ChromID, ORSAB, CHROMagar, and MRSA Select following enrichment (30). However, Cherkaoui et al. found that enrichment broth only marginally improved screening sensitivities for ChromID, ORSAB, and MRSA Select, and the additional reporting delay imposed by its use revealed it to be of questionable benefit (6). In our hands, a strategy of a direct inoculation of surveillance samples on MRSA Select combined with an overnight enrichment (OE) provided the best balance between rapid results and increased sensitivities (20). The direct inoculation allowed detection of 68% of the MRSA after 24 h, and the false-negative results could be resolved by plating the enrichment broths, resulting in an almost 30% increase in sensitivity (20).

**(iii) Effect of sampling site.** The anterior nares are the predominant site of MRSA carriage and expectedly all chromogenic media perform best in detecting MRSA from nasal swabs. Sensitivities for MRSA detection from nonnasal samples decrease either due to a larger amount of competing flora or due to lower levels of MRSA colonization at these anatomical sites. However, 2.6% to 26.9% of MRSA-colonized patients show exclusive groin, rectal, or perianal carriage and would be overlooked on nasal screening, which emphasizes the importance of screening nonnasal sites for MRSA carriage (4, 33, 44). van Hal et al. compared the performances of ChromID, MRSA Select, and CHROMagar with nose, groin, and axilla swabs (39). Combined sensitivities for all swabs and for axilla/groin swabs were higher for ChromID (71% and 68%, respectively) than for MRSA Select (64% and 52%) or CHROMagar (63% and 40%). However, sensitivities for MRSA detection from nasal swabs were higher for CHROMagar (75%) than for ChromID (72%) or MRSA Select (68%) (39). Perry et al. evaluated ChromID, CHROMagar, and ORSAB for detection from nasal, perineal, and throat swabs and found ChromID's performance to be superior to those of the two other media (32). Nahimana et al. used nose, throat, perineum, and other (wound, urine) sample types to compare ChromID, CHROMagar, MRSA Select, and ORSAB and showed higher sensitivities for MRSA Select for each specimen type (nose, 79%; throat, 50%; others, 77%) except for perineal swabs, for which the sensitivity was similar to that of ChromID and CHROMagar (≈60%) (30).

**(iv) Time to detection.** The time to detection is calculated from the time of the inoculation of the sample to the confirmation of the presence of the relevant microorganism, also including tests recommended by the manufacturers. The time to detection for direct cultures was found to be minimum for MRSA Select (1.35 days), followed by ChromID (1.65 days), CHROMagar (1.72 days), and ORSAB (2.31 days). Also, after enrichment, ChromID (2 days) performed better than CHROMagar (2.25 days) and ORSAB (3 days) (MRSA Select was not evaluated postenrichment) (30). Another study also showed faster cumulative processing times for ChromID compared to those for CHROMagar and ORSAB (7).

**(v) Cost effectiveness.** Chromogenic media are expensive, and this presents a major obstacle for their use in routine practice, as prices could be ~2 to 13 times higher than those of conventional media. However, a cost-effectiveness analysis should also take into account the number of subcultures, additional tests/reagents, and the technologist's time required to arrive at a definitive diagnosis.

A comparison of MRSA Select and mannitol-salt agar with 8 μg/ml cefoxitin found the costs incurred to be nearly equivalent for both media (Can$9,758 and Can$9,106, respectively) because of the greater number of suspicious colonies growing on mannitol-salt agar with 8 μg/ml cefoxitin, which required subculture and additional testing to exclude the presence of MRSA. In addition, examination and processing of most chromogenic media (MRSA Select and ChromID) requires less technologist time, which improves work flow efficiency in the laboratory (6, 26).

**A novel culture-based assay for MRSA detection.** That culture-based assays are no longer limited to a macroscopic identification of bacterial colonies is best exemplified by the new Baclite Rapid MRSA test (3M Healthcare, Berkshire, United Kingdom). This assay detects ciprofloxacin-resistant MRSA strains by measuring adenylate kinase activity using bioluminescence. A total assay time of 5 h combines adenylate kinase detection with selective broth enrichment, magnetic microparticle extraction, and selective lysis of *S. aureus* to add target organism specificity. An initial study with nasal swabs has shown promising results (90.4% sensitivity, 95.7% specificity, and 98.7% negative predictive value [NPV]) (22). The Baclite test requires a relatively low level of expertise and material costs are approximately US$9.5 to 12 per test, which is higher than a conventional culture-based method but much lower than most commercial molecular tests. While further evaluation is awaited, a very evident drawback of this assay is the likelihood of missing CA-MRSA as well as the few HA-MRSA strains that are sensitive to ciprofloxacin.

**Chromogenic media for GRE.** Chromogenic media for GRE are shown in Table 2. Development of chromogenic media for GRE has not been as rapid as for MRSA and until very recently, the most effective selective medium available for screening of GRE was bile esculin azide agar supplemented with 6 to 8 μg/ml of vancomycin (BEAV) or Enterococcosel agar (BD Diagnostics). Recent advances in the elucidation of highly specific chromogenic substrates with sensitivity sufficient to identify GRE have led to the development of ChromID VRE (bioMérieux) and CHROMagar GRE (BD Diagnostics) media; however, being rather new on the market, these media have not been extensively evaluated as yet. ChromID incorpo-

TABLE 2. Overview of currently available chromogenic media for GRE

| Chromogenic medium | Sample or site/sample | Gold standard or comparators | Source or reference(s) | Direct inoculation or OE, h of incubation | % Sensitivity | % Specificity | Total assay time (h) |
|---|---|---|---|---|---|---|---|
| ChromID VRE (prototype: VRE-BMX) | Stool | BEAV and PCR | Ledeboer et al., 2007 (23, 24) | Direct, 24 h | 96.4 | 96.6 | 24 |
| | | | | Direct, 48 h | 94.8 | 73.9 | 48 |
| | | BEAV | Kuch et al., submitted | Direct, 24 h | 73.3 | —[a] | 24 |
| | | | | Direct, 48 h | 93.3 | — | 48 |
| | | | | OE in selective broth with vancomycin, 24 h | 100.0 | — | 48 |
| | | | | OE in selective broth with vancomycin, 48 h | 100.0 | — | 72 |
| | Rectum and stool | Enterococcosel agar with 8 μg/ml vancomycin | Delmas et al., 2007 (12) | Direct, 24 h | 54.6 | 98.3 | 24 |
| | | | | Direct, 48 h | 54.6 | 85.1 | 48 |
| | | | | OE in selective broth with vancomycin, 24 h | 100.0 | 95.1 | 48 |
| | | | | OE in selective broth with vancomycin, 48 h | 100.0 | 89.0 | 72 |

[a] —, data not available.

rates chromogens targeted by enzymes specific for *E. faecium* and *E. faecalis*, and their degradation distinguishes the two species as purple and blue-green colonies, respectively. CHROMagar GRE has been developed for the specific detection of GRE with the *vanA* and *vanB* genotypes, while the *vanC* genotype is inhibited.The sensitivities for GRE detection from stool samples are similar for ChromID and BEAV/Enterococcosel agar following direct inoculation or enrichment (24) (A. Kuch, E. Stefaniuk, T. Ozorowski, and W. Hryniewicz, submitted for publication). Although a major advantage with ChromID is that dual colonization with *E. faecalis* and *E. faecium* is easily distinguishable, sensitivities for species-level identification are 85.4% and 90.0% at 24 h and 87.8% and 90.0% at 48 h for *E. faecium* and *E. faecalis*, respectively (24). Specificities for both media are 100% at 24 h and are sustained at 48 h for ChromID; however, they drop to 72% for BEAV (24). An adequate suppression of the abundant stool flora is a major concern while developing media for GRE. For ChromID, the majority of the breakthrough growth is observed in the first quadrant of inoculation, probably because an excessive inoculum overwhelms the vancomycin in the medium, while on BEAV, contaminants are found in all quadrants. Diluting samples in sterile saline prior to plating can help circumvent this problem (23).False-positive colonies on ChromID are most likely to be *Candida* spp. and gram-negative rods, and on BEAV, *Enterococcus* spp. (other than glycopeptide-resistant *E. faecalis*/*faecium*), gram-positive rods, and *Streptococcus* spp. are more commonly observed. At 24 h, BEAV grows 50% more contaminants than ChromID (23). Contaminants on ChromID are frequently colorless and are not likely to be interpreted incorrectly (24). Incubation of ChromID beyond 24 h does not seem to provide any improvement in sensitivity and also reduces the specificity due to breakthrough growth of normal glycopeptide-resistant flora like *Candida* spp. (23). Studies show that for the same response delay, reading ChromID plates after 24 h of incubation with enrichment provides a better performance than reading after 48 h of incubation and a direct inoculation (12) (Kuch et al., submitted). Use of an enrichment broth prior to plating does ensure a 100% detection rate of GRE (12), although the significance of increased sensitivity for the management of outbreaks has been questioned (8). A 24-h incubation for ChromID followed by a Gram stain of characteristically colored colonies might represent the best compromise between sensitivity and specificity (sensitivity, 54.6%; specificity and positive predictive value [PPV], 100%; NPV, 99%) (12). Cost comparisons of ChromID and BEAV, when used in conjunction with broth enrichment, 24 h of incubation, and Gram staining, showed that the numerous subcultures, supplementary identifications, susceptibility tests, and extra technician time required with BEAV actually allowed a saving of €0.62 per sample with the use of ChromID for an annual GRE prevalence of 2.2% (12).

## MOLECULAR DIAGNOSTICS FOR MRSA

Molecular diagnostics for MRSA are shown in Table 3. Since its discovery in the 1980s, PCR has become the cornerstone of molecular biology, and currently available molecular diagnostic tests also rely heavily on this technology. Benefits afforded by PCR over conventional culture include detection limits below those possible with culture, high-throughput screening (96 specimens/run), and, importantly, shorter times to detection. A significant reduction in MRSA transmission upon screening with a same-day PCR in comparison to culture was recently demonstrated by Cunningham and colleagues in an observational cohort study; MRSA transmission incidence decreased from 13.9/1,000 patient days with phenotypic (culture-based) MRSA surveillance to 4.9/1,000 patient days with a PCR screen (10).

In addition to classic PCR, numerous variations in combination with a variety of detection chemistries and automated systems are being developed. Currently available PCR-based molecular tests for MRSA include the LightCycler *Staphylococcus* and MRSA detection kit (LC assay; Roche Diagnostics, Mannheim, Germany), the Hyplex StaphyloResist PCR (BAG, Lich, Germany), the GenoType MRSA direct assay (Hain Lifescience, Nehren, Germany), the IDI-MRSA assay (GenOhm, San Diego, CA; BD Diagnostics), and the recently introduced GeneXpert MRSA assay (Cepheid, Sunnyvale, CA).

The LC *Staphylococcus* assay from Roche amplifies and detects a species-specific internal transcribed spacer region of the

TABLE 3. Overview of currently available molecular assays for MRSA detection

| Assay | Direct inoculation or OE | References | Gold standard or comparators | Sampling sites | % Sensitivity | % Specificity | % PPV | % NPV | Total assay time (h) |
|---|---|---|---|---|---|---|---|---|---|
| Hyplex StaphyloResist | Direct | Daeschlein et al., 2006 (11) | Conventional culture | Nose | 91.7 | 90.0 | 31.4 | 99.5 | 4.5–6 |
| | OE | Wagenvoort et al., 2007 (41) | Conventional culture and ORSAB | Nose, throat, perineum, and wounds | 97.6 | 83.7 | 37.4 | 99.7 | |
| | | Michiels et al., submitted | Conventional culture and in-house real-time PCR | Nose, throat, perineum, and wounds | 98.0 | 96.0 | 83.0 | 99.0 | 3.5 |
| LC *Staphylococcus* kit and LC MRSA detection kit | OE | Levi and Towner, 2005 (25) | Conventional culture | Nose, axilla, perineum, and others | 95.7 | 90.8 | 75.9 | 98.6 | —[b] |
| IDI-MRSA/GeneOhm MRSA | Direct | Paule et al., 2007 (31) | Conventional culture and in-house PCR | Nose | 98.0 | 96.0 | 77.4 | 99.7 | 2 |
| | | Bishop et al., 2006 (4) | Conventional culture | Nose | 90.0 | 91.7 | 56.3 | 98.8 | 2.5 |
| | | | | Groin | 83.3 | 90.2 | 46.9 | 98.1 | 2.5 |
| | | | | Pooled nose/groin | 88.0 | 91.6 | 61.1 | 98.1 | 2.5 |
| | | Rossney et al., 2007 (35) | Direct culture on MRSA Select | Nose | 89.0 | 89.0 | 63.0 | 97.0 | 2.5 |
| | | | Culture on MRSA Select after OE | Nose | 81.0 | 91.0 | 71.0 | 95.0 | 2.5 |
| | | | Culture on MRSA Select with and without OE | Throat | 89.0 | 99.0 | 98.0 | 97.0 | 2.5 |
| | | | | Groin/perineum | 88.0 | 99.0 | 93.0 | 98.0 | 2.5 |
| | | van Hal et al., 2007 (39) | MRSA ID, MRSA Select, and CHROMagar | Nose | 94.0 | 94.0 | 94.0 | 94.0 | 2–4 |
| | | | | Groin/axilla | 80.0 | 97.0 | 90.0 | 94.0 | 2–4 |
| | | Zhang et al., 2007 (44) | MRSA Select | Nose | 100.0 | 96.8 | 69.2 | 100.0 | — |
| | | | | Rectum | 93.1 | 95.4 | 61.4 | 99.4 | — |
| | | | | Open chronic wounds and exit sites | 100.0 | 95.0 | 60.0 | 100.0 | — |
| | NA[a] | Francois et al., 2007 (17) | Conventional culture | Characterized clinical isolates | 94.0 | 64.0 | 71.0 | 92.0 | NA |
| | OE | Desjardins et al., 2006 (13) | Conventional culture | Pooled nose/rectum | 96.0 | 96.0 | 90.0 | 98.0 | — |
| Genotype MRSA Direct | Direct | Holfelder et al., 2006 (19) | Conventional culture | Nose, throat, skin, groin, axilla, and wounds | 94.6 | 98.7 | 85.4 | 99.6 | 4 |
| | | van Hal et al., 2007 (39) | MRSA ID, MRSA Select, and CHROMagar | Nose | 70.0 | 96.0 | 95.0 | 73.0 | 6 |
| | | | | Groin/axilla | 68.0 | 96.0 | 85.0 | 90.0 | — |
| | | Francois et al., 2007 (17) | Conventional culture | Characterized clinical isolates | 90.0 | 53.0 | 66.0 | 84.0 | — |
| GeneXpert MRSA assay | Direct | Mehta et al., 2007 (29) | IDI-MRSA and conventional culture | Nose | 98.5 | 90.4 | — | — | — |

[a] NA, not applicable.
[b] —, data not available.

rRNA operon, and differentiation of *S. aureus* from CoNS is based on a melting curve analysis. *S. aureus*-positive samples are further tested for *mecA* with the LC MRSA detection kit. Levi et al. tested the LC assay on pooled patient screening swabs (two to five body sites) cultured overnight in selective (oxacillin 2 mg/liter) broths in comparison to ORSAB (25). Using a lysostaphin-lysozyme-selective extraction method, the LC assay showed a 95.7% sensitivity, a 90.8% specificity, and NPV and PPV of 98.6% and 75.9%, respectively.

Hyplex StaphyloResist is a multiplex PCR–enzyme-linked immunosorbent assay that detects a species-specific genetic element of *S. aureus* and the *mecA* gene. Additional modules (Hyplex StaphyloResist plus PCR module) also allow the detection of mupirocin resistance (*ileS*; isoleucyl-tRNA synthetase gene) and of CA-MRSA (the *lukS-lukF* genes coding for the Panton-Valentine leukocidin exotoxin). The running time for the Hyplex StaphyloResist PCR is longer than that for an in-house real-time PCR (3 h 25 min versus 2 h 30 min), although hands-on times for both assays are similar (1 h 30 min and 1 h 15 min, respectively) (M. Michiels, H. Jansens, H. Goossens, and M. Ieven, submitted for publication). Daeschlein et al. compared the Hyplex StaphyloResist with conventional culture for direct detection of MRSA carriage from nasal swabs and found the sensitivity of the former to be much higher (91.6% versus 75%) (11). NPVs were similar and high for both methods (99.5%); however, the PPV for the Hyplex StaphyloResist was only 31.4% (11). Wagenvoort et al. showed that pooling specimens (nose, throat, perineum, wounds/infections) followed by an 18-h enrichment in a nonselective broth could increase the sensitivity (97.6%), and to some extent, the PPV (37.4%) of the Hyplex StaphyloResist, while still retaining a high specificity (83.7%) and NPV (99.7%) (41). A markedly low PPV and a high frequency of false-positive results are observed with the Hyplex StaphyloResist assay because this assay detects two unlinked markers (an *S. aureus* species-specific gene and *mecA*), and mixed cultures of MSSA and MRCoNs are likely to give positive results. A solution to this problem might be an OE of pooled specimens in a selective broth with oxacillin (4 μg/ml), which in our hands increased sensitivities and specificities of Hyplex StaphyloResist to 98% and 96%, respectively (Michiels et al., submitted). However, this assay's utility for direct MRSA detection from clinical specimens remains rather limited. The high NPV observed with both the Hyplex StaphyloResist and the LC assays make these suitable for the rapid identification of MRSA-negative individuals in a low-MRSA-prevalence setting. Positive findings can be further confirmed by culture before any individual is definitively determined to be a MRSA carrier.

A superior approach for MRSA detection is based on the detection of a single amplicon, which includes the right junction of the *SCCmec* downstream of the *mecA* gene and a part of the adjacent *S. aureus*-specific *orfX* gene. This has been successfully utilized in commercial assays like the GenoType MRSA Direct, the IDI-MRSA, and the GeneXpert MRSA assay. The Genotype MRSA Direct targets *SCCmec* types I to V in a multiplex PCR using biotinylated primers followed by a reverse hybridization step. Direct detection of MRSA from nose, throat, groin, axilla, wound, and other sites shows a high sensitivity, specificity, PPV, and NPV (94.59%, 98.73%, 85.37%, and 99.57%, respectively) in comparison to culture (19). An updated version of the assay, the Genoquick MRSA dipstick assay, does away with the reverse line hybridization step to reduce the total assay time from 4 h to 2 h 20 min. The only study comparing the GenoQuick assay to the Genotype MRSA Direct and to CHROMagar (BD Diagnostics) has demonstrated an impressive diagnostic sensitivity, specificity, PPV, and NPV for the former assay (100%, 99.4%, 96%, and 100%, respectively) (16).

The IDI-MRSA (also called GeneOhm MRSA) is a multiplex qualitative real-time PCR assay and is an FDA-approved assay for the direct detection of nasal colonization by MRSA. This assay can be semiautomated using the SmartCycler instrument (Cepheid). The assay's most recent version (V3) contains primers targeting the right-junction sequences of *SCCmec* types I, II, III, IVa, IVb, IVc, and V, combined with one consensus primer and three molecular beacons specific for the *orfX* gene. This assay showed a sensitivity, specificity, PPV, and NPV of 98%, 96%, 77%, and 99.7%, respectively, for direct detection from nasal swabs (31). Sensitivities and specificities for direct detection from throat and groin/perineum samples (89%, 99% and 88%, 99%, respectively) were also high (35). Another study comparing IDI-MRSA to the Genotype MRSA and to three chromogenic media (MRSA-Select, ChromID, and CHROMagar) found IDI-MRSA to be the most rapid (2 to 3 h) and sensitive (90% overall) method independent of the sampling site, although the assay's sensitivity declined from 94% with nasal swabs to 80% with swabs from other sites, with groin swabs accounting for 75% of the missed samples (39). While there is a general consensus for multiple-body-site screening to achieve optimal detection of MRSA carriers, the IDI-MRSA's price (US$36.70/test) also necessitates pooling specimens. Bishop et al. showed sensitivities and specificities for pooled nose-groin specimens comparable to those processed separately with the IDI-MRSA (sensitivities, 90.0% for the nose, 83.3% for the groin, and 88.0% for the combined nose-groin specimen; specificities, 91.7%, 90.2%, and 91.6%, respectively) (4). Desjardins et al. assessed the utility of an overnight incubation of pooled nasal and rectal swabs in selective enrichment broth with aztreonam and ceftizoxime and showed a high sensitivity, specificity, NPV, and PPV (96%, 96%, 90%, and 98%, respectively) for the IDI-MRSA assay in comparison to culture (13). Rectal swabs are likely to contain PCR inhibitors, and pooling rectal and nasal specimens in broth decreases the inhibition rate of the IDI-MRSA to <1% (13). The time lag in the reporting of results due to an OE step remains a major drawback; nonetheless, owing to the high sensitivity of PCR, a shorter (3- to 4-h) incubation time might be just as effective and warrants evaluation.

The GeneXpert MRSA is another FDA-approved real-time PCR assay and works on a fully automated GeneXpert platform. The assay proves expensive (US$35 to 55). However, total assay time is short (75 min), and since the assay is fully automated, both the hands-on time (2 min) and the level of expertise required for operation are minimal. A comparison of the GeneXpert and the IDI-MRSA showed similar sensitivities (98.5% and 97.1%, respectively) and specificities (90.4% and 89.2%, respectively) for MRSA detection from nasal samples (29).

In order to highlight the impact of novel emerging *SCCmec*

TABLE 4. Overview of currently available molecular assays for GRE detection

| Assay | Direct inoculation or OE | References | Gold standard or comparators | Sampling site(s) | % Sensitivity | % Specificity | % PPV | % NPV | Total assay time (h) |
|---|---|---|---|---|---|---|---|---|---|
| GeneOhm VRE | Direct | Stamper et al., 2007 (37) | BEAV with 6 μg/ml vancomycin and OE in BEAV broth with 8 μg/ml vancomycin | Stool | 95.4 | 86.5 | 79.0 | 97.2 | —[a] |
| | | | | Rectum/perineum | 98.3 | 87.5 | 71.1 | 99.4 | — |
| LC *vanA*/*vanB* detection assay | Direct | Sloan et al., 2004 (36) | Enterococcosel agar with 6 or 8 μg/ml vancomycin | Stool, perineum | 100.0 | 97.0 | 42.0 | 100.0 | 3.5 |
| | | Young et al., 2007 (43) | PCR | Stool | 100.0 | 36.0 | — | — | — |
| | OE | Young et al., 2007 (43) | PCR | Stool | 100.0 | 30.0 | — | — | — |
| | | Drews et al., 2006 (15a) | Conventional culture | Stool | 100.0 | 100.0 | — | 100.0 | — |
| | | | | Rectum | 84.0 | 96.0 | — | 100.0 | — |

[a] —, data not available.

elements on the diagnostic accuracies of molecular assays targeting the *orfX-SCCmec* junction, Francois et al. evaluated the performance of the IDI-MRSA and Genotype MRSA Direct on well-characterized MRSA isolates from diverse genetic backgrounds (17). The IDI-MRSA and Genotype MRSA Direct showed 94% and 90% sensitivities; however, specificities were surprisingly low at 64% and 53%, respectively. While these assays failed to detect isolates harboring nontypeable or recently described *SCCmec* cassettes (variants of *SCCmec* IV and V) (17, 35), high rates of false-positive results could also be explained by the homology between the *orfX* moieties in *S. aureus* and other coagulase-negative species, such as *S. haemolyticus* or *S. epidermidis*, or with the capsular polysaccharide cluster *SCCcap1*, which also has a similar integration site in the *S. aureus* genome as the *SCCmec* elements (27). Furthermore, MSSA strains carrying a partially excised *SCCmec* and having lost the *mecA* gene can also give false-positive results on "single-locus" assays targeting the *orfX-SCCmec* junction. Hence, as new *SCCmec* variants emerge, iterative design modifications and revalidation of these assays are warranted.

## MOLECULAR DIAGNOSTICS FOR GRE

Molecular diagnostics for GRE are shown in Table 4. Currently available molecular tests for GRE detection include the LC *vanA*/*vanB* detection assay (LC assay; Roche Diagnostics, Basel, Switzerland), and the GeneOhm VanR assay (BD Diagnostics-GeneOhm, San Diego, CA).

The LC real-time PCR assay targets the *vanA*, *vanB*, and *vanB2* and *vanB3* genes for direct detection of GRE from fecal or perianal swab samples, and the time to detection, including nucleic acid extraction, is 3.5 h. Sloan et al. compared the LC assay to Enterococcosel agar for GRE detection from perianal swabs, and the former assay detected twice the number of positive samples as the agar method (36). A large number of these culture-negative samples were true positives and represented *vanB*-harboring enterococci that might have been inhibited by the vancomycin incorporated in agar plates or possibly even nonviable or noncultivable enterococci (36). Because of the speed and ease of performance of the LC assay, it is conceivable that all patients could be screened for GRE before admission to health care institutions, including hospitals and nursing homes. However, it is important to note that the LC assay detects *van* genes rather than GRE per se. Thus, the presence of *vanB* in nonenterococcal species could result in an overestimation of the rate of fecal GRE colonization (15), and decrease the specificity (30% to 36%) of detection of *vanB* GRE in screening specimens by the LC assay (43). A similar problem is observed with the GeneOhm VanR assay, which also detects the *vanA*/*vanB* genes on a real-time PCR. Direct detection of GRE from stool and rectal specimens shows a high sensitivity (96.6%); however, specificity is decreased (87%) largely due to false-positive results observed with the *vanB* part of the assay (37). When used only to detect *vanA* resistance, the GeneOhm VanR assay was 94.4% sensitive and 96.4% specific, with a PPV and an NPV of 91.3% and 97.7%, respectively (37). Hence, both the LC and GeneOhm VanR assays can be used as GRE screening tests in populations that show a predominant colonization with *vanA*-harboring enterococci, while *vanB*-positive samples need to be confirmed by another method for the presence of GRE.

## CONCLUSIONS

Giant steps have been taken during the last decade to increase the diagnostic potential of the microbiology laboratory, mainly by implementing methods for DNA and RNA amplification. PCR-based molecular diagnostics have allowed not only a clinically relevant turn-around time for a diagnostic result but also an increased sensitivity and specificity by linking it to an end-stage hybridization/enzyme-linked immunosorbent assay or real-time detection step. On the other hand, the high cost and relatively high operator skill requirement required for these tests are obstacles to their widespread routine use and have led to the development of simpler culture-based albeit rapid detection methods. Several studies have analyzed and compared these detection methods; however, these studies differ significantly in the design, gold standards used, sampling sites, and enrichment protocols. Choice of gold standards especially bears a large influence on the study results. For instance, using an "inferior" assay as a gold standard could (artificially) boost the performance results of the test method.

Such factors should be taken into account before comparing study results. Our critical assessment of the existing literature identified several assays that perform well; however, there is as yet no single stand-alone rapid assay that can recover all MRSA or GRE strains. In addition, a need for even faster (and cheaper) diagnostic tests is being felt, highlighted by the mortality associated with MRSA/GRE infections, the ever-increasing problem of antimicrobial resistance, and the lack of new antibacterials in the pharmaceutical pipeline. Recognizing this need, the European Commission-funded project MOSAR (*M*astering H*os*pital *A*ntimicrobial *R*esistance and its spread into the community; https://www.mosar-sic.org/) brings together small and medium-sized (<250 employees) biotechnology enterprises and academic groups in order to achieve two major goals: (i) to study the impact of rapid diagnostic interventions on MRSA/GRE colonization and infection rates in hospitals, and (ii) to apply cutting edge technological advances for the development of next-generation rapid diagnostic assays for MRSA and GRE detection.

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We declare that we have no conflict of interest.

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