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Evaluation of Rapid Methyl-Alpha-D-Glucopyranoside Medium (MGP) for the Differentiation of Vancomycin Resistant Enterococci.

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Abstract

Enterococcus spp. is often associated with various types and degrees of antimicrobial resistance. In particular, resistance to glycopeptides (vancomycin) is of great concern for clinicians and infection control committees. *Enterococcus faecalis* and *Enterococcus faecium*, are the two most frequently encountered species of enterococci of clinical significance. On rare occasions, several other miscellaneous species of enterococci such as *E. casseliflavus* and *E. gallinarum* have also been reported as pathogens.

All of these species can present different levels of acquired or intrinsic resistance to vancomycin. *E. faecalis* and *E. faecium* are often associated with a high or moderate level of acquired resistance to vancomycin (*vanA* and *vanB*) while *E. casseliflavus* and *E. gallinarum* usually present a low level of intrinsic resistance (*vanC*). Rapid and accurate identification of these enterococci is important in preventing inappropriate antibiotic therapy and to avoid implementation of costly infection control measures. While molecular methods represent the gold standard in the identification of enterococci and VRE, many laboratories lack the time and resources for such procedures and thus continue to rely on biochemical methods for the differentiation and identification of enterococci species.

MGP Medium, an alternative identification assay with comparable sensitivity to other identification methods, is a simple carbohydrate test that differentiates pathogenic *E. faecalis* and *E. faecium* isolates from non-significant *E. gallinarium* and *E. casseliflavus* species. MGP Medium turns yellow when inoculated with organisms such as E. gallinarium and E. casseliflavus, while remaining blue for E. faecalis and *E. faecium*. Recently, the original formula was modified to decrease the incubation time to five hours. The intention was to develop an accurate, affordable, and rapid method to distinguish *vanA* and *vanB* from *vanC* vancomycin resistant enterococci (VRE). The rapid MGP Medium was evaluated against 70 clinical isolates of vancomycin resistant Enterococci (40 *E. faecium*, 10 *E. faecalis*, 10 *E. casseliflavus*, and 10 *E. gallinarum*), which were previously identified to the species level using traditional biochemical methods. The vancomycin MICs were determined using Etest as a supporting speciation tool.

Rapid MGP showed sensitivity and specificity of 100%. The vancomycin MIC values were in accordance to the species identified. Rapid MGP was shown to be an inexpensive beneficial addition to any clinical laboratory for rapid screening of Enterococci. It is useful in guiding proper therapeutic decisions, reducing unnecessary and costly infection control, and preventing unneeded surveillance measures.

Introduction

The identification of hospitalized patients infected with vancomycin-resistant enterococci (VRE) has become an important component of infection control programs aimed at minimizing patient-to-patient transmission of these organisms. A variety of vancomycin-containing media have been shown to provide a simple and cost-effective means for differentiating VRE from non-VRE, and the use of these media has been adopted in many clinical laboratories. In addition to organisms possessing high-level, transferable vancomycin resistance (*vanA* and *vanB* phenotypes predominantly in *Enterococcus faecalis* and *Enterococcus faecium*), those enterococci that intrinsically express low-level resistance to glycopeptide antibiotics (vanC), namely, Enterococcus casseliflavus and Enterococcus gallinarum, will also grow on vancomycin-containing media. In contrast to vancomycin-resistant isolates of *E. faecalis* and *E. faecium*, isolates of intrinsically vancomycin-resistant enterococci have not been implicated in outbreaks of VRE infection and appear to be of minimal concern from an infection control standpoint. Since occasional isolates of both *E. gallinarum* and *E. casseliflavus* that harbor the transmissible *vanA* gene have been identified, the determination of the level of vancomycin resistance in clinically significant isolates of VRE may still be warranted. The ability to accurately differentiate *E. gallinarum* and *E. casseliflavus* from other VREs, especially *E. faecalis* and *E. faecium*, is nonetheless of considerable importance and, unfortunately, has proven to be somewhat problematic for commercial biochemical identification systems. A number of tests for rapidly and inexpensively identifying VRE have been described in the literature. In the present study we examined the Rapid MGP (readable in five hours) for its ability to differentiate vancomycin resistant isolates of *E. gallinarum* and *E. casseliflavus* from *E. faecalis* and *E. faecium*.

Materials and Methods

- A total of 70 previously identified clinical isolates of *Enterococcus facium* (n=40), *Enterococcus faecalis* (n=10), *Enterococcus casseliflavus* (n=10), and *Enterococcus gallinarum* (n=10) retrieved from Hardy Diagnostics' collection were evaluated in this study.
- All isolates were previously phenotypically determined and confirmed with reduced susceptibility to vancomycin.
- Bacterial suspensions equivalent to 0.5 McFarland were prepared from freshly grown cultures and inoculated on BHI Agar with 6 µg/mL vancomycin as primary screening for reduced susceptibility to vancomycin.
- The MICs of vancomycin were determined by Etest methodology on isolates that grew on BHI agar with 6 µg/mL vancomycin.
- The species differentiation was determined by Rapid MGP Medium (Hardy Diagnostics).
- Isolates that turned the Rapid MGP yellow were considered as positives from MGP acidification, therefore categorized as *E. casseliflavus* or *E. gallinarum*.
- Rapid MGPs that remained blue were interpreted as negative for Rapid MGP, therefore categorized as *E. faecium* or *E. faecalis*.
- Isolates that presented MICs >256 µg/mLwere considered to have high level resistance to vancomycin, suggestive of *vanA* phenotype.
- *E. faecium* and *E. faecalis* isolates that presented MICs 8 to 256 µg/mLwere considered to have intermediate level resistance to vancomycin, suggestive of *vanB* phenotype.
- *E. casseliflavus* and *E. gallinarum* that presented high MICs were considered to harbor additional phenotype (*vanA* or *vanB*) in addition to *vanC*.

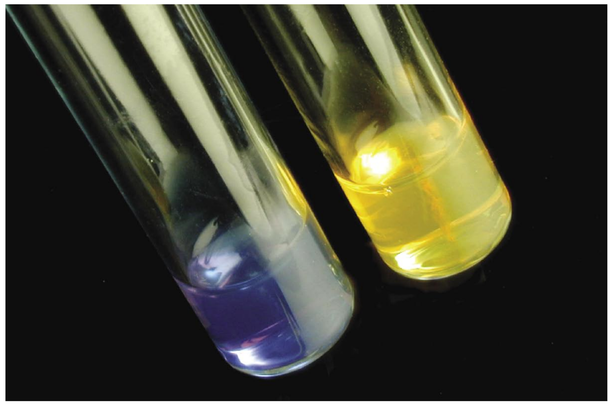
Results

Table 1. Overall Results

Isolate	Growth on BHI Agar with 6 µg/m of vancomycin	Vancomycin MIC (µg/mL)	Rapid MGP Medium	Probable phenotype
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	64	-	*vanB*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	>256	-	*vanA*
E. faecium	+	16	-	*vanB*
E. faecium	+	8	-	*vanB*
E. faecium	+	128	-	*vanB*
E. faecium	+	16	-	*vanB*
E. faecium	+	96	-	*vanB*
E. faecium	+	96	-	*vanB*
E. faecium	+	96	-	*vanB*
E. faecium	+	64	-	*vanB*
E. faecium	+	96	-	*vanB*
E. faecium	+	96	-	*vanB*
E. faecalis	+	32	-	*vanB*
E. faecalis	+	96	-	*vanB*
E. faecalis	+	96	-	*vanB*
E. faecalis	+	96	-	*vanB*

Isolate	Growth on BHI Agar with 6 µg/m of vancomycin	Vancomycin MIC (µg/mL)	Rapid MGP Medium	Probable phenotype
E. faecalis	+	8	-	*vanB*
E. faecalis	+	12	-	*vanB*
E. faecalis	+	12	-	*vanB*
E. faecalis	+	>256	-	*vanA*
E. faecalis	+	>256	-	*vanA*
E. faecalis	+	>256	-	*vanA*
E. gallinarum	+	>256	+	*vanC + vanA?*
E. gallinarum	+	4	+	*vanC*
E. gallinarum	+	12	+	*vanC*
E. gallinarum	+	24	+	*vanC*
E. gallinarum	+	6	+	*vanC*
E. gallinarum	+/-	4	+	*vanC*
E. gallinarum	+	8	+	*vanC*
E. gallinarum	+	8	+	*vanC*
E. gallinarum	+	8	+	*vanC*
E. gallinarum	+	32	+	*vanC*
E. casseliflavus	+	6	+	*vanC*
E. casseliflavus	+	6	+	*vanC*
E. casseliflavus	+	6	+	*vanC*
E. casseliflavus	+/-	4	+	*vanC*
E. casseliflavus	+	6	+	*vanC*
E. casseliflavus	+	6	+	*vanC*
E. casseliflavus	+/-	4	+	*vanC*
E. casseliflavus	+	6	+	*vanC*
E. casseliflavus	+/-	4	+	*vanC*
E. casseliflavus	+	8	+	*vanC*

- Rapid MGP Medium correctly differentiated between all *E. faecium*/*E. faecalis* and *E. casseliflavus*/*E. gallinarum* within five hours of incubation.
- All *E. faecium* and *E. faecalis* were MGP negative.
- All *E. casseliflavus* and *E. gallinarum* were MGP positive.
- 11 of 40 *E. faecium* showed intermediate resistance to vancomycin suggestive of *vanB* phenotype. The remaining 29 presented MICs >256 µg/mL consistent to *vanA* phenotype.
- 7 of 10 E. faecalis showed intermediate resistance to vancomycin suggestive of *vanB* phenotype. Three presented MICs >256 µg/mL consistent of *vanA* phenotype.
- Most of *E. casseliflavus* and *E. gallinarum* showed low resistance to vancomycin suggestive of vanC phenotype.
- One isolate of *E. gallinarum* presented high level resistance to vancomycin suggestive of plasmid-mediated vanA phenotype in addition to *vanC* phenotype.



Discussion/Conclusion

Early detection of patients colonized or infected with VRE is an essential component of any hospital program designed to prevent nosocomial transmission of VRE. Once the prevalence of VRE reaches high levels within an institution, prevention of transmission becomes troublesome. The microbiology laboratory is the first line of defense against the spread of VRE in the hospital. The ability of the laboratory to identify enterococci and to detect vancomycin resistance promptly and accurately is essential. Recognizing VRE colonization and infection avoids complex and costly measures that are required when recognition of the problem is delayed. Isolates with transferable *vanA* or *vanB* genes, usually present in *E. faecium* and *E. faecalis*, are important from an infection control perspective, whereas those with *vanC* (*E. casseliflavus* / *E. gallinarum*) have not been associated with nosocomial outbreaks.

The results of this study show that Rapid MGP Medium was reliable in aiding in the speciation of Enterococci with reduced susceptibility to vancomycin by differentiating what's relevant in terms of infection control intervention.

For more information on Rapid MGP contact Andre Hsiung, MS (email: hsiunga@hardydiagnostics.com)

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