A Novel Chromogenic Agar Medium for the Detection of Vancomycin Resistant Enterococci (VRE) John Merlino, Jonathan Natoli, and Glenn Funnell

Department of Microbiology and Infectious Diseases Concord Repatriation General Hospital, Concord, 2139, NSW Australia

JMerlino@med.usyd.edu.au or merlinoj@email.cs.nsw.gov.au

INTRODUCTION

In clinical laboratories phenotypic detection of low-level vancomycin resistance in enterococci (VRE) is a problem. Chromogenic detection of VRE during screening, especially for those that carry the *vanA* or *vanB* gene, will minimize the risk and spread of colonization and infections, a major infection control goal in Australia.

AIM

This is a two part study to determine the likely effectiveness of a novel chromogenic agar medium CHROMagar VRE (CHROMagar, Paris) for the screening of rectal swabs collected from hospitalized patients for the presence of Vancomycin Resistant Enterococci (VRE).

METHODS AND RESULTS

Part 1.

59 isolates of VRE (3 VanA, 44 VanB and 12 VanC) were cultured onto CHROMagar VRE (CVRE) and onto Enterococcosel Agar (EA) (Becton Dickenson; code: 212205) at a concentration of 10⁵ cfu/ml using a replicator technique¹. The EA supported the growth of all isolates, including the 12 VanC, whilst the CVRE only supported the growth of the VanA and VanB isolates. The VanC isolates were inhibited. On CVRE, VanA and VanB VRE isolates grew as pink to mauve in colour at 24h and the two types could not be differentiated by colour or colony morphology.

<u>Chromogeni</u> VRE	c Detectio	on of V	RE on CHROM	agar <u>De</u> <u>6n</u>	tection of VI	<u>RE on Ente</u> lycin	erococc	osel agar with	
	VanA n=3	Van n=4	B VanC 4 n=12			VanA n=3	Vanl n=44	B VanC 4 n=12	
Positive	3	44	0		Positive	3	44	9	
Negative	0	0	12		Negative	0	0	3	
	Enterococcus Enterococcus					Enterococcus		Enterococcus	
	VanA/B		VanC			VanA/B		VanC	
Positive	47		0		Positive	47	7	9	
Negative	0		12		Negative	0		3	
CHROMagar V	/ <u>RE :</u> Ser	sitivity	/ 100%	Entero	ococcosel ag	l <u>ar</u> Sei	nsitivity	100% PPV 84%	
	Spe	cificity	100%	6mg/L	Vancomycii	<u>n:</u> Sp	ecificity	25% NPV 100%	

Part 2.

A pilot study of 27 rectal swabs on which VRE screens were requested was undertaken to further evaluate the usefulness of the CVRE plate in comparison to the EA. The agars were incubated for 24 and 24-48 hours respectively as per manufacturer's instructions. Suspicious colonies (pink to mauve on CVRE and black on EA) were further processed to determine their genotype by real-time (Rotorgene, Corbett) and gel-based PCR systems. Further processing was required on only one of the 27 swabs plated to CA, but to 9 of the swabs plated to the EA. One VanB *E.faecalis* was isolated from one of the swabs using both agars. The remaining 8 possible VRE isolates detected on the EA were found to be neither VanA nor VanB (hence VanC), and were subsequently identified as *E.casseliflavus* (n=3) and *E.gallinarum* (n=5).



Data presented in this study shows that CVRE provides a viable alternative medium for screening patients colonised with VanA and VanB VRE genotypes. The new chromogenic medium was more specific in detecting VanA and VanB VRE when compared to EA, which also detected the VanC genotype. The suppression of the VanC genotype by CVRE should provide significant savings in both scientist time and follow-up testing costs, and as it is a 24 hour culture, it should also allow for a decreased turn-around time compared to the 24-48 hour culture time recommended for EA.

References: (1) Merlino, J et al. Enzymatic chromogenic identification and differentiation of enterococci. Aust. J. Med. Sci. 1998; 19:76-80.