Rapid Identification And Confirmation Of Carbapenem-Resistant Enterobacteriaceae Using *Brilliance* CRE Agar And Sensititre Gram-Negative Plate Format

Butler, D. A.[1], Powell, K.[1], Oleksiuk, M.[2]

[1]Thermo Fisher Scientific, East Grinstead, West Sussex, UK. [2]Thermo Fisher Scientific, Basingstoke, Hants, UK.

Overview

Purpose: The purpose of the study was to determine whether Thermo Scientific™ Sensititre™ GN4F antimicrobial susceptibility panels could be used directly with Thermo Scientific™ *Brilliance*™ CRE Agar for the MIC determination of suspected carbapenem-resistant *Enterobacteriaceae* (CRE).

Methods: A collection of 48 CRE clinical isolates were each inoculated onto *Brilliance* CRE Agar and Columbia Blood Agar (CBA). Sensititre GN4F panel inocula were prepared from colonies grown on both *Brilliance* CRE Agar and CBA plates. The GN4F panels were read automatically using the Thermo Scientific™ Sensititre™ ARIS™ 2X and visually using the Thermo Scientific™ Vizion™ System. For each isolate, the carbapenem MIC results on the Sensititre GN4F panel inoculated directly from growth on *Brilliance* CRE Agar were compared to the MIC results on the GN4F panel inoculated from growth on CBA.

Results: MIC results can be determined directly from growth on *Brilliance* CRE Agar using the Sensititre GN4F plate format. In the majority of cases the MIC was unaffected by inoculation directly from *Brilliance* CRE Agar compared to the non-selective Columbia Blood Agar (CBA).

Introduction

As a result of increased resistance amongst the *Enterobacteriaceae*[1], the use of carbapenem antibiotics has grown and in recent times the emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) has become a major concern for patient safety and public health[2]. The need for an accurate, easy-to-use screening method to help identify colonized patients more quickly is of great importance. Early detection of CRE will allow faster implementation of appropriate strategies to limit the spread of these pathogens.

Brilliance CRE Agar (see figure 1) is a chromogenic medium designed for the screening of patients for CRE. The Thermo Scientific™ Sensititre™ system is a microbroth dilution method that provides qualitative (susceptible, intermediate or resistant) and quantitative minimum inhibitory concentration (MIC) results in a dried plate format. Each plate contains antimicrobial agents at appropriate dilutions. The GN4F plate format contains four carbapenem antibiotics (doripenem, ertapenem, imipenem and meropenem) that allow confirmation of CRE status (see Figure 2 for plate layout).

The ability to report susceptibility results as rapidly as possible is extremely important to clinical laboratories. If direct susceptibility testing can be performed from a primary isolation plate, the time to results reporting will be significantly reduced thus saving time, money and possibly reducing morbidity and mortality.

Methods

Forty eight CRE including 30 *Klebsiella pneumoniae*, 10 *Escherichia coli*, 6 *Enterobacter cloacae* and 2 *Enterobacter spp.* were tested. The CRE were inoculated onto CBA with a Thermo Scientific™ ertapenem 10µg disc in the primary bed. After 18-24 hrs incubation at 36±1°C, colonies from the edge of the inhibition zone were subcultured onto CBA and *Brilliance* CRE Agar. All plates were incubated at 36±1°C for 18-24 hrs.

Inocula for the Sensititre GN4F panels were prepared from colonies grown on both *Brilliance* CRE Agar and CBA plates. The Sensititre GN4F panels were dosed with 50µl per well using a Thermo Scientific™ Sensititre™ AIM™ automated inoculation delivery system. After 18-24 hrs incubation at 36±1°C, the panels were read both visually on a Sensititre Vizion system and using the Sensititre ARIS 2X automated fluorometric plate reading system.

FIGURE 1. *Brilliance* CRE Agar

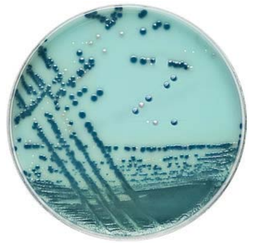
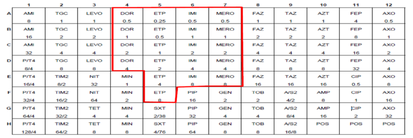


FIGURE 2. Layout of the Sensititre Gram-negative plate format (GN4F), showing the antimicrobic code and antimicrobic concentration (µg/ml) of each well



	1	2	3	4	5	6	7	8	9	10	11	12
A	AMI 8	TGC 1	LEVO 1	DOR 0.5	ETP 0.25	IMI 0.5	MERO 0.5	FAZ 1	TAZ 1	AZT 1	FEP 4	AXO 0.5
B	AMI 16	TGC 2	LEVO 2	DOR 1	ETP 0.5	IMI 1	MERO 1	FAZ 2	TAZ 2	AZT 2	FEP 8	AXO 1
C	AMI 32	TGC 4	LEVO 4	DOR 2	ETP 1	IMI 2	MERO 2	FAZ 4	TAZ 4	AZT 4	FEP 16	AXO 2
D	P/T4 8/4	TGC 8	LEVO 8	DOR 4	ETP 2	IMI 4	MERO 4	FAZ 8	TAZ 8	AZT 8	FEP 32	AXO 4
E	P/T4 16/4	TIM2 8/2	NIT 32	MIN 1	ETP 4	IMI 8	MERO 8	FAZ 16	TAZ 16	AZT 16	CIP 0.5	AXO 8
F	P/T4 32/4	TIM2 16/2	NIT 64	MIN 2	ETP 8	PIP 16	GEN 2	TOB 2	A/S2 4/2	AMP 8	CIP 1	AXO 16
G	P/T4 64/4	TIM2 32/2	TET 4	MIN 4	SXT 2/38	PIP 32	GEN 4	TOB 4	A/S2 8/4	AMP 16	CIP 2	AXO 32
H	P/T4 128/4	TIM2 64/2	TET 8	MIN 8	SXT 4/76	PIP 64	GEN 8	TOB 8	A/S2 16/8	POS	POS	POS

Carbapenem antibiotics tested during this study are located within the red outline. Key: DOR = Doripenem, ERT = Ertapenem, IMI = Imipenem, MERO = Meropenem

Quality control was assured by testing QC organisms *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 each day according to the manufacturer's instructions. All QC results were checked to be within the ranges as stated by CLSI[3] (see table 1).

TABLE 1. Quality control strains and expected ranges for each of the carbapenems tested.

Antimicrobial agent	ATCC® number	Organism ID	CLSI MIC range (µg/ml)
Doripenem	29212	*Enterococcus faecalis*	1-4
Meropenem			2-8
Imipenem	27853	*Pseudomonas aeruginosa*	1-4
Ertapenem			2-8

For each CRE isolate, the difference between the susceptibility results (for doripenem, ertapenem, imipenem and meropenem) of colonies from the *Brilliance* CRE Agar plate and of colonies from the CBA plate was evaluated.

The susceptibility results for an isolate read using the Sensititre Vizion or Sensititre ARIS, are in essential agreement when the MIC on the Sensititre GN4F panel inoculated directly from growth on *Brilliance* CRE Agar is ± one 2-fold dilution compared with the MIC result on the Sensititre GN4F panel inoculated from growth on CBA. The essential agreement was calculated only for isolates where the MIC result for both *Brilliance* CRE Agar and CBA were on-scale. The results are in categorical agreement when the category, (susceptible or intermediate/resistant) as determined by CLSI or EUCAST breakpoints is the same for an isolate where the inoculum has been prepared from colonies grown on *Brilliance* CRE Agar and CBA.

Results

Table 2 shows the total percentage essential agreement and the total percentage categorical agreement for CLSI and EUCAST, comparing the susceptibility results of CRE isolates when grown on a *Brilliance* CRE Agar and CBA. For all four carbapenem antibiotics on the Sensititre GN4F broth micro dilution panel read using a Sensititre Vizion or Sensititre ARIS, there was 100% essential agreement between the MIC of CRE isolates grown on *Brilliance* CRE Agar and CBA. The number of isolates from which the essential agreement was calculated is indicated in table 2.

Table 2 shows that the categorical agreement is ≥85% for EUCAST and ≥87% for CLSI between the susceptibility results of CRE isolates grown on *Brilliance* CRE Agar and CBA.

TABLE 2. The comparative essential & categorical agreement between the results from *Brilliance* CRE Agar and CBA

Antimicrobial agent	Read Method	% Essential agreement (No. of isolates with on-scale MIC results)	% Categorical agreement CLSI/EUCAST
Doripenem	ARIS	100.0 (n=21)	97.9/97.9
	Vizion	100.0 (n=19)	95.8/95.8
Ertapenem	ARIS	100.0 (n=21)	97.9/97.9
	Vizion	100.0 (n=18)	100.0/100.0
Imipenem	ARIS	100.0 (n=25)	95.8/85.4
	Vizion	100.0 (n=25)	91.7/89.6
Meropenem	ARIS	100.0 (n=25)	93.8/97.9
	Vizion	100.0 (n=25)	87.5/97.9

Conclusion

- The MIC of doripenem, ertapenem, imipenem and meropenem for CRE colonies grown on *Brilliance* CRE Agar can be determined directly from the agar plate using the Sensititre GN4F plate format.
- A high level of essential and categorical agreements were obtained between the results from *Brilliance* CRE Agar and CBA (See table 2). This suggests the MIC results were unaffected by inoculation directly from *Brilliance* CRE Agar compared to the non-selective CBA.

References

1. Hawkey, PM. and Livermore, DM. (2012) Carbapenem antibiotics for serious infections. British Medical Journal 344:e3236
2. Schwaber, MJ., Carmeli, Y. and Harbarth, S. Controlling Hospital-Acquired Infection due to Carbapenem-Resistant *Enterobacteriaceae* (CRE). In 'Antibiotic Policies' ed Gould, IM and van der Meer, JMW; published by Springer Science. ISBN 978-1-4419-1734-8. 2012, p105-115.
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility testing: Twenty Third Informational Supplement. CLSI Document M100-S23. January 2013.

LT2121A/April 2014