International Pathology Day 2024

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#IPD2024

Mast Group

Development of **Mast Uri®** *Plates* for Urinary Tract Infections AMR Screening: Carbapenemase-Producing Enterobacteriacae (CPE)

Nicholas King and Louis Ellwood

Introduction

Carbapenemases are β-lactamase enzymes, produced by bacteria, that inactivate certain antibiotics (including penicillins, cephalosporins, monobactams and carbapenems).[1] Identification of Carbapenemase-producing bacteria is of clinical importance due to higher mortality rates associated with resistance mechanisms.[1]

Mast Uri® *Plates* aid in the diagnosis and treatment of Urinary Tract Infections (UTIs). An improved method of screening for Carbapenemase-producing Enterobacteriacae (CPE) would be of benefit to the management of UTIs.

The aim is to develop a **Mast Uri®** *Plate* for Urinary Tract Infections (UTIs) which can:

- Screen for Antimicrobial Resistance (AMR) caused by Carbapenemase-producing Enterobacteriacae (CPE).
- Inhibit growth of non-target organisms with the use of a selective supplement.

EUCAST Screening Cut-offs

Minimum Inhibitory Concentrations (MICs) for some CPE may be below clinical breakpoint values (i.e. reported as susceptible). EUCAST recommends screening cut off MIC and disc diffusion criteria, which are independent of clinical breakpoints, for the screening of CPE.[1] These values are shown in the table below.

Carbapenem	MIC (mg/L)	Disk diffusion (mm) 10µg
Meropenem	>0.125	<25
Ertapenem	>0.125	<25

Method

Trial formulations tested against an organism panel (n=10) consisting of American Type Culture Collection (ATCC®) and/or National Collection of Type Cultures (NCTC) with known Carbapenemase resistance profiles. Target organisms, known CPE, should give a positive result (growth) and non-target organisms, non-CPE, should give a negative result (no growth).

A bacterial suspension with the turbidity equivalent of a 0.5 McFarland standard (10^0 dilution) was prepared for each organism. A tenfold serial dilution was performed, from 10^0 dilution (containing approximately $1x10^8$ to $1x10^7$ CFU/mL) down to 10^{-3} dilution (approximately $1x10^5$ to $1x10^4$ CFU/mL). Dilutions were inoculated onto trial formulations and incubated at 36°C for 18 to 24 hours before reading. Performance reported in terms of sensitivity and specificity.[2]

Positive Organisms:
Klebsiella pneumoniae NCTC 13340: MβL (VIM)
Klebsiella pneumoniae NCTC 13442: OXA-48
Klebsiella pneumoniae NCTC 13438: KPC
Klebsiella pneumoniae ATCC®BAA-2472: MβL (NDM-1)
Escherichia coli NCTC 13476: MβL (IMP)

Negative Organisms:
Escherichia coli ATCC®25922: non-CPE
Escherichia coli ATCC®8739: non-CPE
Escherichia coli NCTC 13351: non-CPE (ESβL)
Enterococcus faecalis ATCC®29212: non-CPE (Gram-positive)
Staphylococcus aureus ATCC®29213: non-CPE (Gram-positive)

Trial Formulations

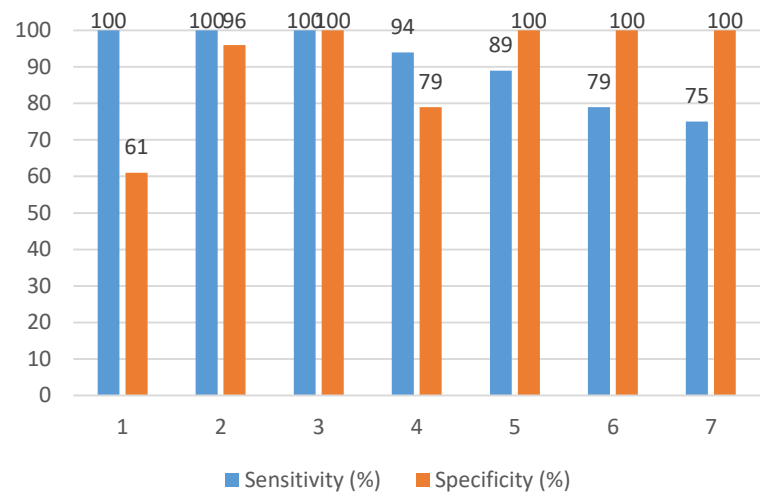
1. Mast Mueller-Hinton Agar + Carbapenem
2. Mast Mueller-Hinton Agar + Carbapenem + Selective Supplement
3. Mast Mueller-Hinton Agar + Carbapenem + Selective Supplement + Nitrocefin
4. Mast Chromogenic Agar + Carbapenem
5. Mast Chromogenic Agar + Carbapenem + Selective Supplement
6. Mast Chromogenic Agar + Carbapenem + Selective Supplement + Nitrocefin
7. CHROMAgar™ mSUPERCARBA™ (competitor comparison)

Sensitivity/Specificity

		Reference value		Total
		(-) no growth	(+) growth	
Trial formulation	(-) no growth	A True Negative	B False Negative	A+B
	(+) growth	C False Positive	D True Positive	C+D
	Total	A+C	B+D	Sum (A,B,C,D)

Sensitivity (%) = 100 x [D / (D+B)]
Specificity (%) = 100 x [A / (C+A)]

Results



Discussion

- F1 Mast Mueller-Hinton + Carbapenem reported a low specificity (61%) due to growth (false positives) of Gram-positive organisms.
- F2 Mast Mueller-Hinton + Carbapenem + Selective Supplement and F3 Mast Mueller-Hinton + Carbapenem + Selective Supplement + Nitrocefin both reported a sensitivity and specificity of >95%.
- F4 Chromogenic Agar + Carbapenem reported a low specificity (70%) due to growth (false positive) of a single Gram-positive organisms.
- F5 Chromogenic Agar + Carbapenem + Selective Supplement reported a low sensitivity (89%) due to no growth (false negative) of a single organism.
- F6 Chromogenic Agar + Carbapenem + Selective + Nitrocefin reported a low sensitivity (79%) due to no growth (false negative) of a single organism.
- F7 CHROMAgar™ mSUPERCARBA™ reported a low sensitivity (75%) due to no growth (false negative) of *Klebsiella pneumoniae* ATCC®BAA-2472.

Two formulations (F2 and F3) reported sensitivity and specificity of over 95%. The use of Mast Mueller-Hinton Agar, carbapenem antibiotic and a selective supplement (with and without Nitrocefin) can be used as a method of AMR screening for CPE in urine cultures.

References

1. EUCAST. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiologic importance v2.0. July 2017.
2. ISO 20776-2: 2021 Clinical laboratory testing and in vitro diagnostic systems…BSI Standards Limited. 2021