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Evaluation of three selective chromogenic media, CHROMagar ESBL, CHROMagar CTX-M and CHROMagar KPC, for the detection of *Klebsiella pneumoniae* producing OXA-48 carbapenemase

Michael Hornsey,[1] Lynette Phee,[1,2] Neil Woodford,[1,3] Jane Turton,[3] Daniele Meunier,[3] Claire Thomas,[4] David W Wareham[1,2]

[1]Antimicrobial Research Group, Centre for Immunology and Infectious Disease, Blizard Institute, Barts & The London School of Medicine and Dentistry, Queen Mary University London, London, UK
[2]Division of Infection, Barts Health NHS Trust, London, UK
[3]Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Health Protection Agency, Microbiology Services, Colindale, London, UK
[4]Hammersmith Hospital NHS Trust, London, UK

Correspondence to
Dr David W Wareham, Antimicrobial Research Group, Centre for Immunology and Infectious Disease, Blizard Institute, 4 Newark Street, Whitechapel, London E1 2AT, UK; d.w.wareham@qmul.ac.uk

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ABSTRACT

Three selective chromogenic culture media (CHROMagars ESBL, CTX-M and KPC) were evaluated for their ability to support the growth of nine *Klebsiella pneumoniae* isolates producing OXA-48 carbapenemase in combination with other β-lactamases. CHROMagar ESBL and CHROMagar KPC were the most sensitive media, supporting growth of all isolates with a detection limit as low as < 100 CFU/ml. Five isolates failed to grow on CHROMagar CTX-M, and five were recovered on CHROMagar KPC only at counts > 10^6 CFU/ml. Both CHROMagar ESBL and CHROMagar KPC may be useful for enhanced isolation of *K pneumoniae* producing OXA-48-like carbapenemases.

Carbapenemase producing *Enterobacteriaceae* (CPE) present a major threat to public health and are rapidly disseminating globally,[1] aided by the spread of successful bacterial clones, successful plasmids and international travel. The most prevalent CPE include those which produce the class A carbapenemase KPC, the metallo-β-lactamases of the IMP, NDM and VIM families, and the class D OXA-48-like enzymes. As treatment options are limited, attention is increasingly focused on methods for the rapid detection of CPE that may direct effective infection prevention and control strategies. Some countries (eg, France) have introduced comprehensive screening of all 'high risk' patients for CPE on admission to hospital,[2] particularly those with a history of hospitalisation in foreign countries, and many others (UK Health Protection Agency, http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1294740725984; US Centers for Disease Control, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5810a4.htm) also advise that this is done.

Strains of CPE producing OXA-48 carbapenemase raise several challenges. First, the gene is carried on plasmids (IncL/M) that have spread into multiple sequence types of *Klebsiella pneumoniae*,[3] which appear to be highly successful and capable of causing nosocomial outbreaks.[4 5] Second, detection of OXA-48 producers can be difficult unless there is co-carriage of additional β-lactamases, especially when using automated systems.[6] OXA-48 is an unusual enzyme as, unlike other carbapenemases, it is able to hydrolyse penicillins and carbapenems, but not cephalosporins.[7] This potential for a carbapenem-resistant but cephalosporin-susceptible phenotype may subvert standard laboratory detection protocols and expert rules used to infer carbapenem resistance mechanisms.[8] In this study, we assessed the ability of three commercially available chromogenic agars to detect carbapenem-resistant *K pneumoniae* producing the OXA-48 enzyme.

The nine *K pneumoniae* isolates used in this study were isolated from patients involved in an outbreak of carbapenem-resistant *K pneumoniae* in a London hospital renal unit.[9] Susceptibility testing was performed by the British Society for Antimicrobial Chemotherapy agar dilution methodology and molecular typing was performed by pulsed-field gel electrophoresis (PFGE).[10] Molecular mechanisms of β-lactam resistance were determined using a series of multiplex PCRs for the detection of class A (TEM, SHV, CTX-M-1,2,9,8/25-like, VEB, PER, GES, KPC), D (OXA-1/4/30,48), plasmidic AmpC (ACC, FOX, MOX, DHA, CIT, EBC) and metallo-β-lactamases (IMP, VIM, NDM).[11] Genes encoding variants of TEM, SHV and CTX-M enzymes known to have ESBL activity were identified by sequencing following PCR amplification of the entire coding sequence.

All of the isolates were resistant to ertapenem (MIC ≥2 μg/ml) and imipenem (MIC ≥4 μg/ml) according to the 2011 Clinical Laboratory Standards Institute breakpoints, but exhibited variable susceptibility to cephalosporins, aztreonam and meropenem (table 1). MICs of cloxacillin and piperacillin/tazobactam were >256 and >64 μg/ml, respectively, for all isolates tested. Each contained a $bla_{\text{OXA-48-like}}$ gene in combination with bla_{SHV} (n=8), bla_{TEM}-1 (n=3), $bla_{\text{CTX-M-15}}$ (n=3) or $bla_{\text{OXA-1}}$ (n=2) genes (table 1). Six carried $bla_{\text{SHV-11}}$ (non-ESBL) and two had $bla_{\text{SHV-1}}$. No plasmidic AmpC or metallo-carbapenemase genes were detected. PFGE showed that the isolates belonged to three distinct strains; strain A lacked a coresident ESBL, whereas strains B and C both produced a CTX-M-15 enzyme.

The three selective media, CHROMagar ESBL, CHROMagar CTX-M and CHROMagar KPC, were obtained directly from the manufacturer (CHROMagar, Paris, France). All components were provided as dehydrated powders and media were prepared in-house according to the accompanying instructions; CHROMagar Orientation[12] was the base medium used for the preparation of CHROMagar ESBL and CHROMagar KPC plates,

Table 1 Characteristics of *Klebsiella pneumoniae* isolates used

Isolate	β-lactamase genes	Co-resident ESBL	PFGE profile	MIC (μg/ml)									Lowest limit of detection on CHROMagar		
				CTX	CTXC	CAZ	CAZC	FEP	AZT	ERT	IMP	MEM	ESBL	CTX	KPC
KP52	bla_{OXA-48}, bla_{SHV-11}	No	A	2	1	1	0.5	2	0.25	8	8	1	9.8×10^5	1.2×10^7	6.1×10^6
KP55	bla_{OXA-48}, bla_{TEM-1}, bla_{SHV-11}	No	A	2	2	1	0.5	4	0.25	8	4	2	1.2×10^4	$>3.5\times10^9$*	1.4×10^2
KP56	bla_{OXA-48}, bla_{TEM-1},bla_{SHV-11}	No	A	16	32	8	32	2	8	8	4	2	1.3×10^6	3.4×10^7	1.6×10^6
KP58	bla_{OXA-48}, bla_{SHV-11}	No	A	2	1	0.5	0.5	4	≤0.125	8	4	2	1.4×10^6	6.7×10^6	2.2×10^6
KP60	bla_{OXA-48}, bla_{TEM-1},bla_{SHV-11}	No	A	1	1	1	0.5	2	≤0.125	8	8	2	4.4×10^6	$>2.5\times10^9$*	1.2×10^6
KP61	bla_{OXA-48}, bla_{SHV-11}	No	A	4	2	1	0.5	4	0.25	8	4	2	3.3×10^1	$>3.1\times10^9$*	2.3×10^1
KP53	bla_{OXA-48}, $bla_{CTX-M-15}$	Yes	B	>256	>32	128	>32	>64	>64	>16	128	>32	1.0×10^1	$>4.4\times10^9$*	1.0×10^1
KP54	bla_{OXA-48}, bla_{SHV-1}, $bla_{CTX-M-15}$, bla_{OXA-1}	Yes	C	>256	32	64	4	>64	>64	8	16	8	1.0×10^1	$>4.9\times10^9$*	2.0×10^1
KP59	bla_{OXA-48}, bla_{SHV-1}, $bla_{CTX-M-15}$, bla_{OXA-1}	Yes	C	256	4	128	2	>64	>64	8	4	0.5	1.0×10^1	1.0×10^1	5.0×10^6

*No growth at the inoculum plated.
AZT, aztreonaml; CAZ, ceftazidime; CAZC, ceftazidime plus clavulanic acid; CTX, cefotaxime; CTXC, cefotaxime plus clavulanic acid; ERT, ertapenem; FEP, cefepime; IMP, imipenem; MEM, meropenem; PFGE, pulsed-field gel electrophoresis.

and CHROMagar ECC was used to prepare CHROMagar CTX-M.[13] *K pneumoniae* colonies were metallic blue on CHROMagar Orientation, but red on CHROMagar ECC. Selective supplements were added to the prepared base media, cooled to 50°C, at final concentrations of: ESBL supplement, 570 μg/ml; CTX-M supplement, 250 μg/ml; and KPC supplement, 400 μg/ml.

Each *K pneumoniae* isolate was grown at 37°C for 18 h in 3 ml of IsoSensitest broth. Ten-fold serial dilutions of each overnight culture were then made in 100 μl of sterile saline and plated onto each of the selective chromogenic media. Following incubation for 18 h at 37°C, colonies were counted and the number of bacteria recovered expressed as colony forming units per ml (CFU/ml). Dilutions were also plated onto unsupplemented CHROMagar Orientation and CHROMagar ECC base media. The difference in colony counts between these control plates and the supplemented plates was used to determine the lowest number of bacteria within the population required for growth under selection (the lowest limit of detection) (table 1). The antibiotic-susceptible type strain *K pneumoniae* NCTC 9633 was used as a control for growth/non-growth on each batch of agar.

All isolates were recovered at <100 CFU/ml on unsupplemented base media alone. CHROMagar ESBL was the most sensitive selective medium, with all of the nine isolates forming characteristic blue colonies, although only four were detected when diluted to <100 CFU/ml. All isolates were also able to grow on CHROMagar KPC, but with only three detected at <100 CFU/ml. Two isolates (KP53 and KP54) recovered on both media at <100 CFU/ml produced OXA-48 in combination with a CTX-M-15 enzyme. Of note, KP59, which displayed a meropenem MIC 16-fold lower than KP54 despite belonging to the same PFGE-defined strain, was only recovered at 10^6 CFU/ml on CHROMagar KPC. On CHROMagar CTX-M five of the nine isolates were unable to grow even using an inoculum of 10^9 CFU/ml, including two with $bla_{CTX-M-15}$. KP59, which was highly resistant to cephalosporins, was the only isolate supported at <100 CFU/ml in contrast to its growth on CHROMagar KPC which was only supported at 10^6 CFU/ml.

The poorer performance of CHROMagar CTX-M was surprising, especially for those strains exhibiting resistance to cefotaxime (KP53, KP54, KP56). This medium was initially developed for veterinary use, and designed to be selective for CTX-M ESBL producing *Enterobacteriaceae* in the presence of organisms producing AmpC enzymes. Sensitivity of 100% was reported when it was evaluated against a large panel of Gram-negative bacteria of animal and human origin, although only a small number of *K pneumoniae* and no OXA-48 producers were included.[13] No *ampC* genes were present in the OXA-48 producers tested here.

The potential for CPE with OXA-48 enzymes to be missed if media designed for detecting ESBL producers are used for screening has been highlighted in a recent report.[14] In this case, an OXA-48 producing *Escherichia coli* isolate, which did not harbour a co-resident ESBL, consistently failed to grow on another chromogenic ESBL medium, ChromID ESBL (bioMérieux). This isolate was readily recovered when an enrichment method using ertapenem-supplemented brain heart infusion broth and subculture onto Drigalski agar was employed. The limited ability of ChromID ESBL to detect isolates producing OXA-48 carbapenemase alone was also reported in a study that compared its performance with that of CHROMagar KPC.[15]

Of the three media studied here, only CHROMagar KPC was designed to be specifically selective for CPE. Most studies have assessed the ability of this medium to recover *K pneumoniae* producing KPC enzymes from clinical samples, with higher rates of sensitivity and specificity compared with MacConkey agar

and carbapenem disks.[16] Sensitive detection of CPE and non-fermentative bacteria producing IMP, VIM and OXA-48-like carbapenemases has also been described.[17] However, the medium performed less well in the detection of KPC producing *Escherichia coli*[18] versus MacConkey agar supplemented with 1 μg/ml imipenem.[19] In our study, CHROMagar KPC was able to support the growth of all *K pneumoniae* isolates producing OXA-48 carbapenemase. Of note, the isolates belonged to three different PFGE-defined strains and displayed widely differing MICs of carbapenems and other β-lactams, reflecting variable presence of other β-lactamases, including in some cases CTX-M-type ESBLs. Although the performance of this medium may be generalisable to other OXA-48 producing *K pneumoniae*, there was wide variation (up to 5 logs) in the lower limit of detection even in strains belonging to the same PFGE-defined clone despite only small differences in carbapenem MICs.

In summary, both CHROMagar ESBL and CHROMagar KPC were able to detect *K pneumoniae* producing OXA-48 carbapenemase. However, given the wide difference in limits of detection further studies using clinical samples (rectal swabs, spiked stool) are warranted before either can be recommended for routine use to investigate asymptomatic carriage or in the context of an outbreak.

Key messages

- Klebsiella pneumoniae isolates producing OXA-48 carbapenemase can be difficult to detect phenotypically.
- The selective chromogenic media CHROMagar ESBL and CHROMagar KPC may be useful for detecting Klebsiella pneumoniae producing OXA-48 carbapenemaseWide differences in limits of detection were observed; further studies using clinical samples are warranted.

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