JOURNAL OF MEDICAL MICROBIOLOGY

RESEARCH ARTICLE

Abdul Momin *et al.*, *Journal of Medical Microbiology*

DOI 10.1099/jmm.0.000602

MICROBIOLOGY SOCIETY

CHROMagar COL-*APSE*: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens

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Abstract

Purpose. A selective chromogenic culture medium for the laboratory isolation and differentiation of colistin resistant *Acinetobacter, Pseudomonas, Stenotrophomonas* and *Enterobacteriaceae* spp. (CHROMagar COL-*APSE)* was developed, evaluated and compared to an existing selective bacterial culture medium (SuperPolymyxin).

Methodology. The medium was challenged with 84 isolates, including polymyxin B (POL B)-susceptible and -resistant type strains and colistin (COL)-resistant organisms recovered from human and animal samples. Susceptibility to COL and POL B was determined by agar dilution and broth microtitre dilution. The lower limit for the detection of COL-resistant organisms was also calculated for both CHROMagar COL-*APSE* and SuperPolymyxin media. The ability to isolate and correctly differentiate COL-resistant organisms within mixed cultures was also assessed and compared using both media.

Results. Using CHROMagar COL-*APSE,* Gram-negative pathogens (*n*=71) with intrinsic (*n*=8) or acquired COL (*n*=63) resistance were recovered with 100 % specificity down to the lower limit of detection of 10^1 colony-forming units (c.f.u.). The growth on SuperPolymyxin was similar, but notably weaker for COL-resistant non-fermentative bacteria (*Acinetobacter, Pseudomonas* and *Stenotrophomonas*). CHROMagar COL-*APSE* was also more sensitive in supporting the growth of *Enterobacteriaceae* with COL resistance associated with the carriage of *mcr-1.*

Conclusion. CHROMagar COL-*APSE* is a sensitive and specific medium for the growth of COL-resistant bacterial pathogens. Due to the low limit of detection (10^1 c.f.u.), it may be useful as a primary isolation medium in the surveillance and recovery of COL-resistant bacteria from complex human, veterinary and environmental samples, especially those with plasmid-mediated MCR-1 or novel mechanisms of polymyxin resistance.

INTRODUCTION

Polymyxin B and E (colistin) are increasingly used in the treatment of multi-drug-resistant bacterial infections. Both are cationic polypeptides that bind the lipopolysaccharides (LPS) of Gram-negative bacteria and disrupt the outer membrane. Polymyxin resistance (PR), although intrinsic amongst Gram-positive and some Gram-negative species (*Proteus, Morganella* and *Serratia* spp.), is an emerging problem in a number of other pathogens (*Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichiacoli, Salmonella enterica* and *Klebsiella pneumoniae).* Resistance can occur due to mutations/insertions in genes involved in LPS biosynthesis (*lpx*, *pmrA/B, mgrB* and *phoP/Q)* and/or can be acquired by the horizontal transmission of genes encoding phosphoethanolamine transferase (pEtN) enzymes. Plasmid-mediated colistin (COL) resistance due to the *mcr-1* pEtN gene was recently identified in China [1]. Since it was first described, bacteria producing MCR-1 have been found in a wide range of food-producing animal, human and environmental sources. Moreover, MCR-1-producing isolates are often pan- (PDR) or extensively

Received 22 March 2017; Accepted 6 September 2017

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Keywords: CHROMagar COL-*APSE*; colistin; resistance; MCR-1.

Abbreviations: COL, colistin; MIC, minimum inhibitory concentration; pEtN, phosphoethanolomine transferases; PNPG, p-nitrophenyl glycerol; POL B, polymyxin B.

(XDR) drug resistant, which significantly limits the therapeutic options for these organisms.

A sensitive and specific means for the isolation of PR organisms is critical for any strategy aimed at monitoring the spread of these isolates and identifying new resistance determinants. Furthermore, polymyxin resistance may often be low level or unstable (heteroresistance), and there are well-established difficulties (e.g. cation concentrations in broth, adsorption of colistin to plastics, poor diffusion in agar) in the accurate determination of COL MICs [2]. Molecular detection is limited to the small number of mutations (*mcr-1–1.8*) and MCR-like alleles (*mcr-2, mcr-3* and *mcr-4*) discovered to date [1, 3].

Selective bacterial culture media offer a means to rapidly detect and identify organisms with resistance to polymyxins. One such medium, SuperPolymyxin, uses an eosin methylene blue (EMB) agar base, with selection for PR Gram-negative bacteria achieved by the addition of a low concentration of COL (3.5 μg ml^{-1}), along with daptomycin (10 μg ml^{-1}) and amphotericin B (5 μg ml^{-1}) to suppress Gram-positive and fungal growth. This medium has been evaluated and proven to be sensitive and specific in the selective growth of PR-resistant *Enterobacteriaceae*, either from pure cultures or using spiked stool samples [4]. Here we describe an alternative medium, CHROMagar COL-*APSE,* designed to be selective in the isolation and differentiation of all strains of *Acinetobacter* (*A*), *Pseudomonas* (*P),* *Stenotrophomonas* (*S*) and *Enterobacteriaceae* (*E*) with either intrinsic, acquired or uncharacterized mechanisms of resistance. A potential advantage compared with SuperPolymyxin would be the ability to recover and differentiate PR non-fermentative Gram-negative organisms as well as *Enterobacteriaceae*.

METHODS

Media preparation

CHROMagar COL-*APSE* plates were prepared in-house using dehydrated CHROMagar base media (CHROMagar, Paris, France) supplemented as in Table 1 with the CHROMagar COL-*APSE* supplement (X192). This contains antimicrobials (colistin sulfate/oxazolidonones) at concentrations designed to enhance the growth of PR Gram-negative species and suppress the growth of Gram-positive bacteria, whilst avoiding significant synergy between the active compounds. This +medium was not autoclaved, but instead sterilized by boiling at 100 °C in order to preserve the chromogenic compounds included in the mixture, prior to the addition of all of the supplements. All of the additional antibiotics used (colistin sulfate, polymyxin B, daptomycin and amphotericin B) were sourced from Sigma-Aldrich (Gillingham, UK) or Cambridge Bioscience (Cambridge, UK) and prepared as stock solutions (10 000–100 μg ml^{-1}) in distilled water. Those containing COL or polymyxin B (POL B) were only handled in glass tubes and used within 24 h. SuperPolymyxin plates were prepared exactly as described by Nordmann *et al.* [4] using EMB manufactured by Thermo Fisher Oxoid (Basingstoke, UK). CHROMagar COL-*APSE* media for use with samples containing *Proteus* spp. were prepared with the addition of 50 mg l^{-1} p-nitrophenyl glycerol (PNPG).

Table 1. Preparation of CHROMagar COL-*APSE* plates

Compound	Amount/L*
CHROMagar base medium	42.5 g
Distilled water	1000 ml
Sterilize by heating to 100 °C	
CHROMagar growth supplement S1	2 ml
CHROMagar COL-*APSE* supplement (X192)	4 ml

*For the production of 50×20 ml CHROMagar COL-*APSE* plates.

Bacterial isolates and determination of polymyxin minimum inhibitory concentrations (MICs)

Eighty-four isolates were used in the evaluation of CHROMagar COL-*APSE* media (Table 2). These included 8 isolates with intrinsic COL resistance (1 clinical human and 7 type strains), 13 COL susceptible isolates (6 human clinical, 3 veterinary clinical and 4 type strains) and 63 isolates with acquired COL resistance (5 human clinical and 58 veterinary clinical) contained within our collections.

The MICs of COL and POL B (0.006–256 μg ml^{-1}) were determined for each isolate by agar dilution on Mueller–Hinton II (MH 2) agar [5]. The plates were seeded with 10^4 c.f.u. using a multi-point inoculator and examined for growth after incubation at 37 °C for 24 h. Susceptibility ($\leq$2–4 μg ml^{-1}) and resistance (>2–8 μg ml^{-1}) to either COL or POL B were interpreted according to current CLSI and/or EUCAST species-specific breakpoints, where available [6, 7]. Susceptibility to COL was also determined by broth microtitre dilution (BMD) to enable the MICs to be compared to those obtained on solid media.

All clinical isolates were additionally screened for the presence of *mcr*-related genes by PCR using the CLR5-F (5′-CGGTCAGTCCGTTTGTTC-3′), CLR5-R (5′-CTTGG TCGGTCTGTAGGG-3′), MCR_2-F (5′-TGGTACAGCCCC TTTATT-3′) and MCR_2-R (5′-GCTTGAGATTGGGTTA TGA-3′) primers for the detection of *mcr-1–1.8* and *mcr-2*, respectively [1, 3].

Comparative growth on selective media

The ability of CHROMagar COL-*APSE* and SuperPolymyxin to support or suppress growth was first assessed using a fixed inoculum of 10^3 c.f.u. of each strain. Overnight cultures were used to prepare a 0.5 McFarland standard (1.5×10^8 c.f.u.) further diluted in 0.85 % saline (OXOID, United Kingdom) and used to inoculate supplemented and unsupplemented CHROMagar and SuperPolymyxin (EMB) base plates.

Table 2. Characteristics of bacterial isolates

Isolate	MIC (mg l^{-1})/Agar dilution		Polymyxin resistance mechanism	Lowest limit of detection (c.f.u.)	
	Colistin	Polymyxin B		CHROMagar COL-*APSE*	SuperPolymyxin
Intrinsic resistance to polymyxins					
P. mirabilis NCTC 13376	>256	>256	Intrinsic	10^1	10^1
S. marcescens NCTC 10211	>256	>256	Intrinsic	10^1	10^1
M. morgannii MM2	>256	>256	Intrinsic	10^1	10^1
E. faecalis ATCC 2912	>256	256	Intrinsic	$>10^9$	$>10^9$
E. gallinarum ATCC 49573	>256	>256	Intrinsic	$>10^8$	$>10^8$
C. albicans ATCC 10231	256	256	Intrinsic	$>10^7$	$>10^7$
E. cloacae NCTC 10005	128	256	Intrinsic	10^2	10^1†
S. maltophilia NCTC 10258	8	64	Intrinsic	10^1*	10^2
Susceptible to polymyxins					
P. aeruginosa ATCC 27853	2	2	NA	10^2*	10^5
Salmonella enterica group D (non-Typhi) Sal3	2	2	N/A	10^4*	10^6
E. coli E17	1	2	MCR-1	10^7	10^7
Salmonella enterica subsp. *diarizonae* Sal1	1	2	N/A	10^5*	10^6
A. baumannii ATCC 19606	1	2	NA	10^6	10^6
E. coli E44	1	1	MCR-1	$>10^9$	$>10^9$
Salmonella enterica subsp. *diarizonae* Sal4	1	1	N/A	10^5*	10^6
K. pneumoniae KP32	0.5	1	NA	10^6	10^6
Salmonella enterica subsp. *diarizonae* Sal2	0.5	1	N/A	10^5*	10^6
Salmonella enterica group D (non-Typhi) Sal5	0.5	1	N/A	10^3*	10^5
E. coli ATCC 25922	0.5	0.5	NA	10^6	10^6
E. coli 408756	0.5	0.5	MCR-1	10^6	10^6
K. pneumoniae ATCC 9633	0.5	0.5	NA	$>10^9$*	10^5
Acquired resistance to polymxins					
A. baumannii AB219	>256	>256	Unknown	10^1	10^1
A. baumannii AB205	>256	32	Unknown	10^1	10^1
A. baumannii AB287	8	4	Unknown	10^1*	10^2
K. pneumoniae KP6	128	256	Unknown	10^1	10^1
K. pneumoniae KP19	64	64	Unknown	10^1	10^1
E. coli 35 095	>256	>256	MCR-1	10^1	10^1
E. coli 35 175	64	32	MCR-1	10^1	10^1
E. coli 29 881	32	32	MCR-1	10^1	10^1
E. coli 33 907	32	32	MCR-1	10^1	10^1
E. coli 34 936	32	32	MCR-1	10^1	10^1
E. coli 35 593	32	32	MCR-1	10^1	10^1
E. coli E10	32	16	MCR-1	10^1*	10^4
E. coli E9	32	16	MCR-1	10^1	10^1
E. coli E33	32	16	MCR-1	10^1	10^1
E. coli E3	32	16	MCR-1	10^1	10^1
E. coli E1	32	16	MCR-1	10^1	10^1
E. coli E35	32	16	MCR-1	10^1	10^1
E. coli E29	32	16	MCR-1	10^1	10^1
E. coli E32	32	16	MCR-1	10^1	10^1
E. coli E13	32	16	MCR-1	10^1	10^1
E. coli E24	32	16	MCR-1	10^1	10^1
E. coli E20	32	16	MCR-1	10^1	10^1
E. coli E25	32	16	MCR-1	10^1	10^1
E. coli E27	16	32	MCR-1	10^1	10^1
E. coli E8	16	16	MCR-1	10^1	10^1
E. coli E11	16	16	MCR-1	10^1*	10^2

Table 2. cont.

Isolate	MIC (mg l^{-1})/Agar dilution		Polymyxin resistance mechanism	Lowest limit of detection (c.f.u.)	
	Colistin	Polymyxin B		CHROMagar COL-*APSE*	SuperPolymyxin
E. coli E39	16	16	MCR-1	10^1	10^1
E. coli E40	16	16	MCR-1	10^1	10^1
E. coli E34	16	16	MCR-1	10^1	10^1
E. coli E2	16	16	MCR-1	10^1	10^1
E. coli 412049521	16	16	MCR-1	10^1	10^1
E. coli E37	16	16	MCR-1	10^1	10^1
E. coli E41	16	16	MCR-1	10^1	10^1
E. coli E36	16	16	MCR-1	10^1	10^1
E. coli E31	16	16	MCR-1	10^1	10^1
E. coli E30	16	16	MCR-1	10^{1}*	10^3
E. coli E14	16	16	MCR-1	10^1	10^1
E. coli E12	16	16	MCR-1	10^1	10^1
E. coli 14042624	16	16	MCR-1	10^1	10^1
E. coli 412016126	16	16	MCR-1	10^1	10^1
E. coli E22	16	16	MCR-1	10^1	10^1
E. coli E19	16	16	MCR-1	10^1	10^1
E. coli E7	16	16	MCR-1	10^1	10^1
E. coli E47	16	16	MCR-1	10^1	10^1
E. coli E49	16	16	MCR-1	10^{1}*	10^3
E. coli E5	16	16	MCR-1	10^{1}*	10^3
E. coli E16	16	16	MCR-1	10^1	10^1
E. coli E18	16	16	MCR-1	10^1	10^1
E. coli E43	16	16	MCR-1	10^{1*}	10^3
E. coli E46	16	16	MCR-1	10^1	10^1
E. coli E28	16	16	MCR-1	10^1	10^1
E. coli E26	16	16	MCR-1	10^1	10^1
E. coli 27 852	16	16	MCR-1	10^1	10^1
E. coli 35 062	16	16	MCR-1	10^{1}*	10^3
E. coli 37 914	16	16	MCR-1	10^1	10^1
E. coli 41 323	16	16	MCR-1	10^1	10^1
E. coli 41 339	16	16	MCR-1	10^1	10^1
E. coli 41 848	16	16	MCR-1	10^1	10^1
E. coli 412044854	16	8	MCR-1	10^1	10^1
E. coli 413040864	16	8	MCR-1	10^1	10^1
E. coli 34 692	16	8	MCR-1	10^{1}*	10^2
E. coli E45	8	16	MCR-1	10^1	10^1
E. coli 32 218	8	8	MCR-1	10^1	10^1

*Enhanced sensitivity of CHROMagar COL-*APSE*.

†Enhanced sensitivity of SuperPolymyxin.

Lower limit of detection (LLD)

The sensitivity was compared by determining the lowest number (c.f.u.) of COL-resistant bacteria in the inocula required for growth on each media. The lowest limit of detection (LLD) was assessed using serial dilutions (10^{-1}–10^{-9}) of overnight cultures grown at 37 °C for 24 h in 3 ml of Luria–Bertani (LB) broth. Tenfold serial dilutions were made in 0.85 % saline (volume/volume) and 20 µl of each dilution was plated onto unsupplemented MH 2 (control), CHROMagar COL-*APSE* and SuperPolymyxin agar plates using the Miles and Misra procedure [8]. Following incubation for 24 h at 37 °C, the colonies were counted and the number of bacteria recovered were expressed as colony-forming units per ml (c.f.u. ml^{-1}). The colony counts after incubation on CHROMagar COL-*APSE* and SuperPolymyxin agar were subtracted from the number recovered on MH 2 agar to quantify the total number of COL-resistant organisms (c.f.u.) within the plated population required for viable growth on each selective media (LLD).

Isolation and differentiation of organisms in mixed culture

Two pools containing mixtures of COL-resistant (R) and COL-susceptible (S) organisms were used to assess the performance of the media with complex polymicrobial samples. Pool one consisted of *Proteus mirabilis* NCTC 13376, *A. baumannii* AB205 (COL R), *E. coli* E7 (COL R), *E. cloacae* NCTC 10005 (COL R), *E. faecalis* ATCC 2912 (COL R), *K. pneumoniae* KP19 (COL R), *M. morgannii* MM2 (COL R), *S. marcescens* NCTC 10211 (COL R) and *P. aeruginosa* ATCC 27853 (COL heteroresistant). Pool two consisted of *A. baumannii* ATCC 19606 (COL S), *E. coli* ATCC 25922 (COL S), *E. faecalis* ATCC 2912 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant) and *K. pneumoniae* ATCC 9633 (COL S). The pools were made by mixing single colonies of each isolate in 3 ml 0.85 % saline adjusted to a 0.5 McFarland standard (1.5×10^8 c.f.u. ml^{-1}), and were confirmed by viable colony counts. Further dilutions were made to achieve a concentration of 10^5 c.f.u. ml^{-1} and CHROM-agar COL-*APSE* plates were then inoculated with 10 µl (10^3 c.f.u.), either spread across the entire plate using a L-shaped spreader, or streaked across the plate using a 10 µl loop (Fig. 2).

The stability of CHROMagar COL-*APSE* following batch production and storage at 4 °C was assessed using media prepared centrally by E and O laboratories (Glasgow, UK) and tested using *P. mirabilis* (NCTC 13376), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 9633) and *E. faecalis* (ATCC 2912) as internal and external quality control and the control pools (10^3 c.f.u.) defined above. The media for use with pools containing *P. mirabilis* were supplemented with PNPG.

RESULTS

The *in vitro* activity (MIC mg l^{-1}) of both COL and POL B when determined by agar dilution was comparable (+/−1 dilution) for all of the *Enterobacteriaceae* isolates tested (Table 2). However, the MICs of COL and POL B differed by at least three dilutions for *S. maltophilia* NCTC 10258 and *A. baumannii* AB205. The *mcr-1* gene was present in 61 of the *E. coli,* 58 (95 %) of which were also phenotypically resistant to COL (MIC >2 µg ml^{-1}). Three isolates containing *mcr-1* had colistin MICs below the pharmacodynamic breakpoint when the MIC was determined by agar dilution. Three COL-resistant *A. baumannii* and two *K. pneumoniae* (MIC 8 ->256 µg ml^{-1}) clinical isolates were also identified and included in the evaluation of both media. The mechanism of resistance in these three strains is yet to be determined, despite sequencing for mutations in *mgrB, pmrA/B* and the *phoP/Q* regulatory genes known previously to be associated with mutational polymyxin resistance [9]. When susceptibility to COL was determined by BMD, the MIC of COL was comparable to that determined by agar dilution (+/−1 dilution vs all strains).

Growth on CHROMagar COL-*APSE* on plates inoculated with 10^3 c.f.u. was observed for all COL R Gram-negative isolates. All of the COL-susceptible *Enterobacteriaceae*, COL-resistant Gram-positive (*Enterococci* spp) and fungal (*Candida* spp) isolates failed to grow at these inocula (Fig. 1). Growth of the *P. aeruginosa* ATCC 27853 type strain (COL MIC 2 µg ml^{-1}), however, was supported on CHROMagar COL-*APSE* at 10^3 c.f.u. Although this isolate is deemed susceptible to COL according to current CLSI breakpoints, and is recommended as a control strain for antimicrobial susceptibility testing, it should be noted that it has frequently been shown to exhibit heteroresistance to COL *in vitro* when population analysis profiling (PAP) is used as the gold standard [10]. The −70 °C stock of ATCC 27853 held in our laboratory and used in this study consistently demonstrates heteroresistance to COL when assessed using the PAP method.

The growth on SuperPolymyxin media with an inoculum of 10^3 c.f.u. was similar, but visibly weaker for each of the COL-resistant non-fermenters. The growth of one strain of COL-resistant *A. baumannii* (MIC 8 µg ml^{-1}) and *S. maltophilia* (MIC 32 µg ml^{-1}) was not supported at all (Fig. 1). It is possible that the antimicrobial synergy known to exist between COL and daptomycin against *A. baumannii* [11] could account for the poor growth or inhibition of these species that we observed on SuperPolymyxin agar. In contrast to growth on SuperPolymyxin, the chromogenic properties of the CHROMagar COL-*APSE* media enabled the clear differentiation of COL-resistant *Enterobacteriaceae* from non-fermenters. Phenotypic identification as either dark pink to reddish (*E. coli*), metallic blue (*Klebsiella, Enterobacter* and *Serratia* spp) or colourless natural pigmentation (*Morganella)* was also possible, whilst on SuperPolymyxin only COL-resistant *E. coli* could be identified by their metallic green appearance. In the limit of detection studies, COL-resistant strains could be recovered on both media at 10^1 c.f.u., whilst the growth of strains deemed susceptible to COL was only possible when using an inoculum of $>10^4$ (Table 1).

The stability of the media confirmed that there was no reduction in performance (10^3 c.f.u. ml^{-1}) for up to 4 weeks. Swarming of *P. mirabilis* NCTC 13376 was observed when it was used to inoculate both CHROMagar COL-*APSE* and SuperPolymyxin plates. The addition of 50 mg l^{-1} p-nitrophenyl glycerol (PNPG) to the CHROMagar COL-*APSE* plates reduced this significantly (Fig. 2).

DISCUSSION

We found that both CHROMagar COL-*APSE* and the SuperPolymxin media were able to support the growth of COL-resistant Gram-negative bacteria whilst suppressing COL-resistant Gram-positive pathogens. Using clinical isolates, CHROMagar COL-*APSE* supported the growth of all of the COL-resistant strains down to an inoculum of as low as 10^1 c.f.u. Growth with an inoculum of 10^1 c.f.u. was also supported on SuperPolymyxin, but only for 50/58 (86 %) of the *E. coli* isolates with COL resistance accompanied by *mcr-1*, suggesting that it may have slightly lower sensitivity

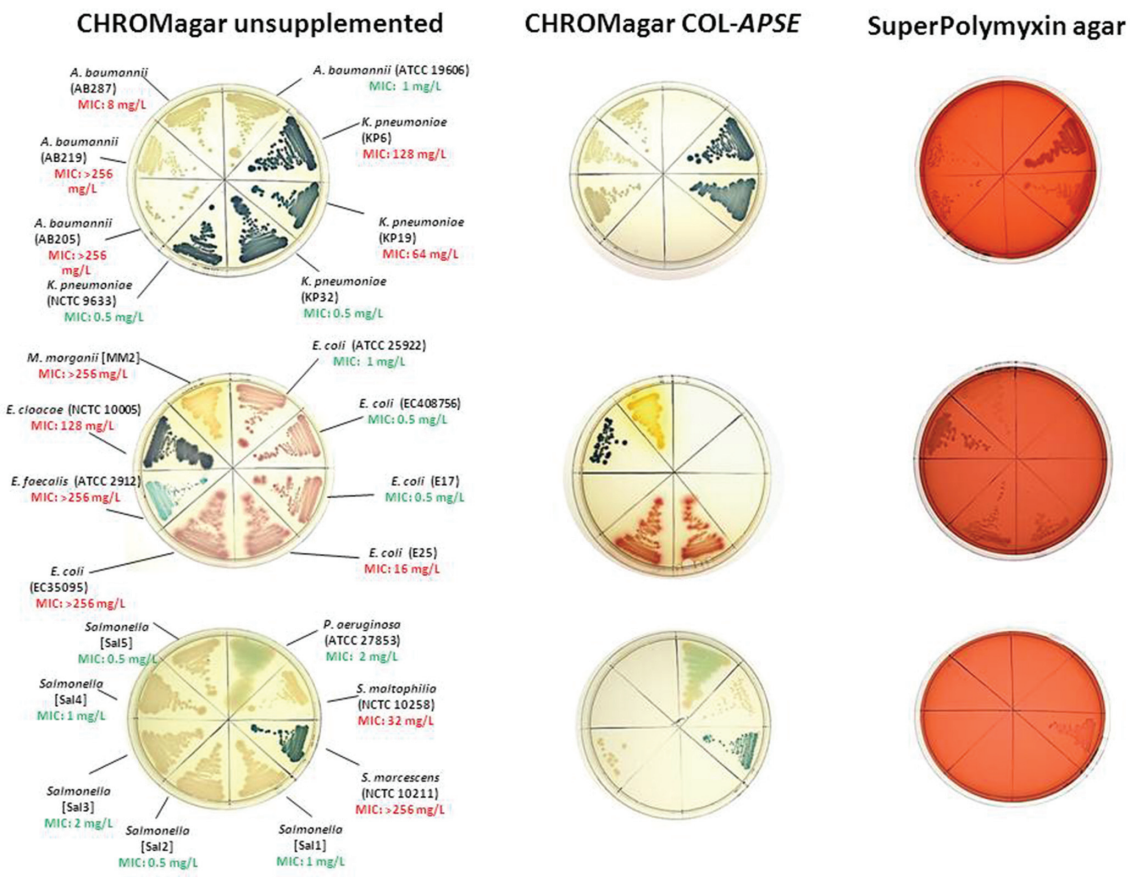

Fig. 1. Comparative growth of COL-susceptible and -resistant isolates on CHROMagar base media, CHROMagar COL-*APSE* and SuperPolymyxin. Strains were streaked on the same segments of corresponding plates.

in the detection of these strains (Table 2). SuperPolymyxin, however, was found to be better (≥1 dilution) at suppressing the growth of COL-susceptible *Salmonella* spp. Reduced susceptibility to polymyxins in *Salmonella enteritidis* and other serogroup 9 strains has been reported, and the epidemiological cut-off (>2 mg l^{-1}) for resistance has been challenged [12]. As the mechanisms for the reduced susceptibility reported in *Salmonella* spp. remain unknown, a more sensitive medium may help in the recovery of COL-resistant strains and aid in the elucidation of alternative resistance mechanisms.

The only other difference we observed between the two media (Table 2) was differential growth of *P. aeruginosa* ATCC 27853 on CHROMagar COL-*APSE* (LLD=10^2 c.f.u.) when compared to SuperPolymyxin (LLD 10^5=c.f.u.). Again, this may represent the heteroresistant properties of this strain and a high frequency of COL resistance by spontaneous mutation. As with *A. baumannii*, synergy between the selective antimicrobials in the media may be responsible for the differential ability of CHROMagar COL-*APSE* and SuperPolymyxin to identify heteroresistant strains. The clinical relevance of heteroresistance to polymyxins is unclear, although the ability to identify such strains easily may be useful in optimizing or selecting COL-based therapies on an individual basis.

Notably, the addition of PNPG was beneficial in suppressing swarming of colistin-resistant *Proteus* spp. without affecting the performance of the media. This provides a more robust method to select out COL-resistant isolates within mixed specimens.

In summary, we developed and evaluated a new chromogenic culture medium for the isolation and identification of COL-resistant Gram-negative bacterial pathogens. CHROMagar COL-*APSE* was similar to SuperPolymyxin as a medium for the selective growth of COL-resistant organisms, although we found CHROMagar COL-*APSE* to be slightly more sensitive in the detection of *Enterobacteriaceae* producing MCR-1, and it also provides the benefit of presumptive chromogenic identification. The low limit of detection (10^1 c.f.u.) of both media suggest either could be used to confirm polymyxin resistance in organisms recovered from other growth media, or if they were employed as a primary isolation medium. Evaluation of the media as tools for the surveillance and recovery of COL-resistant

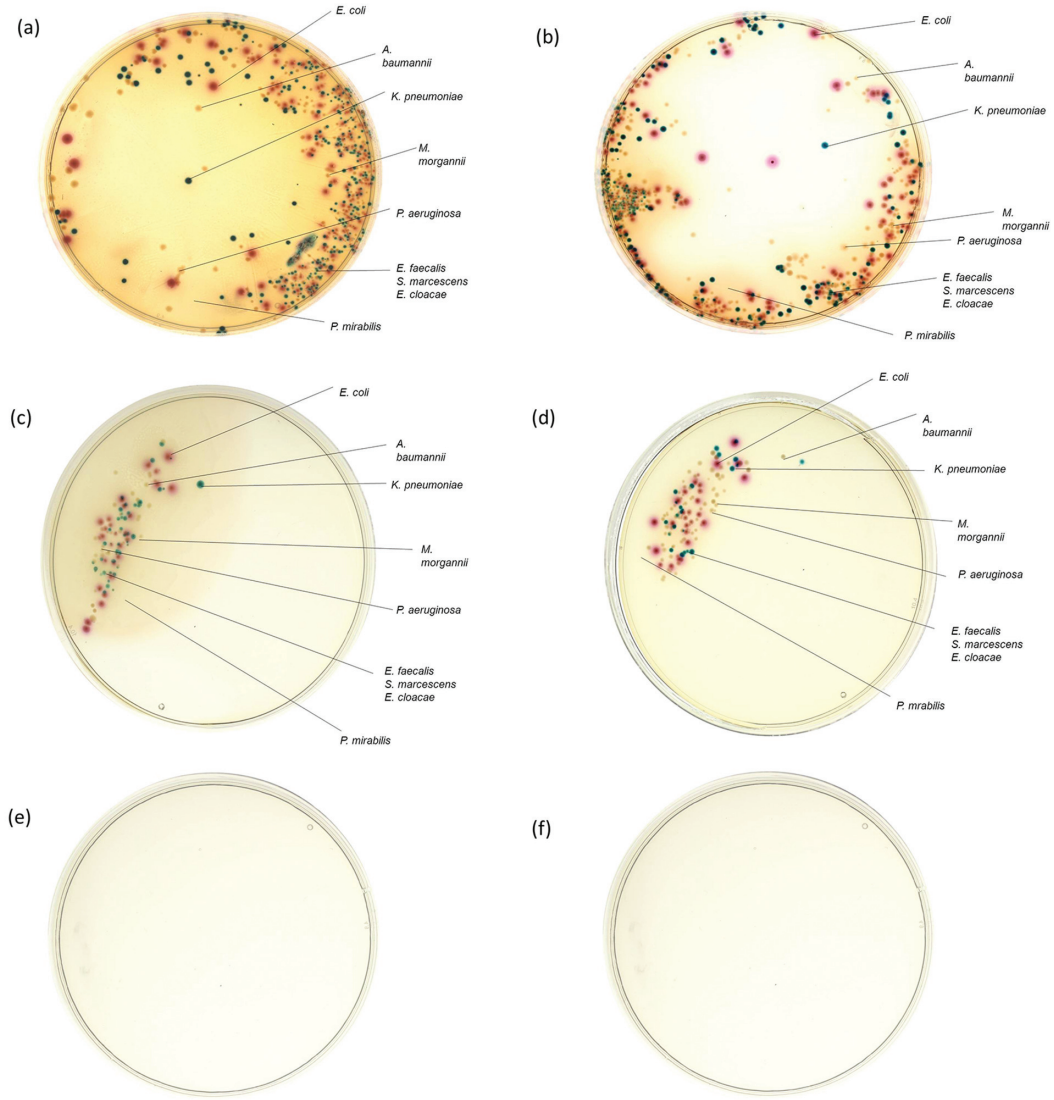

Fig. 2. Growth of simulated pools of COL-susceptible and COL-resistant strains on CHROMagar base media and CHROMagar COL-*APSE*. (a) CHROMagar COL-*APSE* simulated pool containing *A. baumannii* AB205 (COL R), *E. coli* E7 (COL R). *E. cloacae* NCTC 10005 (COL R), *E. faecalis* ATCC 2912 (COL R), *K. pneumoniae* KP19 (COL R), *M. morgannii* MM2 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant), *S. marcescens* NCTC 10211 (COL R) and *P. mirabilis* NCTC 13376 (COL R). The plate was inoculated with an L-shaped spreader. (b) CHROMagar COL-*APSE* + 50 mg l^{-1} p-nitrophenyl glycerol (PNPG) simulated pool containing *A.baumannii* AB205 (COL R), *E. coli* E7 (COL R). *E. cloacae* NCTC 10005 (COL R), *E. faecalis* ATCC 2912 (COL R), *K. pneumoniae* KP19 (COL R), *M. morgannii* MM2 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant), *S. marcescens* NCTC 10211 (COL R) and *P. mirabilis* NCTC 13376 (COL R). The plate was inoculated with an L-shaped spreader. (c) CHROMagar COL-*APSE* simulated pool containing *A. baumannii* AB205 (COL R), *E. coli* E7 (COL R). *E. cloacae* NCTC 10005 (COL R), *E. faecalis* ATCC 2912 (COL R), *K. pneumoniae* KP19 (COL R), *M. morgannii* MM2 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant), *S. marcescens* NCTC 10211 (COL R) and *P. mirabilis* NCTC 13376 (COL R). The plate was inoculated using the streaking method. (d) CHROMagar COL-*APSE* + 50 mg l^{-1} p-nitrophenyl glycerol (PNPG) simulated pool containing *A. baumannii* AB205 (COL R), *E. coli* E7 (COL R). *E. cloacae* NCTC 10005 (COL R), *E. faecalis* ATCC 2912 (COL R), *K. pneumoniae* KP19 (COL R), *M. morgannii* MM2 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant), *S. marcescens* NCTC 10211 (COL R) and *P. mirabilis* NCTC 13376 (COL R). The plate was inoculated using the streaking method. (e) CHROMagar COL-*APSE* simulated pool containing *A. baumannii* ATCC 19606 (COL S), *E. coli* ATCC 25922 (COL S). *E. faecalis* ATCC 2912 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant) and *K. pneumoniae* ATCC 9633 (COL S). The plate was inoculated with an L-shaped spreader. (f) CHROMagar COL-*APSE* + 50 mg l^{-1} p-nitrophenyl glycerol (PNPG) simulated pool containing *A. baumannii* ATCC 19606 (COL S), *E. coli* ATCC 25922 (COL S). *E. faecalis* ATCC 2912 (COL R), *P. aeruginosa* ATCC 27853 (COL heteroresistant) and *K. pneumoniae* ATCC 9633 (COL S). The plate was inoculated with an L-shaped spreader.

bacteria from complex human, veterinary and environmental samples is therefore underway, focusing on the identification of those with MCR-mediated polymyxin resistance.

Funding information
We thank Alberto Lerner for the supply of CHROMagar base media and supplements used in this study, and E and O laboratories for commercial production of the media.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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