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Evaluation of Meropenem, Imipenem and Ertapenem Impregnated MacConkey Agar Plates for the Detection of Carbapenem Resistant *Enterobacteriaceae*

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Abstract

Background: Rapid detection of carbapenem resistant bacteria, in particular, members of the *Enterobacteriaceae* family (CRE), is of utmost importance for the management of infected or colonized patients.

Methods: Three carbapenems; meropenem, imipenem and ertapenem, with two different concentrations (0.5 μg/ml and 1.0 μg/ml), were impregnated in MacConkey agar. The carbapenem impregnated MacConkey agar plates; ([Mac-Mem], [Mac-Imp] and [Mac-Ert]), were then evaluated for the detection of carbapenem resistant Gram-negative bacteria in particular the bla_{KPC} producing *Enterobacteriaceae*. The Limit of Detection (LOD) of the plates was determined in triplicate after serial logarithmic dilution of the bacterial strains in saline. This was followed by inoculating the plates and counting the colonies that grew after 24 hours of incubation. The specificity and the shelf-life of the plates were determined by testing the plates with six Extended Spectrum β-lactamases (ESBL) producing members of the *Enterobacteriaceae* family and one genus with the bla_{AmpC} phenotype. Finally, the 0.5 μg/ml Mac-Mem plates were further challenged by incorporating them in the routine active surveillance program for the detection of carbapenem resistant bacteria.

Results: Of the three carbapenems impregnated plates, Mac-Ert plates gave the lowest LOD; however, its specificity was poor as it failed to inhibit the growth of some of the ESBL producing strains. The Mac-Imp plate LOD and specificity were both unsatisfactory for several strains of the bacterial isolates tested. On the other hand the 0.5 μg/ml Mac-Mem plates showed low LOD for the bla_{KPC} producing bacteria and had excellent specificity as it prevented the growth of all the ESBL and bla_{AmpC} positive isolates tested. Not only that, but the shelf-life of the 0.5μg/ml Mac-Mem plate was long, up to one month at 4°C.

Conclusions: The high specificity and the good selectivity, in addition to the long shelf-life allowed the 0.5μg/ml Mac-Mem agar to be used as a cost effective selective medium for the isolation of carbapenem resistant Gram-negative bacteria, in particular the bla_{KPC} producing members of the *Enterobacteriaceae* family.

Key words: bla_{KPC}, bla_{OXA-48}, Meropenem Agar (Mac-Mem), Carbapenemase, Palestine

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Introduction

Rapid detection of carbapenem resistant bacteria, in particular, members of the *Enterobacteriaceae* family (CRE) is of utmost importance for the management of infected or colonized patients (1). Carbapenems are widely used to treat severe bacterial infections that are often hard to manage. Several carbapenem resistance mechanisms have been implicated in how bacterial strains become resistant to carbapenems. Based on the molecular properties of the β–lactamases, four classes of carbapenemases have been identified (2). These include the molecular classes (A, C, and D) β-lactamases with the amino acid serine at their active site, and the molecular class B where the β-lactamases are all metalloenzymes with an active-site zinc (2). Class A enzymes, an Ambler molecular class enzymes (ex: bla_{KPC}), have been the most common detected carbapenemases with worldwide distribution (1).

For any health care facility, it is important to understand the level of CRE spreading in its different medical departments. It has been recommended by the American Centers of Disease Control and Prevention (CDC), that in areas which are not endemic for CRE, the acute care facilities should review all the microbiology records for the preceding 6-12 months to determine if CRE's have been isolated at the institution (3). Upon the identification of CRE cases, surveillance cultures from high risk areas should be performed to identify any unrecognized cases.

CRE's active surveillance cultures (perianal/rectal swabs), taken from patients upon admission are of great importance for the identification of unrecognized CRE-colonized patients, in order to take immediate and appropriate isolation precautions (4, 5). Studies have shown that in areas where CRE's are endemic, up to 5.4% of hospitalized patients can be carriers of CRE's, of which only one-third had a positive clinical culture (4). Moreover, surveillance swabs have been reported to considerably lower the cost of patient hospitalization (6). Active surveillance cultures taken from patients coming from highly CRE endemic medical settings upon admission have been used as part of a comprehensive strategy to combat the spread of CRE's. Studies have shown a 4.7-fold reduction in the incidence of CRE infection following the activation of the active CRE surveillance program (7, 8).

Several highly sensitive Polymerase Chain Reaction (PCR) assays and Real-Time PCR assays have been developed and validated for the rapid detection and identification of bla_{KPC} positive bacteria (9-12). However, one of the disadvantages of these assays was the need for specialized equipment and well trained personnel. Consequently, screening agar plates, that have incorporated one of the commonly used carbapenems (ertapenem, meropenem or imipenem), have been developed and utilized for the rapid detection of bla_{KPC} producing bacteria. Chromogenic agar, CHROMagar^{TM} KPC (CHROMagar, France), has been evaluated for the detection of bla_{KPC} positive bacteria. However, this agar base medium still missed certain strains of bla_{KPC} positive *Enterobacteriaceae* (1, 13, 14). Similar performance between the CHROMagar^{TM} KPC and MacConkey Agar impregnated with 2 μg/ml imipenem was noted by Adler et al. (15). More recently, the Drigalski agar-based culture medium was reported to have excellent sensitivity and specificity for the detection of bacteria carrying the bla_{KPC} resistance mechanism (14).

In this study, we report the evaluation of cost effective MacConkey Agar plates impregnated with one of the carbapenems (meropenem, imipenem and ertapenem) for the detection of CRE's. In addition, the specificity, analytical sensitivity and the plates' shelf-life were also evaluated. The concentrations of the antibiotics evaluated were 1.0 μg/mL and 0.5 μg/mL. These concentrations were chosen after careful evaluation of the carbapenems lowest minimal inhibitory concentration (MIC) interpretation guidelines published by the Clinical and Laboratory Standards Institute (CLSI) (17).

Materials and Methods

Reagents

Meropenem trihydrate (Catalogue # M2574) and imipenem monohydrate (Catalogue # I0160) were supplied by Sigma-Aldrich, USA. Ertapenem sodium, Invanz (Catalogue # 7290004997960) was supplied by Merck Sharp and Dohme Corp, USA. All reagents were stored and diluted according to the manufacturer's guidelines.

Bacterial Strains

Carbapenem resistant *Enterobacteriaceae* members (N=9), Extended Spectrum β–Lactamases (ESBL) producing *Enterobacteriaceae* strains (N= 6), bla_{AmpC} β-lactamase (N=1) and multi-drug resistant *Acinetobacter baumannii* (N=1), all isolated from patients' samples at Caritas Baby Hospital (CBH) laboratory, were used to evaluate the performance of the carbapenems impregnated MacConkey Agar. The carbapenem resistant isolates included: 3 *Klebsiella pneumoniae* bla_{KPC} positive; 2 *Enterobacter* species bla_{KPC} positive; 2 *E. coli* bla_{KPC} positive; 1 *Citrobacter* species bla_{KPC} positive and 1 *Klebsiella pneumoniae* $bla_{OXA\text{-}48}$ positive. The ESBL positive isolates were (*K. pneumoniae*, *Salmonella* species, *Shigella sonnei*, *E. coli* , *Proteus* species and *Citrobacter* species); while the bla_{AmpC} positive bacteria was an *Enterobacter* species. Moreover, 14 *Klebsiella pneumoniae* $bla_{OXA\text{-}48}$ positive and 2 *E. coli* $bla_{OXA\text{-}48}$ positive were used to evaluate the analyti-

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cal sensitivity of the 0.5 µg/ml Mac-Mem agar plates for the detection of low level carbapenemase producing bla_{OXA-48} *Enterobacteriaceae.*

All bacterial strains were identified according to the Clinical Microbiology Procedures Handbook, and their antimicrobial susceptibility testing was performed in duplicate according to the Clinical and Laboratory Standard Institute guidelines (CLSI) (16, 17). In addition, the Minimal Inhibitory Concentrations (MIC) of meropenem, imipenem and ertapenem for all tested members of the *Enterobacteriaceae* family were determined using the Etest® (bioMérieux, France) on Mueller-Hinton plates (Oxoid, UK). The presence of the carbapenemase enzyme in the members of the *Enterobacteriaceae* family was confirmed by the modified Hodge Test (17), and in duplicate by the Polymerase Chain Reaction (PCR) amplification of either the bla_{KPC} or the bla_{OXA-48} genes (18, 19).

Limit of Detection (LOD) of Carbapenems Impregnated MacConkey Agar Plates

Three types of carbapenem impregnated MacConkey agar plates (Mac-Mem, Mac-Imp and Mac-Ert), with two different concentrations (0.5 µg/ml and 1.0 µg/ml), were prepared and evaluated for the detection of carbapenem resistant bacteria. Bacterial strains were inoculated on 5% sheep blood agar (Hy-Lab, Rehovot) and incubated in ambient air at 35ºC for 18-24 hours. Well isolated colonies of the different strains were used to prepare bacterial suspensions equivalent to 0.5 McFarland turbidity standard (1.5 x 10^8 CFU/ml) using Oxoid Turbidimeter (Oxoid, UK). This was followed by preparing serial logarithmic dilutions of 10 (1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, 1:1,000,000) for each isolate in normal saline. For each bacterial strain, an aliquot (100 µl) from each dilution was inoculated on the 0.5 µg/ml and 1.0 µg/ml Mac-Mem, Mac-Imp and Mac-Ert plates in duplicate. Thus, the total number of plates used for checking each antibiotic concentration was 12 plates. In addition, a bacterial viability control was performed by inoculating all of the bacterial dilutions on MacConkey with no carbapenems in duplicate. The inoculums were spread on the whole plate surface with a sterile plate spreader. All inoculated plates were incubated in ambient air at 35ºC for 18-24 hours. Following incubation, bacterial colonies were counted and the LOD of each plate was determined by calculating the ratio between the number of colonies that grew on the MacConkey agar plates with and without antibiotic. Plates with less than 300 colonies were counted. The two tailed Student t-test was used to determine statistical significance between the LOD's of the plates evaluated.

Determination of the specificity and Shelf-Life of the different Carbapenems Impregnated Plates

MacConkey agar plates (Mac-Mem, Mac-Imp, and Mac-Ert) impregnated with the two different concentrations (0.5 µg/ml and 1.0 µg/ml) of the three carbapenems, were prepared and stored at 4-8ºC. The plates were inoculated with colonies from six members of the *Enterobacteriaceae* family (*K. pneumoniae, Salmonella* species, *Shigella* species, *E. coli, Proteus* species, and *Citrobacter* species), all with the ESBL phenotype and one *Enterobacter* species with the bla_{AmpC} phenotype. The plates specificity was determined after monitoring the growth of the ESBL and bla_{AmpC} positive bacteria on freshly prepared plates; while the plates shelf-life was determined by monitoring bacterial growth on the different plates after 7, 14, 21 and 30 days of plates storage at 4-8ºC. The plates were inoculated and incubated in ambient air at 35ºC for 18-24 hours. Following incubation, the plates were evaluated for the growth of any bacterial colonies.

Detection of low level carbapenemase producing bla_{OXA-48} *Enterobacteriaceae* on 0.5µg/ml Mac-Mem

0.5µg/ml Mac-Mem agar plates were inoculated with colonies from 16 bla_{OXA-48} producing members from the *Enterobacteriaceae* family (14 *Klebsiella pneumoniae* and 2 *E. coli*). The MIC's of the isolates tested to the 3 carbapenems were determined by the E-test®. All inoculated plates were incubated in ambient air at 35ºC for 18-24 hours. Following incubation, bacterial colonies were counted and the LOD of each plate was determined in order to evaluate the analytical sensitivity of the 0.5µg/ml Mac-Mem for detecting bla_{OXA-48} producing CRE's.

Detection of CRE's on 0.5 µg/ml Mac-Mem agar from clinical patient samples

0.5µg/ml Mac-Mem agar plates were incorporated from January 2012 till April 2013 into CBH active surveillance system for the detection of drug resistant bacteria (19). During the study period, 2,784 swabs were collected from three body sites (nose/throat, umbilical/axillary, and rectal) of patients referred to CBH for admission from other medical institutions. All three swabs were inoculated on MacConkey agar with 10µg/ml Cefotaxime (Mac-CTX), 5% Sheep Blood Agar with 6µg/ml vancomycin, 5% Sheep Blood Agar (Hy-Lab Rehovot) and 0.5µg/ml Mac-Mem. Inoculated plates were incubated in ambient air at 35ºC for 18-24 hours. Any bacterial growth on any of the selective media was worked up according to the Clinical Microbiology Procedures Handbook and their antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standard Institute guidelines (CLSI) (16, 17).

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Results

Analytical sensitivity of the 0.5 µg/ml and 1.0 µg/ml Mac-Mem, Mac-Imp and Mac-Ert Agar plates

The lowest number of bacterial colony forming units (LOD) for each of the three plates Mac-Mem, Mac-Imp and Mac-Ert was determined for the two antibiotic concentrations in duplicate and the LOD testing was repeated three times with similar results. Moreover, the MIC's of carbapenem resistant bacteria to the three carbapenems, were also determined (**Table 1**). Of the three carbapenems evaluated, Mac-Ert plates gave the lowest number of CFU's detected regardless of the concentration of the antibiotic used (0.5 µg/ml or 1.0µg/ml). With the exception to the *K. pneumoniae* with the $bla_{OXA\text{-}48}$ carbapenem resistance mechanism which failed to grow on both Mac-Ert concentrations. The Mac-Ert plate lowest LOD for all of the carbapenem resistant bacterial strains tested was <15 CFU's, regardless of their carbapenem MIC's (**Table 1**). There was a statistical significance (p-value <0.05) between the LOD of the Mac-Ert, for the majority of the organisms tested, when compared to the Mac-Mem and the Mac-Imp (**Table 1**).

On the other hand, the 0.5µg/ml Mac-Mem plates detected all 10 strains of carbapenem resistant bacteria, however at a

Table 1. Limit of Detection of the three carbapenem impregnated MacConkey Agar plates.

Bacterial Strain	Bacterial Isolates MIC's (E-Test) mg/L*			Limit of Detection (CFU) of 0.5 µg/ml Plates**			Limit of Detection (CFU) of 1.0µg/ml Plates**		
	Meropenem	Imipenem	Ertapenem	Meropenem	Imipenem	Ertapenem	Meropenem	Imipenem	Ertapenem
Klesiella pneumoniae (*bla_{KPC}*) 57449	≥32	≥32	≥32	1	1	9	1	1	13
E. coli (*bla_{KPC}*) 41807	8	32	4	1.6 x 10^{3} ~	NG	1 #	9.1 x 10^{5}	NG	1
Citrobacter species (*bla_{KPC}*) 46685	32	24	24	2.5 x 10^{5} ~	NG	1 #	2.2 x 10^{8}	NG	1
Enterobacter species(*bla_{KPC}*) 40426	≥32	≥32	≥32	4 ~	1,204	1 #	523	NG	1
Klebsiella pneumoniae (*bla_{KPC}*) 40926	≥32	≥32	≥32	17 ~	108	1 #	5.5 x 10^{3}	1.9 x 10^{4}	1
Klebsiella pneumoniae (*bla_{KPC}*) 59313	≥32	≥32	≥32	43 ~	2.3 x 10^{3}	1 #	3.9 x 10^{3}	NG	1
Enterobacter species(*bla_{KPC}*) 42783	≥32	≥32	≥32	1 ~	310	1	5	NG	1
E. coli (*bla_{KPC}*) 47961	≥32	16	12	290 ~	NG	2 #	1.4 x 10^{5}	NG	2
Klebsiella pneumoniae (*$bla_{OXA\text{-}48}$*)	0.75	2	≥32	1.1 x 10^{6} ~	NG	NG	NG	NG	NG
Acinetobacter baumannii MDR	≥32	≥32	≥32	1	1	1	1	1	1

NG = No Growth; *MIC = Minimal Inhibitory Concentration; ** Limit of Detection is the lowest number of colony forming units that was detected on the different plates.
#Statistically significant difference, p Value (<0.05), between LOD of Mac-Ert and both the Mac-Mem and Mac-Imp.
~Statistically significant difference, p Value (<0.05), between LOD of Mac-Mem and Mac-Imp.

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2013
Vol. 3 No. 3:5
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Table 2. Growth of bacterial strains with the ESBL phenotype and *Enterobacter* species with *bla*$_{AmpC}$ phenotype on the different freshly prepared agar plates.

Bacterial Strain	Growth on Mac-Mem 0.5µg/ml / 1.0µg/ml	Growth on Mac-Imp 0.5µg/ml / 1.0µg/ml	Growth on Mac-Ert 0.5µg/ml / 1.0µg/ml
K. pneumoniae	NG / NG	NG / NG	NG / NG
Salmonella species	NG / NG	NG / NG	NG / NG
Shigella species	NG / NG	NG / NG	NG / NG
E. coli	NG / NG	NG / NG	NG / NG
Proteus species	NG / NG	G / G	G / NG
Citrobacter species	NG / NG	G / G	G / G
Enterobacter species	NG / NG	G / G	G / G

NG = No growth ; G = Growth

higher LOD. The 0.5µg/ml Mac-Mem plates detected 8 out of the 10 strains at a limit of detection of ≤ 1.6 x 10^3 CFU's, while the lowest LOD for the *Citrobacter* species *bla*$_{KPC}$ and the *K. pneumoniae bla*$_{OXA-48}$ were 2.5 x 10^5 and 1.1 x 10^6 CFU's, respectively. Higher LOD was noted for the 1.0µg/ml Mac-Mem agar in addition to its failure to detect the *K. pneumoniae bla*$_{OXA-48}$ (**Table 1**). The worst performance was noted for the imipenem plate regardless of the antibiotic concentration used as a number of *bla*$_{KPC}$ producing bacterial strains in addition to the *Klebsiella pneumoniae bla*$_{OXA-48}$ positive strain failed to grow on the 0.5µg/ml and the 1.0µg/ml plates (**Table 1**). There was a statistical significance (*p*-value <0.05) between the LOD of the Mac-Mem, for the majority of the organisms tested, when compared to the Mac-Imp (**Table 1**). All three plates tested at both antibiotic concentrations had low limit of detection (1 CFU) for the multi-drug resistant *Acinetobacter baumannii*.

Specificity of the Mac-Mem, Mac-Imp and the Mac-Ert Agar plates.

Six members of the *Enterobacteriaceae* family (*K. pneumoniae, Salmonella* species, *Shigella* species, *E. coli, Proteus* species, and *Citrobacter* species), all with the ESBL phenotype, in addition to one *Enterobacter* species with the *bla*$_{AmpC}$ phenotype were used to evaluate the specificity of the plates for detecting carbapenem resistant bacterial strains in triplicate. Mac-Mem plates showed the best specificity among the carbapenem plates tested, as none of the strains grew on either the 0.5µg/ml or the 1.0µg/ml Mac-Mem plates (**Table 2**). On the other hand, the Mac-Ert plates consistently failed to inhibit the growth of the *Citrobacter* and the *Enterobacter* species tested at both antibiotic concentrations and the *Proteus* species tested at the 0.5µg/ml antibiotic concentration. As for Mac-Imp plate, it showed poor specificity as both antibiotic concentrations consistently failed to inhibit the growth of the *Proteus*, *Enterobacter* and *Citrobacter* species evaluated (**Table 2**).

Shelf-life of the 0.5µg/ml and 1.0µg/ml Mac-Mem, Mac-Imp and Mac-Ert Agar plates

Six members of the *Enterobacteriaceae* family (*K. pneumoniae, Salmonella* species, *Shigella* species, *E. coli, Proteus* species and *Citrobacter* species) all with the ESBL phenotype, in addition to one *Enterobacter* species with the *bla*$_{AmpC}$ phenotype were utilized to evaluate the shelf-life of the different plates in triplicate after 7, 14, 21 and 30 days. None of the bacterial isolates grew on the 0.5µg/ml or the 1.0µg/ml Mac-Mem plates after plates storage for 30 days at 4-8°C. On the other hand, bacterial growth was noted on the stored Mac-Imp and Mac-Ert plates after one day of the plates preparation, and after 7, 14, 21 and 31days of plates storage for some of the isolates.

Evaluation of the 0.5µg/ml Mac-Mem agar plates for the detection of low level carbapenemase producing *bla*$_{OXA-48}$ *Enterobacteriaceae*

The analytical sensitivity of the 0.5µg/ml Mac-Mem agar plates was determined on 16 *bla*$_{OXA-48}$ positive *Enterobacteriaceae* (14 *K. pneumoniae* and 2 *E. coli*) in duplicate (**Table 3**). The 16 *bla*$_{OXA-48}$ positive *Enterobacteriaceae* MIC's (mg/L) of the three carbapenems widely varied (**Table 3**). For meropenem, the MIC's were between 0.25 and 16, for imipenem between 0.5 and 8, while for ertapenem between 0.75 and >32. Consistent with the low meropenem MIC's, the limit of detection of the 0.5µg/ml Mac-Mem agar varied between 3.0E+05 (CFU/ml) and ≤ 6.60E+06. One of the isolates growth was completely inhibited (**Table 3**).

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2013
Vol. 3 No. 3:5
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Table 3. Limit of detection of *bla*$_{OXA-48}$ producing *Enterobacteriaceae* on the 0.5 μg/ml meropenem plates and the MIC's to the 3 carbapenems

bla$_{OXA-48}$ Positive Bacteria	Carbapenem MIC mg/L determined by E-Test			Limit of Detection(CFU) of 0.5μg/mL Mac-Mem
	Meropenem	Imipenem	Ertapenem	
K. pneumoniae 1560	0.5	1.5	1.5	6.60E+05
K. pneumoniae 1937	0.5	1	1	3.00E+05
K. pneumoniae 2301	0.5	0.75	>32	1.68E+06
K. pneumoniae 2307	0.5	1	2	2.04E+06
K. pneumoniae 2663	0.5	1	3	ND
K. pneumoniae 2806	4	1	>32	7.8E+05
K. pneumoniae 2213	16	8	>32	3.0E+05
K. pneumoniae 2214	0.38	2	4	1.14E+06
K. pneumoniae 2215	0.75	0.75	2	1.44E+06
K. pneumoniae 2216	0.38	0.75	12	4.68E+05
K. pneumoniae 2217	0.75	2	>32	3.00E+05
K. pneumoniae 3856	0.38	0.75	1	3.00E+05
K. pneumoniae 4451	0.25	0.5	8	6.60E+06
K. pneumoniae 4529	0.38	4	2	3.12E+06
E. coli 3856F	0.38	0.75	2	1.38E+06
E. coli 3856NF	0.5	0.75	0.75	6.60E+05

ND = Not Detected

Detection of CRE in clinical patient samples inoculated on 0.5µg/ml Mac-Mem agar plates

The 0.5µg/ml Mac-Mem Agar plate was included in panel of plates used in the active bacterial drug resistance surveillance program at CBH (19). From the 2,784 inoculated swabs, 59 carbapenem resistant strains of bacteria were isolated on the 0.5µg/ml Mac-Mem plates and on the Mac-CTX. These include *K. pneumoniae bla*$_{KPC}$ (N=12), *Enterobacter cloacae bla*$_{KPC}$ (N = 2), *Enterobacter cloacae bla*$_{VIM}$ (N=1), *Klebsiella pneumoniae bla*$_{OXA-48}$ (N=16), *E. coli bla*$_{OXA-48}$ (N=3), multi-drug resistant *Acinetobacter baumannii* (N=10), multi-drug resistant *Pseudomonas aeruginosa* (N = 10), and *Stenotrophomonas maltophilia* (N=5). Thus, the clinical sensitivity, specificity, positive predictive values and negative predictive values for the Mac-Mem when compared to the Mac-CTX were 100%.

It was of importance to note that other types of Gram-negative bacteria grew on the 0.5µg/ml Mac-Mem plate. These include *Pseudomonas aeruginosa* strains that were not resistant to meropenem but had a meropenem MIC greater than 0.5µg/ml. In addition, bacterial strains (N=20) from the *Enterobacteriaceae* family carrying the *bla*$_{AmpC}$ β-lactamase grew on the primary streak zone of the 0.5µg/ml meropenem. These isolates that hyper produce *bla*$_{AmpC}$ β-lactamase were not included in the study but their isolation was of importance from an infection control point of view.

Discussion

Rapid, sensitive and cost effective ways are of utmost importance for the early detection of CRE's in patient samples. In this study we evaluated the performance of MacConkey agar plates impregnated with different concentrations (0.5µg/ml or 1.0µg/ml) of one of the three commonly used carbapenems; meropenem, imipenem and ertapenem. In addition, the plates' specificity to detect carbapenem resistant bacteria, their shelf-life at 4-8ºC and their cost effectiveness were determined.

The Mac-Ert with either of the two antibiotics concentrations showed the best LOD of the *bla*$_{KPC}$ positive *Enterobacteriaceae*. On both plate concentrations the lowest LOD was between 1 and 13 CFU's (**Table 1**). Surprisingly, both Mac-Ert plates did not support the growth of the *bla*$_{OXA-48}$ producing *K. pneumoniae* strain used in the evaluation (**Table 1**). The overall performance of the Mac-Ert plates was similar to that reported by Nordman et al., who reported an LOD

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2013
Vol. 3 No. 3:5
doi: 10.3823/737

of <500 CFU/mL for some of the *Enterobacteriaceae* members that were cultured on the Supercarba medium (Drigalski agar-base culture medium containing 0.25µg/ml ertapenem, 250µg/ml cloxacillin and 70µg/ml $ZnSO_4$) (14). However, the Supercarba medium supported the growth of the bla_{OXA-48} positive strains (14). Unlike its excellent LOD, the specificity and the shelf-life of the Mac-Ert plates were poor as *Citrobacter* species with the ESBL phenotype and *Enterobacter* species with the bla_{AmpC} phenotype grew on the plates (**Table 2**). Consistent with what was noted on the Mac-Ert plates, some bacterial strains carrying the bla_{AmpC} phenotype grew on the Supercarba medium (14). Similar to the low LOD of Mac-Ert to the bla_{KPC} positive *Enterobacteriaceae,* it was also shown by Lolans et al., that the utilization of two 10µg/ml ertapenem disks on MacConkey agar can facilitate the detection of bla_{KPC} positive bacteria (20). Interestingly, in that study the authors did not evaluate the growth of different ESBL and bla_{AmpC} positive *Enterobacteriaceae* around the ertapenem disks.

Unlike the Mac-Ert plates, both concentrations of the Mac-Imp plates (0.5µg/ml or 1.0µg/ml) gave poor sensitivity, specificity and shelf-life. Both concentrations of the Mac-Imp plates failed to inhibit the growth of *Proteus* species and *Citrobacter* species with the ESBL phenotype, and *Enterobacter* species with the bla_{AmpC} phenotype (**Table 2**). In addition to the poor specificity, the plates' shelf-life was extremely short as the Mac-Imp plates failed to inhibit the growth of two of the ESBL positive isolates and the bla_{AmpC} positive isolate tested. The LOD of the Mac-Imp plates evaluated in this study was similar to that reported by Adler et al. who showed that the MacConkey agar impregnated with 2µg/ml imipenem had a limit of detection between 1.1E+02 and 8.3E+06 (15); however, the authors did not evaluate the shelf-life of the plate.

The 0.5µg/ml Mac-Mem plates gave suitable LOD for the bla_{KPC} positive *Enterobacteriaceae* when compared to the 1.0µg/ml Mac-Mem agar plates as the lowest LOD was less than 2.0E +03 for the majority of the bla_{KPC} positive bacteria (**Table 1**). One *Citrobacter* species gave an LOD of 2.5E+05 CFU/ml in the 0.5µg/ml Mac-Mem plate. The LOD of the 0.5µg/ml Mac-Mem plates for the detection of bla_{KPC} positive *Enterobacteriaceae* was similar to that reported by Nordman et al. who reported an LOD of <500 CFU/ml for some of the *Enterobacteriaceae* members (14). However, in the study conducted by Nordman et al. the authors did not evaluate bla_{KPC} positive *Citrobacter* species (14). Consistent with the excellent sensitivity, the specificity of the 0.5µg/ml Mac-Mem plates was excellent as the plates prevented the growth of both ESBL and bla_{AmpC} positive tested members of the *Enterobacteriaceae* family (**Table 2**). The specificity of the 0.5 and 1.0µg/ml Mac-Mem plates was the best when compared to the Mac-Ert and the Mac-Imp plates.

The 0.5 µg/ml Mac-Mem agar long shelf-life (30 days) was of considerable importance since the laboratory technologist can prepare the medium and stores it at 4-8°C for up to four weeks. With the current work load and the number of employees available in the clinical microbiology laboratory, such long shelf-life is of importance. The four week shelf-life is much longer than the one week shelf-life of the Supercarba medium (14).

The preparation of the 0.5µg/ml Mac-Mem plates in the clinical laboratory was cost effective. In Palestine, the direct cost of preparing the plate was 0.33 USD. The cost is cheaper than the CHROMagar^{TM} KPC and the 1.0µg/ml imipenem MacConkey Agar which cost $2.1 and $0.7, respectively (15). Estimated cost analysis of the Supercarba medium is above the cost of the 0.5µg/ml Mac-Mem agar plates.

The 0.5µg/ml Mac-Mem agar plate excellent analytical sensitivity, specificity, low cost and long shelf-life prompted the utilization of this screening plate on 2,748 patient swab samples for the presence of carbapenem resistant Gram-negative bacteria. The 0.5µg/ml Mac-Mem agar plate inhibited the growth of non-carbapenem resistant bacteria and allowed for the isolation of 59 different bacterial strains with different carbapenem resistant mechanisms. The isolation of these pathogens was at least 24-48 hours faster than isolating them on MacConkey agar impregnated with the third generation cephalosporin Cefotaxime (Mac-CTX). This was of utmost importance since it allowed the microbiology laboratory personnel to report the isolation of these pathogens to the infection control team who took the necessary precautions to prevent the spread of these pathogens in the hospital setting. However, one of the drawbacks that were noted after utilizing the 0.5 µg/ml Mac-Mem was the growth of bacterial strains with the bla_{AmpC} phenotype on the primary streak zone of the plate. The growth of bacterial strains carrying the bla_{AmpC} phenotype was also reported on the Supercarba medium but to a much lower extent. However, the authors added 250µg/ml cloxacillin to reduce the chance that these bacterial strains that hyper produce the bla_{AmpC} will grow (14). In our study we opted not to add the cloxacillin since the detection of bacterial isolates with the bla_{AmpC} phenotype did not occur frequently. The other drawback of utilizing the meropenem instead of ertapenem was the high LOD of the bla_{OXA-48} positive bacteria. Overall, the LOD of the bla_{OXA-48} positive bacteria was around 1.4E+06. This was higher than the LOD reported by Nordmann et al. who showed that depending on the bla_{OXA-48} positive bacteria, the LOD can be as low as 1 CFU/mL to 5.0E+02 CFU/mL (14). The higher LOD of bla_{OXA-48} positive bacteria on the 0.5µg/ml Mac-Mem plate did not compromise the bacterial detection as 16 isolates were detected on both the 0.5µg/ml Mac-Mem and the 10 µg/mL Mac-CTX plates.

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Overall, the 0.5µg/ml Mac-Mem agar plate can be used as a cost effective selective medium for the isolation of carbapenem resistant Gram-negative bacteria in particular the ones induced by the bla_{KPC} carbapenem resistance mechanism. Not only that, but the 0.5µg/ml Mac-Mem agar allows for the detection of other carbapenem resistant mechanisms such as bla_{VIM} and bla_{OXA-48}. In areas where carbapenem resistance mechanism is endemic this plate can be used to rapidly assist the infection control practitioners in controlling the spread of these pathogens.

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