Utilization of MacConkey-Meropenem screening Agar for the Detection of Carbapenem Resistanant Enterobacteriaceae in a Tertiary Care Hospital

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Abstract:
Introduction: *The increasing frequency of Carbapenem Resistant Enterobacteriaceae (CRE) has become a serious threat to public health due to production of different types of carbapenemases, multi-drug resistant (MDR) and limited treatment options. The present study was planned to evaluate the MacConkey agar supplemented with meropenem at 1µg/ml, as a screening agar for the detection of CRE among the clinical isolates of Enterobacteriaceae.*
Methods: *The total 1080 Enterobacteriaceae species isolated from different clinical samples. All the isolates were identified based on standard bacteriological techniques. All these isolates were screened for meropenem resistance by Kirby-Bauer disk diffusion method according to CLSI guidelines. Carbapenem resistant isolates were inoculated on screening agar (MacConkey agar supplemented with meropenem at concentration of 1µg/ml) and also tested for carbapenemase production by phenotypic method (combined disk method).*

Results: *Among the 1080 clinical isolates of Enterobacteriaceae, 50 isolates showed resistance to carbapenem by Kirby Bauer disk diffusion method. All of these 50 isolates were grown on carbapenem screening agar. Of these 50 carbapenem resistant isolates, Eighteen (36%) E.coli, 22(44%) Klebsiella species, 6(12%) Enterobacter species, 3(6%) Citrobacter species and 1(2%) isolate was Proteus vulgaris. Out of 50 Carbapenemase producing Enterobacteriaceae, 18(36%) KPC producers, 15(30%) MBL producers, 13(26%) were both MBL&KPC producers and 4(8%) isolates were negative for MBL&KPC production by combined disk method.*

Conclusion: *MacConkey agar with meropenem 1µg/ml is the most appropriate for detection of carbapenem resistant Enterobacteriaceae (CRE). This screening agar plate provides a rapid, sensitive, convenient and relatively cost effective method for the screening of CRE.*

Keywords: *Screening agar, CRE, Meropenem 1 µg, Cost-effective.*

I. INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) have emerged globally and have become a major threat to public health.[1] The World Health Organization identified antimicrobial resistance as one of the three greatest threats to human health. More importantly, *Enterobacteriaceae* members possess the highest risk to public health, because of their rapid dissemination of resistance to other bacterial strains and species through plasmids and resistant to many drugs.[2,3] *Enterobacteriaceae* are normal flora of intestine and are important causes of most common clinical infections like urinary tract infection, pneumonia, septicaemia, meningitis, peritonitis and device associated infections.[4] Carbapenem-resistant *Enterobacteriaceae* was first described in the early 1990s.[5] *Enterobacteriaceae* are common causes of both community and hospital acquired infections. These problems, combined with the limited treatment options have made CRE of public health important.[6] Detection of CRE is challenging to the wide heterogeneity in resistance levels to carbapenems that depend on the enzyme and the physiological properties of an organism, which also makes it difficult to set up uniform screening and confirmatory tests for detection of carbapenemase producers. An agar screening assay for detection of carbapenem resistance in *Enterobacteriaceae* was initially described by Alder A.et al., they were used MacConkey agar supplemented with imipenem at 1µg/ml as the main screening agar plate for the detection of CRE from rectal swabs.[7] Chromogenic agar based media available in the market for the screening of CRE, in few studies the CHROMagarTM 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*,[8,9,10] Drigalski agar-based culture medium was reported to have excellent sensitivity and specificity for the detection of bacteria carrying the *bla*KPC resistance mechanism[10]. At present scenario, utilization of the carbapenemase inhibitors; phenyl boronic acid and EDTA to detect and differentiate *Klebsiealla* producing carbapenemase (KPC) and metallo-β-lactamase (MBL) from the isolates of *Enterobacteriaceae* was

shown to be highly sensitive and specific method.[11,12] Molecular techniques are the gold standard method for detection of carbapenem resistant genes in *Enterobacteriaceae*, however it is not suitable for routine laboratories due to cost effective, required well setup and trained technical persons.[13]

We are planned the study is to evaluate the usefulness of MacConkey agar supplemented with meropenem at 1µg/ml, as a screening agar for the detection of the Carbapenem Resistant *Enterobacteriaceae (CRE).*

II. MATERIALS AND METHODS

A prospective study was conducted over a period of eleven months (December 2013 to September 2014). A total of 1080 *Enterobacteriaceae* were isolated from specimens like pus, urine, blood, throat swabs and other body fluids of patients admitted to different wards, which were sent to the microbiology laboratory for routine culture identification and sensitivity testing.

All the *Enterobacteriaceae* isolates were identified based on standard bacteriological techniques.[14] All these isolates were screened for meropenem resistance by Kirby-Bauer disk diffusion method according to CLSI guidelines.[15] The Carbapenem screening agar was prepared by dissolving 51.53 grams of dehydrated MacConkey agar (Hi-media) in 1000ml distilled water and heated on hotplate for boiling to dissolve the medium completely; the media was sterilized by autoclaving at 15lbs pressure (121^0C) for 15 minutes. Cooled at 45-50^0C, aseptically added meropenem at 1mg/litre and mixed well and poured into sterile petri plates. All the isolates with a reduced susceptibility to meropenem (diameter of zones of inhibition, ≤ 21mm) were inoculated on to MacConkey agar supplemented with meropenem 1µg/ml and incubated at 37^0C for 24 hours. After 24 hours of incubation if an isolate grown on culture, it was considered as positive, while an isolate has not grown on culture, was considered as negative for carbapenemase resistance. The presence of the carbapenemase enzyme in the carbapenem resistant isolates of *Enterobacteriaceae* was confirmed by combined disc method.[12,13] The *E.coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC BAA-1705 strains are used as negative and positive controls respectively.

III. PHENOTYPIC METHOD FOR DETECTION AND DIFFERENTIATION OF KPC AND MBL

Phenylboronic acid (PBA), EDTA or both along with meropenem disc was used for detection of KPC and MBL, respectively. The stock solution of PBA in the concentration of 20 mg/ml was prepared by dissolving PBA in DMSO. Twenty microliters (400 µg of PBA) from this solution was dispensed onto meropenem discs. The stock solution of EDTA was prepared by dissolving anhydrous EDTA in distilled water at concentration of 0.1M. Ten microliters(292 µg of EDTA) from this solution was dispensed onto meropenem discs. The meropenem discs with inhibitor added was dried and used within 60 minutes.

Test strain inoculated on Muller Hinton agar plate as per the standard protocol, four discs of meropenem were used. One disc of meropenem was used without any inhibitor, one disc has PBA only, one disc has EDTA only and fourth disc of meropenem have both PBA and EDTA. The inoculated plates were incubated at 37^0C overnight and the diameter of the growth inhibitory zone around these meropenem discs with inhibitor added were compared with that around the plain meropenem disc.[11,12]

IV. INTERPRETATION

The isolates were considered KPC producing when the growth inhibitory zone diameter around the meropenem disc with PBA and the meropenem disc with both PBA and EDTA were increased ≥5mm were compared with the growth-inhibitory zone diameter around the disc containing meropenem alone.

The isolates were considered MBL producing when the growth inhibitory zone diameter around the meropenem disc with EDTA and the meropenem disc with both PBA and EDTA were increased ≥5mm were compared with the growth-inhibitory zone diameter around the disc containing meropenem alone.

The isolates were considered producing both KPC and MBL enzyme when the growth-inhibitory zone diameter around the meropenem disc with both PBA and EDTA were increased ≥5mm were compared with the growth-inhibitory zone diameter around the disc containing meropenem alone while the growth-inhibitory zone diameters around the meropenem disc with PBA and the meropenem disc with EDTA were increased <5mm were compared with the growth-inhibitory zone diameter around the disc containing meropenem alone.

The isolates were considered negative for MBL and KPC production, when none of the three combined-disc tests are positive.[11,12]

V. RESULTS

Among 1080 Enterobacteriaceae isolates, 50(4.62%) were resistant to meropenem by Kirby Bauer disk diffusion method. All of these carbapenem resistant Enterobacteriaceae (CRE) isolates were inoculated on MacConkey-Meropenem screening agar and subjected to phenotypic confirmatory test by combined disc method. All of

these 50 isolates were grown on MacConkey-Meropenem screening agar and produced different types of carbapenemases. Among the 50 isolates, 18(36%) *E.coli*, 22(44%) *Klebsiella species*, 6(12%) *Enterobacter species*, 3(3%) *Citrobacter species* and 1(2%) isolate was *Proteus vulgaris*.

Out of 50 Carbapenemase producing *Enterobacteriaceae*, 18(36%) were KPC producers, 15(30%) were MBL producers, 13(26%) were both MBL&KPC producers and 4(8%) isolates were negative for MBL&KPC production by combined disk method. Among the 18 KPC producers, 12(54.54%) were *Klebsieallae species*, 3(16.66%) were *E.coli*, 2(33.33%) were *Enterobacter* and 1(33.33%) isolate was *Citrobacter*. Of these 15 MBL producers, 8(44.44%) *E.coli*, 2(9.09%) *Klebsiellae*, 2(33.33%) *Enterobacter*, 2(66.66%) *Citrobacter* and 1(100%) isolate was *Proteus vulgaris.* Thirteen isolates were positive for both MBL&KPC production and 4 isolates were negative for both MBL and KPC carbapenemase production, probably these four MBL and KPC negative isolates were resistant to some other mechanisms.(Table-I)

VI. DISCUSSION

Carbapenems have the broadest spectrum of antibacterial activity and are increasingly used to treat infections caused by multidrug resistant gram negative bacilli. The implementation and evaluation of a simple and accurate laboratory method to detect carbapenemase production in *Enterobacteriaceae* is useful, particularly in places where sources are limited and multi-drug resistant strains are increasingly reported.[16,4]

Rapid, sensitive and cost effective methods are most important in routine clinical laboratory for the detection of carbapenem resistance in Enterobacteriaceae. More importantly, carbapenem resistant *Enterobacteriaceae* members possess the highest risk to public health, because of their rapid dissemination of resistance to other bacterial strains and species through plasmids and resistant to many drugs.[2,3] The early detection of carbapenem resistance in Enterobacteriaceae is helpful to prevent further spread in the community as well as in the hospitalized patients. In this study we evaluated the usefulness of the MacConkey agar supplemented with meropenem at 1µg/ml, as a screening agar for the detection of the Carbapenem Resistant *Enterobacteriaceae (CRE).*

The preparation of the 1µg/ml MacConkey-Meropenem screening agar is very simple, even though laboratory technologist can prepare the medium. We can also store the medium upto 30 days at 4-8^{0}C[17] and the medium was cost effective compared to other carbapenem screening agar.[7]We can also inoculate multiple clinical isolates for the detection of carbapenem resistance on a single plate.

In the present study 1µg/ml MacConkey-Meropenem screening agar was detected all types of carbapenem resistance mechanisms. In the study conducted by Rula Al-Dawodi et al also reported the specificity of the 1µg/ml Mac-Mem plates was the best for the detection of carbapenem resistance in *Enterobacteriaceae*. However, in the study conducted by Nordman et al. the authors did not evaluate Klebsiellae Producing Carbapenemase (KPC) producing Citrobacter species.[10] Panagea T. et al. also reported 97.8% sensitivity and 100% specificity by using MAC+IPM 1µg/ml for detection of CRE from rectal swab.[18]

MacConkey screening agar plate is useful for the early detection (24-48 hours) of carbapenem resistance in Enterobacteriaceae. Detection of carbapenem resistance has important implications for infection control and for epidemiological purpose.[19]In addition detection and surveillance of CRE has become a major importance for the selection of suitable therapeutic schemes and execution of infection control measures.[13,20]

This study had certain limitations, all the carbapenem resistant clinical isolates cannot be processed by E-test for MICs and carbapenemase production was not detected by gold standard test such as molecular techniques, due to lack of funding. However carbapenemase production was detected by phenotypic test (combined disk test).

The prevalence of carbapenem resistant *Enterobacteriaceae* in our hospital was found to be 4.62%. This is similar to the CRE prevalence rates obtained in studies from other parts of India. Datta et al. reported CRE prevalence rate of 7.87% from a tertiary care hospital in North India[19] while Gupta et al. reported carbapenem resistance varying from 17 to 22% among *Enterobacteriaceae*.[21] The prevalence of carbapenemase producing *Enterobacteriaceae* reported from India range from 7 to 51%.[19,22]

In present study out of 50 carbapenemase producing *Enterobacteriaceae* isolates, 18(36%) *E.coli*, 22(44%) *Klebsiella species*, 6(12%) *Enterobacter species*, 3(6%) *Citrobacter species* and 1(2%) was *Proteus vulgaris*. Hidron AI et al. reported to the National Healthcare Safety Network (NHSN), carbapenem resistance was up to 4.0% of *Escherchia coli* and 10.8% of *K.pneumoniae isolates*.[23]

In our study out of 50 Carbapenemase producing *Enterobacteriaceae*, 18(36%) were KPC producers, 15(30%) were MBL producers, 13(26%) were both MBL&KPC producers and 4(8%) isolates were negative for both MBL&KPC production, possibility of these 4 isolates may produce other than MBL and KPC or other carbapenem resistant mechanism like altering the membrane permeability. In a study conducted by Bansal M et al.[12] reported

55.9% were KPC producers, 29.39% were MBL producers and 14.79% were both KPC&MBL producers. Tsakris A et al[24] reported out of 141 carbapenemase positive *Enterobacteriaceae* isolates 44.68% were KPC producers, 33.33% were MBL producers and 21.98% were KPC&MBL producers. They reported combined disc test 100% sensitive for detection of KPC and MBL with reference to molecular methods.

VII.CONCLUSION

In conclusion, the MacConkey agar with meropenem 1µg/ml can be used as a cost effective selective medium for the screening of Carbapenem Resistant *Enterobacteriaceae* (CRE). This screening agar plate provides a sensitive, convenient and detects all types of carbapenem resistant mechanisms. On screening agar medium, the isolation of CRE was 24-48 hours prior to the routine laboratory method. This was of most 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 further spread of these pathogens in the hospital setting.

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Table-I: Species distribution and differentiation of Carbapenemase producing *Enterobacteriaceae*

Organism	No.of isolates	¥KPC producers (%)	£MBL producers (%)	KPC+MBL producers (%)	Negative for KPC/MBL producer (%)
Escherchia coli	18	03(16.66)	08(44.44)	07(38.88)	-
Klebsieallae	22	12(54.54)	02(09.09)	05(22.72)	03(13.63)

Enterobacter	06	02(33.33)	02(33.33)	01(16.66)	01(16.66)
Citrobacter	03	01(33.33)	02(66.66)	-	-
Proteus	01	-	01(100)	-	-
Total	**50**	**18(36)**	**15(30)**	**13(26)**	**04(8)**

[¥]KPC- Klebsieallae Producing Carbapenemase, [£]MBL- Metallo-β-Lactamase.

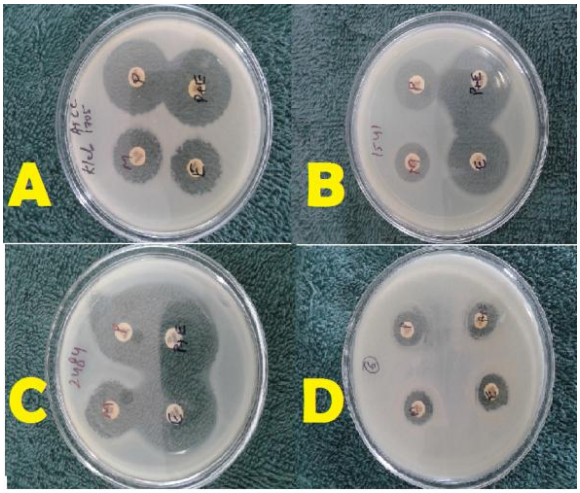

Fig 1: Combined disc test

A- KPC **B-** MBL

C-KPC+MBL **D-** Negative for KPC&MBL