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Research Article

LABORATORY DETECTION OF CARBAPENAM RESISTANT ENTEROBACTERIALES FROM RECTAL SWABS BY DIRECT AGAR AND BROTH ENRICHMENT CULTURE TECHNIQUES

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A R T I C L E I N F O

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A B S T R A C T

Gastrointestinal tract of patients colonized with Carbapenem-resistant Enterobacteriales (CRE) constitutes a major reservoir for transmission of CRE in healthcare facilities. Rapid horizontal spread of these CRE and paucity of available treatment options, accentuates the need for a simple and sensitive screening method for rapid identification and isolation of CRE colonized patients to improve the infection control measures.
Methods: The study was conducted in the Department of Microbiology from February to July 2019. Rectal swabs from 365 patients admitted to Intensive care units were collected. Swabs were subjected to four different culture based screening protocols. All isolates presumptively identified as CRE by screening methods were subjected to modified carbapenam inactivation method detection of carbapenamase production and confirmed by multiplex PCR.
Result: The overall prevalence of colonization of CRE was 19.7% (n =72). Out of 72 CRE 30 isolates were detected only by direct ertapenam disc method and MacConkey broth enrichment technique. Both were 100% sensitive and 98% specific. Direct ertapenam disc method require 24 hrs and enrichment method needs 48 hrs for final result.
Conclusion: Direct ertapenem disk method is a simple, sensitive, and specific protocol with short turn around time than selective enrichment methods for screening rectal swab specimens for detection of carbapenamase producing CRE.

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INTRODUCTION

Carbapenem-resistant *Enterobacteriales* (CRE) have become a significant cause of healthcare infections in many parts of the world. They are considered as an important public health threat by the Centre for Disease Control and Prevention.[1]

The alarming warning is the emergence and dissemination of CRE in specific closed environments like intensive care units of acute care hospitals. Gastrointestinal tract of patients in ICU create an ecological space for potentially pathogenic and resistant bacteria through selection pressure.[2]Consequently, colonized patients constitutes a major reservoir for transmission of CRE in the healthcare setting. Unless rectal screening of patients is in practice, they may remain unidentified. In india Fecal CRE carriage rate in wards and ICUs ranges from 18 to 73% and the risk of CRE infections following colonization is reported as 16.5%. Rapid horizontal spread of these CRE and paucity of available treatment options, accentuates the need for a simple and sensitive screening method for rapid detection and isolaton of CRE colonized patients to improve the infection control measures.[3&4]

Eventhough Polymerase Chain Reaction (PCR)-based methods are found to be highly sensitive and reliable, these methods require expertise and they are quite expensive. So, culture-based methods are still essential for the initial detection of CRE from rectal swabs Therefore performance of different culture based screening methods for detection of intestinal colonization of carbapenamase producing Carbapenem-Resistant *Enterobacteriales (*CP-CRE) were assessed.[5]

MATERIAL AND METHOD

The prospective observational study was conducted in the Department of Microbiology from february to July 2019. The study was ethically cleared by institute ethics committee [IEC/BMCRI/PS/66/2018-19]

Rectal swabs from 165 patients admitted to trauma, medical and respiratory ICUs and 200 patients admitted to pediatric ICU were collected after obtaining informed consent. Rectal swabs were then placed in the transport tube containing sterile Cary-Blair medium and brought to the Microbiology laboratory and swabs were vortexed for 1 minute. Swabs were subjected to four different culture based screening protocols for detection of CPCRE. To ensure an equal inoculum for each method, 100 µl of the transport medium with the sample was placed into 3 ml of saline and used for further processing.
The screening protocols were:

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1. CETP- CDC recommended broth enrichement protocol was carried out by adding 100µl of the saline mixture in 5 ml of tryptic soy broth containing a 10µg disk of ertapenem. Following an overnight incubation at 37°C, it was streaked onto MacConkey agar and incubated for 18-24 hours. The presence or absence of bacterial growth was recorded.[6]
2. METP - MacConkey broth enrichment protocol was performed similar to CETP except for replacement of tryptic soy broth with 5 ml Mac Conkey broth.[7]
3. TETP - For Tryptic soy broth without ertapenem protocol 100 µl of the saline suspension was placed in 5 ml of tryptic soy broth and incubated overnight at 37°C.Then, it was inoculated onto MacConkey agar and streaked to four quadrants. An ertapenem disk was placed at the junction of first and second quadrant. Colonies growing within the zone diameter of ≤ 21mm were recorded.[8]
4. DETP - Direct ertapenem disc method, a single step direct process wherein saline suspension was directly inoculated onto MacConkey agar and streaked to 4 quadrants. An ertapenem disk was applied at the junction of quadrants 1 and 2. Isolates growing within a zone diameter of ≤21 mm were recorded.[8&9]

Lactose-fermenting colonies with different morphotypes grown in methods CETP, METP and within a zone diameter of ≤21mm in TETP, DETP were picked up and subjected to Gram stain. Further identification and antimicrobial susceptibility testing of all Gram negative bacilli was done by Vitek 2 automated system (bioMérieux, Marcy l'Étoile, France).

Table 1 Performance characteristics of screening protocols for detection of CP-CRE

Methods	Nos Tested	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	Accuracy
DETP	365	42	30	293	00	100%	90.7%	58.3%	100%	91.7%
METP	365	42	28	295	00	100%	91.3%	60%	100%	92.3%
TETP	365	30	00	323	12	71.4%	100%	100%	96.4%	96.7%

All these isolates were tested phenotypically for carbapenamase production by modified carbapenem inactivation method.[10] This was further confirmed by Multiplex PCR for the presence of of blaVIM, blaIMP, blaNDM, blaKPC and blaOXA-48 like genes by using previously published primers.[11]

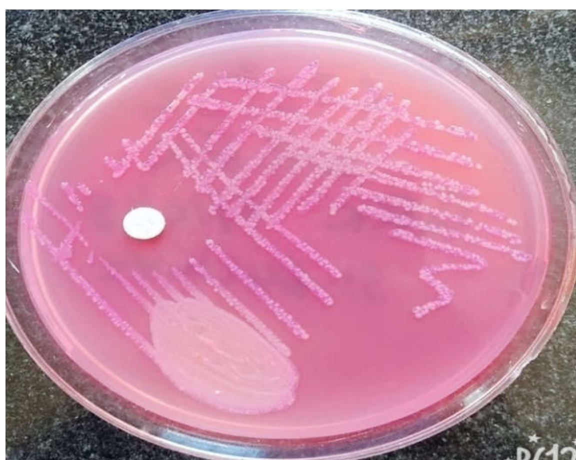

Fig 1 Direct ertapenam disc method for screening of carbapenam resistant Enterobacteriales from rectal swabs. Test result shows negative for the presence of carbapenam resistant isolates.

Data Analysis

The sensitivity and specificity of different culture based methods for CRE screening was calculated by considering CDC recommended broth enrichement(CETP) as the reference method. Further the results are correlated with the results of mCIM and multiplex PCR. The agreement between the two tests was established by Cohen's kappa index. The kappa index was interpreted as follows: 0.01–0.20, poor agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1, almost perfect agreement. Statistical significance was calculated by Pearson's chi-square test, and values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Out of 165 ICU patients, 60 were females and 105 were males. Among 200 PICU patients 122 were male and 68 were female. CRE isolates were identified in 72 of the 365 swabs by at least one of the tested protocols. The overall prevalence of colonization of CRE among the studied patient populations was 19.7% (*n* =72). CRE carriage in ICU patients was 25% (n=42) and in PICU it was 15% (n=30). Each patient was positive for carriage of only one carbapenem resistant enterobacteriales. Out of 72 CRE isolates recovered, 26were *E. coli* (26/365,7.1%) and 46 were *Klebsiella pneumoniae* (46/365, 12.6%).

By DETP protocol all 72 CRE isolates, METP protocol 70/72, CETP protocol 42/72 and TETP protocol 30/72 CRE isolates were detected. DETP and METP detected (30&28) more CRE isolates than CDC reference method with 100% sensitivity and specificity of 90.7% & 91.3% respectively. TETP protocol detected 12 CRE isolates less than CETP with 71.4% sensitivity and 100% specificity. Performance characteristics of the all the screening methods are summarized in Table-1

Additionally the results of screening protocols were compared with the results of mCIM and PCR, then the specificity of DETP and METP increased to 98% but sensitivity of CETP and TETP decreased to 58% and 42% respectively. The DETP and METP protocols were statistically more sensitive (p=0.001) than CETP along with detection of more number of CRE isolates. There was almost perfect agreement found between DETP and METP (kappa index- 0.98) whereas CETP and TETP showed a substantial agreement (kappa index – 0.69 - 0.70) with DETP and METP.

Results of mCIM and PCR confirm the presence of carbapenamase genes in 66 out of 72 presumptively identified CRE. Among the 06 PCR negative isolates, the MIC range for carbapenems for two *E.coli* isolates remained between ≥0.25 µg to ≥0.5µg and were sensitive to all the three carbapenams. The other 02 *E.coli* and 02 *K.pneumoniae* isolates were resistant [MIC ≥8 µg - ≥16 µg] to all the carbapenems tested. mCIM tested negative for 02 carbapenem sensitive isolates. Remaining 04 isolates were found positive by mCIM protocol. These isolates were detected as CRE by two type [METP+DETP] of screening methods.

The most common resistant gene detected was bla NDM [34/66] followed by OXA-48 like gene [23/66]. 09/66 isolates were positive for both NDM and OXA genes. Other target genes bla- VIM, bla-IMP and bla-KPC were not detected in any of the isolates.

DISCUSSION

Implementation of adequate infection control policies to prevent transmission of CRE through its intestinal carriers requires prompt identification of fecal CRE. In order to achieve this, an accurate method with quick turn around time is required. In this regard, several culture and molecular based techniques have been developed. A reference method based on selective enrichment for screening of CRE carriers has been proposed by CDC. In the study, we compared the performance of three screening methods for detection of CRE from the rectal swabs with CETP and multiplex PCR.

CRE colonization in ICU and PICU was 25% and 15% respectively. Similar colonization rate of 29% and 10.67% PICU were found by Mohapatra *et al*[4] ,Mohan *et al*[12] and Saxena *et al.*[13]

Among the executed protocols DETP, METP exhibited 100% sensitivity and 91%specificity and TETP was 71.4% sensitive and 100% specific for the detection of CP-CRE , when compared with the CDC recommended method. Landman *et al.*[7] stated that, broth enrichment method with a carbapenem disc was more sensitive than the plating of overnight growth of rectal swab culture onto MacConkey agar with a carbapenem disc. In the present study also METP was found to be more sensitive than TETP. On the other hand Simner *et al.*[9] Lolans *et al*[8] found that direct MacConkey plate method was more sensitive than broth enrichment methods. Adler *et al.*[14] found that MacConkey agar supplemented with imipenem at 1µg/ml was superior than MacConkey agar with carbapenem disc. In our study, DETP and METP performed equally well with almost perfect agreement but METP is two step protocol and requires 48 hours.

Out of 72 CRE 30 isolates were detected only by DETP and METP protocols. These protocols allowed us to detect 30 more CRE from rectal swabs. Further PCR results demonstrated that except 06, all 66 isolates found positive for the presence of one of the target genes. Among the PCR negative isolates, the false positive results of 04 CRE isolates might be due to the presence of other carbapenem resistance mechanisms or the presence of other variants of target genes that were not detected by the primers used in our study.[5&15]Detection of 02 carbapenam susceptible isolates by METP might be due to incomplete release of ertapenam into the broth.[7]

More number of false negative results obtained by CETP and TETP in comparison with PCR, may possibly due to overgrowth of Gram positive cocci and ertapenam resistant non fermenting gram negative bacteria which might have masked the existence of CRE.[8] Segarra *et al.*[16] stated that the CETP is highly sensitive for the detection of KPC carbapenamases. Similar observations were found by Olivgeris *et al.*[17], Landman *et al.*[7],Vrioni *et al*[18] and Saegeman *et al.*[19] Absence of KPC type carbapenamases in our study could be the other reason for low sensitivity of CETP. Even though direct detection of CRE by molecular assays is found to be more sensitive for the surveillance of CPCRE from rectal swabs, these method may not be available in many laboratories and might have the disadvantages of detecting specific genes.[18]

CONCLUSION

In conclusion, the regions, where NDM and OXA-48 like carbapenamases are more prevalent, direct ertapenem disk method is a simple, sensitive, and specific protocol with short turn around time than selective enrichment methods for screening rectal swab specimens for detection of CRE. Direct ertapenam disc method followed by mCIM test confirmation could be implemented in clinical microbiology laboratories for screening of rectal swab specimen for early detection of CRE carriers and proper implementation of infection control policies.

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