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COMPARATIVE EVALUATION OF AN IN-HOUSE AND COMMERCIALLY AVAILABLE MEDIUM FOR DETECTING CARBAPENEM RESISTANT GRAM NEGATIVE BACILLI IN SURVEILLANCE RECTAL SWAB AND IDENTIFICATION OF PREDISPOSING RISK FACTORS FOR COLONIZATION

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ABSTRACT

Carbapenem resistant Gram negative bacilli (CR-GNB) are an increasing problem worldwide, and rectal swab surveillance is recommended as a component of infection control programs. The performance of an in-house medium (modified CDC protocol) for detecting CR-GNB in surveillance rectal swab was evaluated and compared with a chromogenic medium (ChromID CARBA). 40 rectal swab specimens were taken from different patients hospitalized in ICU and screened. Identification of isolates was done using standard biochemical tests and sensitivity using Kirby Baur disk diffusion method. Sensitivity (95.83%) and PPV (92 vs. 88.46%) of the in-house and CARBA agar were comparable whereas specificity of in-house medium was slightly higher than CARBA agar (88.89 vs. 83.33%). Colonization with CR-GNB was independently associated with exposure to carbapenem (p = 0.015). In conclusion, in-house medium as per CDC protocol (modified) has the potential to provide useful tool for convenient and inexpensive screening method for CR-GNB surveillance.

KEYWORDS: **Carbapenem resistance, Gram negative bacilli, surveillance, chrom agar**

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INTRODUCTION

Carbapenems are used as a last-resort antibiotic class for the treatment of infections due to multidrug-resistant organisms. However, during the last decade carbapenem resistance has been increasingly reported and global dissemination of carbapenem resistant Gram negative bacilli (CR-GNB) has become a major public health concern [1,2]. CR-GNB are usually multidrug resistant pathogens, making them even more worrisome, since the treatment options are very restricted. Furthermore,Carbapenem resistant pathogens have been associated with high rates of morbidity and mortality, particularly among critically ill patients with prolonged hospitalization [3]. Patients colonized with CR-GNB are thought to be a source of transmission in the healthcare setting [4]. Consequently the prevention of spread of CR-GNB relies on early and accurate detection of carriers in hospital units or on admission/discharge either to the hospital or to a specific unit. The aim of this study was to compare the suitability of in-house medium and commercially available medium for screening of CR-GNB carriers in the intensive care unit (ICU) of a tertiary care hospital as well as describe the risk factors associated with CR-GNB colonization among patients admitted in ICU.

MATERIALS AND METHODS

(i) Patients and specimens of the study

Rectal swab specimens were collected from 40 patients who were at risk for colonization with CR-GNB hospitalized in November 2013 to January 2014 in ICU of a 700-bed tertiary care hospital in Mumbai, India. Patients were differentiated into four different groups (with 10 patients under each group respectively.) (i) The first group (New admission group) consisted of patients that were new admission (less than 48 hours) in ICU and may be transferred from ward of the same hospital or from another hospital. (ii) The second group (Infected group) consisted of patients in ICU (long term stay) with CR-GNB grown in culture in their clinical sample. (iii) The third group (Carbapenem exposed group) comprised of patients admitted in ICU (long term stay) undergoing treatment with carbapenem for a period of more than 4 days or may have undergone a complete course of carbapenem previously. (iv) The fourth group (Control group) was of the patients who were admitted to ICU since long time, however who had not undergone carbapenem treatment. Two rectal swabs were collected from each patient. The tip of the sterile swab, pre-moistened with sterile saline, was inserted approximately 1 inch beyond the anal sphincter and carefully rotated, in order to sample the external rectal orifice, withdrawn, and placed in the tube. Samples were immediately transferred to the laboratory and processed.

(ii) Culture screening methods

Both the swabs were processed by following two different methods Method 1: Swabs were incubated in 5 ml tryptic soy broth with a 10 µg meropenem disk (final meropenem concentration, 2 µg/ml) for 4 hours. Followed by plating them onto MacConkey agar (Hi-media, Mumbai) [Centers for Disease Control and Prevention(CDC) protocol – modified] (MacM) [5] Method 2: direct plating onto chromID CARBA Agar medium (bioMérieux), which consists of a nutrient base combining different peptones, three chromogenic substrates enabling the detection of activities of specific metabolic enzymes for *Escherichia coli*, *Klebsiella/Enterobacter/Serratia/Citrobacter* (KESC group), and *Proteeae*, and a proprietary mixture of antibiotics favoring the selective growth of carbapenemase-producing *Enterobacteriaceae*. On each medium, evaluation of bacterial growth was made after 18 to 24 h of incubation at 37°C in ambient air by two different observers. Carbapenemase-negative *Escherichia coli* ATCC 25922 and New Delhi metallo-β-lactamase (NDM) positive *K. pneumoniae* were used as control strains.

(iii) Detection and identification of CR-GNB colonies

With the CDC-modified method, presumptive CR-GNB colonies were considered those

growing as lactose-fermenting or lactose-nonfermenting colonies on MacConkey plates. On CARBA agar plates, presumptive CR-GNB colonies were considered those with a color appearance according to the manufacturer's instructions (bluish-green to bluish-grey – KESC group and pink to burgundy or brown colonies – *E. coli*). Suspected CR-GNB colonies were subcultured from respective screening plates onto MacConkey plates and were identified using biochemical tests and susceptibility to imipenem and meropenem were performed on Muller Hinton Agar using Kirby Bauer disk diffusion method[6] and interpreted using CLSI guidelines [7].

(iv) Sensitivity and specificity

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated for both the screening methods. True-positive strains were defined as all presumptive CR-GNB growing on the media and confirmed to be carbapenem resistant. False-positive strains were defined to be all presumptive CR-GNB growing on the media that were confirmed to be carbapenem sensitive. No growth by all screening methods was characterized as a true-negative result. No recovery of a confirmed carbapenem resistant strain using a particular screening method was characterized as a false-negative result for that specific screening method.

(v) Environmental sampling

Environmental sampling was coordinated and supervised by the infection control team at the hospital. Samples were collected from bed railing and cardiac trolley (5 cm X 5 cm area) surrounding each patient colonized with CR-GNB. Environmental samples were immediately (within 30 min) transferred to the laboratory for further workup.

(vi) Culture method for environmental samples

Swabs were immediately inoculated with nutrient broth for enrichment and incubated at 37°C for 4 h. Subsequently, the swab was vortexed and cultured on Nutrient agar plate, which was then incubated at 37°C for 48 hours. Gram staining was carried out to differentiate between Gram positive and Gram negative organisms. Identification was carried out using standard biochemical tests.

(vii) Patients Data collection

Data, including patient demographics, co-morbid conditions, previous history of infection with CR-GNB, origin of patient at the time of hospital encounter (e.g. home, transferred from the ward of same institution or from another hospital), invasive procedure carried out during hospital stay or previously undergone, previous exposure to antibiotic were collected from the electronic medical records, laboratory data and medication administration records from the date of admission till discharge. Patients were followed for 15 days post screening for CR-GNB carriage to determine development of infection with CR-GNB if any. Final disposition was defined as death, discharged during illness or transferred to ward or discharged.

(viii) Statistical analysis

Variables were compared using Fisher's exact test. All tests were two-tailed and a p-value <0.05 was considered significant.

RESULTS

A total of 24 (60%) patients were identified as CR-GNB carriers from 40 rectal swabs collected from patients admitted in ICU during the study period. It included 4 patients from the new admission group, all 10 from the infected group, 8 from the carbapenem exposed group and 2 from the control group. CR-GNB were identified in 24 (60%) samples on at least one out of two screening plates. A total of 49 isolates were obtained from these 24 samples; these were identified as *E. coli* (17, 35%), *Klebsiella pneumoniae* (14, 29%), *Pseudomonas aeruginosa* (9, 18%) and *Acinetobacter* spp. (9, 18%). Along with Gram negative organism, there were few Gram positive cocci obtained on CARBA agar plate having small size and blue color, which were suspected to be vancomycin resistant *Enterococci* as per manufacturer's instructions.

The performances of different screening method are summarized in Table 1. MacM and CARBA agar showed similar sensitivities and NPV, but MacM had superior specificity and PPV, thus greater over-all accuracy. The turnaround times were comparable.

Table 1
Summary of CR-GNB screening method performances

Method	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
MacM	95.83	88.89	92	94.11	92.85
ChromID CARBA	95.83	83.33	88.46	93.75	90.47

MacM – ***inoculation of swabs in tryptic soy broth with a 10 µg meropenem disk followed by plating to MacConkey agar (after 4 h enrichment)***

Environmental sampling was done from area surrounding 24 CR-GNB carriers to check for possible environmental contamination with CR-GNB in the hospital setting. Coagulase negative *Staphylococci* were isolated from two samples collected from bed railing and one sample collected from cardiac trolley, whereas one *Enterococci* was isolated from one bed railing sample. None of the sample indicated presence of CR-GNB, indicating absence of environmental contamination with CR-GNB.

Medical records of 40 patients were reviewed. The median age was 57 years and 25 (62.5%) patients were male. All the patients of the study cohort had undergone invasive procedures. Compared to patients newly admitted in ICU (*n* = 10), higher proportion of CR-GNB carriers were identified from the group of patients who had long term stay in ICU (*n* = 30) (66.7% vs. 40%, *p* = 0.14). However, the *p*-value indicated statistically insignificant effect. Colonization with CR-GNB was significantly associated with previous exposure to carbapenem group of drugs as higher proportion of carriers were identified from the group of patients who were on carbapenem treatment or had previous exposure (*n* = 17), compared to patients not exposed to carbapenems earlier (*n* = 23) (82.4% vs. 43.5%, *p* = 0.015). A total of 8 out of 24 carriers developed infection with CR-GNB in the next 15 days post screening, of these 5 were transferred to wards, 1 was discharged during illness and 2 patients died during the course of treatment. Of the remaining 16 carriers, 7 were discharged, 7 transferred to wards and 2 were discharged against medical advice.

DISCUSSION

Spread of CR-GNB is rising in several parts of the world. Carbapenem resistance is predominantly conferred by carbapenemases, such as serine carbapenemases, metallo-β-lactamases (MBLs) and oxacillinase (OXA)-type enzymes [8]. Some are plasmid-mediated, thus have the ability to transfer their resistance genes to other pathogens in the hospital environment as well as to cause large mono or multiclonal hospital outbreaks [9]. Thus, the control of these pathogens is imperative and clinical laboratories are facing the challenge of screening surveillance specimens for CR-GNB. Moreover, detection of these pathogens should be accomplished in a short time interval from hospital admission in order to prevent further dissemination in the hospital environment.

High prevalence of *Enterobacteriaceae* (64%) observed in rectal swab screening in this study may be due to rectal swab being a representative of normal gastrointestinal flora which majorly comprises of *Enterobacteriaceae* family. However, carbapenem resistance in *Enterobacteriaceae* spp. is a cause of concern as there is an added risk of its dissemination in the community. Thus the spread of carbapenem resistant *Enterobacteriaceae* is deeply disconcerting in a country such as India with a reservoir size of more than 1.4 billion people[10].

In the previous study the performance of CHROMagar KPC was compared with MacConkey agar containing imipenem at 1 µg/ml (MacI). MacI was found to exhibit the greater overall accuracy for the detection of carbapenem-resistant *Enterobacteriaceae*,

even though MacI and CHROM agar KPC had similar sensitivities and NPVs [11]. Also, Wilkinson et al. [12], compared the performance of different chromogenic media with CDC protocol, CDC protocol demonstrated comparable sensitivity for the detection of CR-GNB with high inoculum, although the specificity was low. In this study, we modified the CDC protocol to shorten the time period of screening to 24 h upon receipt of surveillance rectal swab and compared to the ChromID CARBA agar plate. The performance of CARBA and MacM protocol was comparable. The specificity of MacM was found to be slightly higher than ChromID CARBA in the present study, however the difference was not much, suggesting both these media may give us effectively similar results of surveillance screening for CR-GNB. Also, cost effectiveness of MacM being an in-house media compared to commercially available CARBA agar make it more advantageous for routine surveillance screening on a large scale in hospitals. CARBA agar allows the growth of certain carbapenem resistant Gram positive bacteria picked up by rectal swabs, thus showing increased non-specificity of direct plating, which is avoided in case of MacM, as MacConkey allows the selective isolation of only Gram negative organism. Several studies suggest that one of the major contributing factors for colonization with CR-GNB is environmental contamination [13, 14]. Although in our study, no CR-GNB were isolated on environmental screening indicating adequate infection control practices in the ICU followed by hospital.

Present study demonstrated an association between colonization with CR-GNB and exposure to carbapenem antibiotics as a predisposing risk factor. Patients with long term stay indicated a higher risk of colonization with CR-GNB but it was statistically insignificant. Smaller studies have reported similar risk factors[15]. These risk factors likely represent severe underlying illness, susceptibility to colonization and antibiotic selection pressure, leading to increased risk of infection with multidrug resistant organisms [3]. Infection with multidrug resistant organisms have been reported to increase the length of stay [3], present study also supports this view as 44% carriers were discharged post screening, whereas none of the patients were discharged who developed infection with CR-GNB in that period. The study has certain limitation. Firstly, the small sample size that may not allow results to reach statistical significance. Second, the study represents a tertiary care hospital with ongoing CR-GNB outbreak, therefore generalization to other clinical setting is limited.

CONCLUSION

The study provides some insight into the performances of ChromID CARBA agar and CDC-protocol that have been recommended for recovery of CR-GNB. Performance of ChromID CARBA and in-house media was comparable in this study when considering both sensitivity and specificity. In-house media as per CDC protocol with shorter enrichment period propose to have the potential to provide useful tool for convenient and inexpensive screening of patients for CR-GNB carriage, thus necessitating further evaluation. Prevalence of carbapenem resistant *Enterobacteriaceae* colonizing critically ill patients is a worrying trend as it carries an added risk of its dissemination in the community. Exposure to carbapenem was one of the major risk factor for colonization with CR-GNB, hence antibiotic stewardship efforts along with stringent infection control practices should be directed to control the spread of CR-GNB.

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