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A discussion on Carbapenemase presence in Central Kerala

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ABSTRACT

Carbapenem-resistant *Enterobacteriaceae* (CRE) is a challenge in the ICU as well as in the Clinical Microbiology laboratory. Carbapenems are best used as reserve drugs but they are being used in several settings without proper justification. Therefore, emphasis must be placed on its use after a proper clinical microbiology laboratory report rationalizing its use. Several simple testing alternatives have come up such as Rapidec Carba NP. Once CRE is confirmed, carbapenems should be spared and other options should be chosen such as Ceftazidime/Avibactum alone or with Aztreonam.

Keywords: Carbapenem-resistant *Enterobacteriaceae* (CRE), beta lactums(BL), serine carbapenemase, metallo-betalactamase (MBL).

INTRODUCTION

Carbapenems are beta-lactam antibiotics which have a broad spectrum of activity against Gram-negative aerobic and anaerobic organisms and are used for treating serious infections not responding to standard antibiotic therapy. Carbapenems bind to PBPs disrupting the integrity of the bacterial cell wall thus causing cell death. The mechanism of action is similar to beta-lactam antibiotics and cephalosporins but carbapenems have a fused beta-lactam ring structure which is resistant to most beta- lactamases produced by bacteria to inactivate penicillin like antibiotics.[1,2,3]

Carbapenems or broad spectrum beta-lactams possess a carbapenem ring along with beta-lactam ring makes them stable against most beta lactamases including Amp C as well as ESBLs. Their importance is due to the fact that they are the last resort in most life-threatening ICU infections.

The Carbapenemases

Even though carbapenem resistance is intrinsic in Stenotrophomonas maltophila, it is not the case in most Gram-negative pathogens in ICU infections. Here, carbapenem resistance is usually acquired via horizontal gene transfer or by mutations.

Gram-negatives become resistant to Carbapenems through mechanisms such as diminished permeability of their outer membrane, porin loss and efflux pumps. Most important is the resistance by the enzyme Carbapenemase which hydrolyzes almost all beta-lactams and results in high carbapenem MICs.

As per most studies, the relevant Carbapenemases included according to Ambler classification are KPC in class A; the metallobetalactamases- IMP, VIM, VDM, SPM, GIM, SIM, AIM, DIM, FIM in class B and OXA-type in class D. Class C are not included as Carbapenemases in this context.

Regarding the hydrolysis and the ability to disseminate, the important Carbapenemases are KPC, VIM, IMP, NDM and OXA-48 types. While the KPCs hydrolyze all BLs and are only inhibited partially by BLIs such as Clavulanic acid, Boronic acid and Tazobactum; the MBLs hydrolyze all BLs except Aztreonam (ATM) as they possess the metal Zinc in their active centre and are inhibited by metal chelators such as EDTA.[4,5,6,7]

Laboratory Surveillance of Carbapenemases

Rapidec Carba NP test reliably detects Carbapenemase in 30 mts to 2 hrs. By carrying out additional tests such as mCIM

and eCIM, it is possible to further identify whether the enzyme present is a serine Carbapenemase or a Metallobetalactamase. The synergy of Ceftazidime/Avibactum with Aztreonam can be determined by strip stacking, strip crossing methods, E strip and disc diffusion methods and broth microdilution methods. E-test strips determine the MIC of Ceftazidime/Avibactum. Rectal swab surveillance go a long way in informing the prevalence of carbapenem resistance, particularly in ICU patients.[8,9,10,19]

METHODOLOGY

30 MDR isolates were collected from various clinical samples and the testing was carried out as per CLSI 2016 edition. In this study, all the isolates were Klebsiella species.

Initially, a 12 disc screening test was done with the 30 isolates to screen for ESBL, AmpC and carbapenemase presence. The discs were Aztreonam, Ceftazidime, Ceftazidime +Clavulanate, Cefotaxime, Cefotaxime +Clavulanate, Cefoxitin, Cefotetan, Ceftriaxone, Cefepime, Ertapenem, Imipenem and Meropenem. An isolate is detected to be positive for ESBL if it shows =>5mm zone diameter with Clavulanate than for Ceftazidime or Cefotaxime alone. An isolate is AmpC positive if it is resistant to all Cephalosporins, beta-lactamase drug, Cephamycins but susceptible to Cefepime. Carbapenem resistance was defined as resistant to atleast anyone Carbapenem- Imipenem or Meropenem or Ertapenem.

Rapidec Carba NP is a phenotypic method to detect CRE. Even though, it fails to detect other resistance mechanisms such as porin loss and efflux pump, this test was found to be useful in this study. This in-vitro diagnostic test is for the qualitative detection of Carbapenemase enzyme in Enterobactericeae and Pseudomonas aeruginosa by a colorimetric positive signal in 30 mts to 2 hrs. In this study, Rapidec NP was positive in 7 out of 30 isolates (23.33%).

In case of mCIM, a loopful of the isolate taken from overnight blood agar is emulsified in trypticase soy broth; a meropenem disc is immersed in it and incubated for 4hrs. A 0.5 McFarland suspension of E coli ATCC 25922 is prepared and inoculated on a Mueller Hinton plate. The meropenem disc is placed on this plate and incubated for 18 hours. A positive result is inhibition zone around meropenem disc to be => 6 to 15mm or presence of pin point colonies within 18mm zone size.

In eCIM, the only difference is that the loopful of isolate is emulsified in trypticase soy broth with 20µL of 0.5 EDTA added to it and incubated for 4hrs. A 0.5 McFarland suspension of E coli ATCC 25922 is prepared and inoculated on a Mueller Hinton plate. The meropenem disc is placed on this plate and incubated for 18 hours. An increase in 5mm zone size between eCIM and mCIM indicate the isolate to be a Metallobetalactamase producer. If less than 4mm diameter, it is a serine Carbapenemase producer.

RESULTS

A small study was conducted with 30 MDR isolates from respiratory tract of critical care ICU patients in tertiary centre over a period of 2 months. All the isolates tested were Klebsiella species.

11 isolates were positive for ESBLs ,6 for AmpC and mCIM – eCIM zone diameter was greater than 5mm only in 2 isolates. Therefore, this study detected Metallobetalactmases (classB) only in 2 isolates. The other isolates could belong to class A such as KPC or class D- OXA-type. Based on the revelations, it was possible to initiate Ceftazidime-Avibactum alone or with Aztreonam in 5 patients and in these, 2 had a positive outcome.[11,12,13,19]

DISCUSSION

In India, the prevalence of Carbapenemases is slated to be over a range of 18 to 32% with lower rates in Kerala, and this study reveals 22%. Nevertheless, the small number of isolates makes it difficult to get a real picture and further studies are needed. Several studies in Kerala have detected using various methods the presence of NDM, OXA, KPC, VIM and IMP in Gram negative bacteria. The prevalence of Metallo-betalactamases is as high as 75% and serine Carbapenemases are 60% in major big hospitals in India and relatively lower rates in Kerala. KPC has been reported to be around 26.5% overall from Gram negatives.[2,3,4,5,6,10,17,18]

Challenges posed by Carbapenemases

Once the lab reports Carbapenemases, the alternatives for Gram-negatives include Colistin, Tigecycline, Fosfomycin, Polymyxins and of late, Ceftazidime/Avibactum alone or with Aztreonam. Nephrotoxicity has been reported with the use of Colistin and Polymyxins. Fosfomycin can attain bactericidal concentrations in cystitis. Resistance to Tigecycline is gradually increasingly being reported.[14,15,16]

The geographic pattern of Carbapenemases show that generally, KPCs are prevalent in USA and China; MBLs in India and Southeast Asia and OXA-types in the Middle East and Africa. Just like humans, bacteria evolve and they become resistant to antibiotics because of the selective pressure imposed. Furthermore, unregulated prescription by specialists themselves, without being supported by an antibiotic susceptibility report, easy access to antibiotics, non-compliance to recommended infection control measures and the use of such antibiotics in the animal sector- all these contribute to the unchecked spread of carbapenem resistance.[17,18,19, 20,21]

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