Original Article

Prevalence of Carbapenem Resistant Enterobacteriaceae in Gwalior Region in Blood Culture Isolates

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ABSTRACT

Introduction: Carbapenems are broad spectrum β Lactam antibiotics & are the last resort to control infections caused by gram negative bacteria. The increasing resistance to these antibiotics is an alarming sign. CRE (Carbapenem resistant Enterobacteriaceae) is reported due to acquisition of carbapenemase genes or association with decreased outer membrane permeability with β lactamases with weak carbapenemase activity.

Methods: Total 123 culture positive blood samples from our tertiary care hospital were included in which carbapenem resistance was identified and the resistant strains were assessed for carbapenemase production by Modified Hodge Test (MHT) & Modified Carbapenemase inactivation method (mCIM) simultaneously.

Results: Out of total 896 blood samples received, 123 isolates were members of Enterobacteriaceae family. The maximum isolates were Escherichia coli 39(31.70%) next was Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella aerogenes, Citrobacter freundii. Carbapenem resistance was seen in 19(15.44%) isolates. The positivity rate by MHT was 11 (57.89%) while by mCIM was 17(89.47%).

Conclusion: Prevalence of carbapenem resistance was 15.44%.

Key words- CRE, MHT, mCIM

INTRODUCTION

The ability of bacteria to produce enzymes that destroy the β-lactam antibiotics began even before penicillin was developed. The first β-lactamase was identified in an isolate of Escherichia coli in 1940.[1]

The first carbapenemase identified in Enterobacteriaceae was the chromosomally encoded NmcA from an Enterobacter cloacae clinical isolate in 1993.Since then, carbapenem resistant Enterobacteriaceae (CRE) have been reported worldwide.[2]Increasing resistance to carbapenems, which are most often the last line of therapy, is now frequently being observed in many hospital-acquired and several community-acquired gram-negative rods. Resistance in bacteria to carbapenems is due to production of carbapenem hydrolyzing enzymes called carbapenemase and is associated with alarming mortality rates. This may be related either to an association of decreased outer membrane permeability with over expression of β-lactamases possessing very weak carbapenemase activity, or due to expression of carbapenemase. These genes offer a stable and transferable form of resistance enabling spread via clonal expansion or by horizontal transfer of genes to naive bacteria. Carbapenemase defy geographical boundaries, making the prevention of Carbapenemase Producing Organism (CPO) a significant public health concern requiring international coordination. The Enterobacteriaceae family, mostly Escherichia coli and Klebsiella pneumoniae are important causes of serious hospital and community acquired bacterial infections in humans.[3]

A large variety of carbapenemase have been identified in Enterobacteriaceae, belonging to three classes of β-lactamase, the Ambler class A, B and D β-lactamases these are: the KPC types belonging to class A, the Metallo-β-Lactamase (MBL) belonging to class B, and the oxacillinases to class C.[4]

These enzymes exhibit broad spectrum of hydrolytic activity, including all penicillin's, cephalosporins and carbapenems sparing only monobactams and their activity is not inhibited by commercially available by β- Lactamase inhibitors (clavulanic acid, tazobactam, sulbactam).The mechanism of hydrolysis is dependent on interaction of β- Lactam with zinc ion in their active site explaining their inhibition of activity by EDTA, chelator of divalent cations, or dipicolinic acid.[5]

Clinical isolates rarely show phenotype of resistance attributable to carbapenemase expression alone, because they often have other co-resident β-lactamases such as ESBLs, AmpC etc. leading to more composite resistance profile.[5]

Current recommendations for detection of Enterobacteriaceae producing carbapenemase taken from Clinical and Laboratory Standard Institute (CLSI) detect all clinical important resistant mechanisms including majority of carbapenemase.

In the current study, prevalence of carbapenem resistant Enterobacteriaceae and comparison of different phenotypic methods for carbapenemase detection after routine Kirby-Bauer disc diffusion test following CLSI guidelines and further confirmation by 2 tests-MHT (Modified Hodge Test) and mCIM (Modified Carbapenemase inactivation method) in the blood sample is studied.[7,9]

MATERIAL AND METHODS

The prospective study was conducted in the Department of Microbiology, Gajra Raja Medical College, Gwalior from January 2019 to June 2019.

A total of 896 blood samples received in the bacteriology section of Department of Microbiology from J. A. Group of Hospitals (JAH, KRH, Neurology ward, ICU, Madhav Dispensary) were studied.

Blood was collected under aseptic conditions in Brain Heart Infusion (BHI) broth in the dilution of 1:5. Well labeled blood culture bottles were transported to bacteriology laboratory as early as possible.[8]

The samples were processed for Gram staining and for culture on Blood agar and Mac Conkey agar after overnight broth incubation. Identification by standard biochemical tests and Antimicrobial Susceptibility Testing was done according to CLSI guidelines. Carbapenem resistant isolates were taken up for phenotypic tests like MHT, mCIM.

RESULTS

In the current study out of total 123 isolates belonging to Enterobacteriaceae family 19(15%) were resistant to carbapenem. Amongst these 19 resistant isolates maximum were from ICU 11 (57.89%) followed by wards 6 (31.57%) and 2(10.52%) in OPD (Table 1) and 10(81%) were E. Coli followed by K. Pneumoniae 4(21.05%), K. Aerogenes and K. Oxytoca both 1(5.26%) and Citrobacter freundii 3(24%) [Table 2].

All the 19 resistant samples were then subjected to MHT & mCIM tests where the positivity rates were 11(58%) & 17(89.47%) respectively (Figure 3)

Figure 1: Phenotypic test- Modified Hodge Test (MHT) showing positive (clover leaf/ indentation), negative results and two test isolates

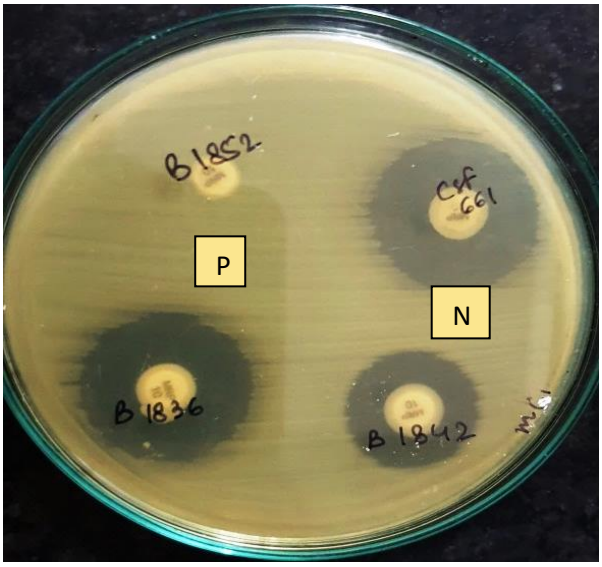

Figure 2: Modified carbapenem inactivation method-(mCIM) showing positive (P) and negative(N) results

Table 1: Distribution of cases at various locations in the hospital

Location	No of cases	Percentage
ICU	11	57.89
Ward	6	31.57
OPD	2	10.52

Table 2: Distribution of Resistant cases according to isolates

Organism isolated	No of cases	Percentage
Escherichia coli	10	52.63
Klebsiella pneumoniae	4	21.05
Klebsiella oxytoca	1	5.26
Klebsiella aerogenes	1	5.26
Citrobacter freundii	3	15.79
Total	19	100

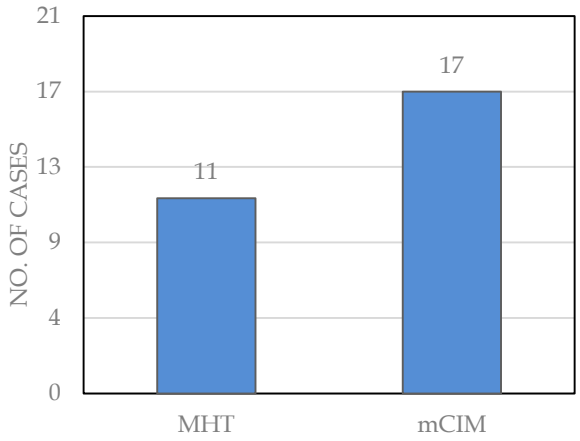

Figure 3: Cases distribution of carbapenem resistance by phenotypic tests

DISCUSSION

The resistant rate in the current study (15%) is similar to study conducted by Ekta Gupta et al **(2006)** with resistance being 17.32%.[12]

Maximum resistant isolates were from ICU 11(57.89%) which is similar to study in Jaipur (**2013),** where ICU positivity rate was 66%.[10]and to study conducted in North East India where it was seen maximum in ICU patients (57.2%) followed by wards (33.3%).[11]

In our study most resistant isolates were E. coli 10(52.63%) which is discordant to study conducted by S Nagaraj et al **(2011)** where K. Pneumoniae resistant rate being 75% while that of E. Coli being 66.6%.[9]

The MHT results in the current study were 11(57.89%) which is similar to results obtained by Ph. Henkhoneng et al (**2013**), where MHT positivity rate was 60.4%[11]while in another study by Prasanna L. Kakaria et al it was 72.3%.[13]

The wide variations in MHT results can be due to the subjective errors as well as false positive and negative results. mCIM is considered better due to its improved sensitivity and specificity. Though, not much comparative studies are available on it.

CONCLUSION

This study will help the clinicians in formulating a revised treatment plan for patients suffering from carbapenemase producing Enterobacteriaceae. For the improvement in diagnosis, national authorities or international institutions should provide proper education to the concerned staff. Screening of contacts for CP-CRE infected patients is essential to curb transmission and control outbreaks, use of promising antibiotic combination like avibactam with ceftazidime or aztreonam is promising CRE treatment.

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