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Original Article

Correlation between Mechanism of Carbapenem Resistance and in vitro Efficacy of Ceftazidime-Avibactam and Meropenem-Vaborbactam on Carbapenem-Resistant Enterobacterales and Pseudomonas Aeruginosa

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ABSTRACT

Objective: To determine the mechanism of Carbapenem resistance, type of Carbapenemase produced and in vitro efficacy of Ceftazidime-Avibactam (CAZ-AVI) and Meropenem-Vaborbactam (MEV) against Carbapenem-resistant Enterobacterales and in vitro efficacy of Ceftazidime-Avibactam against Carbapenem-resistant Pseudomonas aeruginosa.

Study Design: Cross-sectional study.

Place and Duration of Study: Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Mar to Aug 2020.

Methodology: The mechanism of Carbapenem resistance in Enterobacterales and *P.aeruginosa* was determined by mCIM and eCIM methods. In vitro, the susceptibility of isolates to Ceftazidime-Avibactam and Meropenem-Vaborbactam was determined by disk diffusion technique according to CLSI 2020 guidelines.

Results: Out of 249 Carbapenem -resistant isolates, there were 192(77.1%) Enterobacterales and 57(22.9%) *P.aeruginosa*. From 192 Enterobacterales, 174(90.6%) were Carbapenemase producers while 18(9.4%) used 'other mechanisms. From 174 Carbapenemase producers, metallo-β-lactamases were produced by 141(73.4%) while serine Carbapenemases by 33(17.2%). Out of 33 serine Carbapenemase producers,19(57.6%) were sensitive to CAZ-AVI and 6(18.2%) to MEV. Out of 141 MBL producers, 31(22%) were sensitive to CAZ-AVI and 18(12.8%) to MEV. Out of 57 *P.aeruginosa*, 30(52.6%) were Carbapenemase producers and 1(3.4%) were sensitive to CAZ-AVI while 27(47.4%) were non-Carbapenemase producers and 13(48%) were sensitive to CAZ-AVI. MBL production predominated.

Conclusion: The in vitro efficacy of these antibiotics against MBL producers and serine Carbapenemase producers was not satisfactory.

Keywords: Carbapenem -resistant enterobacterial, Carbapenem -resistant pseudomonas aeruginosa, Ceftazidime-avibactam, meropenem-vaborbactam, EDTA-modified Carbapenem inactivation method, Modified carbapenem inactivation method.

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INTRODUCTION

Carbapenems are broad-spectrum antibiotics which are highly effective in treating infections caused by multi-drug resistant bacteria. They are the drugs of choice against Extended Spectrum β-lactamase (ESBL) producing Gram-negative bacilli.[1]

The situation is even worse in a resource-limited country like Pakistan. The lack of knowledge and practice of antimicrobial stewardship has led to the injudicious use and an over-reliance on antibiotics, resulting in the emergence of antimicrobial-resistant Gram-negative bacilli with limited treatment options.[2,3]

Over the past two decades, no new class of antibiotics has been discovered. Several years ago, the US Food and Drug Administration Authority (FDA) approved novel non-β-lactam-β-lactamase inhibitors such as avibactam in combination with a third-generation Cephalosporin (Ceftazidime) and Vaborbactam with a Carbapenem (Meropenem)for the treatment of adults with infections of blood-stream, intra-abdominal, urinary tract, lower respiratory tract and other hospital-acquired infections.[4,5]

Ceftazidime-avibactam contains a synthetic β-lactamase inhibitor active against serine Carbapenem ases belonging to Ambler class A, C and D produced by Enterobacterales and *P.aeruginosa* members.[6] Meropenem-vaborbactam contains a boronic acid β-lactamase inhibitor active against Ambler class A and C serine Carbapenemases. It is effective only against members of family Enterobacterales.[7,8]

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It would be valuable to determine focal and current mechanisms of Carbapenem resistance and the primary Carbapenemases produced by Carbapenem -resistant pathogens and to determine the in vitro efficacy of ceftazidime-avibactam and Meropenem-Vaborbactam on these isolates.[9,10] Despite recommendations by CLSI, studies of this kind have yet to be performed in Pakistan. Hence, it is crucial to perform such a study on our indigenous Carbapenem -resistant pathogens.

METHODOLOGY

The cross-sectional study after receiving the institutional review board certificate (MP-MIC19-3/ READ-IRB/21/119) at Microbiology Department, Armed Forces Institute of Pathology Rawalpindi Pakistan, from March-August 2020.

Inclusion Criteria: Non-repetitive, non-duplicate samples received from Combined Military Hospital & Pak Emirates Military Hospital (PEMH) Rawalpindi Pakistan and other hospitals in Rawalpindi, Islamabad and surrounding areas were included.

Exclusion Criteria Repeat samples of the same patient were excluded.

The samples such as blood, urine, pus and pus swabs, lower respiratory tract specimens, tissue and fluid were inoculated onto appropriate culture media and incubated for 18-24 hours at 35oC±2oC in ambient air. Initial bacterial identification was done according to the growth characteristics on culture media, Gram staining and other rapid biochemical identification procedures like catalase and oxidase tests. Antibiotic susceptibility testing by disk diffusion technique on Mueller Hinton Agar was carried out according to CLSI 2020 guidelines for members of Enterobacterales and *P.aeruginosa*. To identify Carbapenem -resistant pathogens, Carbapenems, Meropenem and Imipenem 10µg each were also part of the antibiotic panel. For each member of Enterobacterales, API 10s was also set up, and API Web was used for the bacterial identification. Isolates showing resistance to either or both Carbapenems were considered the test isolates.

A colony suspension of Carbapenem-resistant isolates equivalent to 0.5 McFarland was used to inoculate MHA. Ceftazidime-avibactam 30/20µg. Meropenem-vaborbactam 20/10µg were then applied, whereas Ceftazidime-Avibactam 30/20µg was applied for Carbapenem-resistant *P.aeruginosa* and then incubated. According to CLSI 2020, Enterobacterales Ceftazidime-Avibactam has an interpretive category of sensitive (S) with a zone diameter of ≥21mm and a resistant (R) category with a zone diameter of ≤20mm. Meropenem-vaborbactam has interpretive categories of sensitive (S) with a zone diameter of ≥18mm, Intermediate (I) with a zone diameter of 15-17mm and resistant (R) with a zone diameter of ≤14mm. For *P.aeruginosa,* Ceftazidime-Avibactam has interpretive categories of sensitive (S) with a zone diameter of ≥21mm and resistant (R) with a zone diameter of ≤20mm. Quality Control for the antimicrobial disks was set up using E.coli ATCC®25922 and *P.aeruginosa* ATCC®27853.7

For Enterobacterales, mCIM along with eCIM was used to detect whether the isolate produced a Carbapenemase and, if yes, the type of Carbapenemase produced and for *P.aeruginosa* only mCIM detected the mechanism of Carbapenem resistance (Figure-1). For mCIM, 1µL loopful of bacteria for Enterobacterales or 10µL for *P.aeruginosa* from an overnight BAP culture was emulsified in 2 mL Tryptic Soy Broth. The suspensions were then vortexed for 10-15s. A 10µg meropenem disk was then added to each of the suspension. For eCIM in Enterobacterales only, 1µL loopful of bacteria was added in 2ml TSB plus 20µL of 0.5M EDTA to make 5mM EDTA. Meropenem disk 10µg was added after the suspension was vortexed. It was then incubated at 35oC±2oC in ambient air for 4hours±15minutes. A 0.5 McFarland suspension of E.coliATCC®25922(Carbapenem sensitive) was inoculated on MHA. Meropenem disks from each TSB-Meropenem disk suspension were placed on the plates, which were then incubated at 35oC±2oC in ambient air for 18-24 hours.[7]

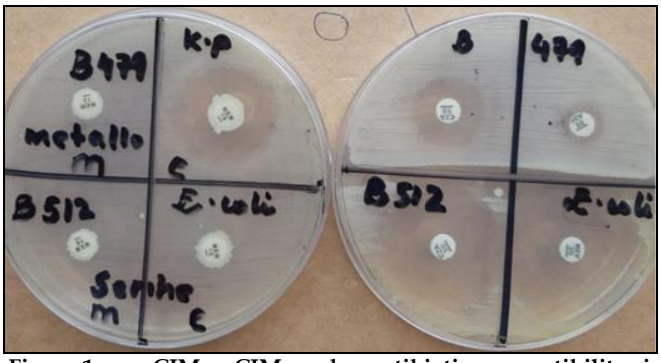

Figure-1: mCIM, eCIM and antibiotic susceptibility in Enterobacterales

A zone diameter of 6-15mm or 16-18mm with pinpoint colonies around the Meropenem disk showed that the isolate was a Carbapenemase producer as the enzyme hydrolyzed Meropenem, so either no or

limited inhibition of growth of Meropenem-susceptible E.coli occurred. A zone of inhibition of ≥19mm indicated that the isolate was not a Carbapenemase producer, as Meropenem remained active.

For Enterobacterales with positive mCIM, eCIM was also interpreted to determine the type of Carbapenemase produced. A metallo β-lactamase producer showed ≥5mm increase in zone diameter for eCIM as compared to mCIM, as EDTA in the eCIM tube chelated the zinc ions required for the MBL to become active, rendering it ineffective thus Meropenem remained active and enhanced zone of inhibition due to hampered growth of Meropenem-susceptible E.coli ATCC® 25922. A serine Carbapenemase producer showed ≤4mm increase in the zone of inhibition between mCIM and eCIM as EDTA did not affect the activity of serine Carbapenemase, so Meropenem was hydrolyzed resulting in no or marginal increase (≤4mm) in zone size. A false-ve eCIM result was obtained for organisms co-producing MBL and serine Carbapenemase. EDTA could inactivate MBL but not alter the serine Carbapenemase, resulting in Meropenem hydrolysis.

For *P.aeruginosa*, only mCIM was performed. Carbapenemase producers exhibited a zone of inhibition of 6–15 mm or 16-18 mm with pinpoint colonies (Figure-2). In isolates showing Carbapenem resistance due to 'other mechanisms', the zone of inhibition was ≥19 mm. Two in-house QC strains were used: Serratia marcescens (serine Carbapenem ase producer) and Klebsiella *P.neumonaie* (metallo β-lactamase producer).

Figure-2: mCIM and Antibiotic Susceptibility in *P.aeruginosa*

Statistical Package for Social Sciences (SPSS) version 26.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages.

RESULTS

Our study included 249 Carbapenem-resistant Gram-negative bacilli, 192(77.1%) Enterobacterales and 57(22.9%) *P.aeruginosa*. Figure-3 shows various Carbapenem-resistant Gram-negative pathogens yielded from clinical samples.

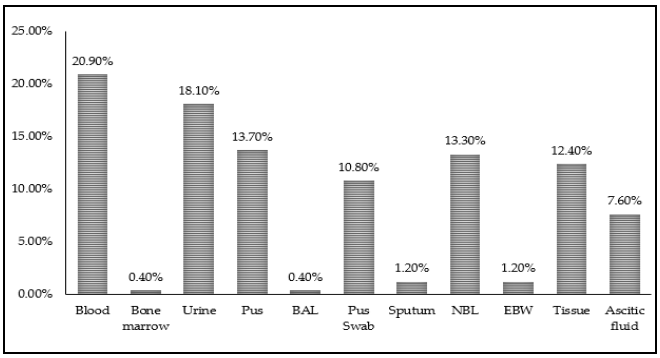

Figure-3: Carbapenem-resistant Gram-negative Pathogens yielded from various Clinical Samples (n=249)

The specimens included 52(21%) blood, 45(18.1%) urine, 33(13.3%) non-directed bronchial lavage, 34(13.7%) pus, 27(10.8%) pus swab, 31(12.4%) tissue, 19(7.6%) ascitic fluid, 3(1.2%) endobronchial washings, 3(1.2%) sputum and 1(0.4%) bronchoalveolar lavage fluid.

Among Enterobacterlaes, MBL was produced by 141(73.4%) and serine Carbapenemase by 33(17.2%) isolates. 'Other mechanisms' of Carbapenem resistance were demonstrated by 18(9.4%) isolates. Among 192 Enterobacterales, 60(31.2%) were sensitive and 132(68.8%) resistant to CAZ-AVI. While, 33(17.2%) were sensitive and 159(82.9%) resistant to MEV.

Of 141 MBL producers, 31(22%) were sensitive, and 110(78%) were resistant to CAZ-AVI. Whereas 18(12.8%) were sensitive, and 123(88.2%) were resistant to MEV. Out of 33 serine Carbapenemase producers, 19(57.6%) were sensitive to CAZ-AVI, but only 6(18.2%) were sensitive to MEV. Out of 18 isolates exhibiting 'other mechanisms' of Carbapenem resistance, 10(55.6%) were sensitive to CAZ-AVI and 9(50%) showed sensitivity to MEV.

Of 57, *P.aeruginosa* isolates, 30(52.6%) were Carbapenem ase producers, and 27(47.4%) employed 'other mechanisms' of Carbapenem resistance. There were 14(24.6%) isolates sensitive to and 43(75.4%) resistant to CAZ-AVI. Of 30 Carbapenemase producers, only 1(3.4%) was sensitive, and 29(96.6%) were resistant to CAZ-AVI. Out of 27 non-Carbapenemase producers, 13(48%) were sensitive, and 14(52%) were resistant to it.

DISCUSSION

With increasing antimicrobial resistance among Gram-negative bacilli and only a handful of antimicrobials introduced to combat this grave situation, novel treatment options like Meropenem-Vaborbactam and Ceftazidime-Avibactam have become more important. These antibiotics have their limitations as they are not active against MBL producers.[11,12]

In our study, the most abundant CRE were K. *P.neumoniae* 66.7%, followed by E. coli 23.4%. A small number of other bacteria, such as Citrobacter braakii 2.6%, Enterobacter cloacae 2.1%, Proteus mirabilis 1.6%,Klebsiella oxytoca 1.6%, Citrobacter freundii 1%,Providencia retgerii 0.5%, and Serratia marcescens 0.5% were also isolated.

Our study demonstrates that the main attributor of Carbapenem resistance was the production of Carbapenemases seen in 174 (90.6%) CRE isolates, 141(81%) of which produced MBLs and 33(19%) produced a serine Carbapenemase. Mechanisms of Carbapenem resistance other than enzyme production were seen in 18(9.4%) isolates. According to a study by Javed *et al*.,99% of CRE isolates were Carbapenemase-producing, and MBLs were detected among 97% of Carbapenemase-producing isolates.[10]

In this study, the results of the in vitro efficacy of CAZ-AVI on CRE could have been more encouraging. As, 60(31.3%) isolates were susceptible and 132(68.8%) were resistant to the antibiotic. In a study conducted by Sonnevend *et al*., 53.3% of CRE were susceptible while 46.7% were resistant.[13] Alatoom *et al*. described 45% susceptibility to the antibiotic among their CRE isolates. However, according to the INFORM global surveillance program (2015-2017), 73% of CRE isolates were susceptible to the compound.[14]

In the case of in vitro efficacy of MEV against Enterobacterales, 33(17.2%) isolates were sensitive, and 159(82.9%) were resistant. In a study conducted by Wilson *et al*. 98% of CRE isolates were susceptible to the drug.[15] According to Castanheira *et al*. MEV was active against 95.4% of CRE isolates.[16]

It is an interesting finding that the rate of resistance among Enterobacterales for both drugs is just the same, 68.8%. According to a study conducted by Pogue *et al*. 99% of Enterobacterales were susceptible to MEV, whereas 98% of isolates were susceptible to MEV.[17]

In our study,31(22%) MBL-producing Enterobacterales showed in vitro susceptibility to CAZ-AVI and 18(12.8%) isolates showed sensitivity to MEV. According to Dhillon *et al*. 3.8-18.6% in vitro susceptibility to MEV was observed among MBL-producing CRE.[18]

These compounds have shown excellent in vitro efficacy against serine Carbapenemase-producing Enterobacterlaes in other parts of the world. The question arises as to why such compounds are ineffective against our serine Carbapenemase-producing isolates. One of the reasons is that the phenotypic methods of mCIM and eCIM have a limitation. It gives a false negative eCIM result, which means that if metallo and serine Carbapenemase are co-produced by an isolate, then exact differentiation between enzymes is impossible.[19] So, the serine Carbapenemase-producing isolates showing resistance to both these compounds could be co-producers of class A and class B Carbapenemases. However, the prevalence of such isolates is <1% in the US, Europe, Latin America and Asia Pacific.[20] To resolve this issue, another method called CIMplus can be employed, which uses another inhibitor, phenylboronic acid (PBA), in addition to EDTA to determine the co-production of both enzymes.[21]

This study demonstrated the increasing burden of Carbapenem-resistant Enterobacterales & *P.aeruginosa* in our hospital settings and their non-responsiveness to novel treatment options like MEV and CAZ-AVI, which is even more worrisome. Antibiotic options like Plazomicin and Cefiderocol with activity against all Ambler class β-lactamases can prove advantageous in the fight against the ever-rising burden of antimicrobial resistance.

LIMITATIONS OF STUDY

The phenotypic method of eCIM has limitations. A false negative eCIM result can be obtained, which means the inability to determine whether a single Carbapenemase or more than one Carbapenemase type is produced. Another limitation of the method is the inability to differentiate between the type of serine Carbapenemase produced (class A or/and class D serine Carbapenemase. As facilities to perform MIC were not available, the study was based on the disk diffusion technique. For CAZ-AVI, CLSI 2020 recommends confirmatory MIC testing for isolates with zones of 20 to 20 mm to avoid reporting false susceptible and false resistant results.

CONCLUSION

The in vitro efficacy of these antibiotics against MBL producers and serine Carbapenemase producers was not satisfactory.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

FA: & IAM: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

HW: & WH: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

UK: HZ: & MS: Concept, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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