Original Research Paper

Volume - 13 | Issue - 04 | April - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Medical Microbiology

ASSESSING PHENOTYPIC SYNERGISTIC ACTIVITY OF CEFTAZIDIME-AVIBACTAM AND AZTREONAM AGAINST CARBAPENEMASE PRODUCING MULTIDRUG RESISTANT GRAM-NEGATIVE ORGANISMS IN A TERTIARY CARE HOSPITAL, NAVI MUMBAI.

Dr. Ramya Vaidhyswaran	Resident Microbiologist
Dr. Ravishekhar Karnam	Consultant Microbiologist
Dr. Jyoti Sonawane*	Associate Professor Microbiology*Corresponding Author
Dr. Abhay Chowdhary	Professor and Former Head of Department

ABSTRACT **Introduction:** Multidrug resistance organisms have become a great concern globally. Ceftazidime-avibactam (CAZ/AVI) has a broad-spectrum activity against Ambler molecular Class A (narrow-spectrum & extended-spectrum beta-lactamases (ESBLs), serine carbapenemase including KPC). It also has activity against some Class C (KPC) and Class D carbapenemase including OXA-48-like enzymes in Enterobacteriaceae.**Aim:** The study aims to identify clinically relevant synergy between CAZ/AVI+Aztreonam (ATM) for multidrug resistant isolates (MDR) using E-test/disc method. **Material & Methods:** A total of 60 non-duplicate clinical isolates of different clinical specimens (blood, pus, sputum, urine, and sterile body fluids) were collected prospectively over 3-month period (January – March 2022). Antimicrobial susceptibility determined by Kirby-Bauer disc diffusion method. CAZ/AVI+ATM synergy was determined using E- test/disc method. NMIC 500 BD phoenix automated system confirmed the presence of class of enzymes. **Results:** Of the 60 MDR isolates, Klebsiella (n=35; 58.3%) was the most common followed by E. coli (n=17; 28.3%). CAZ/AVI was susceptible for 25% (n= 15) of the isolates and the remaining 45(75%) were resistant. CLSI guidelines used for interpretation. Using qualitative E-test/disc diffusion method, addition of aztreonam to CAZ/AVI showed susceptibility for 66% (n=30/45) of CAZ/AVI resistant isolates demonstrating a Reverse D synergy. The remaining 15 (out of 45) isolates showed resistance to the above combination and thereby showing no synergy. The most common class of enzymes were Class D (n=25/60) followed by Class B (n=19/60). **Conclusion:** In screening for CZA/AVI +ATM synergy, the E-test/disc method provides a quick and practical approach for the real world screening and for the targeted use in the patients. This approach would be more relevant considering NDM and its coproduction prevalence is on the rise with limited antibiotic options for such infections.

KEYWORDS : Ceftazidime, Drug synergism, Aztreonam, Multi Drug Resistance, In Vitro

INTRODUCTION

According to World Health Organization (WHO) and Centres for Disease Control and Prevention (CDC) Antimicrobial Resistance (AMR) is one of the paramount global health threats causing an annual death over 35,000 in the US alone. [1,2]ESKAPE pathogens are common cause of Multi Drug Resistance (MDR) in a tertiary care hospital.[3] In HAI (Hospital Acquired Infections) CRE (Carbapenem-resistant Enterobacteriaceae) and CR-PA (Carbapenem Resistant Pseudomonas aeruginosa) has become a great threat that leads to resistance to most of the treatment options.[4] Gram negative organisms have a broad spectrum of activity against the beta lactam group of drugs particularly the cephalosporins such as Ceftazidime and Ceftriaxone. They are safe and well tolerated towards treatment. However because of the high occurrence of enzymes like ESBLS (Extended Spectrum Beta Lactamases), MBL's (Metallo Beta Lactamases), AmpC beta lactamases and OXA-48 producing pathogens their role towards MDR pathogens are reducing. In many hospitals, MDR organisms are in combination of these enzymes making the treatment options very limited and difficult.[5] The therapeutic option for these MDR gram negative organisms are carbapenems and because of their increased use led to rapid emergence of resistance. The reasons for the sudden increase in the carbapenem use are due to long term stay in the hospital, exposure to carbapenem antibiotics continuously, contamination of surgical equipment and the use of low doses than the approved dose.[6]

In Pseudomonas aeruginosa even though some isolates produce carbapenemases, carbapenem resistance is commonly mediated by non- carbapenemase mechanisms that include porin loss, drug efflux and overproduction of AmpC.[4] Multiple studies have shown that there are high rates of blaNDM in Klebsiella pneumoniae in India. However, blaOXA-48-like enzymes have also been increasingly reported in the present times. The therapeutic management of these CRKp (Carbapenem Resistant Klebsiella pneumoniae) has challenges such as drug limitations where only few agents like Colistin, Fosfomycin, Tigecycline and Minocycline. For these reasons combination therapy is preferred over monotherapy.[7]

In India, several studies have reported high rates of blaNDM-1 in Gram-negative organisms. Clavulanic acid, Tazobactam or Sulbactam are the currently available first-generation beta-lactamases that have a narrow spectrum and they do not inhibit carbapenemases. Whereas, Avibactam, Zidebactam and Relebactam are some of the second-generation beta lactamases that have a broader spectrum of activity.[8] They have activity against Class A (ESBLs) and KPC's, Class C (AmpC's) and some Class D (OXA-48 like) beta lactamases but not against the Class B (MBLs) such as NDM, VIM and IMP.[9]

Aztreonam is a monobactam antibiotic that targets MBL's which is not covered by Ceftazidime-Avibactam (CZA/AVI) (combination of a cephalosporin and a beta-lactamase inhibitor). Thus, a combination of CZA/AVI with Aztreonam (ATM) is useful especially when we are targeting the MBL's with ESBL's and other CRE enzymes. This combination works against all beta- lactamase producing gram negative organisms. If the treatment is effective, it is a best option in high ESBL, MBL prevalent hospital centres.[10]There are only very few papers and case reports regarding the combination therapy being successful and a great option for treatment for MDR organisms. In the Indian scenario currently, there is no approved method for in-vitro susceptibility by the CLSI or any other organization for testing this methodology.[11] Hence the current study aims at assessing and supporting the use of Ceftazidime Avibactam with Aztreonam for clinical decision making. Also, this study aims to develop a practical laboratory method to screen for clinically relevant synergy between CAZ/AVI+ATM in NDM producing isolates using E-strip/disc method.

MATERIALS AND METHODS

1.Bacterial isolates- Multidrug-producing organisms from different isolates were categorised and taken for this study (Ethics Number-DYP/IECBH/2022/020). It is a prospective and observational study. Inclusion criteria for the isolates are patients above 18 years of age and the organisms should be Multidrug resistant. A total of 60 non-duplicate samples were taken and collected over a period of three months from January to March 2022.

2.Susceptibility testing- Isolates were subjected to antimicrobial susceptibility testing determined using CLSI-recommended disc diffusion. Identification methods (BD Phoenix), and the class of antimicrobial resistance was determined by the NMIC 500 BD Phoenix automated panel which is according to ambler classification.
3.Modified E-test/ disc diffusion method- CAZ/AVI+ATM synergy were determined using the CAZ/AVI E-test/ATM disc diffusion method. Innocula with turbidities of 0.5 McFarland, were determined using spectrophotometry (range 0.08-0.13), and are platted on Muller Hilton agar (Himedia). A CAZ/AVI E-strip (Pfizer, New York, NY) with fixed AVI concentration at 4µg/L were placed on the agar plate. An ATM disc (30µg Himedia) is placed at 15mm from center of the strip to center of the disc. It was placed at the level of the CAZ/AVI breakpoint (8µg/mL) according to CLSI guidelines 2022. Plates were incubated and read after 16-18 hrs. A qualitative approach was taken with an inverse-D defined as demonstration of synergy. This qualitative approach was extrapolated as the inverse observation of inducible clindamycin resistance that is often screened for as part of routine antimicrobial susceptibility testing in the laboratory.

RESULTS-

Of the 60 MDR isolates, Klebsiella species (n=35; 58.33%) was the most common followed by E. coli (n=17; 28.33%) as shown in Figure 1. All the isolates were resistant to most of the antibiotics including the carbapenems and sensitive to only Colistin. In the class of carbapenems according to Ambler classification it was found that the highest class was Class D (n=25;41.66%) followed by Class B (n=19; 31.66%) and Carbapenem producer due to other mechanisms like porin loss or due to efflux pump (n=8; 13.33%).In co-production ESBL with a Non-CPCRE (n=4; 6.66%) ,ESBL with Class B (n=2; 3.33%) and ESBL with an AmpC producer (n=1; 1.66%) were shown. There was only one Class A carbapenem (n=1; 1.66%) observed. CAZ/AVI was susceptible for 25% (n= 15) of the isolates and the remaining 45(75%) were resistant. CLSI guidelines was used for interpretation. Using the qualitative E-test/disc diffusion method, the addition of aztreonam to CAZ/AVI showed susceptibility for 66% (n=30/45) of CAZ/AVI resistant isolates demonstrating a Reverse D synergy (Figure 2). The remaining 15 (out of 45) isolates showed resistance to the above combination and thereby showed no synergy (Figure 3,4).There is a third type of synergy known as CZA/AVI sensitive with aztreonam synergy in CZA/AVI sensitive isolates for the E-strip alone (n=8/15; 53.33%), this type of synergy is important as even though the E-strip shows a sensitive MIC, but with combination of Aztreonam there is a drop of MIC which is useful in clinical practice (Figure 5).

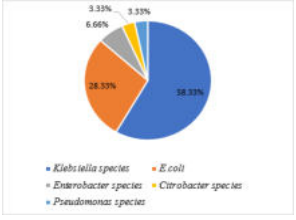

Figure 1: Organism Distribution

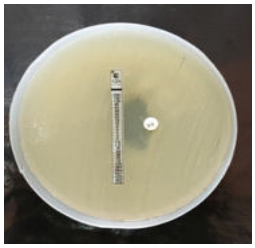

Figure 2: Synergy demonstrated by Reverse-D

Figure 3: Aztreonam sensitive with no synergy

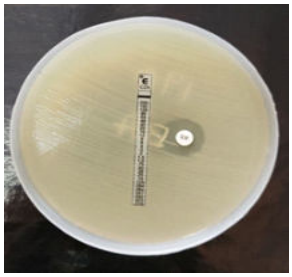

Figure 4: No synergy

Figure 5: CAZ AVI sensitive with Aztreonam synergy

DISCUSSION-

In the last ten years the surfacing and fast identification of Carbapenem- Resistant Organisms (CRO) have been recorded around the world. NDM and OXA-48 like mediated resistance in gram negative organisms are a major concern in India. E.coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa are among the gram negative organisms that commonly have the MBL resistance. Because of the wide range of geographical distribution of Carbapenemases in gram negative organisms its essential to understand the antimicrobial effectiveness against the specific determinants to improve the clinical outcome.[7] During the COVID-19 pandemic there is multiple concerns regarding the escalation of anti-microbial resistance because of the increased use of antibiotics for empirical therapy.[12] Serine carbapenems dominate the most parts of the world and Zinc based carbapenem (MBL) are more prevalent in South Asia particularly in India.[13]

There are various factors that contribute to the problem of the MDR organisms first its occurrence in HAI (Hospital Acquired Infection), co-morbidity status, prior antibiotic use, immunosuppression etc. Carbapenem develop resistance often, which possess a therapeutic challenge especially in health care centers where there is high load of MBL production. The last line of antibiotic that can be used is Colistin and it can't be used often due to its toxicity.[10]

Combination of antibiotic therapy helps in overcoming antibiotic resistance and improving the therapeutic efficacy. Even though combining CZA/AVI with ATM proves theoretically practically it is done individually.[10] There are various methods to determine the synergy between CZA/AVI+ATM in MBL Enterobacterales, yet some of the methodologies have failed to show synergy with the breakpoints for ATM. Khan et al conducted a study where they used disc stacking method, gradient strip stacking and crossing method in 10 MBL producing Enterobacterales by comparing their breakpoints against CLSI. The results of the study showed excellent overall percentage of performance of gradient strip method showing 82%-94% when using these methods. The lowest was disk stacking method showing a percentage of 43%.[4] According to Sreenivasan et al study, 60 isolates were identified as MDR organisms producing MBL which were taken for the in-vitro study against CZA/AVI+ATM. Three methods were used for the identification which included the disk diffusion method, E-test fixed ratio method. MIC of CZA/AVI, and ATM alone and in combination showed 100% correlation with one and other.[11] The E-strip disk diffusion method is a rapid practical method that was done in a study by Rawson et al which demonstrated a reverse-D synergy for a total 43 NDM producing CRE isolates. The study E-test/disc method was correlated with broth dilution method showing a sensitivity of 77% and specificity of 85%. The positive predictive value was 92% and negative predictive valuer was 61%.[14] A similar study was done by Sahu et al showing highest synergy for Klebsiella pneumoniae by Disc-E strip (86%) and E strip- Agar method (84%). Synergy was also

observed among Pseudomonas aeruginosa and E.coli. Molecular testing was also done for identifying the class of enzyme shown in these isolates.[10] In our study, a total of 60 MDR isolates were taken and Klebsiella species were found to be the highest (58.3%) followed by E. coli (28.3%). CZA/ AVI was alone susceptible for 25% of the isolates and the remaining 45% were resistant. The E strip/ disc method was used and found that 66% of the resistant isolates to CZA/AVI showed Reverse-D synergy and the remaining showed no synergy. The most common class of enzyme isolated were the Class D (41.6%) followed by the Class B (31.6%).

As this method was a conventional approach, there was a practical difficulty in performing genotypic profiling and hence it was not done. In a real-world scenario it will be useful in identifying isolates based on the synergy for therapeutic use especially in clinical practice.

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