EFFECT OF BREAKPOINT AND MINIMUM INHIBITORY CONCENTRATION (MIC) RATIO ON OUTCOME OF PATEINTS ADMITTED IN ICU

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I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of her Master of Clinical Pharmacy and Pharmacy Practice studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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ABBREVIATIONS

ICU - Intensive Care Unit

LOS - length of stay

MDR - Multi Drug Resistant

WHO - World Health Organization

ESBL - Extended Spectrum beta-lactamase

APACHE - Acute Physiology and Chronic Health Evaluation

GNB – Gram negative bacteria

MIC – Minimum Inhibitory Concentration

MBC – Minimum Bacterial Concentration

Background and objectives: Infectious disease due to Gram-negative bacteria is a leading cause of morbidity and mortality worldwide. Antimicrobial agents are used to treat Gram-negative infection by inhibiting bacterial growth in vitro in specific minimum inhibitory concentration (MICs). MIC alone cannot predict clinical outcome of an infection. To get a better outcome breakpoints are used to calculate efficacy ratio. Our study assessed the impact of efficacy ratio on outcome of infection due to Gram-negative bacteria.

Methods: It was a prospective observational study, done in ICU and Microbiology department of AMRI Hospitals, Dhakuria, within the period of July, 2018-March, 2019. Database of ICU and Microbiology department of AMRI Hospitals was used as data source for our study. All patients admitted to the ICU during the study period and with documented infection with gram negative pathogens were included in our study. Variables like age, sex, APCHE IV score, antibiotics against isolated Gram-negative organism, MIC values of prescribed antibiotics, ICU mortality and ICU LOS was collected. Efficacy ratio was calculated by this formula **Efficacy Ratio** = $\boldsymbol{Resistant\ Breakpoint} \div \boldsymbol{MIC\ Value}$ and categorized into four groups -<1, 1-2, 2-4 and >4. The data was analyzed by statistical methods.

Results: The total number of patients was 274, majority were male (148, 54%). The mean age was 69.74± (12.87), mean APACHE IV score was 67.88± (27.29). The total ICU mortality rate was 16.8%, and the mean ICU LOS was 6 days (3-6). Among patients who received monotherapy, mortality was significantly less in the Efficacy ratio group >4 compared to the 2 - 4 group (11.1% vs. 50%; p = 0.008). This however did not hold true in the combination therapy group (p=0.3). Mean LOS was lowest in the >4 efficacy ratio groups (7.30 days, in Mono-therapy).

Conclusion: Efficacy ratio is associated with better outcome in Mono-therapy.

INTRODUCTION

Infectious disease due to Gram-negative bacteria is a leading cause of morbidity and mortality worldwide. Antimicrobial agents are the major implicated tools to treat this disease. [1] Resistant strains of Gram-negative bacteria, in particular Enterobacteriaceae and non-fermenters develop due to the misuse of antimicrobial agent. [1] They affect not only individual humans but impose public health burden as the bacteria use their resistance mechanism to spread infections in hospital environment and the community outside hospitals by means of mobile genetic elements. [1] The resistance in Gram-negative bacteria leads to increased resistance to antimicrobial agents. Several mechanisms cause gram negative bacteria to withstand the antimicrobials. The developed mechanism is the production of Extended- spectrum β-lactamases (ESBLs) and carbapenemases; furthermore, Gram-negative bacteria are now capable of spreading such resistance between members of the family Enterobacteriaceae and non-fermenters using mobile genetic elements as vehicles for such resistance mechanisms rendering antibiotics useless. [1] Innovative approaches to the use of antimicrobial therapy become a necessity due to increasing bacterial resistance, and the subsequent burden to society in terms of morbidity, mortality, and increased health care expenditures. [2] Considering the shortage of availability of innovative approaches, recognition to the appropriate utilization of antimicrobials is becoming highly important, particularly as there are fewer new antibiotics in development. [2] During therapy the design of more effective dosing regimens has been facilitated by the elucidation of relationship between pharmacodynamic parameters and organism resistance. [2]. Unfortunately there is little relevant pharmacodynamic examination on the relationship between antibiotic dosing and resistance in patients. [2]

It has been demonstrated that antimicrobials can inhibit bacterial growth in vitro in

specific minimum inhibitory concentration (concentration (**MICs**); since then, MIC value has been used to identify the susceptibility in vivo and to guide clinical practice. [3] **MICs** are defined as the lowest concentration of antimicrobial agent that will help to inhibit the visible growth of a micro-organism after overnight incubation. [4] **MIC** are used as a diagnostic tool in laboratories, mainly to confirm resistance, but most often it is used in research purpose to determine **MIC** breakpoints. [4] **MICs** are considered the 'gold standard' to determine the susceptibility of organism to antimicrobials and are therefore used to judge the performance of all other methods used in susceptibility testing. [4] **MIC** plays a major role to confirm unusual resistance and give a definite answer when a borderline result is obtained by other method of testing, or when disc diffusion methods are not appropriate.[4] The in vitro susceptibility of bacteria to antibiotics have been tested in laboratories since the discovery of penicillin and though most literature report respective approaches of antimicrobial susceptibility testing (AST), [7, 8] there still remains an urgent need to harmonize and further understand the MIC breakpoints used in susceptibility testing. [6]

Breakpoints are discriminatory antimicrobial concentrations used in the interpretation of results of susceptibility testing to define isolates as susceptible, intermediate or resistant. Clinical, pharmacological, microbiological and pharmacodynamic considerations are important in setting **breakpoints**. [5] Realizing the need for standardization of testing methodologies, the World Health Organization (WHO) initiated efforts to develop a method that could be used by all laboratories. [6] Currently, a number of organizations provide instruction for providing AST and these methodologies have been published both nationally and internationally. [6] The Clinical and Laboratory Standard Institute (CLSI) [9, 10, 11] and the European Committee on

Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org) can be considered as the major international organization for AST, while at a national level, bodies such as British Society for Antimicrobial Chemotherapy (BSAS), the Deutsches Institute fűr Normung e.V. (DIN) and the Comité deľ Antibogramme de la Société Française de Microbiologie (CA-SFM) make ongoing and valuable contributions. [6] Moreover, different breakpoints have been listed in the respective AST documents [6, 13].

The breakpoints for classifying organisms as susceptible or resistant to different antimicrobial agents, as determined by the Clinical and Laboratory Standard Institute (CLSI) [15] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), have an extraordinary impact on the observation of antimicrobial resistance as well as on the treatment of infections worldwide. [14] Hence it is important to analyze the effect of inconsistency and changes in the breakpoints recommended by these organizations because any change or discrepancy could be significant for bacteria (such as extended-spectrum β-lactamases (ESBLS)) producing specific mechanisms of resistance [1] as therapeutic options for infections caused by these isolates are limited. [14] In India we follow the CLSI guideline.[15] AST [7, 8], breakpoints [5], and quality control (QC) parameters are established by the CLSI subcommittee on antimicrobial susceptibility testing reviews data from a variety of sources and studies (e.g., in vitro, pharmacokinetic-pharmacodynamic, and clinical studies). [15] CLSI provides three categories of identification: Susceptible, Intermediate and Resistant. [6] The definitions are presented as follows:

Susceptible (S): This is the category which implies that infection due to the isolate may be

appropriately treated with dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated. [6]

Intermediate (I):A category which signify that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used; also indicates a 'buffer-zone' that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations. [6]

Resistant (R): Resistance isolates are those which are not inhibited by the usually achievable concentrations (MIC) of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely (e.g. β-lactamases), and clinical efficacy had not been reliable in treatment studies. [6] Historically, susceptibility 'S' was largely obtained by an evaluation of the MIC distributions of the pathogen(s) under study. [6]

The CLSI guideline M37-A3 [10], which indicates setting breakpoints, identifies that such susceptibility breakpoints should be weighted towards microbial population distributions rather than clinical outcomes in relation to MIC. [6] MIC can be determined from clinical or epidemiological databases, which can examine MIC distribution pattern; through this pattern one can determine a distinct population differentiated by the presence of wild-type or high-MICs. [6] A recent study based on clinical outcomes associated with infections caused by Enterobacteriaceae stratified by carbapenem MIC, reported a statistically significant higher mortality rate and longer ICU length of stay (LOS) as well as numerically longer total hospital LOS and 30-days hospital readmission in the high-MIC group than in the low-MIC group. [18] Although the proportion of the MIC distribution above or below the wild-type population can change over time, there is no rationality to expect a negative on clinical efficacy when choosing

to treat a susceptible strain. [6] These susceptibility breakpoints are, obviously, disease-indication and target-animal-species specific. [6] As per the guideline in CLSI m37-a3 [10], by establishing criteria for correlating the necessary level of drug exposure and the probability of an effective course of therapy, clinically derived susceptibility breakpoints can also minimize the risk of repeated exposure to insufficient antimicrobial drug concentration, which is one of the elements thought to contribute to development of resistance bacteria. [6]

There many factors which contribute to an antimicrobial producing a positive clinical outcome, including:

- Inherent activity of the antimicrobial, measured by the MIC (determined under standard condition). [6]
- Pharmacodynamic properties of the antimicrobial (cidal versus static effect, rate of kill). [6]
- Property of the host-pathogen response, including host immune status. [6]
- Pharmacokinetics of the drug in the administered dosage form. [6]
- Dosage regimen. [6]
- Tendency of inflicting organism to develop resistance.[6]

All these elements are taken into the consideration in the determination of a breakpoint-MIC ratio which is known as Efficacy Ratio, through a review of MIC distribution data and breakpoint listed in CLSI, pharmacokinetics and clinical response. [6] Various studies can help assessing patient outcomes upon MIC and breakpoint ratio known as Efficacy Ratio; illustrate the need to continuously reassess pre-established susceptibility breakpoints. [18] Bhat and

colleagues [19] estimated outcomes of patients with Gram-negative bacteremia treated with cefepime stratified by MIC. A classification and regression tree (CART) analysis conducted in their study reported that a cefepime MIC of ≥8mg/liter was associated with increased mortality (58.4% compared to 21.4%, P = 0.001), despite the fact that an MIC of 8mg/liter was evaluated susceptible at the time of the study. [18, 19] There are many changes that have been observed regarding the susceptibility breakpoints for the Enterobacteriaceae from ≤8mg/L to ≤1 in the case of cefotaxime, and from ≤ 8mg/L to ≤4 in the case of ceftazidime in CLSI, 2010; in addition the interpretation of the breakpoint was reported as found, irrespective of whether there was ESBL production. [14, 16] The breakpoints for carbapenem, determined by the CLSI in June 2010, also changed from ≤4mg/L to ≤1 for imipenem or meropenem, and from ≤2mg/L to ≤0.25 for ertapenem. [14, 17] The whole of the rationale for obtaining a clinical breakpoint MIC ratio is predicted on the fact that an organism nominated as 'susceptible' should respond to the usual dose of the agent. [5] A 'resistant' organism should not respond and an 'intermediate' one may or may not be respond to standard doses, still there are increased chances of responding to a greater dose if the infection is at a site where the actively concentrated antimicrobial are concentrated. [5]

In a developing country like India, where the resistance mechanism of Gram-negative bacteria are on the increase, it is a major concern that such valuable information on MIC-breakpoint ratio is lacking. [5] Such a study will be able to provide important information on clinical response rates for groups of pathogens treated in testing situations where knowledge of the MIC and breakpoint ratio of the pathogen is known. [5] If convincing evidence regarding the outcome of implication of Efficacy ratio is presented, it will be easier to alter the breakpoint

value assessed in the CLSI guideline [15] and it will be helpful for the healthcare system to choose the most appropriate antimicrobial agent for the specific organism considering the circumstances of patients and its adverse event. [5, 15] The CLSI, having close links with the drug licensing authority, the FDA, has a greater ability to capture such clinical information and to change the treatment pattern in hospitals. [5] Many studies conclude that the greater MIC value, the higher is the mortality rate; [18] therefore it can be established that higher the Efficacy ratio greater the patient outcome and lower the Efficacy ratio higher the mortality rate and ICU-LOS. [5] As there is no any strong document on the impact of Efficacy ratio on outcome of patients admitted in ICU due to the infection caused by particularly Gram-negative bacteria, I would like to establish convenient and helpful evidence on 'Effect of Breakpoint and MIC Ratio on Outcome in Patients Admitted in ICU'.

LITERATURE REVIEW

The treatment of serious infectious disease due to Gram-negative bacteria in clinical practice is often complicated by antibiotic resistance. [12] The interpretation of relationship between pharmacodynamic parameters and organism persistence or resistance during therapy will facilitate the design of more effective dosing regimen. [2] WHO initiated an effort to improve the dosing regimen by considering the **MIC** and **Breakpoint** value. [6] Different literatures providing a number of AST methodologies to get a standard MIC value and better patient outcome against increased antibiotic resistance mechanism. [6] In India CLSI updated their breakpoint values and reassess them due to increasing resistance mechanism in Gram-negative bacteria. [9, 10, 15] Through various studies it is noticed that higher MIC value indicate higher mortality rate. [18] From the beginning, there are various studies which represent the need of implementation of Efficacy Ratio. From the following articles, the initial stage of relationship between MIC value and Breakpoint and its effectiveness in health care system will give a better idea for further improvements against antimicrobial resistance in Gram-negative bacteria.

A study was conducted on 'Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients' by ***Thomas et.al (1998)*** [2]**.** The study included patients from nosocomial lower respiratory tract infection clinical trials. They evaluated database based on 107 acutely ill patients, 128 pathogens, and five antimicrobial regimens. Overall, in 32 of 128 (25%) initially susceptible cases resistance developed during therapy. Combination therapy resulted in much lower rates of resistance than monotherapy, probably because all of the combination regimens examined had an $AUC_{0\text{-}24}$(Area Under Curve) /MIC ratio in excess of 100. They concluded that, the selection of antimicrobial resistance appears to

be strongly associated with suboptimal antimicrobial exposure, defined as an AUC_{0-24}/MIC ratio of less than 100.

A study on Antimicrobial susceptibility testing; special needs for fastidious organisms and difficult-to-detect resistance mechanisms was regulated by ***JH et.al (2000)*** [29] to apply several different approaches to detect resistance in both common and infrequently encountered bacterial pathogens. Clinical microbiology laboratories were faced with the challenge of accurately detecting emerging antibiotic resistance among a number of bacterial pathogens. In recent years, vancomycin resistance among enterococci had become prevalent, as had penicillin resistance and multidrug resistance in pneumococci. More recently, strains of methicillin-resistant Staphylococcus aureus with reduced susceptibility to vancomycin have been encountered. Therefore, clinical microbiology laboratories might not be able to depend on a single susceptibility testing method or system to detect all those emerging resistant or fastidious organisms. For reliable detection, laboratories might need to employ conventional, quantitative susceptibility testing methods or use specially developed, single concentration agar screening tests for some resistant specie.

Wheat et.al (2001) [8] illustrated the history and development of some methods still in common use for Antimicrobial Susceptibility Testing (AST).Earlier investigators recognized that there were many variables affecting the results of AST tests. Before 10 years, AST techniques focused on phenotypically testing of isolated bacteria. But a genotypic approach has been advocated to increase the speed and reliability of resistance testing. The limitations and benefits of this new approach may help to standardize and improve the methodology of AST.

Watts et.al (2008) [9] conducted a study on development of in vitro susceptibility testing criteria and quality control parameters for veterinary antimicrobial agents. CLSI document VET02-A3, Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial agents; Approved Guideline (Third Edition) offers instruction for developing agar plate diffusion zone of inhibition, dilution MIC breakpoints, and quality control limits for antimicrobial susceptibility testing of aerobic bacteria isolated from animals. It is implemented to be used in establishing interpretive and quality control criteria for CLSI antimicrobial susceptibility testing standards for antimicrobial agents intended for veterinary use. Different variants like host-specific pharmacokinetics, in vitro drug characteristics, distributions of microorganism, and correlation of test results with outcome statistic were addressed from the aspect of interpretation of test results. This article addressed clinical confirmation of interpretive criteria and quality control limits. The 'ideal' data set may not be obtained during development of a new drug. The guidelines conveyed the limitations and the best-educated conclusions. This guideline followed a path of work-flow which consisted of three sequential processes: pre-examination, examination and post-examination. All clinical laboratories follow these processes to provide the laboratory's services, mainly quality control information.

A study was regulated on 'Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated form Animals' by ***Watts, et.al (2008)*** [10]**.** In vitro susceptibility testing of organism isolated from the disease process in animals in case of insufficiency of susceptibility of a bacterial pathogen to antimicrobial agents cannot be predicted based on the identity of the organism alone. Susceptibility testing is necessary in those cases

Where the causative organism belongs to a bacterial species for which resistance to commonly used antimicrobial agents has been documented, or could arise. There are various laboratory techniques present to measure the in vitro susceptibility of bacteria to antimicrobial agents. In this article, the standard agar disk diffusion method, as well as standard both dilution and agar dilution technique has been demonstrated. It included a series of procedure designed to standardize test performance. The performance, applications, and limitations of the current CLSI-recommended methods were described in this report. Due to increasing number compounds where veterinary-specific information becomes available, these changes will be incorporated.

Thomas et.al (2008) [12] conducted a study on Gram-negative antibiotic resistance to point out the need for multidisciplinary effort to combat resistance, which includes improved antimicrobial stewardship. Resistance rates are increasing in several Gram-negative pathogens and resulted as serious nosocomial infections, including *Acineobacter* spp., *Pseudomonas aeruginosa*, and Enterobacteriaceae. The presence of multi-resistant strains of these organisms cause of several consequences like prolonged hospital stays, higher health care costs, and increased mortality rate, particularly when initial antibiotic therapy does not provide coverage of the causative organisms. With high rate of appropriate initial antibiotic therapy, infections due to multi-resistant Gram-negative pathogens do not have negative impact on patient outcomes or costs. The observations from the study showed the importance of a 'hit hard and hit fast' approach to treat serious nosocomial infection due to multi-resistant pathogens. This article recommended increased resources for infection control, and development of new antimicrobial agents with activity against multi-resistant Gram-negative pathogens.

CLSI has published another study on Performance Standard for Antimicrobial Susceptibility Testing. In this article, ***Wayne et.al (2010)*** [16] updated the pervious guidelines on Performance Standards for Antimicrobial Susceptibility Testing. This update includes new interpretive criteria for doripenem for Enterobacteriaceae, revised interpretive criteria for ertapenem, imipenem, and meropenem for Enterobacteriaceae, guidance for use of the modified test (MHT) with revised interpretive criteria for carbapenem.

A study based on 'Assessing the antimicrobial susceptibility of bacteria obtained from animals' by ***Schwarz et.al (2010)*** [13] reported that AST data intended for the recommendation of therapy and it should be interpreted and reported using clinical breakpoints, whereas AST data intended for surveillance purpose may be reported using epidemiological cut-off values. A review of published literatures disclosed a number of recurring errors such as higher MIC value with regard to methodology, quality control, appropriate interpretive criteria, and circulation of MIC_{50} and MIC_{90} values. The editorial highlighted the major difficulties and provided guidance on the correct performance of antimicrobial susceptibility testing.

Franklin et.al (2011) [11] regulated a study on 'Performance Standards for Antimicrobial Susceptibility Testing' to give a supplemental information about implementation of susceptibility testing procedures published in the CLSI. The standards contain information about both disc and dilution for aerobic bacteria. Clinicians depend on the information from the clinical microbiology laboratory for treatment of theirs seriously ill patients, so the harmonization in standards is important to obtain. The clinical importance of antimicrobial

susceptibility test is performed under optimal conditions and that laboratories have the potential to provide results for the newest antimicrobial agents.

Rodriguez-Baño et.al (2011) [14] conducted a study on impact of changes in CLSI and EUCAST breakpoints for susceptibility in bloodstream infections due to extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E.coli*). In Spain, the impact of recent changes between the breakpoints for cephalosporin and antimicrobials, as determined by CLSI and EUCAST in case of ESBL producing *E.coli* were noticed. After studying a cohort study of 191 episodes of bloodstream infections caused by ESBL producing *E.coli* in 13 Spanish hospitals; the susceptibility of isolates of different antibiotics was investigated by micro-dilution, it was interpreted according to recommendations established in 2009 and 2010 by CLSI and by EUCAST in 2011. Overall, 58.6% and 14.7% of isolates were susceptible to ceftazidime, and 35.1% and 14.7% to cefepime using the CLSI-2010 and EUCAST-2009/2011 guidelines, respectively. The result varied depending upon the ESBL producing micro-organisms. No significant differences were found in the percentage of patients classified as receiving appropriate therapy, following the different guidelines. The study concluded that, by using current breakpoints, extended-spectrum cephalosporin could be regarded as active for a significant proportion of patients with bloodstream infections caused by ESBL-producing *E.coli*.

Salabi et.al (2012) [1] conducted a study on different resistance mechanisms like ESBLs, carbapenemases encoded by genes carried by mobile genetic elements, which are used by Gram-negative bacteria to escape antimicrobial effect. Gram-negative bacteria are now capable of spreading such resistance mechanism between Enterobacteriaceae and non-fermenters family.

Such resistance mechanisms convert antibiotics useless by using their mobile genetic elements as vehicles. Therefore, the authors recommended addressing mechanisms of antimicrobial resistance as one of the most urgent priorities.

A study on Multidrug-resistant and extensively drug-resistant Gram-negative pathogens; current and emerging therapeutic approaches was regulated by ***Karaisskos et.al (2014)*** [24] to improve the treatment protocol in case of multidrug-resistant. In the circumstances of multidrug-resistant, extensively drug-resistant (XDR) and even drug-resistant Gram-negative microorganisms, the medical community was facing the threat of untreatable infections particularly those caused by carbapenemase-producing bacteria, that is *Klebsiellapneumonia, Pseudomonas aeruginosa and Acinetobacter baumannii*. Therefore, all the presently available antibiotics, as well as for the near future compounds, are presented and discussed.

Raman et.al (2015) [20] regulated a study on appropriate initial antibiotic therapy in hospitalized patients with gram-negative infections. In the present scenario the rapid global spread of multi-resistant bacteria and loss of antibiotic effectiveness increases the risk of initial inappropriate antibiotic therapy (IAT) and poses a serious threat to patient safety. A systemic review and meta-analysis of published studies were conducted to summarize the effect of appropriate antibiotic therapy (AAT) or IAT against gram-negative bacterial infections in the hospital setting. AAT was associated with lower risk of mortality and treatment failure. IAT increased risk of mortality in health care system. Using a large set of studies the article showed that IAT is associated with various consequences, including an increased risk of hospital mortality. Infections caused by drug-resistant, Gram-negative organisms represent a considerable

financial expenditure to healthcare systems due to the increased costs associated with the resources required to manage the infection, particularly longer hospital stays.

A study was performed on Performance Standards for Antimicrobial Susceptibility Testing by ***Patel et.al (2017)*** [15] to update the pervious guideline provided by CSLI and to incorporate the new data about breakpoints and its interpretation. It should be considered that M02-A12, M07-A10, and the M100−these information supplement are reference methods. These methods are may be used in routine antimicrobial susceptibility testing of clinical isolates, for evaluation of commercial devices that will be used in laboratories, or by drug or device manufactures for testing a new agents or systems. CLSI breakpoints may differ from other approved organizations for many reasons. Differences also exist as CLSI protectively evaluates the need for changing breakpoints. Decision of changing existing breakpoints, regulatory authorities may also review data in order to determine how changing breakpoints may affect the safety and effectiveness of antimicrobial agent for the approved indications. A delay of one or more years may be required if there is any implementation of interpretive breakpoint change by a device manufacture.

Alasdair et al., (2005) [5] regulated a study on MIC breakpoints and the interpretation of in vitro susceptibility tests. The purpose of focusing susceptibility testing, was to attempt to integrate the drug potency against a population of potential pathogens with the pharmacokinetics of the antimicrobial and, whenever possible, to review this relationship in the light of clinical experience following therapy in clinical trials. Breakpoints are the preferential antimicrobial concentrations use in the interpretation of results of susceptibility testing to define isolates in

three categories as susceptible, intermediate or resistant. Breakpoints depend on several factors like clinical, pharmacological, microbiological, and pharmacodynamic considerations. The authors recommended using one international method of susceptibility testing and breakpoint determination, and using the CLSI. They also recommended that the CLSI guideline should review as bacteria become more resistant to antimicrobial agent. This study tried to establish the need to reassess how clinical breakpoints are defined, and summarize the future activities of different organizations who conducting AST.

A study was conducted to determine Minimum Inhibitory Concentration by ***Andrews JM (2006)*** [4]**,** in department of Microbiology, City Hospital NHS Trust. The study showed different uses of MIC values and the role of MIC in determination of Breakpoints. MICs are used mainly in diagnostic laboratories to confirm resistance. Standardized method for determining MICs are discussed in this paper. The method gives information about the storage of standard antibiotic powder, preparation of stock antibiotic solutions, media, and preparation of inoculums, incubation conditions, and reading and interpretation of results. This paper brought a concept about MIC values and its different uses in antimicrobial therapy.

Wayne et.al (2009) [17] conducted a study on Performance Standards for Antimicrobial Susceptibility Testing to update the CLSI guidelines. The study included the latest recommendations for detecting emerging resistance of aerobic bacteria− arranged in tabular form. The 'breakpoints' include in the supplement are defined as a specific values on the basis of which bacteria can be assigned to the clinical categories of susceptible, intermediate, or resistant. This articles updated the new antimicrobial agents and quality control ranges, improved and

explained methods for ease of use, appendixes for detection of resistance for several organism groups were clarified and combined into one table for each organism, merged disk diffusion and minimal inhibitory concentration (MIC) tables for suggested groups in antimicrobial agents; and appendix for screening and confirmatory tests for suspected carbapenemases production in Enterobacteriaceae.

A study on comparison of European committee on antimicrobial susceptibility testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum β-lactamase production in clinical Enterobacteriaceae isolates, was conducted by ***Polsfuss† et.al (2011)*** [22] to compare the performance of EUCAST and CLSI breakpoints following their revisions in 2010, for the detection of extended-spectrum β-lactamase production in Enterobacteriaceae. 236 well characteristic isolates were investigated. EUCAST non-susceptible breakpoints for ceftazidime and cefpodoxime noticed more ESBL producing Enterobacteriaceae isolates compared with corresponding CLSI ESBL screening breakpoints.

Mouton et.al (2011) [26] regulated a study on the role of pharmacokinetics/pharmacodynamic in setting clinical MIC breakpoints; the EUCAST approach. Clinical breakpoints used in clinical microbiology laboratories to categorize microorganisms as clinically susceptible (S), intermediate (I) or resistant (R) dependent on the quantitative antimicrobial susceptibility as indicated by the MIC value determined in a well-defined standard test system. The laboratory report, with the designations of S, I or R for each antimicrobial agent, provided guidance to clinicians with respect to the potential use of agents in the treatment of patients, and clinical breakpoints should therefore distinguish between patients that were likely

or unlikely to respond to antimicrobial treatment. In Europe, clinical breakpoints were set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), following a defined procedure. The literature provided an overview of the EUCAST process and considerations for setting pharmacokinetic/pharmacodynamic breakpoints. The breakpoints in the EUCAST breakpoint tables were referred to as 'non-species-related breakpoints'.

A study to set interpretive breakpoints for antimicrobial susceptibility testing using disk diffusion was conducted by ***Kronvall et.al (2011)*** [7]. Antimicrobial susceptibility testing plays a major role in clinical microbiology. The disk diffusion test started from 1940s and became a standardized method from 1950s; with the International Collaborative Study (ICS) and National Committee for Clinical Laboratory Standards (NCCLS) are the two major standards. In the late 1970s, large-scale use of species-specific breakpoints was introduced in Lund (Sweden). At the same time, Scientist P. Mouton [MD, MS of Meharry College] constructed species-specific relapse lines and pointed out the difficulties with narrow ranges of minimal inhibitory concentrations (MIC) values. This method used to calibrate the disc test in an individual laboratory. A recent method, 'MIC-coloured zone diameter histogram-technique' has proven convenient for the validation of species-specific interpretive breakpoints. A method for the reconstruction of wild-type zone diameter populations, namely normalized resistance interpretation, was described in this article.

Falagas et.al (2012) [3] carried out a study on the impact of MIC values within the susceptible range of antibiotics on the outcomes of patients with Gram-negative infections. Infections due to *Salmonellaenterica* strains with high fluroquinolone MICs were associated with

more mortality rate than those due to strains with low MICs (relative risk [RR], 5.75; 95% confidence interval [CI], 1.77 to 18.71). More treatment failures were reported for patients infected with non-fermentative Gram-negative bacilli when strains had high MICs (RR, 5.54; 95% CI, 2.72 to 11.27). The mortality rate for patients with infections due to Gram-negative non-fermentative bacilli with high MICs was also higher than for those with low MICs (RR, 2.39; 95% CI, 1.19 to 4.81). The limited obtained data suggest that there is an interpretation between high MICs, within the susceptible range, and adverse outcomes for patients with Gram-negative infections.

Silley (2012) [6] conducted a study on Susceptibility testing methods, resistance and breakpoints to harmonize the different values provided by different international organization. The Clinical Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing are considered the major international contributors to antimicrobial susceptibility testing. This report considered the differences between the respective organizations, examined the terminology used in antimicrobial susceptibility testing. In this article, attention was given to the trend for 'resistance' to be defined by the epidemiological cut-off value, rather than by the long-established clinical breakpoint. The paper discussed susceptibility testing methodologies and presented an approach to setting clinical breakpoints.

Patel et al., (2015) [18] conducted a study on Clinical outcomes of Enterobacteriaceae Infections by Carbapenem MICs to show the mortality rate against the MIC values of carbapenem. The CLSI changed the MIC breakpoints for meropenem and imipenem from

4mg/liter to 1 mg/liter for Enterobacteriaceae in 2010. The changes in breakpoints improve the probability of pharmacodynamic target attainment and eliminate the need for microbiology labs to perform confirmatory testing for *Klebsiellapneumonia* carbapenemase production. A single-centered retrospective matched-cohort analysis was conducted among adult patients with Enterobacteriaceae infections treated with meropenem, imipenem or doripenem. A total 36 patients were included in the study. The group with carbapenem MICs of 2 to 8 mg/liter had a remarkably higher 30-day mortality than the groups with carbapenem MICs of≤1 mg/liter (38.9% compared to 5.6%, P = 0.04). Total hospital length of stay (LOS) and intensive care unit (ICU) LOS were longer in the group with MICs of 2 to 8 mg/liter than in the group with MICs of≤1 mg/liter (57.6 days compared to 34.4days [P = 0.06] and 56.6 days compared to 21.7 days [P<0.01], respectively). Patients infected with Enterobacteriaceae with a carbapenem MIC of 2,4, or 8 mg/liter had higher mortality rates and longer ICU LOS than matched cohorts with carbapenem MICs of≤1 mg/liter, which supports CLSI's guidelines to lower susceptibility breakpoints for carbapenem.

Heil et.al (2016) [25] conducted a study on Impact of CLSI Breakpoint Changes on Microbiology Laboratories and Antimicrobial Stewardship Programs. In 2010, the Clinical and Laboratory Standards Institute (CLSI) lowered the MIC breakpoints for many β –lactam antibiotics to enhance detection of known resistance among *Enterobacteriaceae*. The decision to implement these new breakpoints, including the changes announced in both 2010 and 2014, can have a significant impact on both microbiology laboratories and antimicrobial stewardship programs. In this literature, they discussed the changes and how implementation of these updated CLSI breakpoints required partnership between antimicrobial stewardship programs and the

microbiology laboratory, including data on the basis of changes of antibiotic usage at own institution.

Vostrov SN et.al (2000) [28] was conducted study on Comparative pharmacodynamic of gatifloxacin and ciprofloxacin in an in vitro dynamic model; prediction of efficient doses and the breakpoints of the area under the curve/MIC ratio. The study demonstrated the impact of the pharmacokinetics of gatifloxacin (GA) relative to those of ciprofloxacin (CI) on the antimicrobial effect (AME), the killing and regrowth kinetics of two differentially susceptible clinical isolates each of *Staphylococcusaureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The method of generalization of data obtained with specific organisms to other representatives of the same species described in the report might be useful for prediction of AME values of new quinolones.

A study was regulated by ***Bhat et.al (2007)*** [19] on Failure of current Cefepime breakpoints to predict clinical outcomes bacteremia caused by Gram-negative organisms. In case of commonly encountered Gram-negative bacilli, a MIC of cefepime of 8µg/ml or less was defined by the CLSI as 'Susceptible' prior the commercial release of antibiotic. The cefepime MIC breakpoint derived by the classification and regression tree (CART) software analysis to describe the risk of 28-day mortality was 8µg/ml. Patients who are infected with gram-negative organisms treated with cefepime at a MIC of >8 µg/ml had a mortality rate of 54.8% (17/31 died), compared to 24.1% (35/145 died) for those treated with a cefepime MIC of <8 µg/ml. The rate of mortality for those treated with a cefepime MIC of 8 _g/ml was 56.3% (9/16 died), compared to 53.3% (8/15 died) for those treated with cefepime at a MIC of >8 µg/ml. There was

no remarkable difference between outcomes of patients according to the dosage regimen utilized. According to previous pharmacodynamic assessments, cefepime treatment (particularly a dosage of 1g every 12 h) has a low probability of target attainment associated with successful in vivo outcome when the cefepime MIC is ≥8 μg/ml. The authors recommended that based on pharmacodynamic and clinical grounds, breakpoints for cefepime can be lowered in countries where the cefepime dosage of 1 to 2 g every 12 h is the licensed therapy for serious infections, so that organisms with a cefepime MIC of 8 μg/ml are no longer regarded as susceptible to the antibiotic.

A study was conducted on Susceptibility breakpoints and target values for therapeutic drug monitoring of voriconazole and *Aspergillus fumigatus* in an in vitro pharmacokinetic/pharmacodynamic model by ***Siopi et.al (2014)*** [23] to optimize voriconazole therapy against *Aspergillus fumigatus*. Although the voriconazole discovered as antimicrobial against *Aspergillus fumigatus* ten years ago and become the standard care in the treatment of intensive aspergillosis, reliable clinical breakpoints are still in high demand due to recent emergence of azole resistance. The susceptible/intermediate/resistant breakpoints were determined to be 0.25/0.5–1/2 mg/L for CLSI, 0.5/1–2/4 mg/L for EUCAST and 0.25/0.375–1/1.5 mg/L for MIC test strip (MTS). The results of the in vitro PK/PD model were comparable to the in vivo outcome of voriconazole therapy in a nonneutropenic model of experimental aspergillosis using the same A. fumigatus strains and the same as the in vivo outcome of voriconazole therapy in neutropenic models of disseminated candidiasis thus providing an in vivo correlation of the present in vitro model.

Shuklaet.al (2016), [21] conducted a study on influence of minimum inhibitory concentration in clinical outcomes of *Enterococcus faecium* bacteremia treated with daptomycin, which showed the need of modification in the daptomycin breakpoint for enterococci should be considered. Daptomycin played a major role in multidrug-resistant *Enterococcus faecium* bloodstream infections. It was observed that patients with *E. faecium*, treated with daptomycin belonging to MIC values 3-4 µg/ml are more likely to have worse clinical outcomes than those exhibiting that exhibiting daptomycin MICs ≤2 µg/ml. A total of 62 patients were included in this study. 31 patients were infected with isolates that presented daptomycin MICs of 3-4 µg/ml. Overall, 34 patients had microbiologic failure and 25 died during hospitalization. This observation clearly showed that a modification regarding breakpoint changes should be considered.

A study was conducted on Empirical third-generation cephalosporin therapy for adults with community-onset Enterobacteriaceae bacteremia; Impact of revised CLSI breakpoints by ***Hsieh et.al (2016)*** [27] to focus on patients with community-onset Enterobacteriaceae bacteremia receiving empirical therapy. The present study provided clinically critical evidence to validate the proposed reduction in the susceptibility breakpoint of CTX to MIC≤1mg/L. Third-generation cephalosporins (3GCs) [ceftriaxone (CRO) and cefotaxime (CTX)] have remarkable potency against Enterobacteriaceae and are commonly prescribed for the treatment of community-onset bacteremia. However, clinical evidence supporting the updated interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) was limited. Adults with community-onset monomicrobial Enterobacteriaceae bacteremia treated empirically with CRO or CTX were recruited. Clinical information was collected from medical records and CTX MICs

were determined using the broth micro dilution method. The study showed that isolates with a CTX MIC≤8mg/L (indicated as susceptible by previous CLSI breakpoints) were not associated with mortality. Furthermore, clinical failure and 28-day mortality rates had a tendency to increase with increasing CTX MIC (γ=1.00; P=0.01).

The above articles represent the necessity of Efficacy Ratio in regular practice of treatment through antimicrobial agent against Gram-negative bacteria. It may be established that the higher the Efficacy ratio greater the patient's outcome.

AIMS AND OBJECTIVES

The purpose of the study is to assess effect of breakpoint and MIC ratio on outcome in patients admitted in ICU.

STUDY RATIONALE

Our current state of knowledge about each of the aspects of breakpoint setting is imperfect. It is not an exact science. It requires knowledge of the wild-type distribution of MICs, assessment of the PK/PD of the antibacterial, and study of the clinical outcome of infections when the antibacterial is used.

Breakpoint-setting organizations must utilize experts in microbiology, PD, and clinical infectious diseases in order to come to a consensus regarding the most appropriate breakpoint to be utilized. If appropriately developed and revised, breakpoints have greater relevance to the prescriber than phenotypic detection of resistance mechanisms; as this information may have epidemiologic and clinical importance.

METHODOLOGY

Study Design: This was an observational study based on the data taken from the database of ICU and Microbiology laboratory of AMRI hospital. AMRI Hospitals is a tertiary care super specialty hospital in Kolkata.

Study Setting: ICU of AMRI Hospitals, Dhakuria—a tertiary care hospital. ICU of AMRI is a well equipped department with 23 beds. The Microbiology Laboratory of AMRI is furnished with latest equipments.

Study Period: 1st July, 2018– 31st March, 2019.

Study population:

Patients admitted to the medical and surgical adult ICU during the study period.

Inclusion criteria: All patients admitted in ICU who received antibiotic and was positive for gram Negative pathogen during the study period.

Exclusion criteria: The data which includes the OPD patients is omitted. The patients, who did not receive antibiotic and patients from whom no Gram negative pathogen was isolated, were not included in the study.

Study procedure:

Step 1: The initial step of the study was collection of information about culture, coming in the Microbiology department. Bio-specimens were usually sent to the Microbiology department for culture sensitivity tests from patients suspected of having infections. This study had collected data after the culture reports were prepared in the Microbiology of AMRI Hospitals.

Step 2: After identification of a gram negative culture positive report, detailed data had taken from the ICU database with the help of the patient ID. The prescribed antibiotic to the patient had been noted down. Whether the empiric antibiotic conforms to the appropriate antibiotic as per the culture report was noted. Other collected data was included data regarding all antibiotics prescribed to patient, patient's demography [age, sex, admission date of the patient in ICU, Acute & Chronic Health Evaluation (APACHE) score]. Patient outcomes such as ICU discharge status (alive or dead) and ICU length of stay was collected.

Step 3: Efficacy Ratio had been calculated using the following equations:

$$\textbf{Efficacy Ratio} = \frac{\boldsymbol{Resistant\ Breakpoint}}{\boldsymbol{MIC\ value}}$$

OR, Efficacy Ratio = $\boldsymbol{Resistant\ Breakpoint} \div \boldsymbol{MIC\ Value}$

MIC values noted from the laboratory reports. The resistant MIC breakpoints of the drug against the particular organism were collected from the CLSI guideline **M 100 2018**. All data was collected on a paper data sheet (attached in annexure-1) and uploaded on a pre-structured excel data sheet.

Step 4: Data had been analyzed using appropriate statistical tests to assess whether there any relationship between the Efficacy Ratio of the drug used and Patient's outcome, or not.

Statistical analysis Methods: Categorical variables are expressed as Number of patients and percentage of patients and compared across the groups using Pearson's Chi Square test for Independence of Attributes/ Fisher's Exact Test as appropriate. Continuous variables are expressed as Descriptive Statistics and compared across the groups using Mann-Whitney U test/ Kruskal Wallis Test as appropriate. The statistical software SPSS version 20 has been used for

the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

Ethical approval:

Ethical approval has been taken from the AMRI Ethics Committee before data collection process.

RESULT

Study Selection: During the 8 month study period (July, 2018-March, 2019), 274 episodes of Gram-negative bacterial infection in the ICU of the tertiary care AMRI Hospital, Dhakuria, were included in the study.

Table 1: DEMOGRAPHIC POPULATION

CHARACTERISTIC	VALUES
Age (years), mean ± SD	69.74 (12.87)
Gender n(%) MALE	148 (54)
APACHE IV, mean ± SD	67.88 (27.79)
ICU LOS, median (IQR)	6 (3-6)
ICU mortality, n(%)	46 (16.8)

Demographic population: Among 274 patients the mean age was 69.74 years (12.87). Most of the patients were from Male category (148, 54%). The mean APACHE IV was 67.88 (27.79). The median ICU LOS was 6 days and the ICU mortality rate was 46 episodes around 16.8%.

Table 2: GENDER DISTRIBUTION

SEX	NUMBER	PERCENTAGE (%)
Female	126	46.0
Male	148	54.0
Total	274	100.0

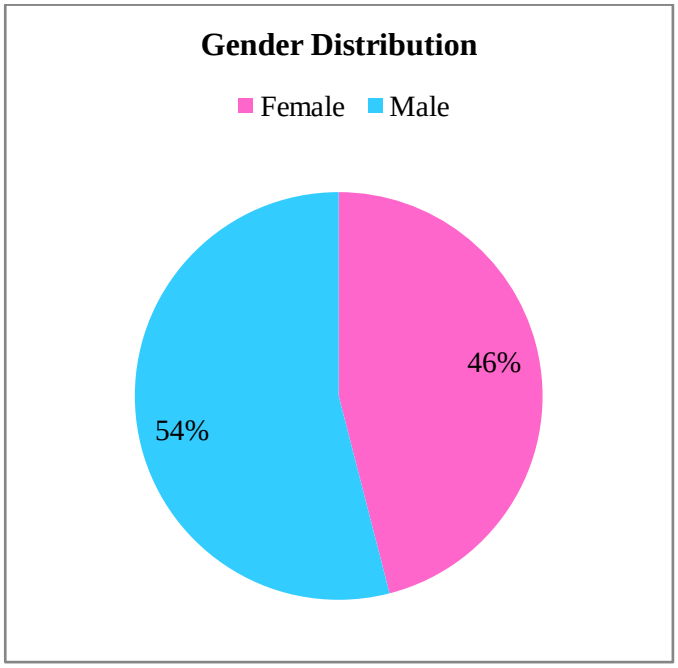

Fig 1: Gender Distribution

Gender Distribution:

Among **274** episodes **148** (54.0%) were **male** and **126** (46.0%) were **female**.

Table 3: AGE DISTRIBUTION

AGE	NUMBER	PERCENTAGE (%)
18-40	8	2.9
41-60	64	23.4
61-80	142	51.8
>80	60	21.9
Total	274	100.0

Age Distribution: Most of the cases were from the age group of **61-80** yrs which included **142** episodes around **51.8%,** followed by **41-60** yrs which included **64** episodes around **23.4%,** followed by **>80** yrs which included **60** episodes, around **21.9%,** lastly least being from age group **18-40**yrs which included **8** episodes, around **2.9%.**

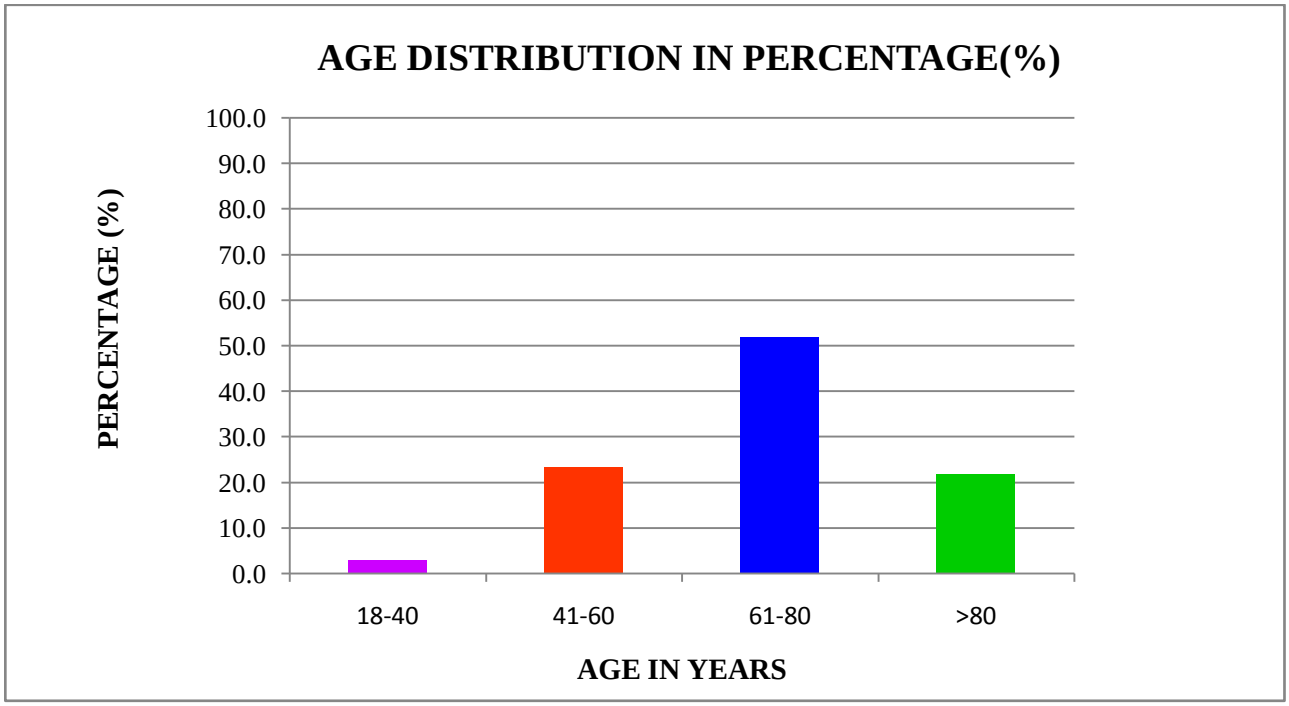

Fig 2: Age Distribution

Table 4: SAMPLE DISTRIBUTION

SAMPLE	NUMBER	PERCENTAGE (%)
Respiratory	111	40.5
Wound	15	5.5
Urine	102	37.2
Blood	27	9.9
Others	19	6.9
Total	274	100

Sample Distribution: Most of the samples were of **Respiratory origin (**E.T Suction, Throat swab, Sputum, BAL fluid, Tracheotomy suction, Nasal swab**)** which included 111 episodes, around 40.5%, followed by **Urine** which included 102 episodes, around 37.2%, followed by **Blood** which included 27 episodes, around 9.9%, and **Other samples (**Vaginal Swab, Rectal Swab, HD Line Tip, Central Line Tip, PUS from Kidney, Right Lower Limb Tissue, Catheter Site PUS, Aerobic Bacterial CS from left leg Cellulites, Bile**)** which included **19** episodes, around 6.9%, least were samples from **Wound (**Wound Swab, Abdominal Wound Swab, Bed Sore**)** which included 15 episodes, around 5.5%.

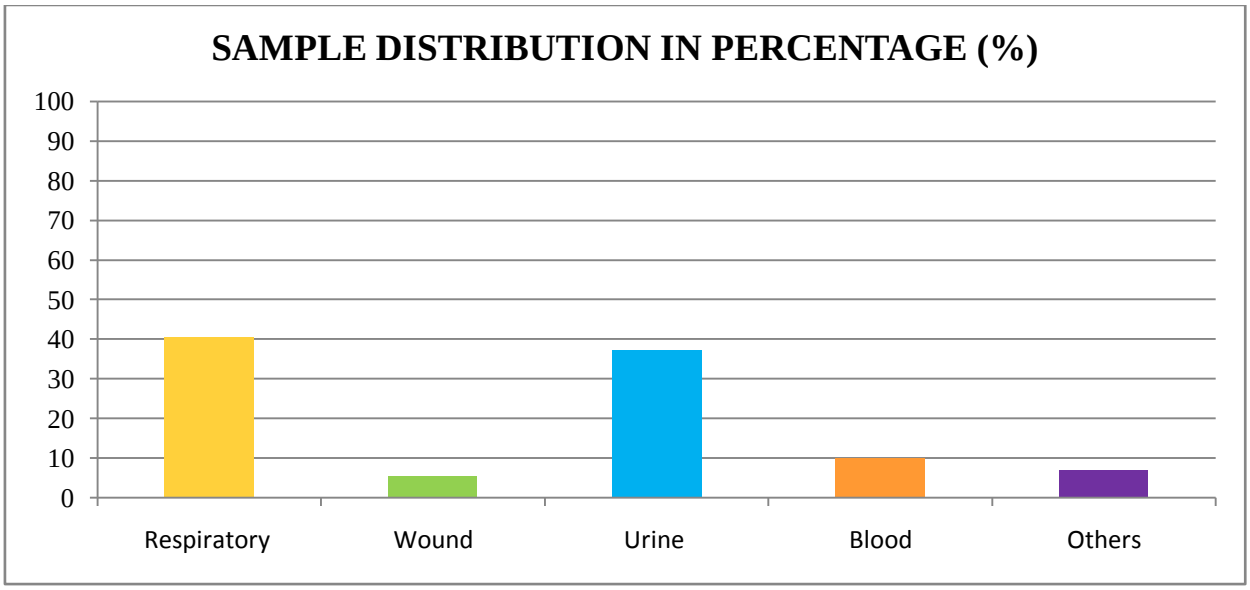

Fig 3: Sample Distribution in Percentage

Table 5: ORGANISM DISTRIBUTION

NAAME OF THE ORGANISM	NUMBER	PERCENTAGE (%)
ESCHERICHIA COLI	**65**	**23.7**
KLEBSIELLA PNEUOMONIAE	**113**	**41.2**
PSEUDOMONAS AERUGINOSA	**24**	**8.8**
PSEUDOMONAS PUTIDA	3	1.1
ENTEROBACTER CLOACAE	3	1.1
ELIZABETHKINGIA MENINGOSEPTICA	4	1.5
PSEUDOMONAS STUTZERI	1	0.4
CHRYSEOBACTERIUM INDOLOGENES	2	0.7
STENOTROPHOMONAS MALTOPHILIA	2	0.7
BUKHOLDERIA CEPACIA	4	1.5
PROTEUS MIRABILIS	4	1.5
ACINETOBACTER LWOFFI	2	0.7
ENTEROBACTER AEROGENES	2	0.7
ACHROMOBACTER XYLOSOXIDANS	2	0.7
SERRATIA MARCESCENS	2	0.7
ENTEROBACTER CLOACAE	2	0.7
SPHINGOMONAS PAUCIMOBILIS	1	0.4
PROTEUS PENNERI	1	0.4
PROVIDENCIA STUARTII	1	0.4
PROVIDENCIA RETTGORI	1	0.4
SERRATIA FONTICOLA	2	0.7
MORGANELLA MORGANNII	1	0.4
CITROBACTER KOSERI	1	0.4
ACINETOBACTER JUNII	1	0.4
ACINETOBACTER BAUMANNII	25	9.1
Total	274	100

Organism Distribution: Most common organism was found from the group of **Klebsiella** (113 episodes around 41.2%), followed by **E.coli** (65 episodes around 23.7%, followed by

Acinetobacter (Acinetobacter Lwoffi, Acinetobacter Junii, Acinetobacter Baumannii) (28 episodes around 10.2%), followed by **Pseudomonas** (Pseudomonas Aeruginosa, Pseudomonas Putida, Pseudomonas Stutzeri) (28 episodes around 10.3%) and **Enterobacter (**Enterobacter Cloacae, Enterobacter Aerogenes) (7 episodes around 2.5%), **Proteus (**Proteus Mirabilis, Proteus Penneri) (5 episodes around 1.9%), **Serratia (**Serratia Marcescens, Serratia Fonticola) (4 episodes around 1.4%), **Elizabethkingia** which included 4 episodes around 1.5%, **Providencia (**Providencia Stuartii, Providencia Rettgori) (2 episodes around 0.8%), **Chryseobacterium, Stenotrophomonas and Achromobacter** (2 episodes around 0.7% each), **Sphingomonas, Morganella and Citrobacter** which included 1 episodes around 0.4% each .

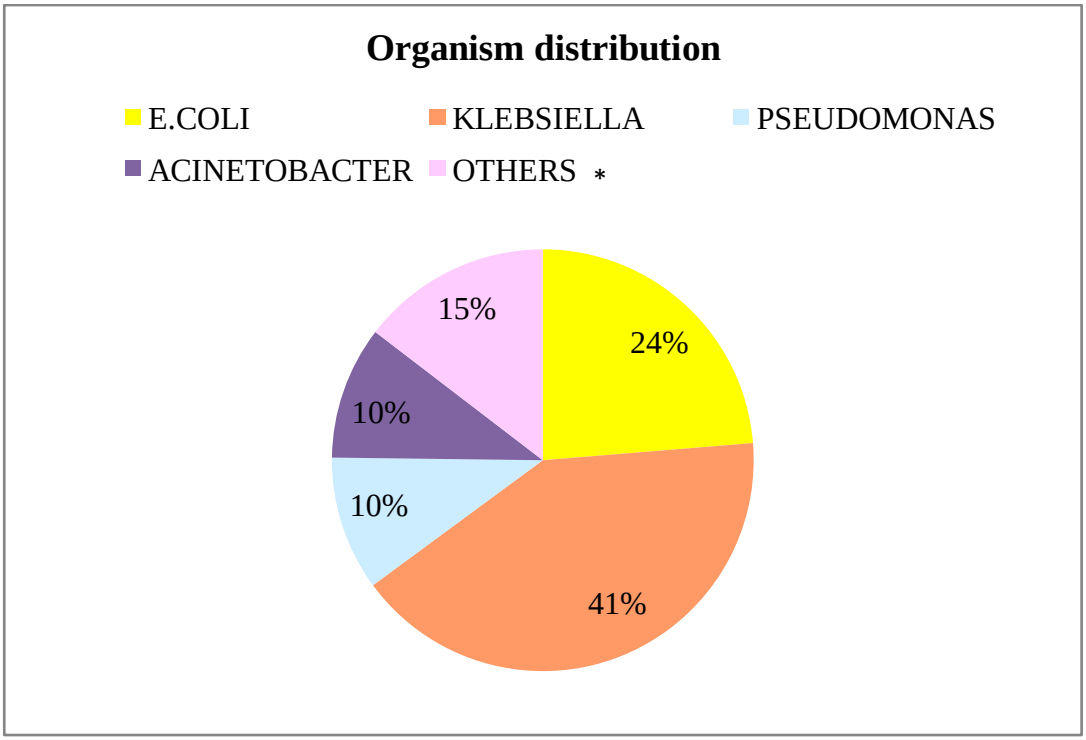

Fig 4: Organism Distribution in Percentage

***** Other group contains **Proteus, Serratia, Elizabethkingia, Providencia, Chryseobacterium, Stenotrophomonas and Achromobacter, Sphingomonas, Morganella** and **Citrobacter** species**.**

Table 6: ORGANISM DISTRIBUTION IN RESPIRATORY SAMPLE

NAAME OF THE ORGANISM	NUMBER	PERCENTAGE (%)
ESCHERICHIA COLI	**5**	**4.5**
KLEBSIELLA PNEUOMONIAE	**48**	**43.2**
PSEUDOMONAS AERUGINOSA	**15**	**13.5**
PSEUDOMONAS PUTIDA	2	1.8
ENTEROBACTER CLOACAE	3	2.7
CHRYSEOBACTERIUM INDOLOGENES	2	1.8
STENOTROPHOMONAS MALTOPHILIA	2	1.8
BUKHOLDERIA CEPACIA	2	1.8
PROTEUS MIRABILIS	2	1.8
ACINETOBACTER LWOFFI	2	1.8
ENTEROBACTER AEROGENES	2	1.8
SERRATIA MARCESCENS	1	0.9
ENTEROBACTER CLOACAE	1	0.9
PROVIDENCIA STUARTII	1	0.9
MORGANELLA MORGANNII	1	0.9
CITROBACTER KOSERI	1	0.9
ACINETOBACTER BAUMANNII	21	18.9
Total	**111**	**100**

Organism distribution in Respiratory sample: Most common organism was **Klebsiella** (**48** episodes around **43.2%)**, followed by **Acinetobacter** (**23** episodes around **20.7%**), followed by

Pseudomonas (13 episodes around 15.3%), and **Acinetobacter** (6 episodes around 5.4%), **E.coli** which included 5 episodes around 4.5%.

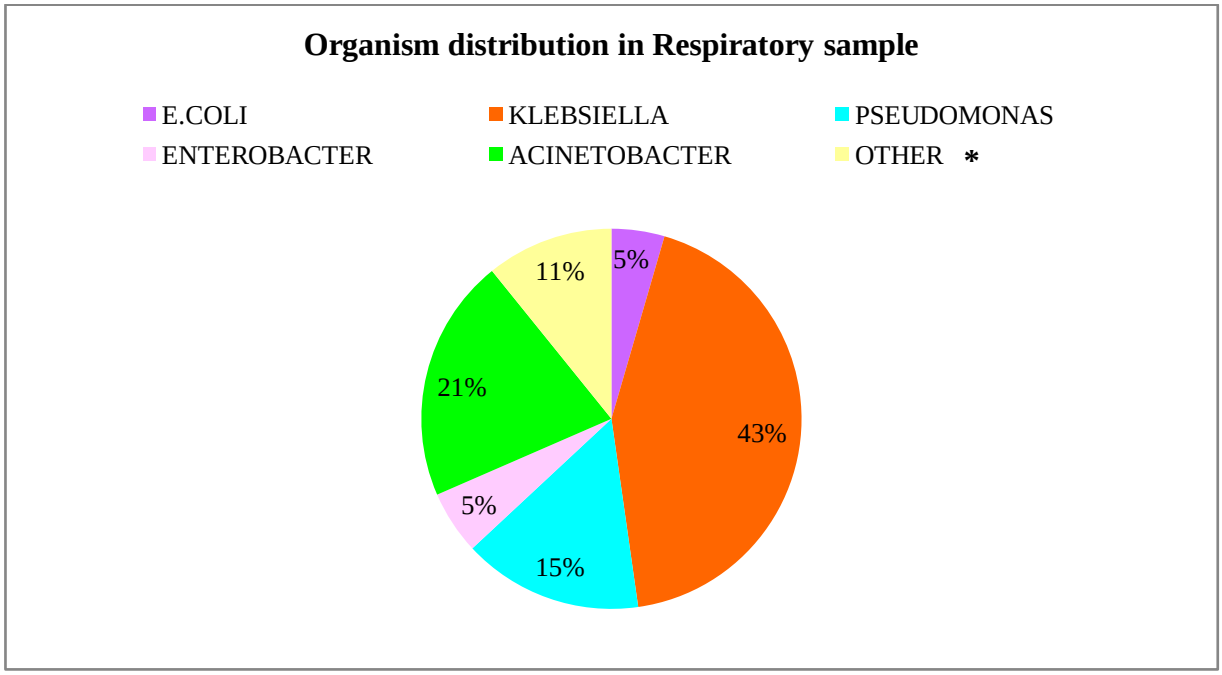

Fig 5: Organism Distribution in Respiratory sample Percentage

***** Other group contains **Chryseobacterium, Stenotrophomonas, Proteus, Serratia, Providencia, Morganella** and **Citrobacter** species.

Table 7: Organism Distribution in Wound sample

NAAME OF THE ORGANISM	NUMBER	PERCENTAGE (%)
ESCHERICHIA COLI	5	33.3
KLEBSIELLA PNEUOMONIAE	7	46.7
PROTEUS PENNERI	1	6.7
SERRATIA FONTICOLA	1	6.7
ACINETOBACTER BAUMANNII	1	6.7
Total	**15**	**100**

Organism distribution in Wound sample: Most common isolated organism from Wound sample was **Klebsiella** (7 episodes around 46.7%), followed by **E.coli** (5 episodes around 33.3%).

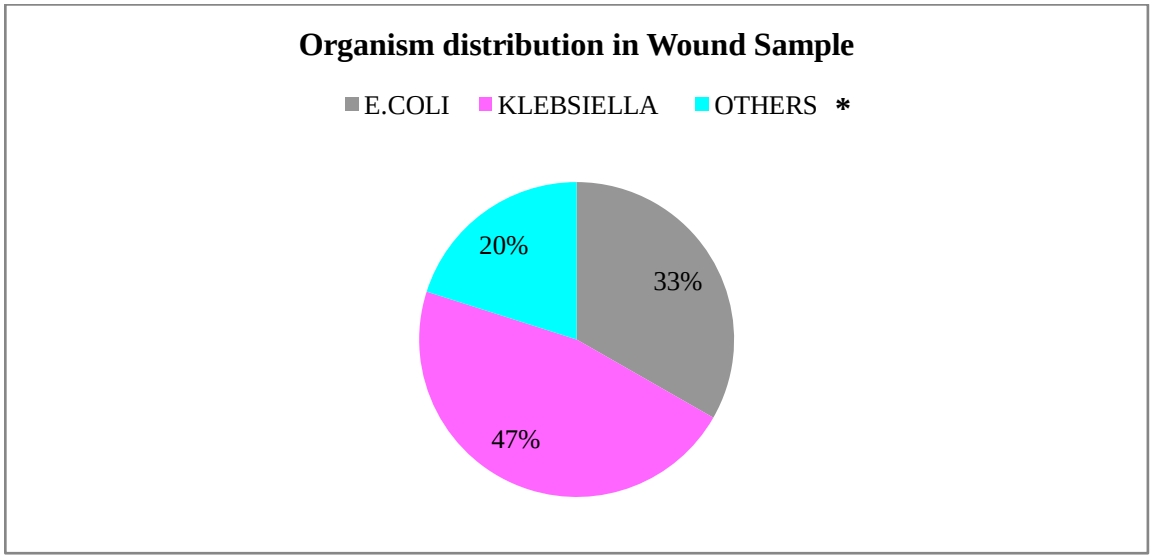

Fig 6: Organism Distribution in Wound sample Percentage

***** Other sample contains **Proteus, Acinetobacter** and **Serratia** species.

Table 8: Organism Distribution in Urine sample

NAAME OF THE ORGANISM	NUMBER	PERCENTAGE (%)
ESCHERICHIA COLI	**44**	**44.4**
KLEBSIELLA PNEUOMONIAE	**42**	**42.4**
PSEUDOMONAS AERUGINOSA	**7**	**7.2**
PSEUDOMONAS PUTIDA	1	1
PSEUDOMONAS STUTZERI	1	1
PROTEUS MIRABILIS	2	2
ACINETOBACTER JUNII	1	1
ACINETOBACTER BAUMANNII	1	1
Total	**99**	**100**

Organism distribution in Urine sample: Most common organism isolated from Urine was **E.coli** (44 episodes around 44.4%), followed by **Klebsiella** (42 episodes around 42.4%), followed by **Pseudomonas** (9 episodes around 8.9%).

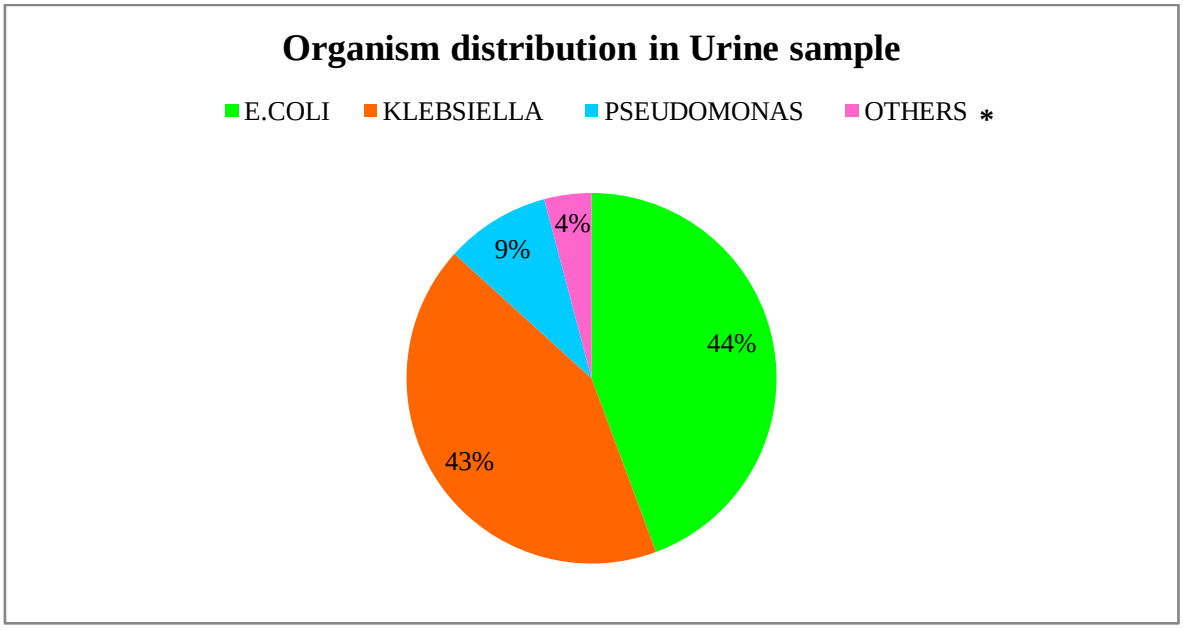

Fig 7: Organism Distribution in Urine sample

***** Other sample contains **Acinetobacter** and **Proteus** species.

Table 9: Organism in Blood sample

NAAME OF THE ORGANISM	NUMBER	PERCENTAGE (%)
ESCHERICHIA COLI	**8**	**32**
KLEBSIELLA PNEUOMONIAE	**6**	**24**
PSEUDOMONAS AERUGINOSA	1	4
ELIZABETHKINGIA MENINGOSEPTICA	2	8
BUKHOLDERIA CEPACIA	2	8
ACHROMOBACTER XYLOSOXIDANS	2	8
SPHINGOMONAS PAUCIMOBILIS	1	4
PROVIDENCIA RETTGORI	1	4
SERRATIA FONTICOLA	1	4
ACINETOBACTER BAUMANNII	1	4
Total	**25**	**100**

Organism distribution in Blood sample: Most common isolated organism from Blood was **E.coli** (8 episodes around 32%), followed by **Klebsiella** (6 episodes around 24%).

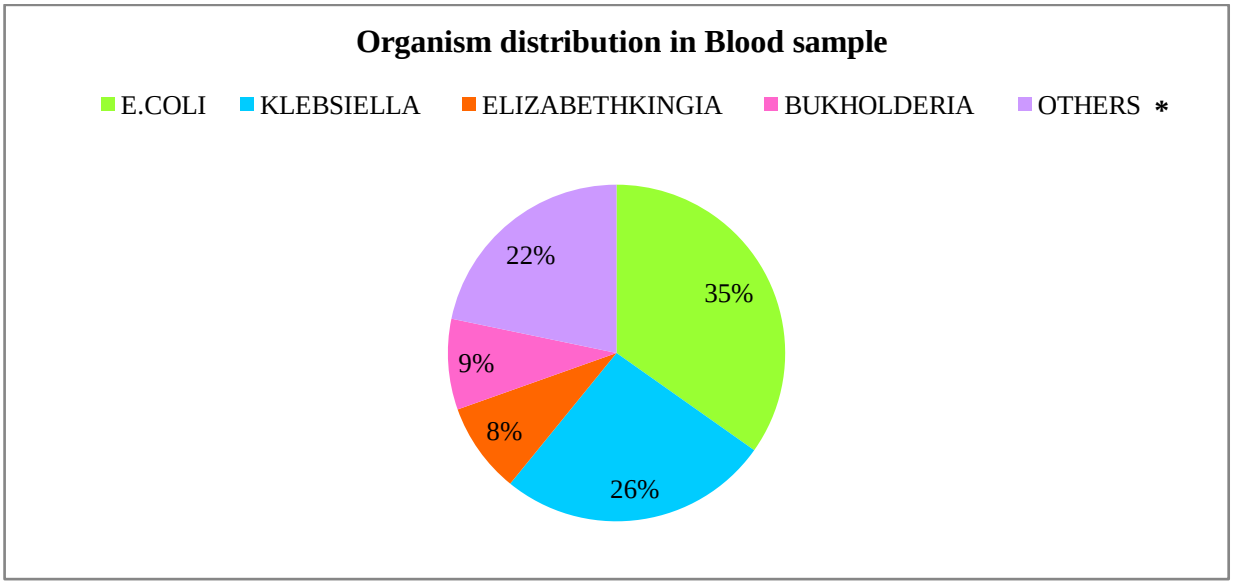

Fig 8: Organism Distribution in Blood sample Percentage

* Other sample contains **Pseudomonas, Sphingomonas, Providencia, Serratia** and **Acinetobacter** species**.**

Table 10: Organism distribution in Other sample

NAAME OF THE ORGANISM	NUMBER	PERCENTAGE (%)
ESCHERICHIA COLI	**3**	**15.8**
KLEBSIELLA PNEUOMONIAE	**10**	**52.6**
PSEUDOMONAS AERUGINOSA	1	5.3
ELIZABETHKINGIA MENINGOSEPTICA	2	10.5
SERRATIA MARCESCENS	1	5.3
ENTEROBACTER CLOACAE	1	5.3
ACINETOBACTER BAUMANNII	1	5.3
Total	**19**	**100**

Organism distribution in Other sample: Most common isolated organism from samples other than blood, urine, respiratory and wound was **Klebsiella** (10 episodes around 52.6%), followed by **E.coli** (3 episodes around 15.8%).

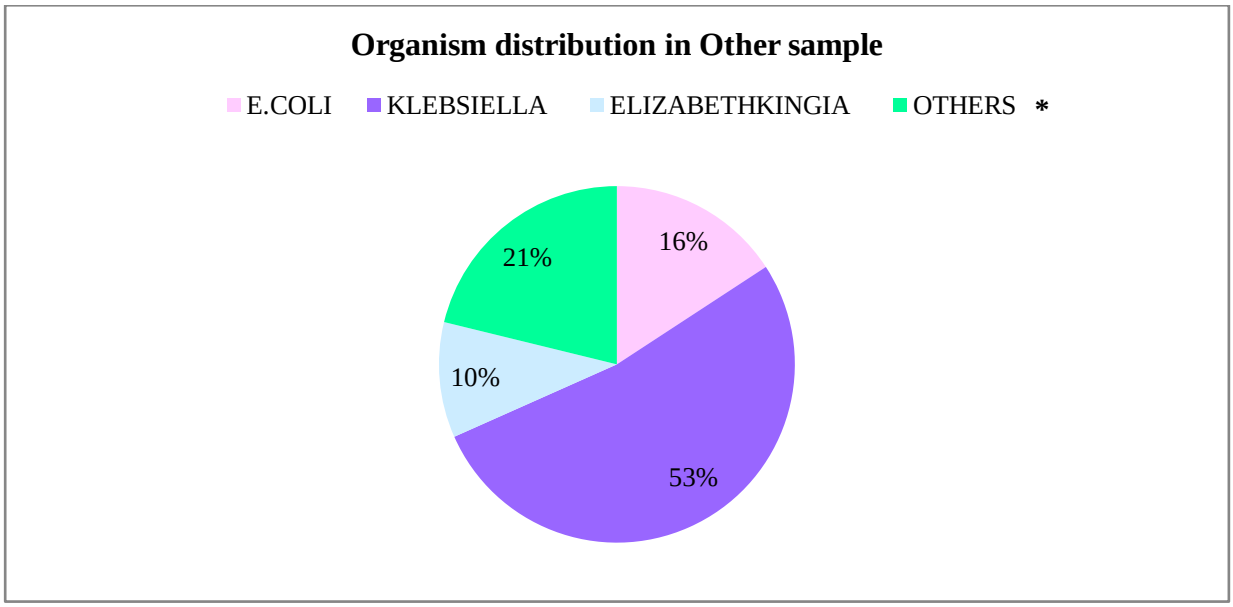

Fig 9: Organism Distribution in Other sample Percentage

* Other sample contains **Serratia**, **Enterobacter** and **Acinetobacter**.

Table 11: ANTIBIOTIC DISTRIBUTION

NAME OF THE DRUG	NUMBER	PERCENTAGE (%)
MEROPENEM	**123**	**44.9**
PIPERACILLIN / TAZOBACTUM	**29**	**10.6**
COLISTIN	**35**	**12.8**
TRIMETHOPRIM / SULFAMETHOXAZOLE	6	2.2
MINOCYCLIN	8	2.9
TIGECYCLINE	**30**	**10.9**
CEFTAZIDIME	7	2.6
AMOXICILLIN / CLAVULANIC ACID	2	0.7
CEFOPERAZONE / SULBACTUM	1	0.4
CEFEPIME	1	0.4
AMIKACIN	1	0.4
FOSFOMYCIN	**25**	**9.1**
CEFTRIAXONE	3	1.1
DORIPENEM	2	0.7
AZTREONAM	3	1.1
Total	**274**	**100**

Antibiotic Distribution:

Most commonly used antibiotic was **Meropenem** which was used in **123** episodes, around **44.9%**, followed by **Colistin** which was used in **35** episodes, around **12.8%**, followed by **Tigecylin** which was used in **30** episodes, around **10.9**%, followed by **Piperacillin/Tazobactum** in **29 episodes,** around **10.6%**, followed by **Fosfomycin** which was used in **25,** around **9.1%**,

followed by **Minocyclin** in **8** episodes around **2.9%**, **Ceftazidime** in **7** episodes around **2.6%**, **Trimethoprim/Sulfamethoxazole** in **6** episodes around **2.2%**, both **Ceftriaxone** and **Aztreonam** in **3** episodes around **1.1% each**, both **Amoxicillin/Clavulanic acid** and **Doripenem** which included **2** episodes **0.7%**, lastly each **Cefoperazone/Salbactum**, **Cefepime** and **Amikacin** was used in **1** episode **0.4%**.

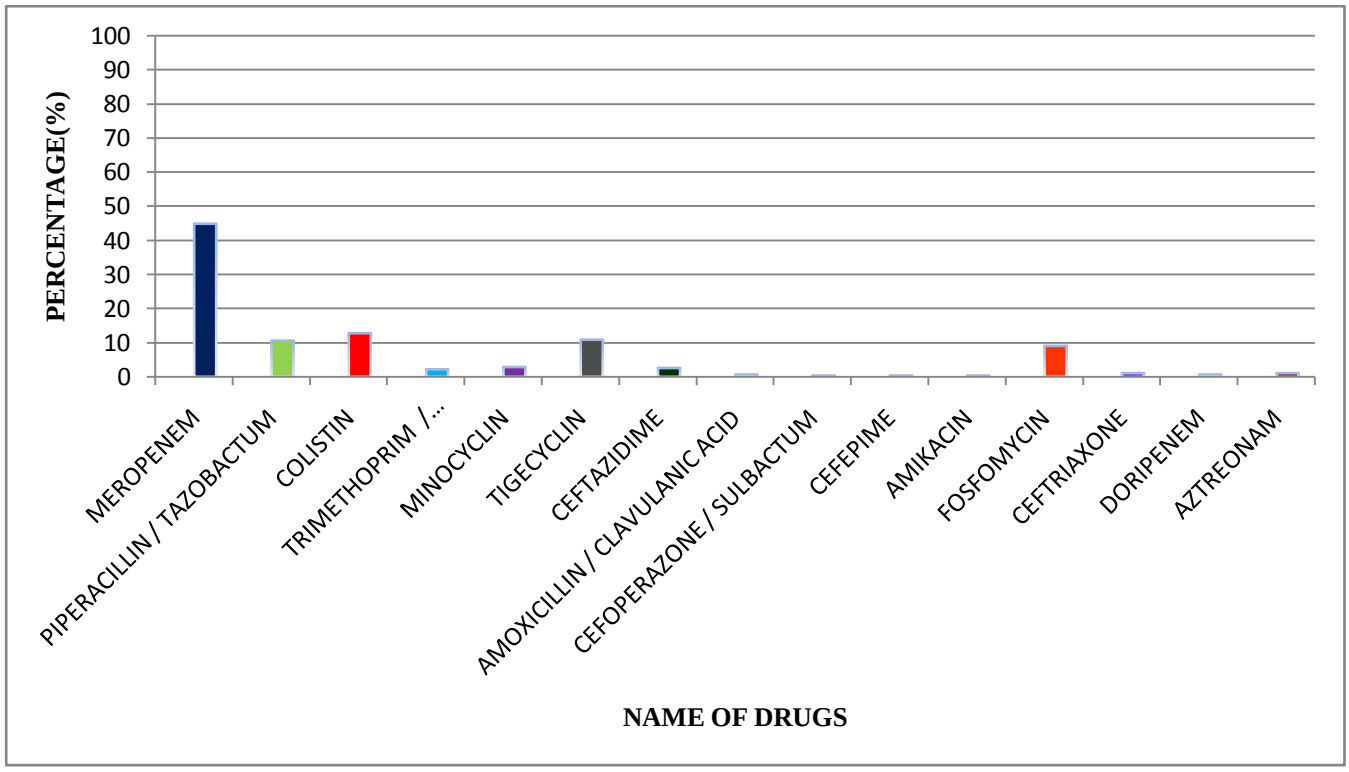

Fig 10: Graph representing the Drug-used Distribution in Percentage

Therapy Distribution: Patients received either Mono-therapy or Combination therapy.

Mono Therapy: When a single antibiotic is used to treat any disease or infection then the incident has been described as Mono-therapy.

Combination Therapy: When more than one antibiotic was used to treat a patient, the episode has been described as Combination therapy.

Table 12: MONO-THERAPY VS COMBINATION THERAPY

THERAPY	NUMBER	PERCENTAGE (%)
Mono	169	61.7
Combination	105	38.3
Total	274	100.0

Therapy Distribution: Most of the patients were having Mono-therapy which included **169** cases around **61.7%** and the rest of the patients were having Combination therapy which included **105** episodes around **38.3%**. So, in our study significantly more patients received Mono-therapy than combination therapy (**P value 0.017**).

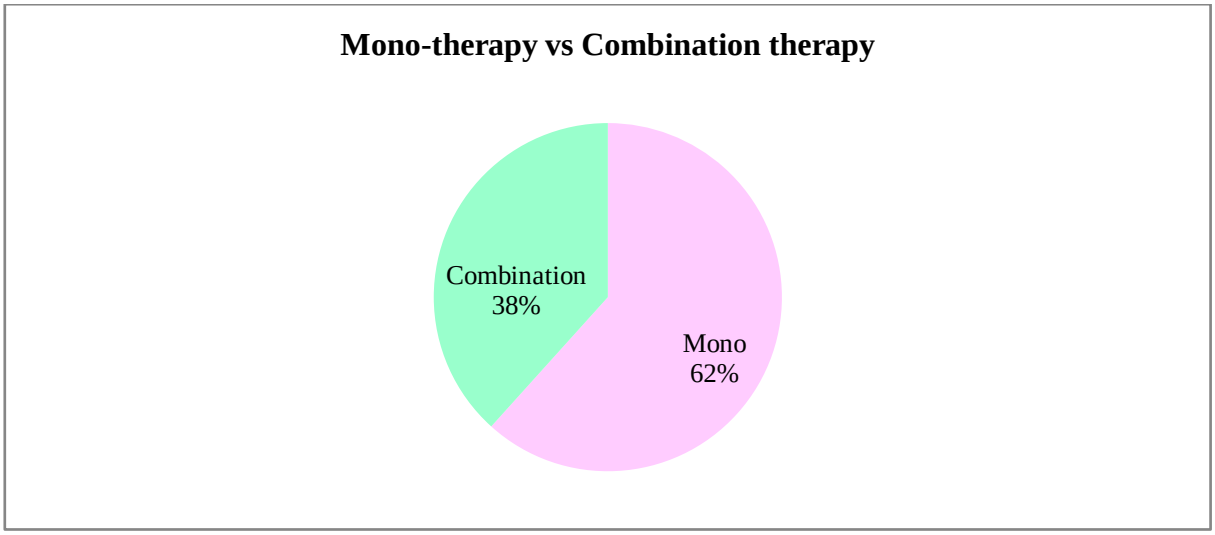

Fig 11: Mono-therapy vs Combination therapy

Table 13: ANTIBIOTIC USED AS MONO-THERAPY

DRUG USED	NUMBER	PERCENTAGE (%)
MEROPENEM	**84**	**49.7**
PIPERACILLIN / TAZOBATUM	**20**	**11.8**
COLISTIN	**25**	**14.8**
TRIMETHOPRIM / SULFAMETHOXAZOLE	1	0.5
MINOCYCLIN	1	0.5
TIGECYCLINE	**17**	**10**
CEFTAZIDIME	5	2.9
AMOXICILLIN / CLAVULANIC ACID	2	1.1
AMICACIN	1	0.5
FOSFOMYCIN	**8**	**4.7**
CEFTRIAXONE	2	1.1
DORIPENEM	1	0.5
AZTREONAM	2	1.1
TOTAL	169	100

Antibiotic used as Mono-therapy: Most common antibiotic used in Mono-therapy was Meropenem (84 episodes around 49.7%), followed by Colistin (25 episodes around 14.8%), followed by Piperacillin/Tazobactum (20 episodes around 11.8%, followed by Tigecycline (17 episodes around 10%), Fosfomycin (8 episodes around 4.7%), Ceftazidime (5 episodes around

2.9%), Amoxicillin/Clavulanic acid, Ceftriaxone and Aztreonam (2 episodes around 1.1%) and lastly Trimethoprim/Sulfamethoxazole, Minocyclin, Amikacin and Doripenem (1 episode around 0.5% for each).

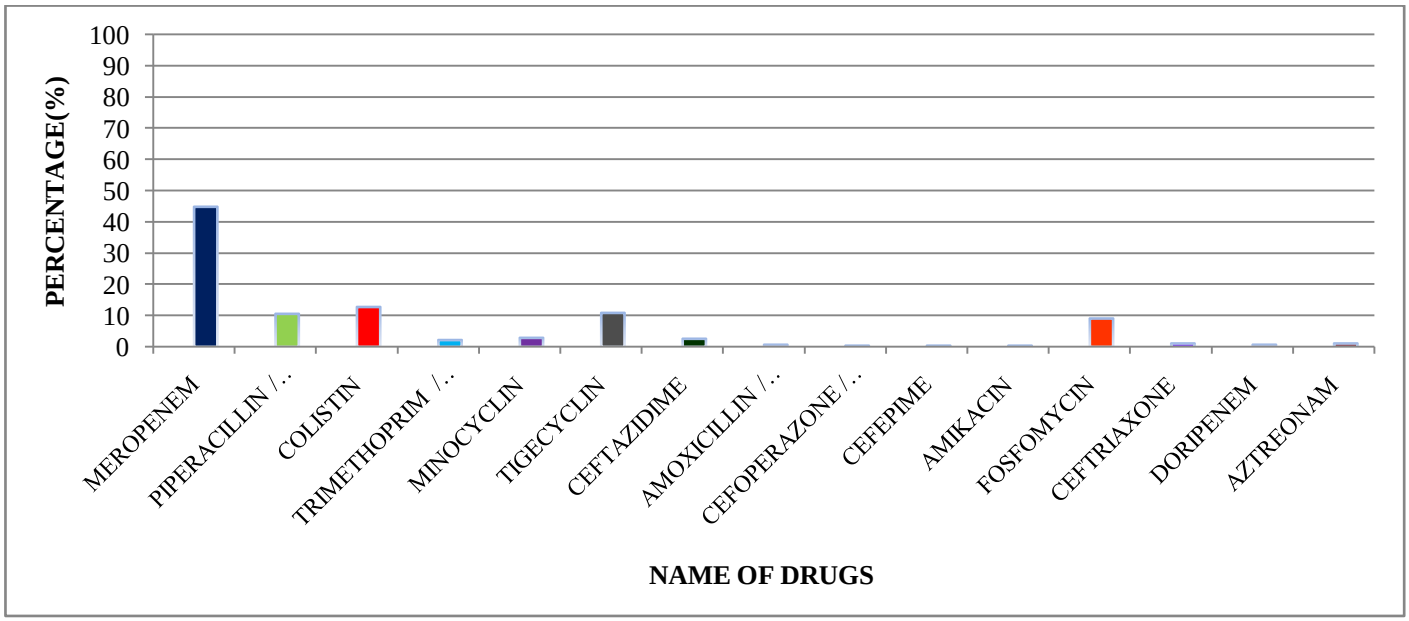

Fig 12: Drug distribution in Mono-therapy

Table 14: ANTIBIOTIC USED IN COMBINATION THERAPY

DRUG USED	NUMBER	PERCENTAGE (%)
MEROPENEM	**39**	**37.4**
PIPERACILLIN / TAZOBATUM	**9**	**8.5**
COLISTIN	**10**	**9.52**
TRIMETHOPRIM / SULFAMETHOXAZOLE	5	4.7
MINOCYCLIN	6	5.7
TIGECYCLINE	**13**	**12.6**
CEFTAZIDIME	1	0.9
CEFPERAZONE / SALBACTUM	1	0.9
CEFEPIME	1	0.9
AMICACIN	0	0
FOSFOMYCIN	**17**	**16.1**
CEFTRIAXONE	1	0.9
DORIPENEM	1	0.9
AZTREONAM	1	0.9
TOTAL	**105**	**100**

Antibiotic used in Combination therapy: Most used antibiotic in Combination therapy was **Meropenem** (39 episodes around 37.4%), followed by **Fosfomycin** (17 episodes around 16.1%),

followed by **Tigecycline** (13 episodes around 12.6%), followed by **Colistin** (10 episodes around 9.5%), **Minocyclin** (6 episodes around 5.7%), **Trimethoprim/ Sulfamethoxazole** (5 episodes around 4.7%) and lastly **Ceftazidime, Cefoperazone/ Salbactum, Ceftriaxone, Doripenem** and **Aztreonam** (1 episode around 0.9% for each).

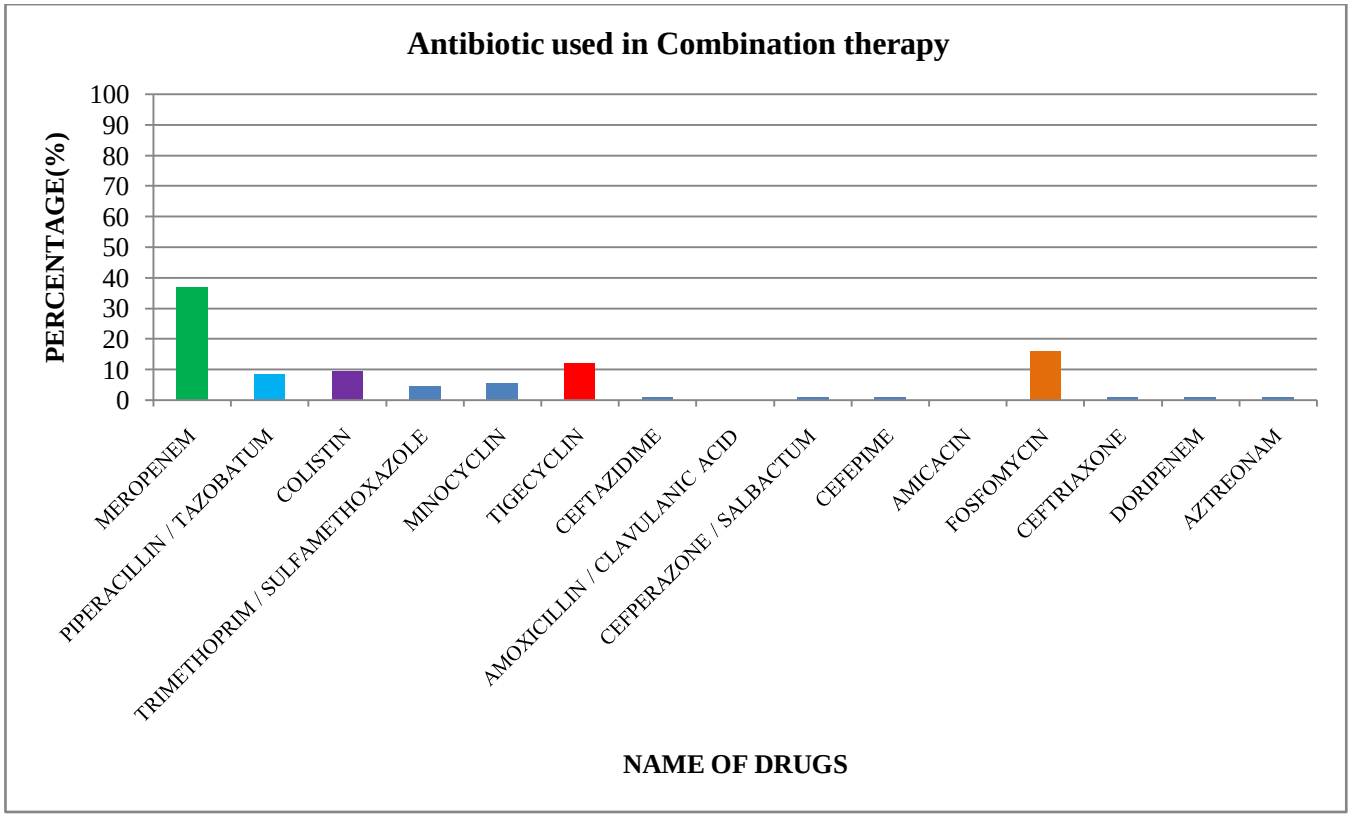

Fig 13: Antibiotic used in Combination therapy in percentage

Table 15: EFFICACY RATIO IN MONO-THERAPY

EFFICACY RATIO	NUMBER	PERCENTAGE (%)
<1	25	14.8
1-2	19	11.2
2-4	8	4.7
>4	117	69.2
Total	169	100

Efficacy ratio distribution in Mono-therapy: Most of the patients were receiving Mono-therapy had efficacy ratio of **>4** for the antibiotic used (117 episodes around 69.2%), followed by **<1** Efficacy ratio (25 cases around 14.8%), followed by **1-2** Efficacy ratio (19 episodes around 11.2%) and lastly from **2-4** Efficacy ratio (8 episodes around 4.7%).

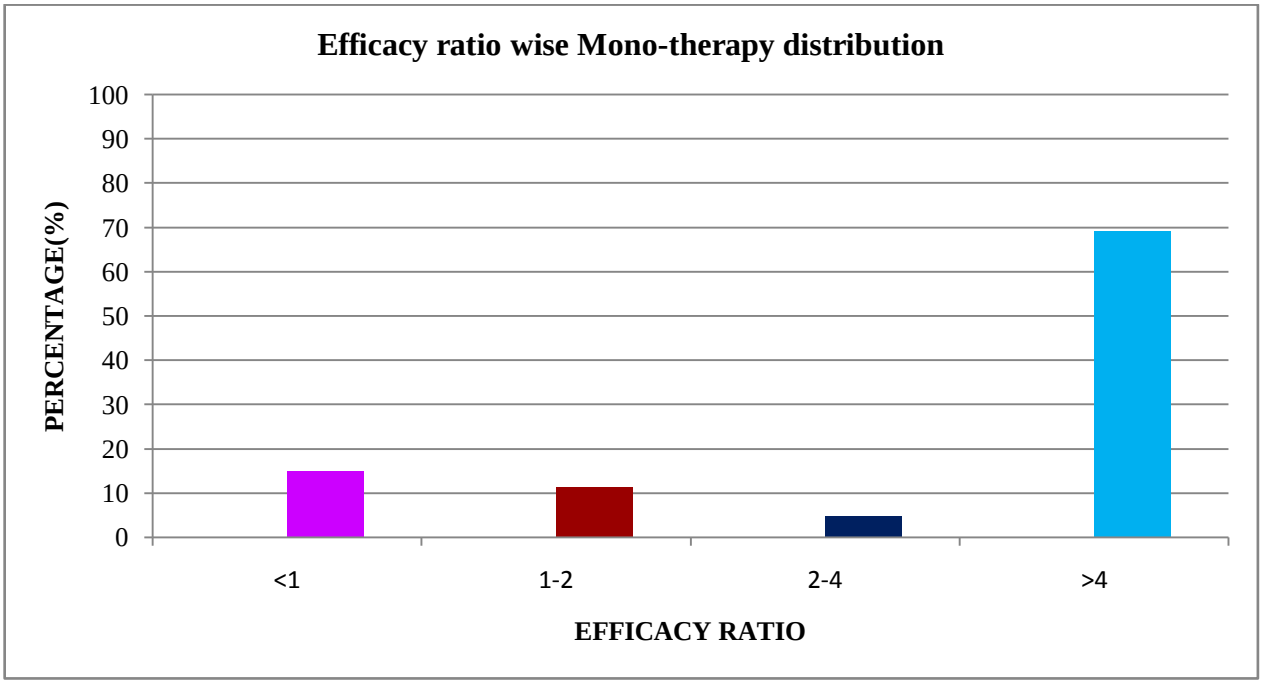

Fig 14: Efficacy ratio in Mono-therapy

Table 16: EFFICACY RATIO IN COMBINATION THERAPY

EFFICACY RATIO	NUMBER	PERCENTAGE (%)
<1	30	28.6
1-2	18	17.1
2-4	5	4.8
>4	52	49.5
Total	105	100

Efficacy ratio in Combination therapy: 49.5% (52 episodes) of patients, who received combination therapy, had an isolate which has efficacy ratio of > **4** to at least one of the antibiotics used. Around 28.6% (30 episodes) patients had an isolate having efficacy ratio of < **1** to all antibiotics used. 17.1% (18 episodes) and 4.8% (5 episodes) patients have their organism with Efficacy ratio of **1-2** and **2-4** to at least one antibiotic used respectively.

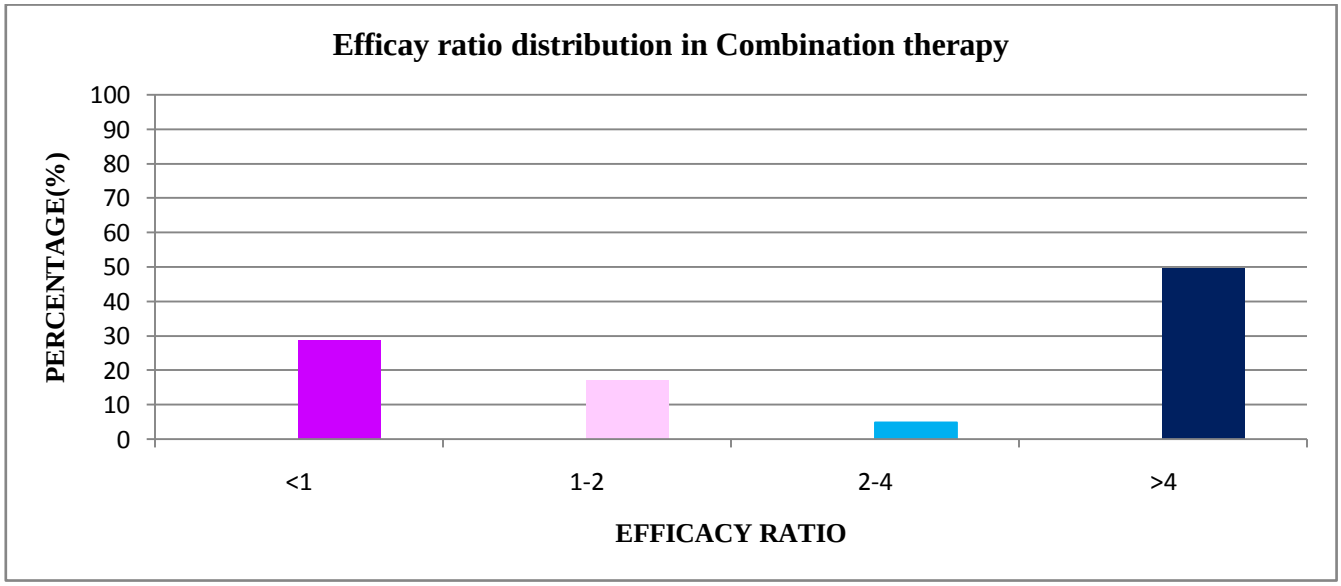

Fig 15: Efficacy ratio in Combination-therapy in percentage

OUTCOME: The outcome is considering with respect to ICU Mortality and ICU Length of Stay (LOS).

Table 17: ICU MORTALITY

ICU MORTALITY	NUMBER	PERCENTAGE (%)
EXPIRED	46	16.8
ALIVE	228	83.2
TOTAL	274	100

Outcome distribution with respect to ICU Mortality: Out off 274 patients in 83.2% cases patients were alive and 16.8% patients had expired.

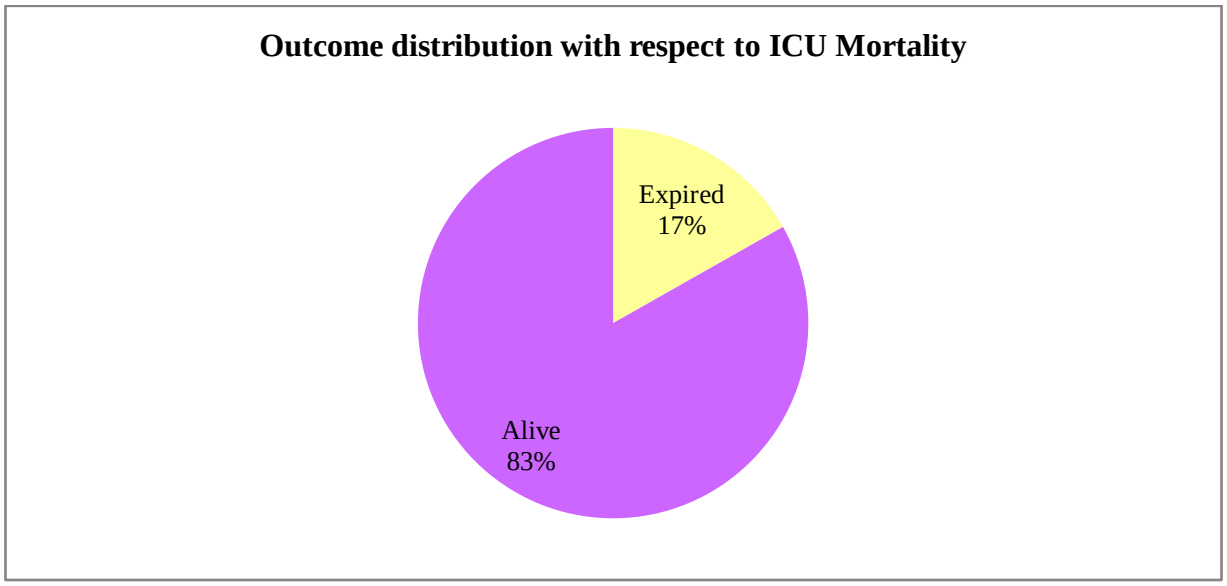

Fig 16: Outcome distribution with respect to ICU

Table 18: ICU LENGTH OF STAY (LOS)

ICU LENGTH OF STAY (LOS)	Frequency	Percentage (%)
1	5	1.8
2	44	16.1
3	40	14.6
4	18	6.6
5	29	10.6
6	16	5.8
7	17	6.2
8	10	3.6
9	8	2.9
10	4	1.5
11	3	1.1
12	9	3.3
13	10	3.6
14	1	0.4
15	1	0.4
16	8	2.9
17	9	3.3
18	8	2.9
19	13	4.7
21	2	0.7
22	9	3.3
23	1	0.4
29	3	1.1
31	4	1.5
47	1	0.4
50	1	0.4
Total	274	100.0

Outcome distribution with respect to ICU LOS: Most of the patients were having 2 days of LOS (44 episodes around 16.1%), followed by 3 days of LOS (40 episodes 14.6%), followed by 5 days of LOS (29 episodes around 10.6%). Mean ICU LOS was 6 days.

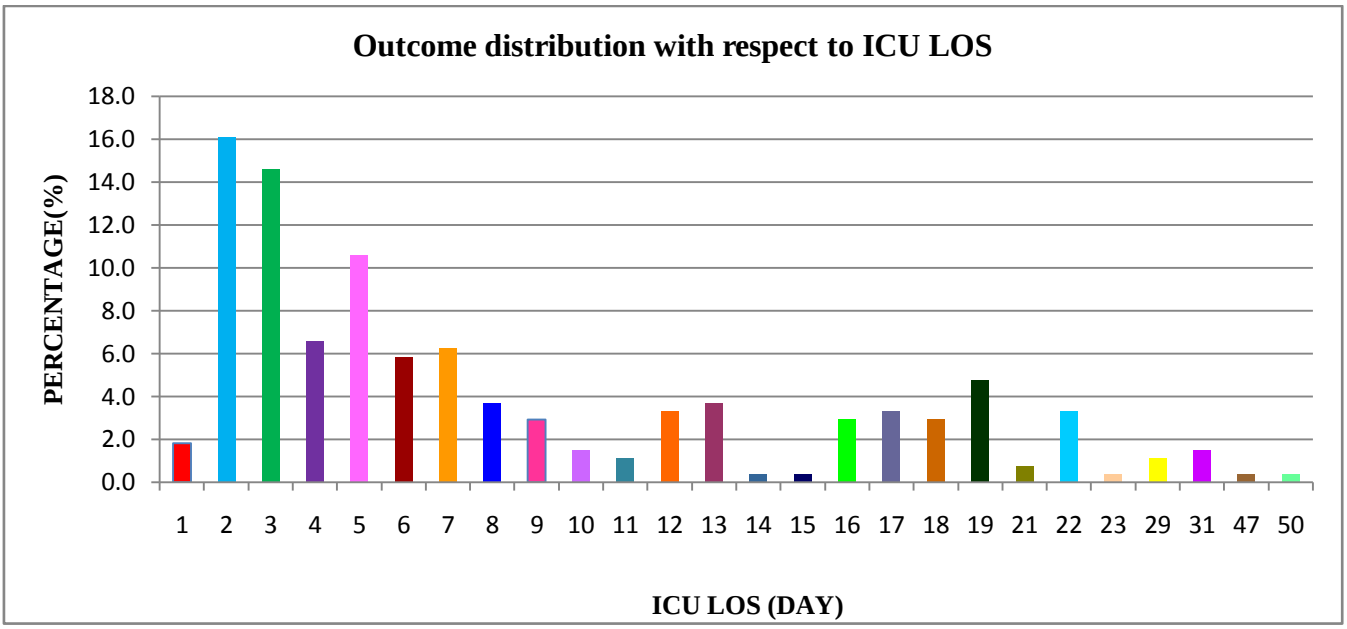

Fig 17: Outcome distribution with respect to ICU LOS

Table 19: ICU MORTALITY IN DIFFERENT EFFICACY RATIOS IN MONO-THERAPY CASES

THERAPY				EFFICACY RATIO			
				<1	1-2	2-4	>4
MONO THERAPY	ICU MORTALITY	EXPIRED	Count	5	3	4	13
			% within EFFICACY RATIO	20.0%	15.8%	50.0%	14.2%
		ALIVE	Count	21	16	4	104
			% within EFFICACY RATIO	81.0%	79.9%	50.0%	88.9%
	Total		Count	25	19	8	117
			% within EFFICACY RATIO	100.0%	100.0%	100.0%	100.0%

ICU mortality in different efficacy ratios in Mono-therapy cases: The mortality rate is lowest (around 14.2%) in >4 efficacy group and the rate has increased in <1 efficacy group (5, 20%,p value 0.145 in respect to > 4 group) and 1-2 efficacy group (3, 15.2%, p value 0.191 in respect to > 4 group) and highest 2-4 efficacy group (4, 50%, p value 0.008 in respect to > 4 group).

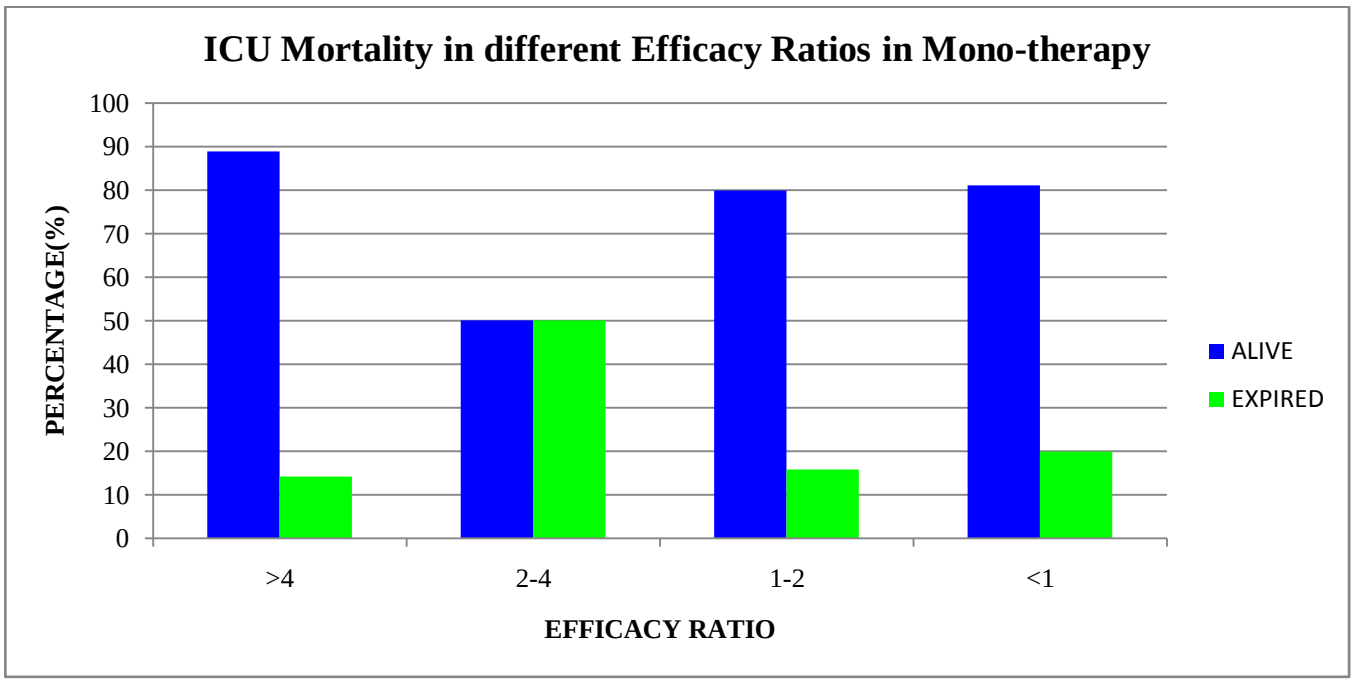

Fig 18: Graph representing ICU mortality in different efficacy ratio in Mono-therapy

Table 20: ICU MORTALITY IN DIFFERENT EFFICACY RATIOS IN COMBINATION THERAPY CASES

THERAPY				EFFICACY RATIO			
				<1	1-2	2-4	>4
COMBINATION THERAPY	ICU MORTALITY	YES	Count	5	6	0	11
			% within EFFICACY RATIO	16.70%	33.30%	0.00%	21.20%
		NO	Count	25	12	5	41
			% within EFFICACY RATIO	83.30%	66.70%	100.00%	78.80%
	Total		Count	30	18	5	52
			% within EFFICACY RATIO	100.00%	100.00%	100.00%	100.00%

ICU mortality in different efficacy ratios in Combination therapy cases: In combination therapy group the mortality rate is lowest in <1 efficacy group (5, 16.70%, p value 0.206 in respect to > 4 group). The mortality rate has increased in >4 efficacy group (where at least one antibiotic has the efficacy ratio 4 or >4) around 21.2%, followed by 1-2 efficacy group (6, 33.3%, p value 0.107 in respect to > 4 group).

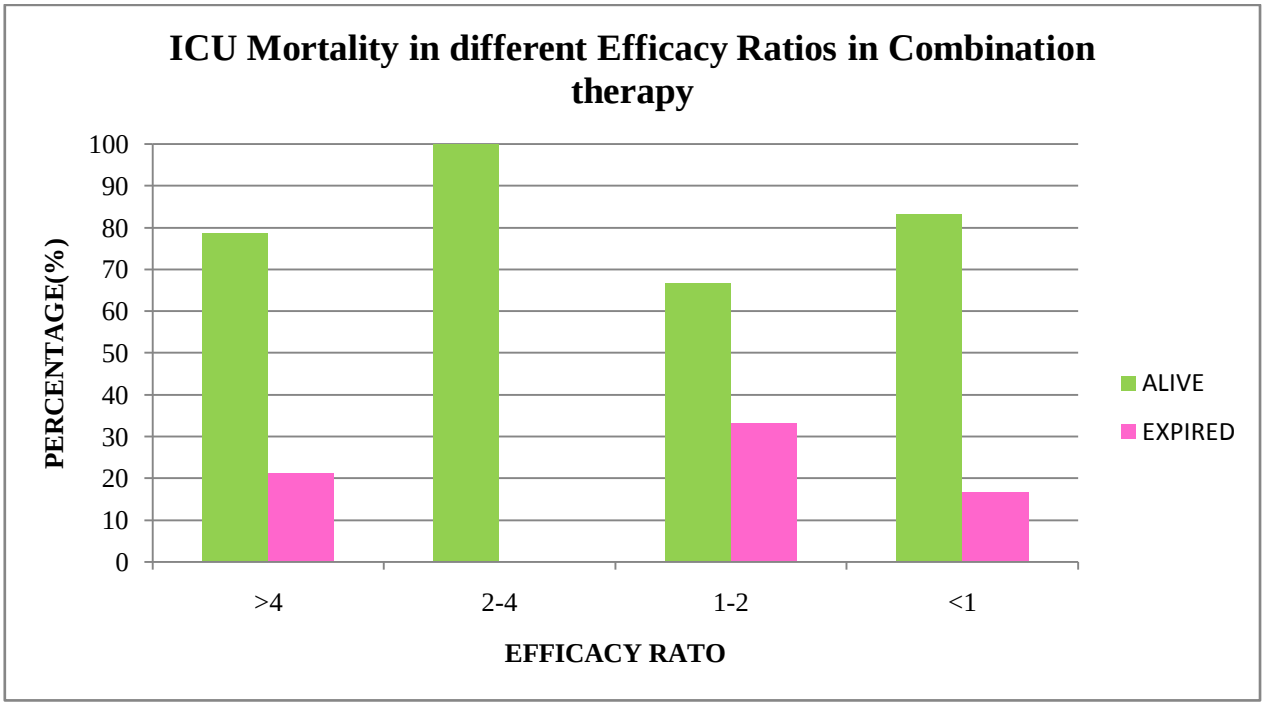

Fig 19: ICU mortality in different efficacy ratio in Combination therapy

Table 21: ICU Length of stay in different efficacy ratios in Monotherapy cases

MONO-THERAPY (EFFICACY RATIO)	Minimum (days)	Maximum (days)	Mean (days)	Median (days)	Std. Deviation
<1	2	47	7.80	5.00	9.482
1-2	2	19	8.32	6.00	6.609
2-4	2	16	10.00	10.50	5.606
>4	1	50	7.30	5.00	7.069
Total	1	50	7.62	5.00	7.331

ICU Length of Stay in different efficacy ratios in Mono-therapy: The lowest mean of LOS is observed from >4 efficacy ratio group (7.3 days) and it is increased in <1 efficacy group (7.80%), followed by 1-2 efficacy group (8.3 days) and 2-4 efficacy group (10 days).

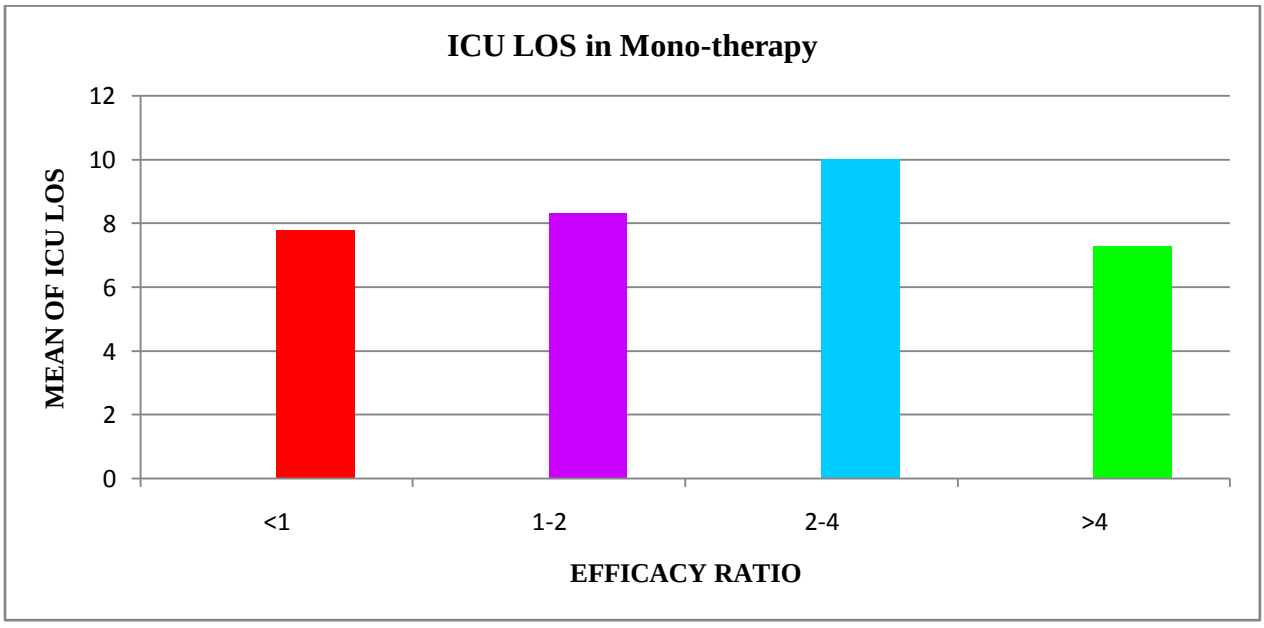

Fig 20 ICU LOS in different efficacy ratios in Mono-therapy

Table 22: ICU Length of stay in different efficacy ratios in Combination therapy cases

COMBINATION-THERAPY	Minimum (days)	Maximum (days)	Mean (days)	Median (days)	Std. Deviation
<1	2	31	12.37	10.00	8.704
1-2	2	29	11.56	11.00	8.205
2-4	2	12	7.00	5.00	4.301
>4	2	31	9.21	5.00	8.050
Total	2	31	10.41	7.00	8.199

ICU Length of Stay in different efficacy ratios in Combination therapy: The lowest mean of LOS is observed from 2-4 efficacy ratio group (7 days) and it is increased in >4 efficacy group (9.21%), followed by 1-2 efficacy group (11.56 days) and 2-4 efficacy group (12.37 days).

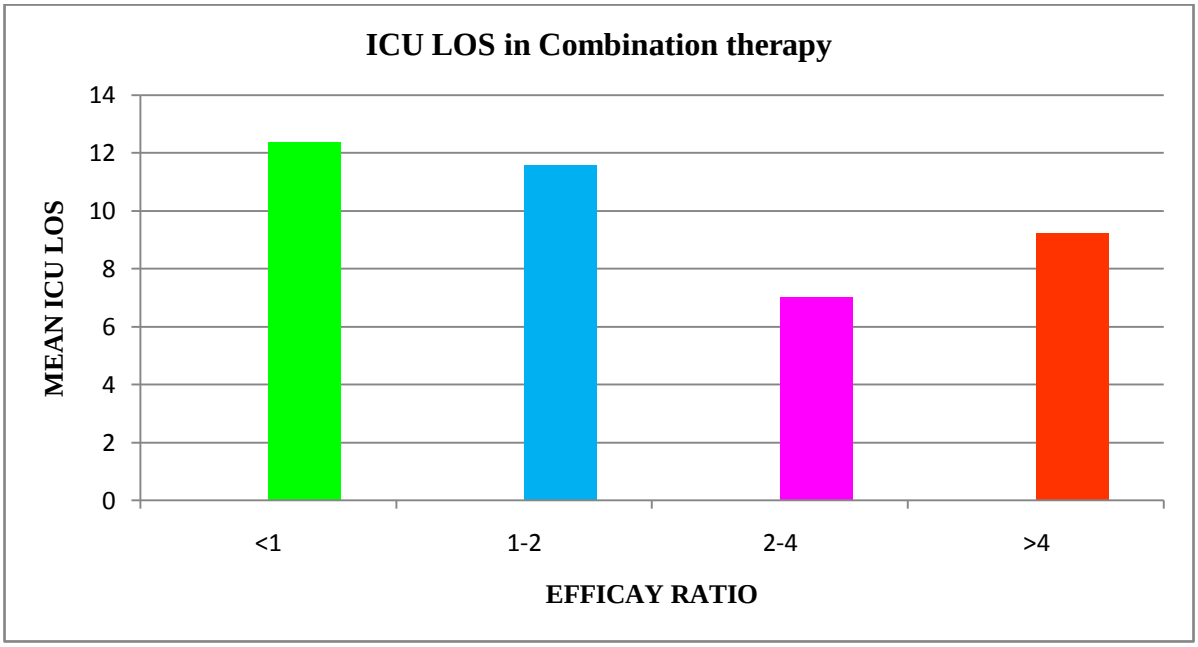

Fig 21: ICU LOS in different efficacy ratios in Combination therapy

DISCUSSION

The number of Gram-negative bacteria exhibiting multidrug resistance is increasing worldwide. Recent studies from India showed an alarming rise of incidence of Carbapenem resistant Enterobacteriaceae, Pseudomonas and Acinetobacter and established them as the most important cause of mortality due to infection in critical care unit. [30] A number of recent studies have shown a statistically significant higher mortality rate and longer ICU Length of Stay (LOS) in patients infected with high MIC organism than low MIC group. [31] Bhat and Colleagues evaluated outcome of patients with Gram-negative bacteremia treated with Cefepime stratified by MIC. They found Cefepime MIC ≥8 mg/lit was associated with increased mortality (58.4% compared to 28.4%), despite the fact that Cefepime MIC of 8gm/lit was consider susceptible at the time of the study. Recent PK/PD studies have proved that MIC values are valuable as they can be used to compare the relative efficacy of different drugs and to calculate doses that may be needed to be customized in particularly challenging infections.

In our study, majority (52%) of the patients were in the age group of 60-80 years. Respiratory samples from suspected patients have been sent for culture and sensitivity testing most frequently (40.5%), followed by Urine sample (37.2%). Klebsiella pneumoniae was the most (41.2%) common organism isolated followed by E.coli (23.7%). Among the non-fermenters Acinetobacter baumannii was most common (9.1%), followed by Pseudomonas (8.8%). Meropenem was the most common antibiotic used in study population either as Mono-therapy or as a part of Combination therapy (around 45%), followed by Colistin (%), Tigecycline (around 11%), Fosfomycin (around 10%) and Piperacillin/Tazobactum (around 8.5%). Antibiotic has been used more frequently as Mono-therapy in comparison to Combination therapy (61.7% VS 38.3%, p value 0.017). Meropenem again most frequently used in Mono-therapy (49.7%),

followed by Colistin (14.8%). In Combination therapy Meropenem-Fosfomycin combination has been used most frequently, followed by Meropenem-Tigecylin. This antibiotic prescription pattern is similar to that found in mini review by Christian G. Giske et.al. We have divided the resistant breakpoint of a drug against an organism with the obtained MIC value to derive an Efficacy ratio which is a measure of how far the measured MIC is from the resistant breakpoint for that drug. A drug with a higher efficacy ratio should be more effective than a drug with a lower efficacy ratio. So this might be a measure to choose the appropriate antibiotic to treat a serious infection by Gram-negative organism. This can also be the reason of getting a worst outcome in patient treated with a particular antibiotic with high MIC values though within the currently acceptable susceptible range [32].

In our study we observed the association of efficacy ratio of an antibiotic used for a particular patient and their outcome in terms of ICU mortality and ICU LOS. ICU mortality rate in our study population was 16.8%. In our study, among patients who got Mono-antibiotic therapy most of the patients (117, 69.2%) had an efficacy ratio >4 and only in 8 cases (4.7%) the efficacy ratio was within 2-4, Mono-antibiotic therapy was used in around 25% of cases where it was actually resistant according to in-vitro antibiogram (efficacy ratio is 1 or <1), when we observed the outcome of these patients we found only 14.2% mortality in the >4 efficacy ratio group in comparison to 20% and 15.8% mortality in the <1 or 1-2 efficacy ratio group respectively. The 2-4 efficacy ratio group had a significantly higher mortality around 50% (p value 0.008 in respect to > 4 group). So we could conclude that mortality wise outcome was significantly better in the >4 efficacy ratio group. Very high mortality in the 2-4 efficacy ratio group could not be explained and warrants further study as the number of patients of this group very low (only 4).

In combination or multiple antibiotic therapy group, in 50% cases at least one antibiotic had an efficacy ratio of >4. In around 46% cases of combination antibiotic therapy group, antibiotics were prescribed though all the antibiotics were resistant to the particular organism according to in-vitro susceptibility testing. Reason behind this is high prevalence of extremely drug resistance and pan-drug resistance. In the combination therapy group when we considering the outcome we found the mortality in <1 efficacy ratio group is lowest 16.7%, it was 22.4% in >4 efficacy ratio group. According to ICU LOS in the Mono-therapy group mean LOS was lowest (7.3 days) in >4 efficacy ratio group and we got the similar picture in the combination therapy group.

To our knowledge it was the first in this kind of study in India. The reason that mortality in combination therapy group is lowest in efficacy ratio <1, maybe due to other reason like more severe infection, more critically ill patient (high APACHE 4 Score) etc. Our study has a number of limitation first evaluating outcomes for organisms and antibiotic used according to their efficacy ratio is challenging as the patients are more likely to have multiple co-morbidities and advance age which increase the risk of mortality. So Matched cohot study is required to get a further information regarding this. Secondly the dose of the antibiotic received by the patient has not been evaluated and considered in this study which might have affected the outcome. We have not studied whether dose of the antibiotic was adjusted according to efficacy ratio. The rate and the way of antibiotic administration (bolus, extended infusion technique etc) were not studied. The number of patient in the 2-4 efficacy ratio was very small, so the outcome of this group could not be properly evaluated.

CONCLUSION

So we can conclude from our study that in cases where single antibiotic has been used if the efficacy ratio is >4, the outcome is better. However in the combination therapy group, multiple factors may have taken a role including in-vivo synergistic effect of the antibiotics used and the role of efficacy ratio in selecting antibiotic could not be established. Further Matched cohot studies are required to establish the use of efficacy ratio in the treatment of patient who need combination therapy.

- Mono-antibiotic therapy has been used more frequently than dual or combination antibiotic therapy (16.7% VS 38.3%, %, p value 0.017).
- Meropenem was the most frequently used antibiotic (49.7%) in Mono-therapy as well as in combination therapy group followed by Colistin, Tigecycline, Piperacillin/Tazobactum and Fosfomycin.
- Meropenem-Fosfomycin was the most frequently used combination, followed by Meropenem-Tigecycline.
- In Mono-antibiotic therapy group, majority of the patient had an efficacy ratio >4 (69.2%) and in combination therapy group at least one antibiotic had a efficacy ratio >4 in 49.5% cases.
- In Mon-antibiotic therapy group ICU mortality as well as ICU LOS was lowest in the >4 efficacy ratio group (14.2%, 7.3 days) and this is significantly higher in 2-4 efficacy ratio group (p value 0.008 in respect to > 4 group).
- In the combination therapy group mortality was lowest in <1 efficacy ratio group. However the ICU LOS is lower in the >4 efficacy ratio.

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ANNEXURE-1

Annexure-1

PATIENT'S ID	**DATE OF ADMISSION**	
AGE	**SEX**	**APACHE SCORE IV**
ANTIBIOTIC USED	**ORGANISM IDENTIFIED**	
MIC VALUE	**RESISTANT BREAKPOINT MIC**	**EFFICACY RATIO**
OUTCOME- ICU MORTALITY YES ☐ **NO** ☐		