"The contributed chapters in the book written by the faculties of science stream in the light of the recent thinking and developments in the field of science and education.- Science& Technology is now dominates almost every field of our activities.In summary, The faculties (Science stream) of GEMS Arts & Science college have made an excellent attempt to bring about this book Homo Scientia", covering almost all the important areas from biological sciences to artificial intelligence. Every article has its own merits in both academic and research fronts..I record my grateful appreciation and thanks to the contributors of this book for their untiring efforts."

**Dr. Balagopalan Unni**

Gems Arts & Science College (Affiliated to University of Calicut),Ramapuram, kadungapuram (PO), Malappuram (DT) Pin -679321

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# HOMO SCIENTIA

Dr. Balagopalan Unni,
Dr. Naveen Mohan,
Dr. K Gopalakrishnan,
Smitha Pramod V

GEMS

# HOMO SCIENTIA


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# Brief Biography

Dr. B.G.Unni, (Balagopalan Unni) Ph.D
(Allahabad central University)
FRES (London), FIANSc , FISAgBc, FICCE

Former Chief Scientist and Area Coordinator (Biotechnology & Biological Sciences) DADD and Fulbright Fellow retired from CSIR service in 2015 after 38 years of research career at CSIR North East Institute of Science & Technology Jorhat Assam. Appointed at Assam down town University as Director-Research in March 2015 and continued up to June 2019 and then re-designated as Adviser Research in August 2019). Back in Kerala, Dr.Unni is appointed as Director Academic & Research at GEMS College of Arts & Science affiliated to University of Calicut from August 2019. Both the positions are on honorary basis to strengthen the institutions in research areas. He did his BSc Biology (1972-74, Ewing Christian College, Alld University), MSc in Biochemistry(1974-76)(Second Rank) and Ph.D in Biochemistry from Allahabad University(1976-80) and PDF in Molecular Biology from Texas A&M University, USA(1988-91). Dr. Unni is specialized in Biochemistry, Molecular Biology, and Biotechnology and well established in his area of research and completed more than 40 years of research in both basic and applied fields of research. Dr.Unni got more than 130 research papers, 190 abstracts, 35 papers in proceedings, 7 patents,1 technology.18 chapters in books, edited 3 books and 29 students

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received PhD degrees under his guidance and supervision. Dr. Unni had completed more than 20 projects sponsored by Commonwealth Science Council, London, Ministry of Non conventional Energy Sources, Department of Non conventional Energy Sources Govt of India, North Eastern Council Govt of India, Department of Science &Technology, Department of Biotechnology, Central Silk Board,GB Pant Institute of Himalayan Environment and Development, CSIR and DRDO, Ministry of Defense, Govt of India during his scientific tenure at CSIR NEIST. Dr Unni received- Fulbright Travel Award/ Fellowship (USA) Dr. B.M. Das Memorial Science award, Hebrew University Award , H.R. Cama Memorial Travel Award,COSTED Travel Award,. DAAD- fellowship-Germany, Well Mark International Scholarship (USA) & Technology award in life sciences by CSIR,Govt of India . Best Fulbright Alumni Chapter Leader-South Asia Selected by the United States Education Foundation In India ( USIEF), New Delhi .Nominated to represent India at the International Fulbright Scholars meet at Marrakech, Morocco- Nominated by United States Education Foundation In India, New Delhi . Dr. Unni is in the editorial board of more than eight indexed journal in the country .Dr.Unni was nominated to various state and central committees such as High power committee for development of sericulture activities Muga, Eri,Tassar and Mulberry in Assam nominated by Governor of Assam, .Expert in the area of non mulberry sericulture, Ministry of Textiles, Advisory Board, Post graduate Biotechnology programme, Academic Council, Assam Agricultural University, Research Council, Central Silk Board, Ministry of Textiles , DBT's Nominee for Biosafety Committee ,Vice President SBC (India) Indian Institute of Science Bangalore, Vice President Indian Academy of Neuro-sciences, Member Fulbright Academy of Science & Technology, USA, Board of studies- Botany Nagaland University and Biotechnology Saugar University Madhya Pradesh., Fellow, Indian Academy of Neurosciences & Indian Society of Agricultural Biochemists, Fellow Royal Entomological Society, London UK and Scientific

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Advisor International Foundation of Science, Sweden, Member, Board of Studies Raiganj University ( 2017----), Member Research Review committee Tea Board of India (2016-2019), Member Advisory Committee Cancer Research Advisory Board, North East Cancer Hospital & Research Institute ( 2017--) President, Tea Improvement Consortium, Ltd, Tocklai Assam ( 2018-2020) .

Dr.Unni visited USA, Germany, Israel, Jordan, France, Morocco ,UK, Thailand ,Jordan, Singapore , China and UAE under various exchange program.

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# Preface

I am very happy to learn that, the GEMS Arts & Science College is bringing out a series of books written by the faculty in this academic year. The college is occupying a very important position among the colleges in Kerala, the same way the college is having unique standing in both academic and research fronts too. This is because of the excellent management, faculties and the best performances of the students.. I have full confident that in the course of time, and with the sincere commitment and dedication of the faculties , students and with management , the college will attain high level perfection and excellence and became a model college in the state of Kerala

This book entitled " Homo Scientia" had comprehensive research topics in various aspects in the topics of cyber security, biotechnology, microbiology and geology.A brief description about the cybersecurity, the protection of computer set up such as hardware, software data from several threats have been described in the chapter The best practices for deploying and managing IPS network security tools have been explored. The integration of intrusion prevention system (IPS) solutions, adherence to security policies, regular updates, monitoring and the implementation of incident response procedures are considered to be the essential components of a comprehensive network security framework. The risk management in cyber security, various cyber-attack kinds, malware, and some strategies to tackle these attacks are also explained by the authors. A comprehensive overview of the evolution of computer graphics, exploring the advancements in hardware, software, algorithms, and techniques that have propelled the field from its early pixel-based beginnings to the current state of realism etc also described. Optical character recognition has been extensively investigated in the past few years, and has been proven that high recognition rates can be achieved in specific

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application scenarios using some standard and well-studied methods such as neural network, support vector machine (SVM), etc. The possibility of learning an appropriate set of features for designing optical character recognition (OCR) has been investigated

Biotechnology is an interdisciplinary science using modern technologies to construct biological processes in research, agriculture, formulation of pharmaceutical products and other related fields. The better understanding of advances in plant genetic resources, genome modifications, omics technologies to generate new solutions for food security under changing environmental scenarios etc have been discussed in this chapter. The increasing demand for food had a great impact on the agriculture sector to address the various challenges associated with crop productivity. The tremendous advancement in plant research helps in understanding plant biology for sustainable food security, functional ecosystems, crop improvement and human health. One of the sustainable farming techniques is the use of fertilizer at nano level. Nanomaterials that enhance plant nutrition could be considered as an alternative to the conventional chemical fertilizers. one chapter covered the importance of nano fertilizer to enhance metabolic processes in plants and reviewed the concerns in developing nanotechnological methods in the future. Metabolomics has now emerged as a powerful tool for the comprehensive analysis of metabolites within biological systems. One of the chapters provides a review on metabolomics, encompassing its methodologies, applications, potential impact on personalized medicine ,and discusses further the need for advancements in analytical technologies. The antifungal activity of mangroves, particularly Rhizophora species are one of the main sources for fungicidal compounds due to the presence of high concentration of phenols. The antifungal activity of Rhizophora species has been elucidated, and could be further utilized as biocontrol agents for fungal disease in agricultural crops. One of the chapters discussed the species identification and its impact on economical and ecological level in the species like Nutmeg, one of the important medicinal plants that had a greater attention ,however, it was very difficult to differentiate the sexual identity

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in the seedling stages. But the protein content screening among the studied plantlets had differentiated the sexes in the species as explained by the author.

AI (Artificial Intelligence) or machine intelligence enables farmers to enhance the quality and ensure a quick go-to market strategy for crops, and adoption of these algorithms to improve food industries. Artificial intelligence (AI) has also the potential to revolutionize education, from personalized learning to assessment and grading. Additionally, AI-powered tools can provide greater accessibility to students with disabilities, while also enabling more engaging and interactive content. AI continues to develop and become more prevalent in education, towards responsible and equitable implementation. However the negative and positive part of the AI may also be looked into.

The chapters related to microbiological aspects have also been incorporated in this book . Carbapenem-resistant A. baumannii (CRAb), bacteria that cause multi-infections in humans and resistant to multiple drugs too. The study attempted to isolate and characterize the bacterial species from the clinical specimens using biochemical techniques. The enzyme, carbapenemase produced by the bacteria was isolated and determined by different assays. Another study identified the antibacterial, antioxidant and anticancer activities of Ganoderma lucidum by various chromatographic techniques. Anticancer activity was also assessed on HeLa cell lines using MTT assay and DPPH assay. In one of the chapters,the author discussed L-asparaginase, one of the widely exploited enzymes for the treatment of acute lymphoblastic leukemia (ALL). Also attempted to isolate and characterize the enzyme from soil samples collected from different locations at Kerala. The study indicated that soils can provide a rich source for L-asparaginase which has got ample application in pharmaceutical industries.

The studies on various geological aspects with respect to different geographical areas in Kerala soil has been included in the book. The vertical geochemical variation and elemental mobility of the lateritic terrain in the Makkaraparamba of Malappuram District, Kerala has been very well investigated. Under extremely oxidizing and leaching conditions, laterite


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soil transformed into a variety of rocks and further developed into stable secondary product in the existing humid tropical and subtropical environments. The hydrogeological conditions in Kumbala- Kaliyar river basin, Kasaragod district, Kerala was assessed by means of Vertical Electrical Sounding (VES). The digital spatial data output of the present study would be much helpful for planning and management of surface and sub-surface water resources of Kasaragod River basin in which the Kasaragod township is centrally located

The contributed chapters in the book written by the faculties of science stream in the light of the recent thinking and developments in the field of science and education.Science& Technology is now dominates almost every field of our activities. In summary , The faculties ( Science stream) of GEMS Arts & Science college have made a n excellent attempt to bring about this book Homo Scientia".covering almost all the important areas from biological sciences to artificial intelligence. Every article has its own merits in both academic and research fronts..I record my grateful appreciation and thanks to the contributors of this book for their untiring efforts.

**Dr.Balagopalan Unni**

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# ISOLATION AND CHARACTERISATION OF CARBAPENEM RESISTANT ACINETOBACTER BAUMANNII FROM CLINICAL SAMPLE (PUS)

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Assistant Professor
PG Department of Microbiology

**ABSTRACT**

Antimicrobial agents are substances that kill or inhibit the growth of microorganisms and are suitable for systemic use, i.e. they do not harm the host. Antibiotics, (antibacterials) are a type of antimicrobial drug used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. Carbapenems are antibiotics used for the treatment of infections known or suspected to be caused by multi-drug resistant bacteria. Their use is primarily in people who are hospitalized. Like the penicillins and cephalosporins, they are members of the beta lactam class of antibiotics, which kill bacteria by binding to penicillin-binding proteins and inhibiting cell wall synthesis. *Acinetobacter baumannii* is an emerging nosocomial bacterium that causes a range of health-care-related illnesses and has a high morbidity and fatality rate. *A. baumannii* often acquires or upregulates resistance determinants, resulting in resistance to several drugs. Because of the related therapeutic challenges, carbapenem-resistant *A. baumannii* (CRAb) is regarded as a particularly serious public-health hazard. As a result, the purpose of this work was to isolate Carbapenem-resistant Acinetobacter species from clinical samples and identify the enzyme implicated in resistance.

## INTRODUCTION

Acinetobacter is a genus of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria. *Acinetobacter species* are widely distributed in nature, and commonly occur in soil and water. Their ability to survive on moist and dry surfaces as well as to survive exposure to various common disinfectants allows some *Acinetobacter* to survive in a hospital environment. Furthermore, *Acinetobacter* can grow at a broad range of temperatures, allowing them to survive in a broad array of environments. *Acinetobacter* is regularly identified in nosocomial infections, and it is especially common in intensive care units, where sporadic instances as well as epidemic and endemic occurrences are widespread. *A. baumannii* is a common cause of hospital-acquired pneumonia, particularly' late-onset' ventilator-associated pneumonia. It can also cause skin and wound infections, bacteremia, and meningitis, however *A. lwoffi* is mostly responsible for the latter. *Acinetobacter baumannii* is the most common cause of human illness among the Acinetobacter species, having been linked to a variety of hospital acquired diseases such as bacteremia, urinary tract infections , secondary meningitis, infective endocarditis, and wound and burn infections.

The clinical importance of *A. baumannii* is owing in part to its ability to develop resistance to several current antibiotics. According to reports, it is resistant to broad-spectrum cephalosporins, -lactam antibiotics, aminoglycosides, and quinolones. Resistance to carbapenems is also becoming more common. *A. baumannii* may persist for weeks on human skin or dry surfaces and is resistant to a wide range of disinfectants, making it especially simple to spread in a hospital setting. Antibiotic resistance genes are frequently plasmid-borne, and plasmids found in Acinetobacter strains can be horizontally transmitted to other dangerous bacteria. Acinetobacter colonies on the skin in healthy people correspond with a low prevalence of allergies; Acinetobacter is assumed to be allergy-protective.

Carbapenems are antibiotics used to treat infections caused by multidrug-resistant bacteria that are known or suspected to be MDR. Their primary application is in hospitalised

patients. They are beta lactam antibiotics, like penicillins and cephalosporins, that kill bacteria by binding to penicillin-binding proteins and blocking cell wall formation. When compared to cephalosporins and penicillins, they have a larger spectrum of action. They are less susceptible to numerous prevalent mechanisms of antibiotic resistance than other beta lactams. Merck & Co. created carbapenem antibiotics from carbapenem thienamycin, a naturally occurring product of *Streptomyces cattleya*.

Many antibiotic groups, including penicillin, chloramphenicol, and frequently aminoglycosides, are intrinsically resistant to Acinetobacter species. Resistance to fluoroquinolones has been recorded throughout therapy, leading to increasing resistance to other medication classes via active drug efflux. The CDC has observed a remarkable increase in antibiotic resistance in Acinetobacter strains, and carbapenems are acknowledged as the gold standard and therapy of last resort. Acinetobacter *species* are rare in that they are sulbactam sensitive; sulbactam is most usually employed to suppress bacterial beta-lactamase, yet this is an example of sulbactam's antibacterial function.

Gram-negative bacteria (e.g., *Pseudomonas spp., spp., and Stenotrophomonas spp.*), as well as Enterobacteriaceae (e.g., Klebsiella spp., *Escherichia coli, and* Enterobacter spp.), as well as Gram-positive bacteria (e.g., s This concerning pattern offers a significant public health risk. Resistance to carbapenems is mediated through the development of -lactamases, efflux pumps, and mutations that change the expression and/or function of porins and PBPs. Carbapenem resistance, on the other hand, occurs as a result of the existence of several mechanisms. Combinations of these processes can result in high levels of carbapenem resistance in specific bacterial species, including *Klebsiella pneumoniae, P. aeruginosa, and A. baumannii.*

There is a contrast between carbapenem resistance in Gram-positive cocci and Gram-negative rods. Carbapenem resistance in Gram-positive cocci is generally caused by substitutions in PBP amino acid sequences or the acquisition/production of a novel carbapenem-resistant PBP. Carbapenem resistance in

Gram-negative rods is related with the expression of -lactamases and efflux pumps, as well as porin loss and changes in PBP. The synthesis of -lactamases has received the greatest attention, and hence it is covered in greater depth here than the other mechanisms of resistance.

β-lactamases are a primary antibiotic resistance mechanism; these periplasmic enzymes hydrolyze β-lactam antibiotics, preventing the medication from reaching the PBP target. Currently, β-lactamases are divided into four separate classes based on structural similarities (classes A, B, C, and D) or four groups based on hydrolytic and inhibitor profiles (classes A, B, C, and D). Class B β-lactamases are carbapenemases that utilise Zn2+ to inactivate β-lactams. To hydrolyze the β-lactam bond, Class A, C, and D β-lactamases employ serine as a nucleophile. Carbapenemases are β-lactamases that have the capacity to hydrolyze carbapenems. Carbapenemases are the most potent β-lactamases, hydrolyzing penicillins, cephalosporins, and carbapenems and causing carbapenems to lose efficacy in treating resistant bacteria

## METHODOLOGY

### Sample collection

This study was carried out in the Department of Microbiology of E.M.S Memorial Co– Operative Hospital & Research Centre Ltd. Perinthalmanna, Malappuram Dt. Kerala, India. Pus was collected from inpatient of the hospital.

Isolation of Carbapenem resistant bacteria

Nutrient agar was prepared and sterilized by autoclaving at 121°C for 15 minutes.The recommended amount of antibiotic stock solution i.e; Meropenem and Imipenem (1 µg per ml) was added to the media after cooling to 55°C and mixed gently, then poured (about 20 ml) into 90 mm diameter sterile Petri dishes making a layer 3-5 mm thick. Collected sample were streaked on to the nutrient agar plates containing antibiotics Meropenem and Imipenem. The plates were then incubated at 37°C for 24 hours.

### Antimicrobial susceptibility testing

The antimicrobial susceptibility was determined by the Kirby Bauer's disc diffusion method on Mueller-Hinton agar, according to the Clinical Laboratory Standard Institute guidelines. The antibiotics tested were: Meropenem (10µg) and Imipenem (10µg). Mueller-Hinton agar were prepared and sterilized by autoclaving at 121°C for 15 minutes. Sterilized MHA was then poured in to sterile petri dishes and were allowed to solidify. A suitable dilution (turbidity matching 0.5 MacFarland standard) of overnight broth culture of the isolated colony on nutrient agar plate was inoculated on the surface of solid MuellerHinton agar as a lawn by spreading with a sterile swab. After drying the plate, Meropenem and Imipenem discs were applied with sterile forceps.The plate is then inocubated at 37°C for 24 hours. After incubation the plates were observed for the formation of zone. The diameter of zone of inhibition on the plate is measured and compared with CLSI standards for interpretation of result.

| | Current Disk Diffusion Zone Diameters (mm)a | | |
|---|---|---|---|
| | Zone Size Interpretationb | | |
| Carbapenems | Susceptible | Intermediate | Resistant |
| Doripenem | ≥23 | 20-22 | ≤19 |
| Ertapenem | ≥22 | 20-21 | ≤18 |
| Imipenem | ≥23 | 20-21 | ≤18 |
| Meropenem | ≥23 | 20-21 | ≤18 |

amm = millimeters
bCLSI. Performance Standards for Antimicrobial Susceptibility Testing Twenty-Sixth Informational Supplement
CLSI document M100-S26, Wayne, PA: Clinical and Laboratory Standards Institute: Jan 2016.

Subculturing of isolated Carbapenem resistant bacteria

Bacteria that have shown resistance to both Meropenem

and Imipenem were subcultured on to sterile Nutrient agar plates containing antibiotics. Plates were incubated at 37oC for 24 hours.

**Identification of isolated Carbapenem resistant bacteria**

The isolated microorganism were identified by Grams staining, subculturing on to MacConkey Agar and Leeds Acinetobacter agar base and by biochemical tests.

**Determination of Minimum Inhibitory Concentration (MIC)**

MIC of Meropenem and Imipenem was determined by broth dilution and agar dilution methods.

**Phenotypic testing for carbapenemase production**

Carbapenemase production by isolated bacteria was phenotypically determined by Modified Hodge Test and Combined disc synergy test.

**Modified Hodge Test (MHT)**

A 0.5 McFarland standard suspension of *E. coli* ATCC 25922 was prepared in broth. Dilute 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB or saline. Streaked a lawn of the 1:10 dilution of E.coli ATCC 25922 on two sterile Mueller Hinton agar plate and allowed to dry for3–5 minutes. Meropenem (10 µg) disk was placed in the center of one plate, similarly Imipenem (10 µg ) disc was placed in the center of other plate. In a straight line, streaked test organism from the edge of the disk to the edge of the plates. Incubate overnight at 35°C ± 2°C in ambient air for 16–24 hours. After 16–24 hours of incubation, examined the plate for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk.

**Combined disc synergy test (CDST)**

As the MHT does not differentiate the type of β-lactamase (carbapenemase) produced by the bacteria, a combination of the carbapenem disc with different inhibitors was used in the inhibition assays. These inhibition tests are based upon the inhibition of different classes of carbapenemase by specific inhibitors. Inhibitors used in this study are:

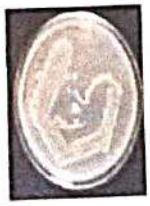
- Class A : Clavulanate
- Class B : EDTA
- Class C : Cloxacillin
- Class D : It is not inhibited by inhibitors

Mueller-Hinton agar were prepared and sterilized by autoclaving at 121°C for 15 minutes. Sterilized MHA was then poured in to sterile petri dishes and were allowed to solidify. A suitable dilution (turbidity matching 0.5 MacFarland standard) of overnight broth culture of the test organism was inoculated on the surface of solid Mueller-Hinton agar as a lawn by spreading with a sterile swab. Meropenem and imipenem discs with respective inhibitors for each class of carbapenemase was placed on the agar plates. Similarly meropenem, imipenem and inhibitor disc alone were also placed. The agar plates were incubated at 37°C overnight and the diameter of the growth inhibitory zone around these meropenem and imipenem discs with inhibitor added was compared with that around the plain meropenem and imipenem disc.

RESULTS AND DISCUSSION

Isolated colonies were observed on meropenem and imipenem containing nutrient agar plates inoculated with sample indicating that the organisms are able to grow even in the presence of carbapenems. The colonies were small, non-pigmented, domed and mucoid.

Sample on nutrient agar with Meropenem



Sample on nutrient agar with Imipenem

The zone of inhibition around meropenem antibiotic was 9mm and for imipenem disc was 8mm. So, the organism isolated from pus is resistant to both merpenem and imipenem. The bacterial isolate was identified as Acinetobacter species based on microscopic, cultural and biochemical analysis according to Bergey's manual of determinative bacteriology.

Colony on Leeds Acinetobacter agar

After incubation there was a gradual decrease in the turbidity and optical density of the culture broth. Test tube with meropenem concentration of 20µg/ml showed complete inhibition of bacterial growth. So 20µg/ml is the MIC of meropenem for the isolated bacteria.

| CONCENTRATION OF MEROPENEM(µg/ml) | OPTICAL DENSITY AT 620nm |
|---|---|
| Control | 0.00 |
| 1 | 0.95 |
| 2 | 0.52 |
| 3 | 0.48 |
| 4 | 0.44 |
| 5 | 0.41 |
| 6 | 0.36 |
| 7 | 0.30 |
| 8 | 0.25 |
| 9 | 0.21 |

| | |
|---|---|
| 10 | 0.19 |
| 11 | 0.18 |
| 12 | 0.15 |
| 13 | 0.13 |
| 14 | 0.12 |
| 15 | 0.09 |
| 16 | 0.06 |
| 17 | 0.05 |
| 18 | 0.03 |
| 19 | 0.01 |
| 20 | 0.00 |

Optical density measurements of culture broths with meropenem

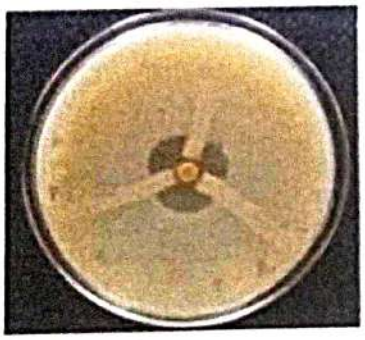
Test tube with imipenem concentration of 21µg/ml showed complete inhibition of bacterial growth. So 21µg/ml is the MIC of imipenem for the isolated bacteria.

| CONCENTRATION OF IMIPENEM(µg/ml) | OPTICAL DENSITY AT 620nm |
|---|---|
| Control | 0.00 |
| 1 | 0.99 |
| 2 | 0.85 |
| 3 | 0.73 |
| 4 | 0.60 |
| 5 | 0.51 |
| 6 | 0.46 |
| 7 | 0.44 |
| 8 | 0.40 |
| 9 | 0.36 |

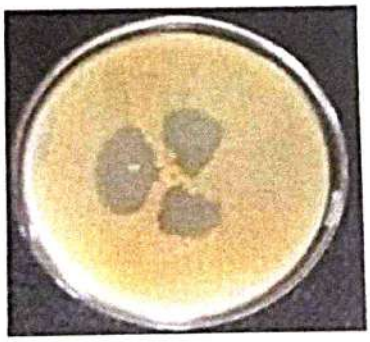
| | |
|---|---|
| 10 | 0.30 |
| 11 | 0.27 |
| 12 | 0.21 |
| 13 | 0.18 |
| 14 | 0.17 |
| 15 | 0.14 |
| 16 | 0.11 |
| 17 | 0.09 |
| 18 | 0.05 |
| 19 | 0.03 |
| 20 | 0.01 |
| 21 | 0.00 |

Mueller-Hinton agar plate with meropenem concentration 20µg/ml showed no bacterial growth and plate with imipenem concentration 21µg/ml were also free from bacterial growth. So MIC of meropenem and imipenem for the isolated bacterial species was 20µg/ml and 21µg/ml respectively.

In Modified Hodge Test, a clover leaf-type indentation was observed at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the meropenem and imipenem discs. The tested clinical isolate was able to produce carbapenemase



Meropenem



Imipenem Modified Hodge Test

Results of Combined disc synergy test (CDST)

| Antibiotic | Inhibitor | Zone of inhibition | Interpretation |
|---|---|---|---|
| Meropenem | No inhibitor | 13mm | |
| Meropenem | Clavulanate | 13mm | There is no increase in zone of inhibition. Absence of Class A (KPC) Carbapenemase |
| Imipenem | No inhibitor | 10mm | |
| Imipenem | Clavulanate | 10mm | |
| No antibiotic | Clavulanate | 8mm | |

| Antibiotic | Inhibitor | Zone of inhibition | Interpretation |
|---|---|---|---|
| Meropenem | No inhibitor | 10mm | There is an increase in zone of inhibition (≥7mm in diameter). Presence of Class B (MBL)Carbapenemase |
| Meropenem | EDTA | 15mm | |
| Imipenem | No inhibitor | 7mm | |
| Imipenem | EDTA | 14mm | |
| No antibiotic | EDTA | No zone | |

| Antibiotic | Inhibitor | Zone of inhibition | Interpretation |
|---|---|---|---|
| Meropenem | No inhibitor | 11mm | There is no increase in zone |
| Meropenem | Cloxacillin | 11mm | |
| Imipenem | No inhibitor | 7mm | of inhibition. Absence of Class C (AmpC)Car-bapen emase |
| Imipenem | Cloxacillin | 7mm | |
| No antibiotic | Cloxacillin | No zone | |

After incubation, a clover leaf-type indentation was observed at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the meropenem and imipenem discs. The tested clinical isolate was able to produce carbapenemase. There was an increase of about 5mm and 7mm in the zone of inhibition around the meropenem and imipenem disc with EDTA. The carbapenemase enzyme produced by the clinical isolate is Class B carbapenemase i.e; Metallo beta lactamases.

Pus inoculated on to Nutrient agar containing imipenem and meropenem showed good bacterial growth indicating that they are highly resistant to carbapenems, "antibiotics of last resort". Antibiotic susceptibility of the isolated bacterial strain was done by Kirby Bauer's disc diffusion method on Mueller–Hinton agar for confirming that the isolated strain were resistant to Carbapenem.

Identification and characterization of the isolated bacterial culture was made by using morphological, culturing on to Mac Conkey& Leeds *Acinetobacter* medium and biochemical tests. Gram staining showed gram-negative cocco bacilli,

and colonies were pink coloured, mucoid on both agar plates. Biochemical tests such as IMViC, Catalase test, nitrate reduction test, oxidase test, urease test, test for utilization of carbohydrates were done. From these tests the isolated bacteria were identified as *Acinetobacter species* according to Bergey's manual of determinative bacteriology.

MIC of Meropenem and Imipenem against the isolated bacteria was determined by broth dilution and agar dilution methods and was found to be 20µg/ml and 21µg/ml. Carbapenemase production by these bacteria were determined by modified hodge test. A clover leaf-type indentation was observed at the intersection of the test organism and the *E. coli* 25922. Carbapenemase class was determined by combined disk synergy test using various inhibitors such as Clavulanate for Class A, EDTA for Class B and Cloxacillin for Class C. The carbapenemase enzyme produced by the clinical isolate was identified as Class B carbapenemase i.e; Metallo beta-lactamases.

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