



Prevalence and Molecular Characteristics of Carbapenem Resistant *Acinetobacter baumannii* Isolates from A Regional Tertiary Care Hospital

Fiji E^{*1}, Anandharaj B² and Jijo G Varghese³

^{*1,3}: Research Scholar, Department of Microbiology, M.R. Government College (Affiliated to Bharathidasan University), Mannargudi-614001, Thiruvallur District, TamilNadu, India

² Department of Microbiology, M.R. Government College (Affiliated to Bharathidasan University), Mannargudi-614001, Thiruvallur District, TamilNadu, India

Abstract: *Acinetobacter* spp., is an emerging opportunistic nosocomial Gram negative bacterial pathogen with increasing prevalence in particular the species *Acinetobacter baumannii*. It infects the most vulnerable immunocompromised hospitalized patients who are critically ill. Significant levels of morbidity and mortality have been reported with outbreaks and the carbapenem hydrolyzing beta lactamases that includes MBLs and oxacillinases are recognized as important contributors of carbapenem resistance in *Acinetobacter* spp., Enzymatic degradation of drugs, target modifications, multidrug efflux pumps and permeability defects are some of the important resistance mechanisms present in *A. baumannii*. Accumulation of various resistance mechanisms made treatment of *A. baumannii* infection very difficult. The major objective of the study was to identify the pattern of antibiotic resistance and its regional prevalence. Hence this study was aimed and conducted to isolate, identify and distinguish the antibiogram of *A. baumannii* from clinical specimens and to study a molecular level identification of resistance mechanisms of the isolates from a tertiary care hospital. Various clinical specimens like blood, urine, abscess, vaginal swab were analyzed and evaluated for the presence of *Acinetobacter*. Four members of *Acinetobacter* species; *A. junii*, *A. lowffii*, *A. ursingii* and *A. baumannii*, were isolated from clinical specimens. *A. baumannii* was the predominant species and 15% of the *A. baumannii* isolates were confirmed to be resistant to carbapenems. A molecular typing was done to identify the genes conferring antibiotic resistance and five major genes were identified in the isolates. The predominant genes present in the isolates were OXA-58, OXA-23 and GIM. Presence of IMP & VIM were also identified.

Keywords: *Acinetobacter baumannii*, Multidrug resistance, Carbapenems, Molecular typing, OXA-23, OXA-58, GIM

*Corresponding Author

Fiji E, Research Scholar, Department of Microbiology, M.R. Government College (Affiliated to Bharathidasan University), Mannargudi-614001, Thiruvallur District, TamilNadu

Received On 28 April, 2022

Revised On 5 May, 2022

Accepted On 13 May, 2022

Published On 15 May, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Fiji E, Anandharaj B and Jijo G Varghese, Prevalence and Molecular Characteristics of Carbapenem Resistant *Acinetobacter baumannii* Isolates from A Regional Tertiary Care Hospital.(2022).Int. J. Life Sci. Pharma Res.12(3), L181-187
<http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.3.L181-187>

This article is under the CC BY- NC-ND Licence (<https://creativecommons.org/licenses/by-nc-nd/4.0>)



Copyright © International Journal of Life Science and Pharma Research, available at www.ijlpr.com

1. INTRODUCTION

Acinetobacter spp., is an emerging opportunistic nosocomial Gram negative bacterial pathogen with increasing prevalence in particular the species *A. baumannii*. *Acinetobacter* genus has undergone significant taxonomic modifications over the last 30 years and the species *A. baumannii* is identified as one of the most troublesome pathogen. It infects the most vulnerable immunocompromised hospitalized patients who are critically ill. Medically relevant species, such as *A. calcoaceticus*, *A. lowffii*, *A. nosocomialis*, and *A. pittii*, have been found on vegetables, meat, dairy products, and human skin². *A. baumannii* strains harbouring extensive antibiotic resistance have contaminated commercial food, including meat, vegetables, and various types of livestock, suggesting multiple environmental routes of transmission into human populations³. *A. baumannii* has emerged to a major nosocomial pathogen from a relatively low virulent commensal bacteria and it is a causative agent for severe infections like bacteraemia, pneumonia, urinary tract infections and wound infections^{4,5}. Significant levels of morbidity and mortality have been reported⁶ with outbreaks and common infections include ventilator associated pneumonia and bacteremia; less frequently burn wounds and urinary tract⁷. *A. baumannii* is also a common cause of bloodstream infections in the intensive care setting⁸ and the lower respiratory tract infections and intravascular devices⁹⁻¹² are reported to be the common sources. The risk factors of the infection with multidrug resistant *Acinetobacter* spp., include prolonged hospital stay, exposure to an intensive care unit, receipt of mechanical ventilation, colonization pressure, exposure to antimicrobial agents, etc.¹³ As the multidrug resistant *Acinetobacter* spp., infection usually occurs in severely ill patients in the ICU, the mortality rate is high up to 68%¹⁴. In recent years, a substantial increase in *A. baumannii* associated with nosocomial pneumonia cases are reported¹⁵. Peleg et al., 2008 reported that the *A. baumannii* ranks with 10th among the organisms causing monomicrobial blood stream infections. There is a need for novel therapeutic options owing to the emergence of isolates resistant to drug choice like carbapenems. In recent studies done by Chang et al., 2015 also revealed high prevalence of CRAB¹⁶, upto 60% of total isolates. Other researchers also found a high prevalence rate of CRAB in nosocomial infections^{17,18}. In a study conducted by Henwood et al., 2002¹⁹, among consecutive *A. baumannii* isolates collected, more than 85% were resistant to cephalosporins, 43% were resistant to gentamicin and 46% were resistant to quinolones, leaving carbapenems as the only drug active against more than 90% of isolates. Carbapenems are the drugs of choice for the treatment of nosocomial infections. But resistance to this class of antibiotic is emerging, and leading to the evolution of pan resistance strains and to the need of new therapeutic options^{20,21}. Carbapenem resistant *Acinetobacter* are becoming widespread in several regions of the world²². Mechanistically, resistance to these potential beta lactams may be due to impaired permeability resulting from altered outer membrane proteins or to alterations in the penicillin binding proteins (PBP)²³. However, the carbapenem hydrolyzing beta lactamases that include MBLs and oxacillinases are recognized as important contributors of carbapenem resistance in *Acinetobacter*. Resistance offered by oxacillinases is more often than MBLs²⁴. There are four major OXA subgroups (OXA-51, OXA-23, OXA-40 and OXA-58) associated with *A. baumannii*. In order to control the spread of *Acinetobacter baumannii* in the hospital, it is necessary to distinguish the outbreak strain and its characteristics. The major objective of the study was to

identify the pattern of antibiotic resistance and its regional prevalence. Hence this study was aimed and conducted to isolate, identify and to distinguish the antibiogram of *A. baumannii* from clinical specimens and to study a molecular level identification of resistance mechanisms.

2. MATERIALS AND METHODS

2.1 Study Design and Area

The study was conducted at Sunrise Institute of Medical Sciences (SIMS), Kerala, India. This study was reviewed and approved by the institutional ethical committee of Sunrise Institute of Medical Sciences (SIMS/IEC/03/2022).

2.2 Sample Collection and Isolation

The samples were collected from patients admitted in the hospital in various departments as well as from Out patients. Various clinical specimens like urine, blood, pus, abscess and endo-tracheal aspirations were screened for the presence of *Acinetobacter* spp., All the samples were collected by aseptic methods²⁵.

2.3 Selective Culture and Biochemical Identification

All samples were inoculated on to two enriched and selective agar media, 5% sheep blood agar (Biomerieux) and on MacConkey agar and incubated at 37° C for 24 to 48 hours. All colonies resembling *Acinetobacter* were initially identified by standard morphological, cultural and biochemical characteristics. And further identification was done by Vitek 2 compact system from Biomerieux India pvt Ltd.

2.4 Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done by disc diffusion on Mueller Hinton agar (Himedia) plates according to the guidelines of Clinical Laboratory Standards Institute²⁶. All the isolates were inoculated in peptone broth and adjusted to McFarland standard and swabbed on Muller Hinton agar. The ready-made antibiotic discs from Himedia were placed on the inoculated plates and incubated overnight at 37°C. The diameter of the zone of inhibition was measured and interpreted using CLSI²⁶ guideline. Along with the disc diffusion method susceptibility was also analysed using AST N280 cards on Vitek 2 compact system. The antibiotics tested include Ampicillin sulbactam (10µg), Cefazidime (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Gentamycin (10µg), Amikacin (30µg), Tobramycin (10µg), Imipenem (10µg), Meropenem (10µg), Piperacillin tazobactam (110µg), Cefepime (30µg), Cefotaxim (30µg), Ceftriaxone (30µg), Tetracyclin (30µg) and Colistin. The quality control of the antibiotic sensitivity was done with *Escherichia coli* ATCC25922.

2.5 Isolation of Genomic DNA

Four Carbapenem resistant *Acinetobacter baumannii* isolates were subjected to screening for various genes. The extraction of total genomic DNA using Mag Genome DNA isolation kit procedure as per the manufacturer instructions. Quality of the genomic DNA was assessed using 0.7 % agarose gel along with 1kb DNA ladder as size standard and the quantity of the genomic DNA was assessed in UV-Vis Spectrometer.

2.6 Screening for Genes Associated With Antibiotic Resistance

Amplification of genes encodes for antibiotic resistance were carried out for the sample using primers. The details of the genes and primers used are mentioned in table I. Presence or absence of expected band was considered as + ve or – ve

genotype of the strains. PCR-generated amplicon was confirmed and purified using GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality of the product was assessed using 2% agarose gel along with 100bp DNA ladder as size standard. The primer sequence used and corresponding genes are listed in table I.

Table I: Primers used for Antibiotic resistance gene extraction²⁷

| Antibiotic Resistance Mechanism | Gene | Primer | Expected Amplicon Size |
|-----------------------------------|-------|--|------------------------|
| Beta Lactamase – CLASS D | OXA23 | F – GATGTGTCATAGTATTCGTCG R – TCACAACAACATAAAGCACTG | 1065 bp |
| Novel Class D Beta Lactamase | OXA58 | F – CGATCAGAATGTTCAAGCGC R – ACGATTCTCCCCTCTGCGC | 528 bp |
| Metallo beta lactamases (class B) | IMP | F – CTACCGCAGAGTCTTTG R – AACCAGTTTTGCCTTACCAT | 587 bp |
| Metallo beta lactamases (class B) | VIM | F – AGTGGTGAGTATCCGACAG R – ATGAAAGTGCGTGGAGAC | 261 bp |
| Metallo beta lactamases (class B) | GIM | F – GATTTCCAAATGAAAAATGTA R – TTAATCAGCCGACGCTTC | 762 bp |

3 RESULTS

3.1 Sample Collection and Isolation

A total of 500 clinical samples were collected aseptically from various patients from Sunrise Institute of Medical Sciences, Kochi and a total of 39 isolates of *Acinetobacter* species were identified. *A. baumannii* was confirmed to be the most abundant species (84 %), followed by *A. lowfii*, *A. junii* and *A. ursingii*. (fig 1)

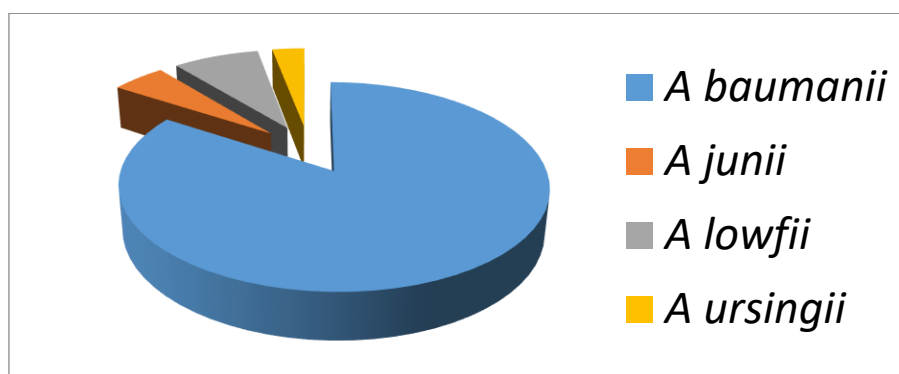


Fig1: Distribution of various genus of *Acinetobacter* among the isolates

3.2 Antibiotic Sensitivity

Out of the 39 isolates, 6 isolates were observed to be multidrug resistant and carbapenem resistant strains. The most susceptible antibiotic was collistin; i.e., 83% of the Carbapenem resistant *Acinetobacter baumannii* (CRAB) isolates

were susceptible to collistin. The observed susceptibility pattern of the CRAB isolated showed that the tobramycin has second highest percentage (77%). Most of the other tested antibiotics were found to be resistant and the pattern of resistance of the isolates were shown in fig 2

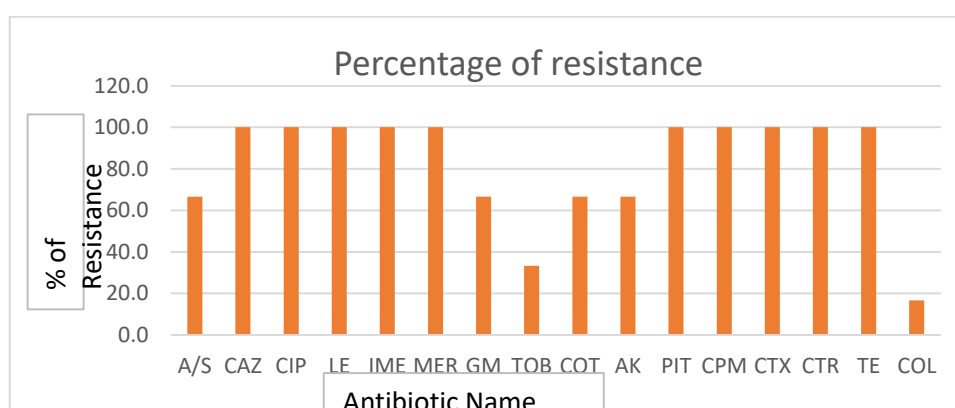


Fig 2: Antibigram of the CRAB isolates. COL - Colistin; TE - Tetracycline; A/S - Ampicillin sulbactam; CAZ – Ceftazidime; CIP - Ciprofloxacin, LE- Levofloxacin; GM – Gentamycin; AK –Amikacin; TOB – Tobramycin; IME – Imepenenem; MER – Meropenem; PIT - Piperacillin tazobactam; CPM – Cefepime; CTX - Cefotaxim, CTR – Ceftriaxone.

3.3 Screening for Genes Associated with Antibiotic Resistance

Four Carbapenem resistant isolates (FEA1, FEA2, FEA3 and FEA4) of *A. baumannii* were subjected to molecular level analysis for the presence of antibiotic resistance associated genes. Screening for the presence of five genes (*OXA23*,

OXA58, *IMP*, *VIM*, *GIM*) were observed in all four CRAB isolates. (Table2 & Fig 3). All the four isolates were screened with positive results in the presence of *OXA28* and *GIM* genes. Among these isolates, *OXA-23* was also observed as a predominant gene followed by other isolates. *IMP* and *VIM* were only present in one isolate.

| Table 2: Antibiotic Resistance genes screened from the CRAB isolates | | | | |
|--|-------|------|------|------|
| Gene | FEA 1 | FEA2 | FEA3 | FEA4 |
| <i>OXA23</i> | - | + | + | + |
| <i>OXA58</i> | + | + | + | + |
| <i>IMP</i> | + | - | - | - |
| <i>VIM</i> | + | - | - | - |
| <i>GIM</i> | + | + | + | + |

Table 2: Four CRAB isolates (FEA1, FEA2, FEA3 and FEA4) and the presence (+) or absence (-) of particular gene

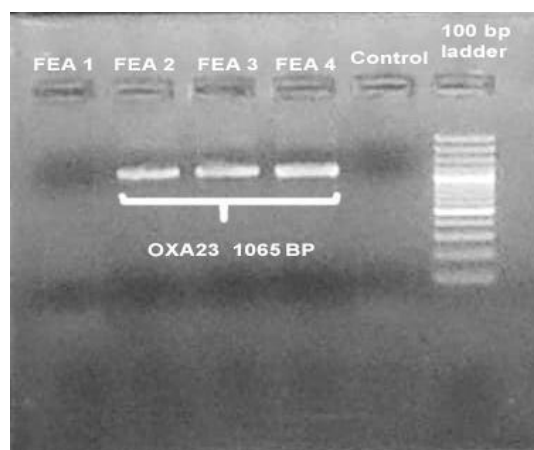


Fig 3. a: Gel image of *OXA23* gene presence of 1065 band revealed that the stains/isolates are +ve for *OXA23* genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder.

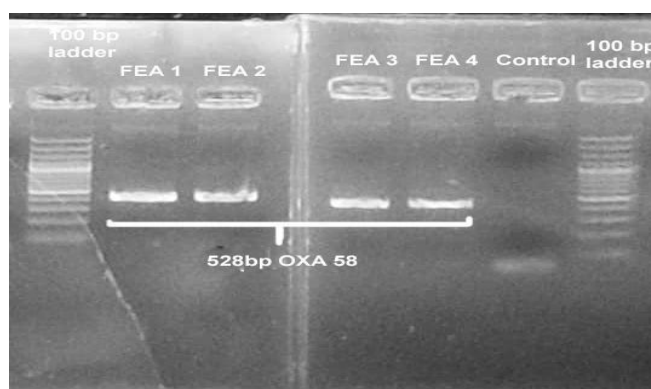


Fig 3. b: Gel image of *OXA58* gene presence of 528 band revealed that the stains/isolates are +ve for *OXA58* genotype lane 2 to 5: isolates 1 to 4; lane 6: -ve control; lane 1 & 6:100 bp ladder.

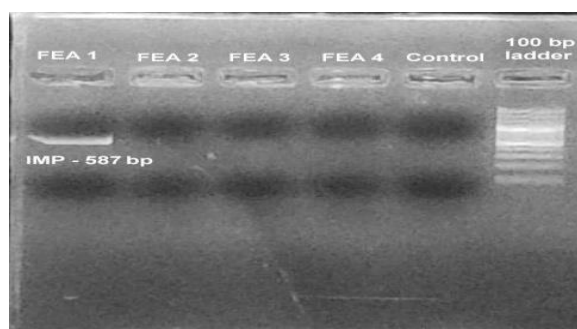


Fig 3. c: Gel image of *IMP* gene presence of 587 band revealed that the stains/isolates are +ve for *IMP* genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder:

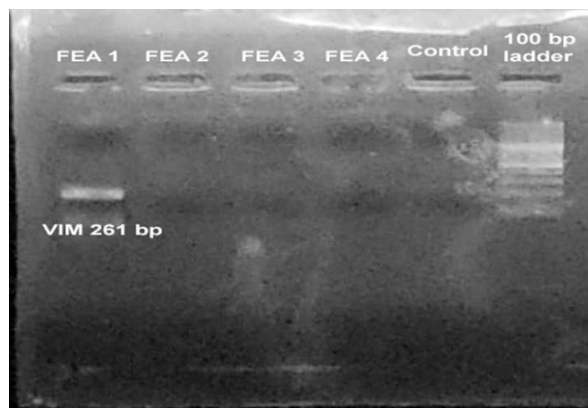


Fig 3. d: Gel image of VIM gene presence of 261 band revealed that the stains/isolates are +ve for VIM genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder



Fig 3. e: Gel image of GIM gene presence of 762 band revealed that the stains/isolates are +ve for GIM genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder.

4 DISCUSSION

This organism has been reported as the most frequent cause of respiratory tract infections and the strains were isolated from 3 to 5% of patients with nosocomial pneumonia. In our study, about 5% of the isolates of *A. baumannii* were from tracheal tube aspiration, and mechanical ventilation was the most important risk factor for these infections. Our present study was confirmed with the work done by Rit and his colleagues in 2012²⁸, they observed *A.baumannii* as the prevalent species. Nearly 75% of the isolates were *A. baumannii* and only 25% of isolates were other types of *Acinetobacter*. About 74% of the isolates of this study was *A baumannii* and which is in accordance with Rit et al., 2012²⁸. Momtaz et al. (2015)²⁹ reported that the *A.baumannii* strains were detected in 121 out of 500 human clinical samples (24.2%) which was lower than our results. It is reported³⁰ in 2012 that the prevalence of *A. baumannii* in various types of clinical infections of 9.4% which was very lower than our results. Siau et al. (1996)³¹ reported that the prevalence of *A. baumannii* in the cases of infections in the Korean hospitals was 11% which was lower than our results. We observed the presence of *A. junii* and a total of 5% of the isolates were *A junii*. Previous reports also showed the presence of *A.junii* in clinical specimens³². *Acinetobacter* spp., are rapidly spreading with emergence of extended resistance to even newer antimicrobials. In this study we observed a high rate (15% of the isolates) of carbapenem resistance among the isolates and this finding is comparable with previous studies conducted by Ayenew et al., 2021 in Ethiopia³³. Our results suggest that the carrying OXA-23 genes is one of the main causes of the carbapenem resistance

phenotype, which is consistent with the previous reports^{34,36}. In a study conducted³⁷ in 2018, it was reported that OXA-23 is a prevalent mechanism for sulbactam resistance in *A. baumannii*. In our present results were also in accordance with their results. A high rate (67%) of sulbactam resistance was observed among the isolates with OXA 23 genes. Previous reports showed that OXA-58 gene as a major reason for carbapenem resistance^{38, 39, 40} and it is similar to our results. All the screened carbapenem resistant isolates were positive for the presence of OXA-58 gene. In this study GIM gene was also a predominant one in CRAB isolates and presence of VIM gene is comparatively low in the screened isolates, only one isolate was positive for VIM gene. But this is in contrast with the previous study⁴¹. In their study VIM (27.45%) was the predominant gene than GIM (11.76%) gene.

5 CONCLUSION

The spread of *Acinetobacter* infections are related to several factors like duration of antibiotic usage, patient co-morbidities, virulence of bacteria, etc. The genomic studies revealed important factors of *Acinetobacter* which are contributing to the survival on the hospital settings and pathogenicity of *Acinetobacter baumannii*. Knowledge regarding such factors are important in preparing proper prevention protocols. A local antibiogram pattern database preparation and implementation of antibiotic stewardship based on the antibiogram might help in prevention of development of antibiotic resistance. Proper disinfection protocol development based on the virulence mechanism of the bacteria can help to restrict the *Acinetobacter* infection spread. Hence this study strongly recommends

implementation of proper sterilization protocol and antibiotic stewardship along with the continuous surveillance of spread.

6 ACKNOWLEDGMENT

The authors acknowledge the management and staffs of Sunrise Institute of Medical Sciences and staff members of MR Government Arts College, Mannargudi, Thiruvavur District, Tamil Nadu, India.

7 AUTHOR CONTRIBUTION STATEMENT

Ms. Fiji E conceived and planned the experiments. Fiji E and Mr. Jijo G Vaghese carried out the experiments with the support from Dr. B. Anandharaj. All authors discussed the results and contributed to the final manuscript. Dr. B. Anandharaj supervised the entire project.

8 CONFLICT OF INTEREST

Conflict of interest declared none.

9 REFERENCES

1. Peleg AY, Jara S, Monga D, Eliopoulos GM, Moellering RC, Mylonakis E. Galleria Mellonella As A Model System To Study Acinetobacter Baumannii Pathogenesis And Therapeutics. *Antimicrob Agents Chemother.* 2009;53(6):2605-9. Doi: 10.1128/AAC.01533-08. PMID 19332683.
2. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical And Pathophysiological Overview Of Acinetobacter Infections: A Century Of Challenges. *Clin Microbiol Rev.* 2017;30(1):409-47. Doi: 10.1128/CMR.00058-16, PMID 27974412.
3. Al Atrouni A, Joly-Guillou ML, Hamze M, Kempf M. Reservoirs Of Non-Baumannii Acinetobacter Species. *Front Microbiol.* 2016 Feb 1;7(49):49. Doi: 10.3389/Fmicb.2016.00049, PMID 26870013.
4. Antunes LCS, Visca P, Towner KJ. Acinetobacter Baumannii: Evolution Of A Global Pathogen. *Pathog Dis.* 2014;71(3):292-301. Doi: 10.1111/2049-632X.12125, PMID 24376225.
5. Roca I, Espinal P, Vila-Farrés X, Vila J. The Acinetobacter Baumannii Oxymoron: Commensal Hospital Dweller Turned Pan-Drug-Resistant Menace. *Front Microbiol.* 2012;3:148. Doi: 10.3389/Fmicb.2012.00148, PMID 22536199.
6. Tak-Chiu WU. Carbapenem-Resistant Or Multidrug Resistant Acinetobacter Baumannii – A Clinician's Perspective. *The Hong Kong. Med. Diar.* 2011;16(4):6-9.
7. Bergogne-Bérézin E, Towner KJ. Acinetobacter Spp. As Nosocomial Pathogens: Microbiological, Clinical, And Epidemiological Features. *Clin Microbiol Rev.* 1996;9(2):148-65. Doi: 10.1128/CMR.9.2.148, PMID 8964033.
8. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial Bloodstream Infections In US Hospitals: Analysis Of 24,179 Cases From A Prospective Nationwide Surveillance Study. *Clin Infect Dis.* 2004;39(3):309-17. Doi: 10.1086/421946, PMID 15306996.
9. Seifert H, Strate A, Pulverer G. Nosocomial Bacteremia Due To Acinetobacter Baumannii. Clinical Features, Epidemiology, And Predictors Of Mortality. *Med (Baltim).* 1995;74(6):340-9. Doi: 10.1097/00005792-199511000-00004, PMID 7500897.
10. Cisneros JM, Reyes MJ, Pachón J, Becerril B, Caballero FJ, García-Garmendia JL Et Al. Bacteremia Due To Acinetobacter Baumannii: Epidemiology, Clinical Findings, And Prognostic Features. *Clin Infect Dis.* 1996;22(6):1026-32. Doi: 10.1093/Clinids/22.6.1026, PMID 8783704.
11. Jang TN, Lee SH, Huang CH, Lee CL, Chen WY. Risk Factors And Impact Of Nosocomial Acinetobacter Baumannii Bloodstream Infections In The Adult Intensive Care Unit: A Case-Control Study. *J Hosp Infect.* 2009;73(2):143-50. Doi: 10.1016/J.jhin.2009.06.007, PMID 19716203.
12. Jung JY, Park MS, Kim SE, Park BH, Son JY, Kim EY Et Al. Risk Factors For Multi-Drug Resistant Acinetobacter Baumannii Bacteremia In Patients With Colonization In The Intensive Care Unit. *BMC Infect Dis.* 2010;10:228. Doi: 10.1186/1471-2334-10-228, PMID 20670453.
13. Fournier PE, Richet H. The Epidemiology And Control Of Acinetobacter Baumannii In Health Care Facilities. *Clin Infect Dis.* 2006;42(5):692-9. Doi: 10.1086/500202, PMID 16447117.
14. Lisa LM, Trish MP. Acinetobacter Baumannii. *Epidemiol Antimicrob Resist Treat Options Antimicrob Resist.* 2008;46:1254-63.
15. Stahl J, Bergmann H, Göttig S, Ebersberger I, Averhoff B. Acinetobacter Baumannii Virulence Is Mediated By The Concerted Action Of Three Phospholipases D. *PLOS ONE.* 2015;10(9):E0138360. Doi: 10.1371/Journal.Pone.0138360, PMID 26379240.
16. Chang Y, Luan G, Xu Y, Wang Y, Shen M, Zhang C Et Al. Characterization Of Carbapenem-Resistant Acinetobacter Baumannii Isolates In A Chinese Teaching Hospital. *Front Microbiol.* 2015;6:910. Doi: 10.3389/Fmicb.2015.00910, PMID 26388854.
17. Hassan KA, Liu Q, Henderson PJ, Paulsen IT. Homologs Of The Acinetobacter Baumannii Acei Transporter Represent A New Family Of Bacterial Multidrug Efflux Systems. *Mbio.* 2015;6(1):E01982-14. Doi: 10.1128/Mbio.01982-14, PMID 25670776.
18. Huang Y, Zhou Q, Wang W, Huang Q, Liao J, Li J, Et Al. Acinetobacter Baumannii Ventilator-Associated Pneumonia: Clinical Efficacy Of Combined Antimicrobial Therapy And In Vitro Drug Sensitivity Test Results. *Front Pharmacol.* 2019;10:92. Doi: 10.3389/Fphar.2019.00092, PMID 30814950.
19. Henwood CJ, Gatward T, Warner M, James D, Stockdale MW, Spence RP Et Al. Antibiotic Resistance Among Clinical Isolates Of Acinetobacter In The UK, And In Vitro Evaluation Of Tigecycline (GAR-936). *J Antimicrob Chemother.* 2002 Mar;49(3):479-87. Doi: 10.1093/Jac/49.3.479, PMID 11864948.
20. Quale J, Bratu S, Landman D, Heddurshetti R. Molecular Epidemiology And Mechanisms Of Carbapenem Resistance In Acinetobacter Baumannii Endemic In New York City. *Clin Infect Dis.* 2003;37(2):214-20. Doi: 10.1086/375821, PMID 12856214.
21. Van Looveren M, Goossens H, ARPAC Steering Group. Antimicrobial Resistance Of Acinetobacter Spp. In Europe. *Clin Microbiol Infect.* 2004, Antimicrobial Resistance Of Acinetobacter Spp. In Europe;10(8):684-704. Doi: 10.1111/J.1469-0691.2004.00942.X, PMID 15301671.

22. Coelho JM, Turton JF, Kaufmann ME, Glover J, Woodford N, Warner M Et Al. Occurrence Of Carbapenem-Resistant *Acinetobacter Baumannii* Clones At Multiple Hospitals In London And Southeast England. *J Clin Microbiol.* 2006 Oct;44(10):3623-7. Doi: 10.1128/JCM.00699-06, PMID 17021090.
23. Bou G, Oliver A, Martínez-Beltrán J. OXA-24, A Novel Class D Beta-Lactamase With Carbapenemase Activity In An *Acinetobacter Baumannii* Clinical Strain. *Antimicrob Agents Chemother.* 2000;44(6):1556-61. Doi: 10.1128/AAC.44.6.1556-1561.2000, PMID 10817708.
24. Poirel L, Lebossi E, Héritier C, Patsoura A, Foustoukou M, Nordmann P. Nosocomial Spread Of OXA-58-Positive Carbapenem-Resistant *Acinetobacter Baumannii* Isolates In A Paediatric Hospital In Greece. *Clin Microbiol Infect.* 2006;12(11):1138-41, ISSN 1198-743X. Doi: 10.1111/j.1469-0691.2006.01537.X, PMID 17002616.
25. Miller J, Miller S. A Guide To Specimen Management In Clinical Microbiology. ASM Press; 2017. Doi: 10.1128/9781555819620.
26. Clinical And Laboratory Standards Institute (CLSI). Performance Standards For Antimicrobial Susceptibility Testing. 30th Ed. CLSI Supplement M100. Wayne,PA: CLSI; 2020.
27. Valenzuela JK, Thomas L, Partridge SR, Van Der Reijden T, Dijkshoorn L, Iredell J. Horizontal Gene Transfer In A Polyclonal Outbreak Of Carbapenem-Resistant *Acinetobacter Baumannii*. *J Clin Microbiol.* 2007;45(2):453-60. Doi: 10.1128/JCM.01971-06, PMID 17108068.
28. Rit K, Saha R. Multidrug-Resistant *Acinetobacter* Infection And Their Susceptibility Patterns In A Tertiary Care Hospital. *Niger Med J.* 2012;53(3):126-8. Doi: 10.4103/0300-1652.104379, PMID 23293410.
29. Momtaz H, Seifati SM, Tavakol M. Determining The Prevalence And Detection Of The Most Prevalent Virulence Genes In *Acinetobacter Baumannii* Isolated From Hospital Infections. *Int J Med Lab;2(2):87-97* (201).
30. Jaggi N. *Acinetobacter Baumannii* Isolates In A Tertiary Care Hospital: Antimicrobial Resistance And Clinical Significance. *J Microbiol Infect Dis.* 2012;2(2):57-63. Doi: 10.5799/Ahinjs.02.2012.02.0043.
31. Siau H, Yuen KY, Wong SSY, Ho PL, Luk WK. The Epidemiology Of *Acinetobacter* Infections In Hong Kong. *J Med Microbiol.* 1996;44(5):340-7. Doi: 10.1099/00222615-44-5-340, PMID 8636948.
32. Linde H-J, Hahn J, Holler E, Reischl U, Lehn N. Septicemia Due To *Acinetobacter Junii*. *J Clin Microbiol.* 2002;40(7):2696-7. Doi: 10.1128/JCM.40.7.2696-2697.2002, PMID 12089313.
33. Ayenew Z, Tigabu E, Syoum E, Ebrahim S, Assefa D, Tsige E. Multidrug Resistance Pattern Of *Acinetobacter* Species Isolated From Clinical Specimens Referred To The Ethiopian Public Health Institute: 2014 To 2018 Trend Analysis. *PLOS ONE.* 2021;16(4):E0250896. Doi: 10.1371/journal.pone.0250896, PMID 33914829.
34. Zowawi HM, Sartor AL, Sidjabat HE, Balkhy HH, Walsh TR, Al Johani SM Et Al. Molecular Epidemiology Of Carbapenem-Resistant *Acinetobacter Baumannii* Isolates In The Gulf Cooperation Council States: Dominance Of OXA-23-Type Producers. *J Clin Microbiol.* 2015;53(3):896-903. Doi: 10.1128/JCM.02784-14, PMID 25568439.
35. Chang Y, Luan G, Xu Y, Wang Y, Shen M, Zhang C Et Al. Characterization Of Carbapenem-Resistant *Acinetobacter Baumannii* Isolates In A Chinese Teaching Hospital. *Front Microbiol.* 2015;6:910. Doi: 10.3389/fmicb.2015.00910, PMID 26388854.
36. Zhao Y, Hu K, Zhang J, Guo Y, Fan Xuecai, Wang Y Et Al. Outbreak Of Carbapenem-Resistant *Acinetobacter Baumannii* Carrying The Carbapenemase OXA-23 In ICU Of The Eastern Heilongjiang Province, China. *BMC Infect Dis.* 2019;19(1):452. Doi: 10.1186/S12879-019-4073-5, PMID 31113374.
37. Yang Y, Xu Q, Li T, Fu Y, Shi Y, Lan P Et Al. OXA-23 Is A Prevalent Mechanism Contributing To Sulbactam Resistance In Diverse *Acinetobacter Baumannii* Clinical Strains. *Antimicrob Agents Chemother.* 2019;63(1). Doi: 10.1128/AAC.01676-18. PMID 30348663.
38. Rahbar M, Hajia M. Detection And Quantitation Of The Etiologic Agents Of Ventilator-Associated Pneumonia In Endotracheal Tube Aspirates From Patients In Iran. *Infect Control Hosp Epidemiol.* 2006;27(8):884-5. Doi: 10.1086/506410, PMID 16874653.
39. Marqué S, Poirel L, Héritier C, Brisse S, Blasco MD, Filip R, Et Al. Regional Occurrence Of Plasmid-Mediated Carbapenem-Hydrolyzing Oxacillinase OXA-58 In *Acinetobacter* Spp. In Europe. *J Clin Microbiol.* 2005;43(9):4885-8. Doi: 10.1128/JCM.43.9.4885-4888.2005, PMID 16145167.
40. Pournaras S, Markogiannakis A, Ikonomidis A, Kondyli L, Bethimouti K, Maniatis AN, Et Al. Outbreak Of Multiple Clones Of Imipenem-Resistant *Acinetobacter Baumannii* Isolates Expressing OXA-58 Carbapenemase In An Intensive Care Unit. *J Antimicrob Chemother.* 2006;57(3):557-61. Doi: 10.1093/Jac/Dkl004, PMID 16431857.
41. Liu WJ, Fu L, Huang M, Zhang JP, Wu Y, Zhou YS Et Al. Frequency Of Antiseptic Resistance Genes And Reduced Susceptibility To Biocides In Carbapenem-Resistant *Acinetobacter Baumannii*. *J Med Microbiol.* 2017;66(1):13-7. Doi: 10.1099/Jmm.0.000403, PMID 27930267.