Detection and characterization of antibiotic resistant and Biofilm producing clinical isolates of *Acinetobacter baumannii.*

By

JARIFA ALAM

ID: 19126062

RUHAMA BINTE FARUK

ID: 19126063

KAMRUL HASAN ANIK

ID: 19126061

A thesis submitted to the Department of Mathematics and Natural Sciences (MNS) in partial fulfilment of the requirements for the degree of

BSc. in Microbiology

Mathematics and Natural Sciences (MNS)

BRAC University

August 2024

Declaration:

It is hereby declared that

1. The thesis submitted is my own original while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

JARIFA ALAM
ID: 19126062

RUHAMA BINTE FARUK
ID: 19126063

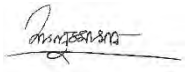
KAMRUL HASAN ANIK
ID: 19126061

Approval:

The thesis titled "Detection and characterization of antibiotic resistant pattern and Biofilm producing clinical isolates of *Acinetobacter baumannii*" submitted by Jarifa Alam (19126062), Ruhama Binte faruk (19126063), Kamrul Hasan Anik (19126061) has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Microbiology in April 2024.

Examining Committee:

Supervisor:
(Member).



Md. Hasanuzzaman
Senior Lecturer,
Department of Mathematics and Natural Sciences
BRAC University

Co-supervisor:
(Member).

Akash Ahmed
Senior Lecturer,
Department of Mathematics and Natural Sciences
BRAC University

Program Coordinator:
(Member).

Dr. Nadia Sultana Deen
Associate Professor,
Department of Mathematics and Natural Sciences
BRAC University

Departmental Head:
(Chair).

Md. Firoze H. Haque, PhD
Associate Professor and Chairperson,
Department of Mathematics and Natural Sciences,
Brac University, Dhaka, Bangladesh

Abstract:

Acinetobacter baumannii is an opportunistic pathogen that affects persons with weakened immune systems and causes nosocomial infections due to its antibiotic resistance. Because of the global proliferation of multidrug resistance bacteria and biofilm development-ability to survive on dry surfaces aid in proliferation in a variety of healthcare environments, this study focuses on identifying the relevant gene for biofilm development and antibiotic resistance. *Acinetobacter baumannii* clinical isolates (150) were recovered from BSMMU in Dhaka. For *A. baumannii* pure strain identification; Biochemical tests and PCR (Polymerase chain reaction) were performed and validated using Agarose gel-electrophoresis. After verifying pure strains, the antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method and biofilm development was assessed using the 96-well microtiter plate method. Furthermore, PCR was used to screen both the carbapenem resistance gene and the biofilm generating gene. Out of 150 isolates, 109 were verified true positive with PCR analysis, enforcing additional research. AST was performed with medicines from several groups, the majority of them were MDR and mostly, Carbapenem resistant. PCR was performed for screening both MDR gene and biofilm producing gene, with 15.59% NDM gene being positive and the others yielding no significant results. Biofilm-related genes such as *bap, bla*$_{PER-1}$*, csuE, and ompA* were reported to be frequent at 88.98%, 56.88%, 84.40%, and 83.48%, respectively, in all biofilm generating isolates (24.77%) strong, (33.02%) moderate, and (10.09%) weak, (31.19) % non-biofilm forming. Our study focused on the prevalence and antibiotic -resistant pattern of the MDR *A. baumannii*, which is more common in clinical isolates, as well as biofilm producing capability and Biofilm-forming genes. It appears that effective surveillance and control actions are required to halt the CRAB outbreak in our country and healthcare settings.

Keywords: *Acinetobacter baumannii*, PCR, Multidrug resistance, Carbapenem resistant gene, Biofilm formation. Biofilm forming gene.

Dedicated to

Our Parents
&
Md. Hasanuzzaman sir.

Acknowledgment:

We would like to begin by expressing our gratitude to Almighty Allah for providing us with the opportunity and strength to complete this research. We acknowledge our esteemed Professor Md.Firoze H. Haque, and Nadia Sultana Deen, Program Coordinator of the Microbiology program for all of their assistance, kindness, and guidance.

Our regards, gratitude, indebtedness, and appreciation go to our respected supervisor Md Hasanuzzaman, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his constant supervision, untiring advice and guidance during our thesis research.

Our sincere appreciation goes out to Laboratory Officers Md. Mahmudul Hasan, Shamima Akhter Chowdhury for their unwavering kindness and advice. Additionally, we would like to express our gratitude to Nishat Tasnim Ananna, Srabon Deb Nath, Nasiha Jahan Muna, Joya Prottasha Das, Jahid Hasan Tushar, Aziz Hossain Shovon, Aditya Banik for all their moral support and help throughout this challenging journey.

Table of Contents :

List of figures:

List of Tables:

List of Abbreviation :

1. ABR	Antibiotic Resistance
1. AMR	Antimicrobial resistance
2. AST	Antimicrobial susceptibility test
3. BAL	Bronchoalveolar lavage
4. BAP	Biofilm associated protein
5. BL	Beta lactamase
6. BP	Base pair
7. CLSI	Clinical and Laboratory Standards Institute
8. CPS	Capsular exo-polysaccharide.
9. CRA	Congo Red Agar
10. CSU	Chaperon-usher pilus
11. DNA	Deoxyribonucleic acid
12. EDTA	Ethylenediamine tetra-acetic acid
13. EPS	Extracellular polymeric substance.
14. ESBL	Extended Spectrum. Beta-Lactamase
15. HAI	Hospital associated Infection
16. ICU	Intensive Care Unit
17. KPC	Klebsiella pneumoniae carbapenemase
18. LB	Luria-Bertani
19. LPS	Lipopolysaccharide
20. MCT	Micro centrifuge tube
21. MDR	Multi Drug Resistant
22. MHA	Mueller Hinton Agar
23. MR	Methyl Red
24. NA	Nutrient Agar
25. NDM	New Delhi metallo beta lactamase
26. OD	Optical Density

27. OMPA	Outer membrane protein
28. PCR	Polymerase Chain Reaction
29. PDR	Pan Drug Resistance
30. QS	Quorum-sensing
31. RNA	Ribonucleic acid
32. RPM	Revolutions per minute
33. SD	Standard deviation
34. SDS	Sodium dodecyl sulfate
35. TBE	Tris Borate EDTA
36. TCP	Tissue Culture plate
37. TE	Tris EDTA
38. TSB	Tryptic Soy Broth
39. TSI	Triple Sugar Iron
40. UTI	Urinary tract Infection
41. UV	Ultra Violet
42. VP	Voges Proskauer
43. XDR	Extensive Drug Resistance

Introduction:

Acinetobacter baumannii is a gram-negative, aerobic, pleomorphic, non-motile Gram-negative bacilli. In recent years, it has been stated as a "red alert" human pathogen, causing concern among medical professionals due to its broad antibiotic resistance spectrum and susceptible hospitalized patients being the primary target for the pathogen. It is one of the most challenging infections for health-care facilities globally. Research indicates that *Acinetobacter baumannii* is responsible for around 12% of hospital-acquired illnesses globally.(Howard et al., 2012). They have been thoroughly investigated in part because they account for at least 65% of all human infections, with a high prevalence in device-related transmission, on body surfaces causing respiratory infections, especially in patients on ventilation devices, urinary tract infections, bloodstream infections, skin infections, and so on. Also its clinical significance in the last 15 years has been fueled by its exceptional ability to acquire resistant determinants, making it one of the most challenging organisms from the current antibiotic era (Howard et al., 2012; Raut et al., 2020; Valcek et al., n.d.).

A recent investigation indicated the ability of *A. baumannii* to rapidly develop resistance to numerous antimicrobials, resulting in the establishment of strains that are resistant to multiple drugs (McConnell et al., 2013). The majority of *A. baumannii* infections are induced by healthcare equipment or interaction with someone who has been exposed to the bacterium from another affected patient. *A. baumannii* features a specialized arsenal of virulence factors that generate physiological benefits at various stages of pathogenesis, beginning with host immune response survival and proceeding to host cell adhesion, internalization, and apoptosis. (Shadan et al., 2023).

This ability to develop resistance facilitates *Acinetobacter baumannii* to persist for extended periods of time in the hospital environment. Basically, increases duration of hospital stay and increase health care expenses. The 2013 antimicrobial resistance report from the Centres for Disease Control classified multi-drug resistant *Acinetobacter* as a "Serious" issue. Even though *Acinetobacter* is not virulent on its own, unwell individuals with multiorgan disease have significant rates of *Acinetobacter* morbidity and fatality.(Wong et al., 2017).

The ongoing changes in the worldwide climate that have arisen over the past several decades as a consequence of multiple human interventions (e.g., the effects of global warming) could result in changes in the epidemiology of community-based *Acinetobacter* infections, increasing the number of cases in other regions of the earth. Worldwide-recognized multidrug-resistant *Acinetobacter baumannii* is transferring to many civilian hospitals by cross-infection of previously infected patients and soldiers. These days, *A. baumannii* strains that are resistant to multiple drugs (MDR) are commonplace around the globe. The Mediterranean area has been shown to have the highest rate of

carbapenem resistance, at over 90%. (Ma & McClean, 2021). Unfortunately, the lack of efficacious treatments has led to high crude mortality ranging from 40% to 80% for infections occurring in sterile sites. (Inchai et al., 2015). It is predicted that the propagation of illness will end up resulting in around 300 million tragic deaths by 2050. (Howard et al., 2012). Up to 70% fatality rates have been recorded in cases of infections caused by resistant strains of *Acinetobacter baumannii*. Although the attributable mortality varies with infection type, prior research showed that the crude mortality rate of *A. baumannii* infection was over 50%. For instance, a multicentre study carried out in eight US metropolitan areas between 2012 and 2015 found that the overall recorded death rate from carbapenem-resistant *A. baumannii* infections was 17.9%; significantly higher mortality rates—41.3%—occurred from infections that occurred at normally sterile sites as opposed to UTIs (8.3%). (Bulens et al., 2018).
One of the main causes of VAP globally, particularly in Asia, Latin America, and the Middle East, is *A. baumannii*(Lynch et al., 2017). Up to 84.3% of VAP cases caused by MDR *A. baumannii* have been reported to die in the intensive care unit(Wisplinghoff et al., 2012). In the United States, individuals with *A. baumannii* bacteraemia had a crude death rate ranging from 37% to 52% (Inchai et al., 2015). While *A. baumannii* meningitis is relatively uncommon, its fatality rate is about 70%, making it a growing risk for patients who have had neurosurgery.(Metan et al., 2007). Following natural catastrophes or wars, such as those that occurred during the Iraq conflicts, the Syrian war, the Wenchuan earthquake, the Marmara earthquake, and the Indian Ocean tsunami, cases of *A. baumannii* skin and soft tissue infections in trauma (wound, burn) patients have also been documented (Diaz, 2016; Oncül et al., 2002; Rafei et al., 2014; Scott et al., 2007). *A. baumannii* was responsible for 14% of wound infections in hospitalised survivors following the Wenchuan earthquake, per a single site investigation(Tao et al., 2009). Notably, hospital-acquired co- or secondary infections in COVID-19 in-patients caused by *A. baumannii* were recorded globally during the Coronavirus illness 2019 (COVID-19) pandemic (Lai et al., 2020; Perez et al., 2020; Sharifipour et al., 2020).

Acinetobacter baumannii is a serious human pathogen that is drawing greater consideration as an issue of public health. It is responsible for an enormous percentage of infections among particular patient groups, primarily critically ill individuals acquiring treatment in the ICU worldwide. In the past few decades, there are major changes in the global epidemiology of *Acinetobacter spp*. Infections. Member of this genus have been recognized as extremely uncommon infectious agents throughout the 1970s, but in the past several years, it has occurred a rise in the frequency of reports of *Acinetobacter* infections, specifically in intensive care units (ICU). Infections caused by Acinetobacter typically occur in four particular populations and settings: (i) infections obtained by patients admitted to ICUs;

(ii)infections associated with healthcare received throughout the ICU setting; and (iii) outbreaks in trauma patients, often after natural disasters such as earthquakes or war epidemics, (iv) also usually followed by community-acquired illnesses, primarily pneumonia, but also bacteraemia, cellulitis, and meningitis, which typically afflict patients with comorbidities in tropical and subtropical settings. Most of these stories originate from tropical or subtropical areas. It has also been proposed that the humid atmosphere in these places contributes people, particularly those with the previously mentioned multiple illnesses, to *Acinetobacter* infections(Anstey et al., 1992).. In recent years, it seems to have an increase in the total number of patients who developed nosocomial *Acinetobacter* infections other than the ICU. The majority of those affected have respiratory-related infections, while some of them have bacterial infection with an uncertain primary location (Ferrara, 2006) (Wisplinghoff et al., 2004). The potential for this pathogen to become a major cause of healthcare-associated infections outside the ICU setting is alarming, thereby expanding the patient population at risk. *A. Baumannii* isolates from patients in intensive care units in the United States, the Netherlands, and the Nordic nations have shown less broad antibiotic resistance patterns (Friedland et al., 2003).

Determining whether this organism is the pathogen causing an infection of interest can be challenging, especially in patients with non-sterile localised infections like pneumonia and wound infections, as it frequently infects patients with comorbidities, antibiotic exposure, and recent hospitalisation (Chusri et al., 2019). *Acinetobacter baumannii* infections have been reported worldwide and are becoming more frequent. In the ICU of the USA and Europe, it is the reason behind 2–10% of all gram-negative infections (Gootz & Marra, 2008). *Acinetobacter baumannii* exhibits resistance to antibiotics through a variety of mechanisms, such as a waterproof cell membrane, an increase in outflow pumps, metallo-β-lactamases (MBL), extended spectrum β-lactamases (ESBL), and carbapenem-hydrolyzing class D β-lactamases (CHDL)(Poirel & Nordmann, 2006; Vahhabi et al., 2021).

During the last few years, increasingly resistant strains of *Acinetobacter baumannii* have emerged, causing necessity of broad-spectrum antibiotics. Inadequate antibiotic medication management frequently results in the establishment of widespread extensively drug resistant and pan-drug resistance (XDR and PDR) bacteria, which provide considerable health difficulties by prolonged hospitalization, treatment failures, and that is associated with a significant mortality and morbidity rate in sick individuals. Being resistant to practically all antimicrobial agents at least once is known as extensive drug resistance (XDR). A person who is resistant to all antimicrobial classes is said to have pan-drug resistance (PDR). (*Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria:*

An International Expert Proposal for Interim Standard Definitions for Acquired Resistance - PubMed, n.d.) (Pattnaik et al., 2019).

Public health is currently facing a major issue as a result of *A. baumannii's* recent fast development of various antibiotic resistance. Because of its capacity to create biofilms, *Acinetobacter* is able to thrive and spread readily inside the hospital setting, attaching itself to a variety of biotic and abiotic surfaces such as Foley's catheter, vascular catheters, and cerebrospinal fluid shunts.*8 A. baumannii* is the most often found opportunistic pathogen in clinical samples. It may colonise hospital environments and acquire resistance, which makes it a risk factor for nosocomial infections, which are challenging to treat (Kasperski et al., 2023). The connection between biofilms and antibiotic resistance is of a considerable interest to biomedical researchers.

Planktonic (free-floating) bacteria are not physiologically similar to biofilms, which are colonies of microorganisms adhering to biotic and/or abiotic surfaces coated in an extracellular polymeric substance (EPS) matrix('Biofilm Formation by *Enterococcus Faecalis* and *Enterococcus Faecium*', 2019). Because the extracellular matrix protects the cells and limits their metabolic activity, biofilm-encased cells are more resistant to innate immune components of the host and antibiotics(Mahmoudi Monfared et al., 2019). The clinical management of *A. baumannii*-related biofilm infections is severely hampered by the fact that the bacteria commonly cause biofilm-related infections, especially ventilator-associated pneumonia and catheter-related infections, which can be extremely resistant to antibiotic therapy. Due to the fast spread of diseases linked to medical devices and antibiotic resistance, *A. baumannii* biofilms have emerged as one of the most significant worldwide concerns (Dijkshoorn et al., 2007; Pour et al., 2011).

Acinetobacter baumannii is an established globally antimicrobial-resistant gram-negative bacteria that particularly enables biofilm-associated infectious diseases. Often via pharmaceuticals, their selective adhering ability is one of the primary causes of resistance to antibiotics. An infection caused by biofilm-producing bacteria is usually harder to treat because biofilms resist both the human immune system and antibiotics(Diaz, 2016). So, to treat this type of challenging sickness, updated vaccines, alternative antibiotics, or therapies could permanently stop the spread of this disease while improving patient health. Also, to inhibit the formation of biofilm(Høiby et al., 2011)

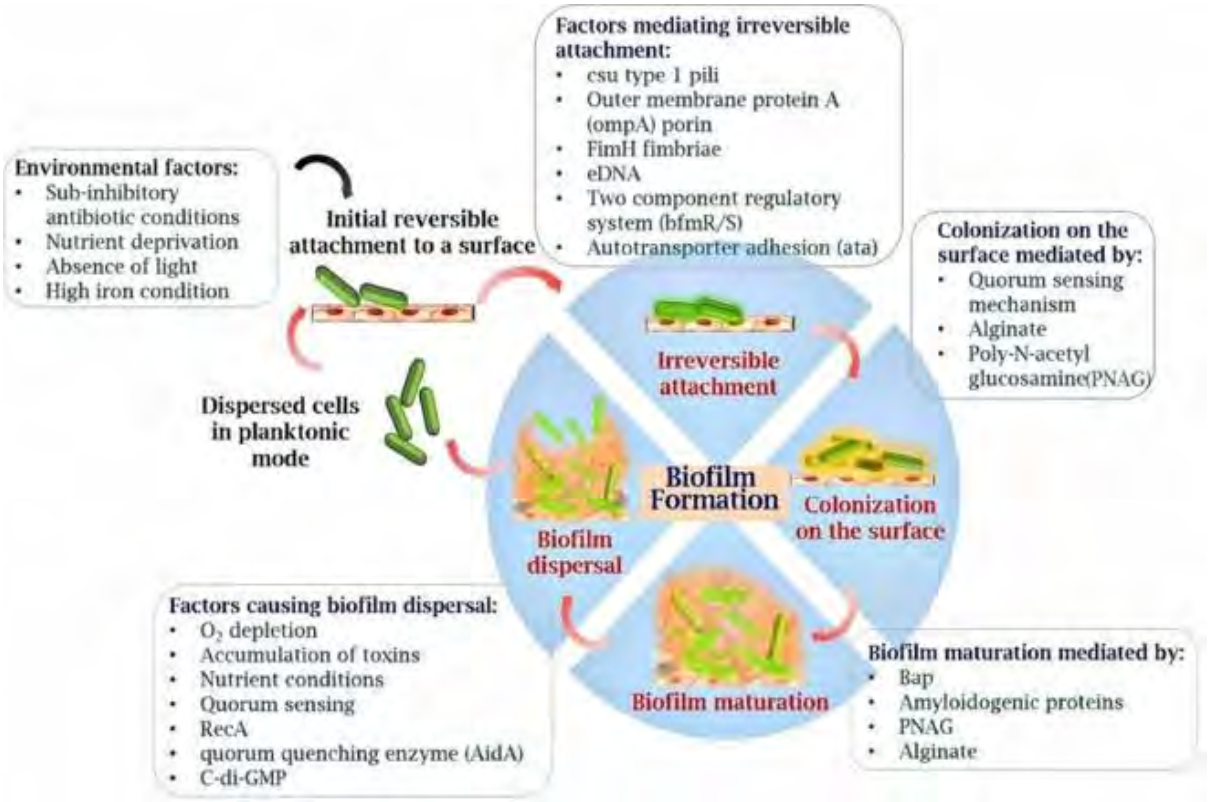


Figure 1: Factors mediating *Acinetobacter baumannii* biofilm formation. (*Factors Mediating Acinetobacter Baumannii Biofilm Formation …*, n.d.)

The growth and development of biofilm is a well-established pathogenic mechanism for such diseases(Roy et al., 2022). *Acinetobacter baumannii's* advanced biofilm machinery gives a survival benefit such as they can thrive in harsh conditions such as desiccation, antibiotic treatments, and the lack of nutrients as well as facilitating its development on various surfaces. Biofilm is a three-dimensional structure created by microbial cells that attach to biotic or abiotic surfaces as a result of a variety of physiological and environmental variables (a few of which remain unknown). Furthermore, these cells continuously grow and create extracellular polymeric substances (EPS), which form a matrix around the microorganisms. The biofilm matrix protects bacterial cells against the action of antibiotics and bacteriophages, and it also helps bacterial cells survive under extreme conditions like desiccation. It may also decrease the permeability of antibiotics to bacteria, exposing patients to MDR and possibly XDR bacteria(Kasperski et al., 2023). Not only Biofilm production helps in various Physicochemical factors such as temperature, growth media, surface hydrophobicity, pH, oxygen concentration but also some biofilm producing genes such as biofilm-associated protein *(Bap*), the outer membrane protein A (*ompA*), chaperon-usher pilus (*csuE)*, *and* bla_{per} is used to describe gene that help development and preserve *A. Baumannii* biofilms by providing multi-resistance to beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems. Carbapenemase

synthesis(Santajit et al., 2023). The functions of Biofilm producing genes are basically; *ompA* is important for *A. Baumannii's* survival and pathophysiology, forming biofilms, invading cells, regulating drug resistance, modulating immune response, and causing cell death through integration into host membranes; *bap* protein stabilizes mature biofilms, influences thickness and biovolume, and contributes to persistence in hospitals and infection. Overexpression may influence biofilm development in low iron conditions. $\underline{bla_{PER}}$: *A. Baumannii* strains exhibit resistance to cephalosporins, monobactams, carbapenems, and penicillin due to Class A β-lactamases like *blaPER-1* gene, which provides multi-resistance to beta-lactam antibiotics; *csuE* gene in *A. baumannii*, is quite significant for biofilm production and can be targeted for therapeutic and infection control, suggesting that incorrect antibiotic use can alter population behaviours and promote planktonic lifestyles.(Zeighami et al., 2019). Lastly, some other numerous microbial features such as adhesins, capsular polysaccharides, surface appendages play key role in production of biofilm as well as bacteria's survival.

In Latin America, several cases of *Acinetobacter baumannii* have been reported(Wisplinghoff et al., 2004). The situation with *Acinetobacter baumannii* CRAB is especially worrying in developing countries like Bangladesh, where hospital conditions and policies are not updated, increasing the nosocomial infection risk. The purpose of this study was to evaluate antibiotic resistance patterns of *A. baumannii* isolated from clinical isolates, largely from the ICU. In addition, clinical and molecular epidemiology of multi-drug resistant *Acinetobacter baumannii* is analysed along with the quantitative approach for biofilm production and the correlation between biofilm development and antibiotic resistance in clinically relevant isolates. Furthermore, observative analysis was initiated in order to study the potential link between the four biofilm-related genes and drug resistance by identifying *csuE ompA, bap and* $bla_{PER\text{-}1}$.

Materials and method;

Sample collection: We have selected Bangladesh Sheikh Mujib Medical University [BSMMU] previously known as Institute of post graduate medical research for our research purpose. Where Different clinical samples were isolated and collected from urine, sputum, tracheal aspirate, wound, pus and automated blood by medical personnel. From them we have collected Samples that resembles *A.baumannii* morphology via following streaking plate method in MacConkey agar plates and transported in BRAC University lab through iceboxes . In where samples were incubated stored for further identification

Sample Processing: Media plate containing desired sample were kept upside down for 24 hours at 37°C. Following incubation the plates were examined to locate cocci shape pink colonies resembling *A. baumannii* morphology Afterwards, the single colonies were chosen for streaking on MacConkey agar plates in order to isolate and grow pure *A.baumannii* colonies . Following the streaking of pure colonies agar plates were incubated for 18 to 24 hours at 37°C. After the incubation period was over biochemical test was performed for further clarification of *A.baumannii* identification.

Bacterial isolates and identification:

This following study consists of a total of 150 samples of *Acinebacter* spp., suspected to be *Acinetobacter baummannni*, that were directly collected from the BSMMU microbiology laboratory from November 1 to May 25. Specimens were accumulated on MacConkey agar and transported to the BRAC university lab by following appropriate cautions. Conventional biochemical methods such as oxidase, citrate, triple sugar iron, catalase, motility, indole, and urease production were used to identify *A. baumannii;* however, the results were not satisfactory, so few samples were left without testing.

Biochemical reactions often provide vital facts for effectively determining species of the different bacteria in a specimen. This test was established to analyze the amounts of bacterial enzymes, and that can be applied for precisely recognizing the particular kind of bacteria that produced it. There are various types of biochemical testing that shows various type of enzymatic reactions. However, for our research we had used MIU test, Citrate test, TSI test, MRVP test, Oxidase test and Catalase test etc (*Biochemical Tests for Microbial Identification*, n.d.)

MIU test: The full form of MIU is MOTILITY INDOLE UREASE TEST. Urease activity, motility, and indole synthesis was detected using the MIU TEST. *Acinetobacter baumannii* is MIU negative that basically symbolizes it is non motile, indole negative and also urease negative.

Citrate test: The citrate test evaluates if particular bacteria can metabolise citrate, meaning an organism's ability to use citrate as its only source of energy and carbon. *Acinetobacter baumannii* is citrate positive so the color will change from green to blue because it can metabolise citrate.

TSI test: The full form of TSI is Triple sugar iron agar. It is a differential media that evaluates an organism for numerous characteristics in a single evaluation. It identifies acid and gas production as the consequence of glucose, sucrose, and/or lactose fermentation, along with hydrogen sulfide formation. *Acinetobacter baumannii* is TSI negative as it does not form bubbles or cracks which indicates no gas or acid formation. It does not produce H_2S so it reduces blackening of the butt. It forms red slant and red butt which indicates no fermentation of sugars also peptone is catabolized.

MRVP test: The full acronym is Methyl Red-Voges Proskauer. This biochemical test actually consists of two separate assays. The methyl red test is used to recognize microorganisms capable of metabolizing glucose via the combination of acid fermentation route. Voges-Proskauer (VP) is a test that observes acetoin in bacterial broth cultures. *Acinetobacter baumannii* is MRVP negative. Negative MR test is indicated by a yellow color that means less acid is produced (pH is higher) from the fermentation of glucose. Additionally negative VP is indicated When the color at the top of the tube is yellow that means tested bacteria does not ferment glucose using the butanediol fermentation pathway.

Oxidase test: Oxidase test is basically done to determine the presence of oxidase enzymes generated by various bacteria. *Acinetobacter baumannii* is oxidase negative. So, it is indicated when the color does not change therefore, *Acinetobacter baumannii* do not have the cytochrome c oxidase that oxidizes the test reagent.

Catalase test: Catalase test is use to evaluate which organisms generate the enzyme catalase. *Acinetobacter baumannii* is catalase positive. Indication of catalase in following bacteria is it forms bubbles.

DNA extraction:

DNA was extracted from the colonies grown in Nutrient agar by boiling method. In a new Eppendorf 150 micro-liter 1XTE buffer had been inoculated with a loop full of colony. Then, boiled using a dry water bath for 15 minutes at 95° C. After that, the tubes were centrifuged for 10 minutes at 40000rpm. Supernatant was separated in sterile microcentrifuge tube, containing DNA, needed for PCR identification.(Ghatak et al., 2013).

Identification of *A. baumannii* by PCR:

Molecular identification of *Acinetobacter baumannii* were confirmed through PCR (Polymerase chain reaction) for identifying bla_{OXA-51} gene. The PCR was carried out with total volume of 13µl Where, 7.5µl master mix (Taq DNA, dNTPs, Buffer, Mg^{2+}), 0.5µl forward primer, 0.5µl reverse primer, 2.5µl nuclease free water and 2µl template DNA has been taken in each PCR tube for all samples. The protocol for amplification was; initial denaturation at 95° for 5 minutes, 30 cycles containing of denaturation at 95° for 25 seconds, annealing at 60° for 40 seconds, extension at 72° for 50 seconds and final extension at 72° for 6 minutes. The PCR product was resolved on 1.5% agarose gel containing ethidium bromide in Tris-borate ethylenediaminetetraacetic acid buffer at 110 V for 1 h.

Antimicrobial Susceptibility Testing:

Susceptibility to the following antimicrobial agents were determined on the Mueller–Hinton agar by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI, 2021) guideline included Gentamicin (GEN), Cefepime (CPM), Ciprofloxacin (CIP), Imipenem (IPM), Piperacillin/Ampicillin (Pi/AMP), and Co-Trimoxazole (COT), Amoxicillin-ClavulanicAcid (AMC), Ceftazidime (CAZ). Zone of inhibition was interpreted per recommendation of the Clinical Laboratory Standard Institute (CLSI) guidelines.

Antimicrobial resistant gene detection:

The majority of the strains were found to be resistant, according to our investigation, although the results of the gene detection were not sufficient. ESBL encoding genes ($bla_{CTX-M,}$ $bla_{SHV,}$ bla_{TEM}) was

nearly absent from all isolates, the percentage were shown in table. Results did not reveal a significant correlation between resistant

patterns and presence of ESBL genes in the isolates. Among all 73 carbapenemase producing isolates, *bla*$_{NDM}$ has shown highest percentage that is 16.51% and other carbapenem encoding genes such as (*bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$) were unidentified in all isolates, then again bla$_{OXA-48}$ has shown minimalist result.

Gene name	Primer sequence ((5'-3')	Product size (bp)	PCR condition:	Reference
bla$_{CTXM}$	5'-ACGCTGTTGTTAGGAAGTG-3' 5'-TTGAGGCTGGGTGAAGT-3'	759	Initial: 94° (5 min) Denaturation: 94° (30sec) Annealing: 58° (30sec) Extension: 72° (30sec) Final extension: 72° (7 min).	• (Ranjbar & Farahani, 2019)
bla$_{SHV}$	5'-TACCATGAGCGATAACAGCG-3' 5'-GATTTGCTGATTTCGCTCGG-3'	450	Initial: 94° (5 min) Denaturation: 94° (30sec) Annealing: 58° (30sec) Extension: 72° (30sec) Final extension: 72° (7 min).	• (Rawat et al., 2018)
bla$_{TEM}$	5' AAAATTCTTGAAGACG-3' 5' TTACCAATGCTTAATCA-3'	1073	Initial: 95° (5 min) Denaturation: 95° (30sec) Annealing: 51° (30sec) Extension: 72° (30sec) Final extension: 72° (7 min).	• (Rawat et al., 2018)
bla$_{KPC}$	5'-CATTCAAGGGCTTTCTTGCTGC-3' 5'-ACGACGGCATAGTCATTTGC-3'	498	Initial: 94° (5 min) Denaturation: 94° (30sec) Annealing: 58° (30sec) Extension: 72° (30sec) Final extension: 72° (7 min).	• (Rawat et al., 2018)
bla$_{NDM}$	5'-ACCGCCTGGACCGATGACCA-3' 5'-GCCAAAGTTGGGCGCGGTTG-3'	621	Initial: 94° (5 min) Denaturation: 94° (30sec) Annealing: 58° (30sec) Extension: 72° (30sec) Final extension: 72° (7 min).	• (Rawat et al., 2018)

bla$_{IMP}$	5′- GAAGGCGTTTATGTTCATAC-3′ 5′- GTATGTTTCAAGAGTGATGC-3′	587	Initial: 95° (5 min) Denaturation: 95° (45sec) Annealing: 60° (45sec) Extension: 72° (60sec) Final extension: 72° (8 min).	• (Rawat et al., 2018)
bla$_{VIM}$	5'-ATTGGTCTATTTGACCGCGTC-3' 5'-TGCTACTCAACGACTGAGCG-3'	780	Initial: 95° (5 min) Denaturation: 95° (45sec) Annealing: 58° (45sec) Extension: 72° (60sec) Final extension: 72° (8 min).	• (Karaman et al., 2024)
bla$_{OXA-48}$	5′-TTGGTGGCATCGATTATCGG-3′ 5′-GAGCACTTCTTTTGTGATGGC-3′	743	Initial: 94° (5 min) Denaturation: 94° (30sec) Annealing: 58° (30sec) Extension: 72° (30sec) Final extension: 72° (7 min).	• (Karaman et al., 2024)

Table 01: Related genes for antibiotic resistant.

Biofilm Formation assay:

Biofilm producing capability was examined using the 96-well microtiter plate method. Fresh bacterial cultures were injected into 10µl of tryptic soy broth (TSB) and incubated at 37 degrees Celsius. 10µl of overnight grown *A.baumannii* culture was injected into each well of a 96 well plate containing 190µl Tryptic Soy Broth (TSB) (Soybean-Casein Digest Broth) and here the exception was done in negative control well where 200µl fresh broth was taken. After 24 hours of incubation at 37 °C, the contents were removed. Initially, cleaned three times with sterile distilled water. After that, the microplate was kept inverted at room temperature to dry. Then, 200 µl of methanol was used for 15 minutes for fixation. Then again, the objects were discarded and dried at room temperature. For 15 minutes, 200 µl of 1% crystal violet was added to each well. Therefore, the wells were cleaned with distilled water and inverted. Lastly, 200 µl of 30% glacial acetic acid was used to liquefy the crystal violet stain on the biofilm cells. Afterwards, the absorbance at 630nm was measured with the help of BioTek ELx808 Absorbance Plate Reader.

The true OD value was calculated by subtracting the OD of the control values. The results were classified into the four given categories: a) OD ≤ ODc = non-biofilm producer; b) ODc < OD ≤ 2ODc = weak biofilm producer; c) 3ODc <OD ≤ 4ODc = medium biofilm producer; d) 4OD < ODc = strong biofilm producer. (Hassan et al., 2011)

Biofilm forming gene identification:

A set of primers were used in polymerase chain reaction (PCR) experiments to detect the genes (*bap, bla*$_{PER-1}$*, csuE, and ompA*) given below. Each isolate's DNA was extracted using the method given before. PCR experiments were performed with 12µl PCR Master Mix, 1µl of each (Forward and Reverse) primer, 5.5µl nuclease free water and 5µl template DNA for each isolate. The PCR conditions were given below in table-02. For observing result, Agarose Gel Electrophoresis was performed.

Gene name	Primer sequence ((5'-3')	Product size (bp)	PCR condition:	Reference
bla$_{PER}$	GCAACTGCTGCAATACTCGG ATGTGCGACCACAGTACCAG	340	Initial: 94° (5 min) Denaturation: 94° (60sec) Annealing: 59° (1min) Extension: 72° (40sec) Final extension: 72°(5 min).	• (Mahmoudi Monfared et al., 2019)
ompA	GTTAAAGGCGACGTAGACG CCAGTGTTATCTGTGTGACC	578	Initial: 94° (5 min) Denaturation: 94° (60sec) Annealing: 60° (1min) Extension: 72° (40sec) Final extension: 72° (5 min).	• (Yang et al., 2019)
bap	TGCTGACAGTGACGTAGAACCA CA TGCAACTAGTGGAATAGCAGCC CA	184	Initial: 94° (5 min) Denaturation: 94° (60sec) Annealing: 62° (1min) Extension: 72° (40sec) Final extension: 72° (5 min).	• (Yang et al., 2019)
csuE	CATCTTCTATTTCGGTCCC CGGTCTGAGCATTGGTAA	168	Initial: 94° (5 min) Denaturation: 94° (60sec) Annealing: 58° (1min) Extension: 72° (40sec) Final extension: 72° (5 min).	• (Yang et al., 2019)

Table-02: Biofilm related genes.

Result:

Isolates and identification:

A total of 150 *Acinetobacter* species were obtained, of which 109 have been identified to be *A. baumannii*. The of male and female were 59.63%and 39.45% respectively. 12.84% of women and 15.6% of men who made up the bulk of patients (28.44%) were admitted to ICU. Of the 109 isolates, 16 (14.68%) were found to be *A. baumannii* in Blood, 10(9.17%) in wound swabs and in pus and in

urine, 25(22.94%) in sputum, and a very few number of samples were collected from other aspects. From tracheal aspirate and sputum, the majority of *A. baumannii* were isolated.

Characteristics		Total	Percentage
Gender	Male	65	59.63%
	Female	43	39.45%
	Unknown	1	0.92%
	Total	109	100%
Age (Years)	0-10	16	14.68%
	11-18	8	7.34%
	19-29	8	7.34%
	30 – 60	50	45.87%
	>60	27	24.77%
	Total	109	100%
Specimen type	T/A	31	28.44%
	Sputum	25	22.94%
	blood	16	14.67%
	Wound swab	9	8.26%
	CV Cathetar line	1	0.92%
	Pus	10	9.17%
	Ascitic fluid	1	0.92%
	Urine	10	9.17%
	W/S	1	0.92%
	E/t tube	2	1.83%
	CSF	1	0.92%
	Aspirate	1	0.92%
	Unknown	1	0.92%
	Total	109	100%

Table-03: Detailed patient's data who is infected with *Acinetobacter baumannii.*

Before Going through the molecular process, Biochemical testing was performed on some isolates. PCR was used to confirm isolates because the results of the other biochemical tests such as Citrate, Oxidate, Catalase, TSI, MIU, and MRVP tests were conducted, but the results did not meet any standard criteria.

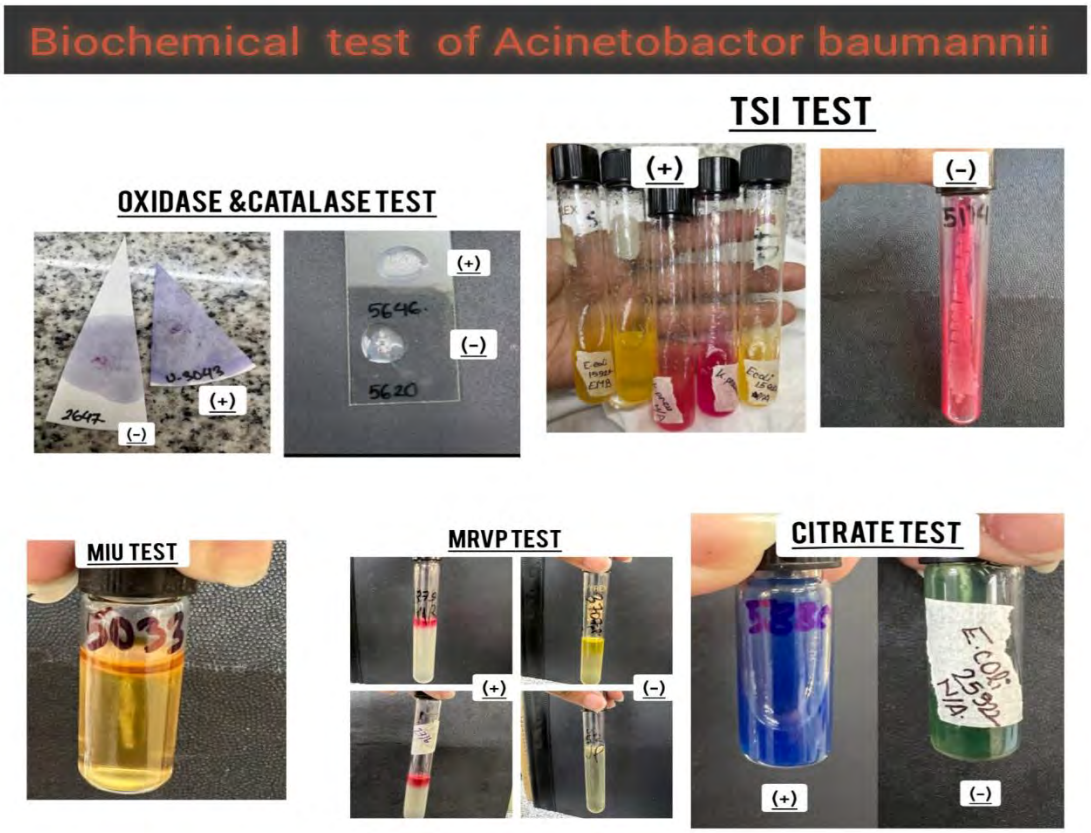


Figure 2: *Suspected results for identifying Acinetobacter baumannii..*

As a result, PCR was ultimately determined to be the most effective method for identifying and detecting bacterial strains. Therefore, out of 150 isolates 109 were confirmed positive strains

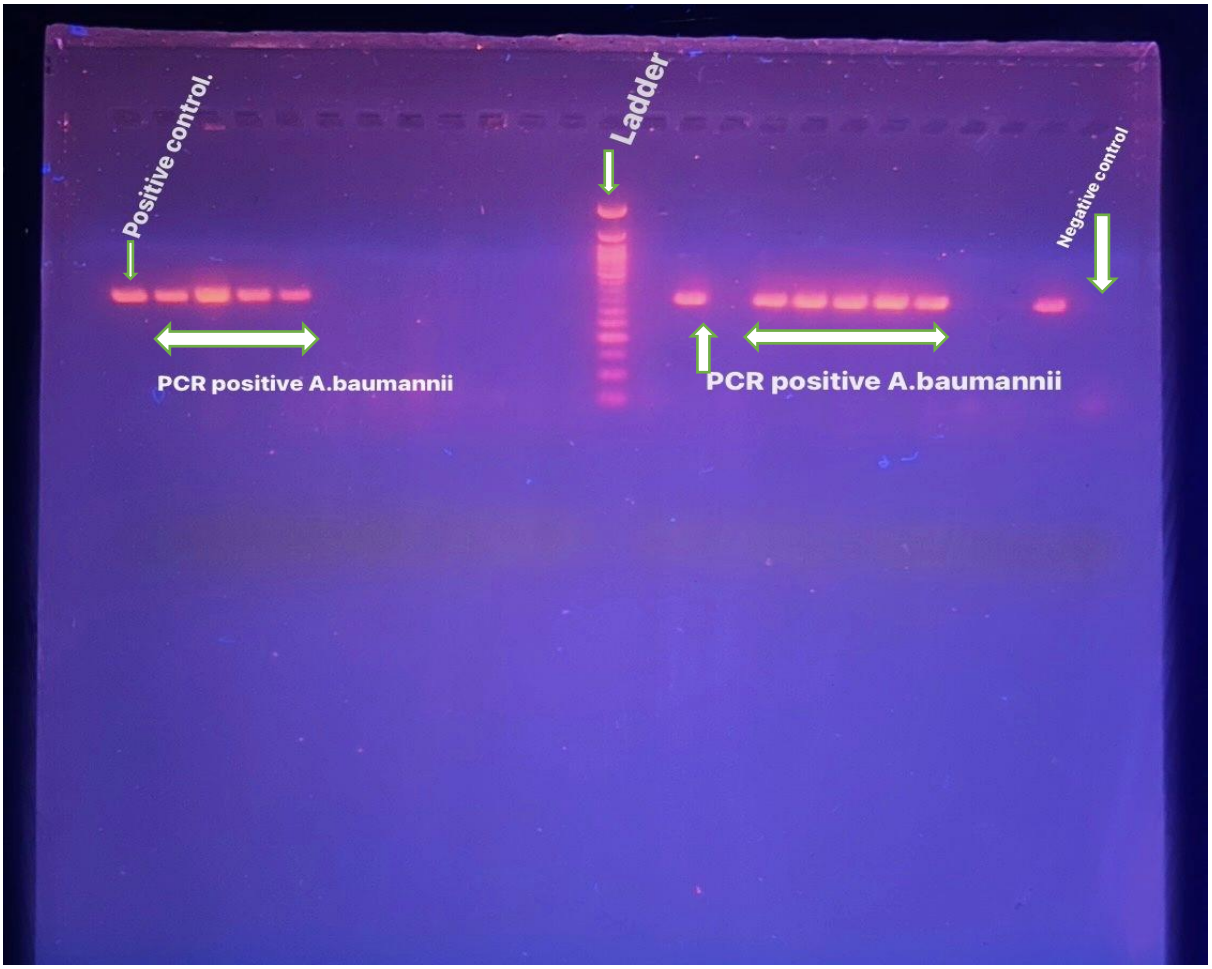


Figure 3: Gel picture of PCR positive *Acinetobacter baumannii.*

Antimicrobial Susceptibility testing (AST):

Using the disc diffusion method, the antibiotic susceptibility of the *A. baumannii* isolates was first identified. For the test, nine antibiotic drugs were chosen from the following categories: aminoglycosides, cephem, carbapenems, Penicillin, Fluoroquinolones, tetracyclines, and Folate Pathway Antagonist. The rates of resistance against cefepime (75.23%), imipenem (70.64%), ceftazidime (88.99%), gentamicin (70.64%), ciprofloxacin (73.39%), Ampicillin/piperacillin (82.56%), cotrimoxazole (33.03%), amoxicillin-clavulanic acid (78.90%), and tetracyclin (65.14%) were found among the 109 *A. baumannii* non-duplicate isolates. The analysis for resistance to different antibiotic classes revealed that 75.22% of the isolates were MDR.

Antimicrobial Agent	Susceptible (%)	Intermediate (%)	Resistance (%)
1. Cefepime	27 (24.77%)	0	82 (75.23%)
2. Imipenem	32 (29.36%)	0	77 (70.64%)
3. Ceftazidime	10 (9.17%)	2 (1.83%)	97 (88.99%)
4. Gentamicin	32 (29.36%)	0	77 (70.64%)
5. Ciprofloxacin	27 (24.77%)	2 (1.83%)	80 (73.39%)
6. Ampicillin/ Pipercillin	17 (15.60%)	2 (1.83%)	90 (82.56%)
7. Co-trimoxazole	71 (65.14%)	2 (1.83%)	36 (32.03%)
8. Amoxicillin/ Clavulanic acid	12 (11.01%)	11 (10.09%)	86 (77.90%)
9. Tetracyclin	30 (27.52%)	8 (7.34%)	71 (65.14%)

Table 04: Antibiotic susceptiblility test result.

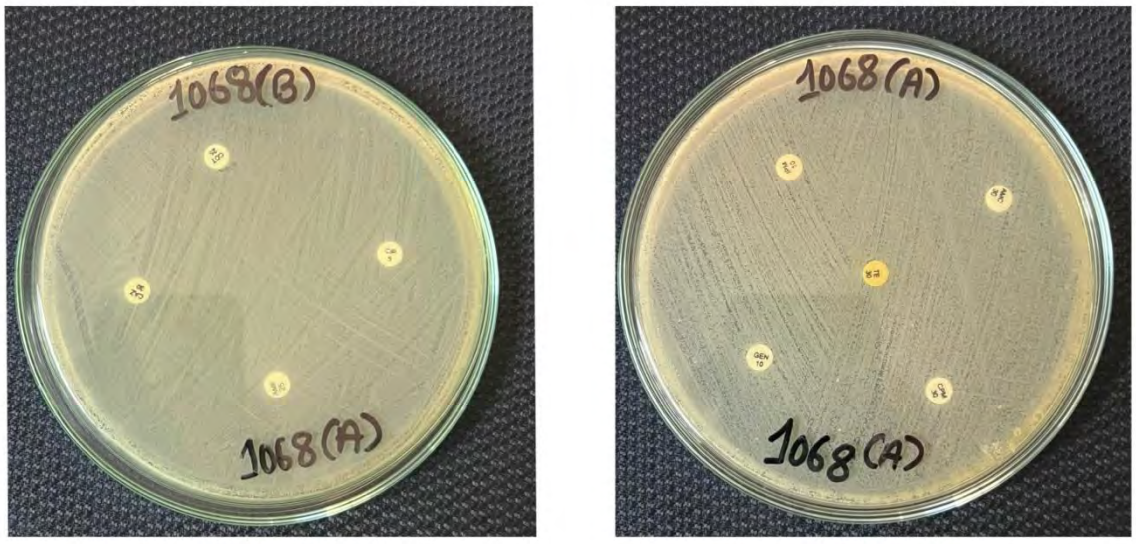


Figure 4: Incubated MHA plates for Antibiotic Susceptibility Test.

Responsible genes for AMR:

Number of gene	Gene name	Total number	Percentage
1	*bla*$_{NDM}$	18	16.51%
2	*bla*$_{CTX-M}$	06	5.50%
3	*bla*$_{SHV}$	01	0.91%
4	*bla*$_{TEM}$	03	2.75%
5	*bla*$_{VIM}$	0	0%
6	*bla*$_{KPC}$	0	0%
7	*bla*$_{IMP}$	1	0.91%
8	*bla*$_{OXA-48}$	03	2.75%

Table 05: Antimicrobial Resistant Gene result.

Biofilm formation:

Among 109 *A. baumannii* isolates, 67.88% strains were biofilm producers and 32.11% strains were non-biofilm producers. On the basis of biofilm forming capacity strains were divided into three categories: 24.77% strains formed a strong biofilm, while 33.02% and 10.09% of these isolates were considered as moderate and weak biofilm-forming isolates, respectively. So, moderate biofilm forming isolates were dominating over other categories.

Table 6: Biofilm formation ability in positive isolates:

	Total sample number (109)	Percentage
Strong	27	24.77%
Moderate	36	33.02%
Weak	11	10.09%
Non-biofilm forming	35	32.11%

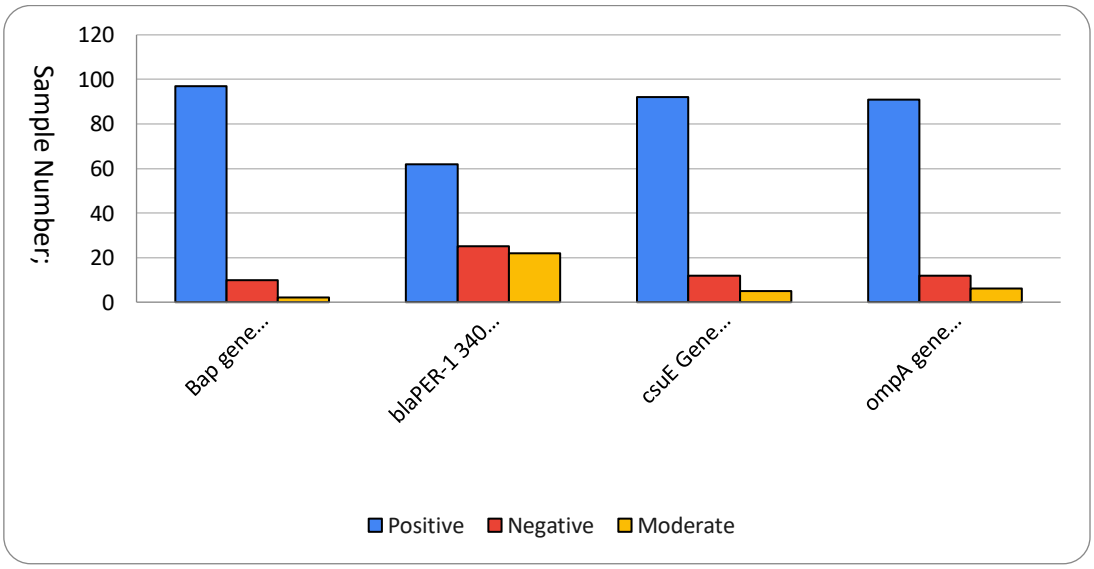
Biofilm forming gene:

Every isolate of *A. baumannii* had at least one gene associated with biofilm. Table 3 displays the frequency of these genes. The *bap* gene was found in the majority of isolates, which is 88.99%; *csuE* (84.40%); bla_{PER-1} (56.88%); *and ompA* (83.48%). These were the most common genes, and *the* bla_{PER-1} gene (56.88%) has shown the least percentage.

Table 7: Biofilm forming genes:

Name of gene	Total positive	Percentage (%)	Total Moderate	Percentage (%)	Total Negative	Percentage (%)
bap	97	88.99%	2	1.83%	10	9.17%
***bla*$_{PER-1}$**	62	56.88%	22	20.18%	25	22.93%
csuE	92	84.40%	05	4.58%	12	11.009%
ompA	91	83.48%	06	5.50%	12	11.009%

Graph 1:



Relationship of biofilm formation and biofilm related genes:

Clinical *A. baumannii* isolates with multidrug resistance produce biofilms because of particular to the distribution of virulence genes (*bap, bla$_{PER-1}$, ompA, and csuE*). This study conducted a polymerase chain reaction to detect the presence of genes relevant to biofilms. The frequencies of *bap, bla$_{PER-1}$, ompA, and csuE* genes in the following table the test isolates, which were 88.99%, 56.88%, 83.48%, and 84.40%, respectively. There were 74 isolates that formed biofilms out of the 109 test strains; of these, 24.77% were strong biofilm formers, 33.02% were moderate biofilm formers, and 10.09% were weak biofilm formers. The results showed that *the bap, blaPER, ompA, and csuE* genes were present in 86% (64/74), 72% (53/74), 86% (64/74), and 88% (65/74) of the biofilm producers, respectively, after examining the relationship between biofilm production and biofilm-related genes.

Table 8: Correlation between biofilm formation and biofilm forming genes:

Biofilm formation	Isolates (Frequency%)	Biofilm related genes			
		bap	*bla$_{PER-1}$*	*ompA*	*csuE*
Non-biofilm	35(32.11%)	35(32.11%)	31(28.44%)	35(32.11%)	32(29.33%)
Weak biofilm	11(10.09%)	10(9.17%)	9(8.26%)	9(8.26%)	9(8.26%)
Moderate biofilm	36(33.02%)	30(27.52%)	23(21.10%)	31(28.44%)	32(29.35%)
Strong biofilm	27(24.77%)	24(22.02%)	21(19.27%)	24(22.02%)	24(22.02%)

Relation between biofilm formation and antibiotic resistance isolates:

The relationship between the capacity to produce biofilms and the degree of antibiotic resistance was examined by comparing the biofilm formation in susceptible and antibiotic-resistant strains using data from the 96-well microtiter plate technique.

Antibiotic resistance was determined from the following categories: aminoglycosides, cephem, carbapenems, Penicillin, Fluoroquinolones, tetracyclines, and Folate Pathway Antagonist. In this study, we observed that the rate of biofilm producing capability were higher in antibiotic resistance isolates and also non-biofilm forming capacity is higher in resistance isolates then antibiotic susceptible isolates. Antibiotics from pipercillin and cephems groups are highly biofilm producers that is 55% and 54% repectively. The isolates that are susceptible to co-trimoxazole had the largest number of biofilm formation (36.69%), despite the fact that co-trimoxazole has the lowest capacity (20.18%) in antibiotic resistant strains. Compared to isolates resistant to antibiotics, isolates sensitive to antibiotics develop less biofilm.

Table 9: Antibiotic Resistance Pattern among Biofilm Producer and Non-Biofilm Producer *A. baumannii* isolates

	Antibiotic Resistance				**Antibiotic Susceptible**			
Antibiotics	Biofilm Producers	Percentage (%)	Non-Biofilm producers	Percentage (%)	Biofilm Producers	Percentage (%)	Non-Biofilm producers	Percentage (%)
Cefepime	50	46.79%	25	22.01%	13	11.93%	09	8.26%
Imipenem	46	42.20%	28	25.69%	17	15.59%	07	6.42%
Gentamicin	44	40.37%	29	26.61%	20	18.35%	6	5.50%
Tetracyclin	42	38.53%	24	22.01%	17	15.59%	08	07.34%
Ceftazidime	54	49.54%	34	31.19%	07	6.42%	01	0.91%
Amoxicillin-Clavulanic acid	49	44.95%	30	27.52%	08	7.33%	03	2.75%
Co-trimoxazole	22	20.18%	11	10.09%	40	36.69%	24	22.01%
Ciprofloxacin	47	43.11%	27	24.77%	15	13.76%	08	07.33%
pipercillin	55	50.46%	28	25.69%	07	6.42%	06	05.50%

Discussion:

It has been determined that *A. baumannii* is a nosocomial infection that spreads among hospitalised patients in recent years. This type of bacteria is known as nonfermenting gram-negative bacilli (NFGNB), and it can colonise the gastrointestinal tract, respiratory system, conjunctiva, skin, and oral cavities. Because this organism may live in the environment for a long period, nosocomial infections are typically spread directly from healthcare staff to patients or through surfaces in the environment. To effectively manage infections in hospitals, especially in the Intensive care unit, an evaluation of the key factors should be conducted in order to provide realistic and helpful techniques that infection control committees can employ as a strategic plan. In addition, physicians should use this information to battle antibiotic resistance, develop better medicines, lower medical expenses, and lower mortality. In order to achieve this, the current study was planned to assess various factors (such as the capacity to produce biofilms, the frequency of biofilm-related genes, resistance to antibiotics and Genes responsible for it etc.) while taking into account the significance of the *genes bap, bla$_{PER-1}$, and csuE* for cell adhesion and pili formation, respectively(Gedefie et al., 2021).
In DMCH, Bangladesh, the average prevalence of *Acinetobacter baumannii* was approximately 14%, slightly higher than in underdeveloped countries such as India (9.5%, 9.4%, and 11%, respectively). The prevalence rates in Japan (18%), Kuwait (22.117), and Saudi Arabia (31.718) were greater than in the current study. (Uddin et al., 2021).

In our study 28.44 % samples were collected from ICU patient, but according an article from Bangladesh journal Online (Abarca-Coloma et al., 2024), In China, percentage of infected ICU patient is up to 71.2%. Numerous investigations conducted worldwide have reported a significant incidence of *A. baumannii* strains that produce ESBLs. ESBL- producing genes are least common in our study, despite multi-drug resistance and prolonged life in hospitals. These days, *A. baumannii* is frequently described as multiple-drug resistant (MDR); Latin America appears to have some of the highest rates of resistance worldwide to imipenem, meropenem, ceftazidime, piperacillin/tazobactam, ciprofloxacin, and gentamicin. In our current study Ceftazidime, Ampicillin/Piperacillin, Cefepime, Ciprofloxacin, Gentamicin, imipenem, cotrimoxazole, tetracyclin, and amoxicillin-clavulanic acid resistance were found to be 88.99%, 82.56%, 75,23%, 73.39%, 70.64%, and 70.64%, respectively(Farzana et al., 2022). Another study in Iran at Tehran hospital showed *A. baumannii* isolates are innately resistant to imipenem (95.5%) and ciprofloxacin (94.5%). *A.baumannii's* Penicillin-Binding Proteins (PBPs) have decreased affinities for cephalosporins, and certain strains carry plasmid-encoded β-lactamases. (Armin et al., 2015). In an anticipated multicenter research

experiment carried out in Turkey for 6 months to assess antimicrobial resistance to several medicines in *Acinetobacter baumannii* transmission, resistance rank results were as follows: Amikacin, 91.8%; ampicillin/sulbactam, 99.4%; ceftazidime, 99.4%; ciprofloxacin, 100%; imipenem, 99.4%; (*Antibiotics | Free Full-Text | Risk Factors Associated with Mortality in Acinetobacter Baumannii Infections: Results of a Prospective Cohort Study in a Tertiary Public Hospital in Guayaquil, Ecuador*, n.d.)

Challenges with *A. baumannii* infection prevention and treatment are linked to bacterial biofilms. The microorganisms themselves produce polymer matrices that maintain biofilms, which are permanently attached to the host's tissues or abiotic surfaces and aid in the development of bacterial communities. The microbial community's resilience can be increased by this viscous matrix, which can isolate bacteria from dangerous external stimuli. The following might be used to explain these observations: (1) Permeation restriction: bacteria in biofilms at high densities can create an extracellular matrix that prevents antibiotics from penetrating the biofilm; (2) Nutrition restrictions: bacteria in biofilms are kept in a low-metabolism and slow-growing state, which reduces their sensitivity to external stimuli like antibiotics; Phenotype inference (3): cellular membranes select or induce strains with resistant traits and boost the prevalence of antibiotic resistance genes and the function of resistance efflux pumps, resulting in drug resistance; (4) immune restriction: *A. baumannii* biofilms are a naturally occurring physical barrier that limit the organism's ability to be killed by the immune system; and (5) quorum sensing: when the number of *A. baumannii* increases, some bacteria use quorum sensing to separate from the biofilm's surface and enter a planktonic growth state, which enables bacteria to adhere to suitable media and promotes infection and recurrence(Mirghani et al., 2022).

However another study in Bangladesh shows ciprofloxacin ,imipenem, gentamicin resistance percentage are much higher in China following 100%, 91.8 % 91.8 percent (Farzana et al., 2022). Numerous investigations conducted worldwide have reported a significant incidence of *A. baumannii* strains that produce ESBLs. ESBL-producing genes are least common in our study, despite multi-drug resistance and prolonged life in hospitals. But the possibility of discovering ESBL-producing genes are prominant, particularly in *Klebsiella* species than *Acinetobacter baumannii(Ran*jbar & Farahani, 2019). The $bla_{CTX\text{-}M}$ *and* bla_{TEM} genes were identified in 5.50% and 2.75% of the ESBL-positive *A. baumannii* strains examined in our investigation using the PCR technique, respectively, whereas the bla_{SHV} gene was only found in 0.91% of the isolates. Our investigations focused on looking into the genes of the major carbapenemases. Among all clinical isolates, the carbapenemase gene was the most common, followed by $bla_{NDM,}$ $bla_{IMP,}$ *and* $bla_{OXA\text{-}48}$-like genes at 16.51%, 0.91%, and 2.75%,

respectively. Unfortunately, bla_{KPC} *and* bla_{VIM} were not found. Additionally, other reports from different nations have demonstrated the dissemination of CRAB containing carbapenem-resistant gene(Alyamani et al., 2015)

The ability of *A. baumannii* to produce hemolysin, lipase, lecithinase, and protease, as well as the formation of biofilms, are among the many virulence factors that are correlated with the pathogenicity and resistance to unfavourable environmental circumstances. One crucial component is thought to be *A. baumannii*'s capacity to colonise and build biofilm on both biotic and abiotic surfaces. Because biofilms are multicellular, these pathways lead to treatment failure and bacterial resistance. Our findings shown that about 67.88% of isolates of *A. baumannii* produce biofilm. Of these, 11 (10.09%), 36 (33.02%), and 27 (24.77%) were weak biofilm producers, moderate biofilm producers, and strong biofilm producers, respectively and 35 (32.11%) isolates were non-biofilm producers. In another similar study, seventy-five biofilm-producing multidrug-resistant *Acinetobacter* species were identified in a study by the microtiter plate method (Liu et al., 2016). Of these 75 isolates 12 (16%), 9 (12%), 30 (40%), and 24 (32%) respectively were weak biofilm producers, moderate biofilm producers, strong biofilm producers, and non-biofilm producers. This study found a clear relationship between *Acinetobacter* isolates' propensity to form biofilm and the development of biofilm and multiple antibiotic resistance(Kasperski et al., 2023)(Yang et al., 2019)

Furthermore, our findings showed that the strong biofilm producer turned out resistant to a variety of antimicrobial agents, including Ceftazidime, Piperacillin, Cefepime, Ciprofloxacin, and Gentamicin. Moreover, The Amoxyclav drugs, a combination of Amoxicillin and Clavulanate frequently prescribed for treating bacterial infections, was also employed to treat the *Acinetobacter baumannii*. Despite of being a powerful combine drug, it was actually resistant in the majority of *A.baumannii* strains. According to a prior study, Piperacillin is a specific antibiotic that causes the formation of strong biofilms. This is quite evident in our study. Yet in our current study, we identified resistance from both the Folate Pathway Antagonist group, such as Co-trimoxasol, and the Aminoglycoside group, such as Tetracycline antibiotics were also associated with biofilm formation because the resistance pattern was lower than other groups of antibiotics, which have not been reported in previous investigations, allowing us to hypothesise that it is due to Folate Pathway Antagonists and Aminoglycosides tend to be ineffective against strains of *A. Baumannii*, therefore combinations involving these two anti-drug along with Carbapenems are commonly employed to produce beneficial effects for the treatment of patients at medical centres. To be more precise Both aminoglycoside and Folate Pathway Antagonist resistance seems to be involved in the formation of biofilm, this could possibly be due to antibiotic

mutual benefit(Yang et al., 2019). In our study, we observed that, stronger biofilm forming isolates showed more resistance, whereas, in some paper the scenario is quite opposite. It showed the stronger the biofilm production, the higher the likelihood of antibiotic sensitivity.(Krzyściak et al., 2017)

In the biofilm-forming *A. baumannii*, the expression of the *bap, ompA, csuE, and bla-$_{PER}$* genes were examined molecularly. The majority of isolates had the genes *ompA and csuE*, with percentages of 91 (83.48%) and 92 (84.40%), respectively. In contrast, 97 (88.99%) and 68 (56.88%) of the isolates contained the genes *bap and bla$_{PER-1}$*. The results of this study indicate that overexpression of bap affects biofilm formation in the context of low iron concentrations. These biofilm-related genes were found in certain isolates of *A. baumannii* that formed mild biofilms, though. Strong biofilm formation was anticipated given the high frequency of biofilm-related genes in *A. baumannii* isolates.Prior research has also documented a high frequency of *csuE* in isolates of *A. baumannii*; in Ghasemi et al. and Youn Sung investigations, for example, *csuE* was found in 100 and 93.8% of isolates, respectively (Ghasemi et al., 2018), (Sung, 2018). Antimicrobial resistance, biofilm formation, and adhesion to human epithelial cells are all likely dependent on *A. baumannii*'s *ompA (Thummeepak et al., 2016)*. In our investigation*, ompA* was found often (81%). Comparable outcomes with 84.4 and 68.8% *ompA* positive isolates, respectively, were reported from Thailand and Korea(Sung, 2018; Thummeepak et al., 2016)

Conclusion:

Although the majority of GNB(Gram negative bacteria) are mainly identical, each has a few distinctive characteristics that make some of these bacteria—like *A. baumannii* is more difficult to deal with than others. The elements that accelerate any given organism to become a successful pathogen are the result of several, varied forces coming together(Nesa et al., 2018). Imipenem is still the recommended treatment for *Acinetobacter* infections that are resistant to multiple drugs. Our research and other recent studies on biofilm-forming *A.baumannii* primarily assess biofilm formation, and evidence supports a relationship between biofilm formation and multiple drug resistance in *A. baumannii*. Biofilms are the reason for the high rate of *A. baumannii* infections connected to medical equipment, which makes infection control and treatment extremely difficult. Hence, in order to prevent device-mediated *A. baumannii* biofilm-related illnesses, health care workers (HCWs) should concentrate on infection prevention and control (IPC) measures or activities. They should also exercise extreme caution while using treatments in conjunction with anti-

biofilms. Thus, one area of interest for future research is the impact of bacteria on biofilms(Kasperski et al., 2023).

References:

- Abarca-Coloma, L., Puga-Tejada, M., Nuñez-Quezada, T., Gómez-Cruz, O., Mawyin-Muñoz, C., Barungi, S., & Perán, M. (2024). Risk Factors Associated with Mortality in Acinetobacter baumannii Infections: Results of a Prospective Cohort Study in a Tertiary Public Hospital in Guayaquil, Ecuador. *Antibiotics*, *13*(3), Article 3. https://doi.org/10.3390/antibiotics13030213
- Alyamani, E. J., Khiyami, M. A., Booq, R. Y., Alnafjan, B. M., Altammami, M. A., & Bahwerth, F. S. (2015). Molecular characterization of extended-spectrum beta-lactamases (ESBLs) produced by clinical isolates of Acinetobacter baumannii in Saudi Arabia. *Annals of Clinical Microbiology and Antimicrobials*, *14*, 38. https://doi.org/10.1186/s12941-015-0098-9
- Anstey, N. M., Currie, B. J., & Withnall, K. M. (1992). Community-acquired Acinetobacter pneumonia in the Northern Territory of Australia. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *14*(1), 83–91. https://doi.org/10.1093/clinids/14.1.83
- *Antibiotics | Free Full-Text | Risk Factors Associated with Mortality in Acinetobacter baumannii Infections: Results of a Prospective Cohort Study in a Tertiary Public Hospital in Guayaquil, Ecuador*. (n.d.). Retrieved 29 June 2024, from https://www.mdpi.com/2079-6382/13/3/213
- Armin, S., Karimi, A., Fallah, F., Tabatabaii, S. R., Alfatemi, S. M. H., Khiabanirad, P., Shiva, F., Fahimzad, A., Rahbar, M., Mansoorghanaii, R., Shirvani, F., Abdolghafoorian, H., & Nia, R. S. S. (2015). Antimicrobial Resistance Patterns of Acinetobacter baumannii, Pseudomonas

aeruginosa and Staphylococcus aureus Isolated From Patients With Nosocomial Infections Admitted to Tehran Hospitals. *Archives of Pediatric Infectious Diseases*, *3*(4), Article 4. https://doi.org/10.5812/pedinfect.32554

- *Biochemical Tests for Microbial Identification*. (n.d.). Retrieved 27 April 2024, from https://www.news-medical.net/life-sciences/Biochemical-Tests-for-Microbial-Identification.aspx
- Biofilm Formation by Enterococcus Faecalis and Enterococcus Faecium: Review. (2019). *International Journal of Research Studies in Biosciences*, *7*(4). https://doi.org/10.20431/2349-0365.0704002
- Bulens, S. N., Yi, S. H., Walters, M. S., Jacob, J. T., Bower, C., Reno, J., Wilson, L., Vaeth, E., Bamberg, W., Janelle, S. J., Lynfield, R., Vagnone, P. S., Shaw, K., Kainer, M., Muleta, D., Mounsey, J., Dumyati, G., Concannon, C., Beldavs, Z., … Kallen, A. J. (2018). Carbapenem-Nonsusceptible Acinetobacter baumannii, 8 US Metropolitan Areas, 2012–2015. *Emerging Infectious Diseases*, *24*(4), 727–734. https://doi.org/10.3201/eid2404.171461
- Chusri, S., Chongsuvivatwong, V., Silpapojakul, K., Singkhamanan, K., Hortiwakul, T., Charernmak, B., & Doi, Y. (2019). Clinical characteristics and outcomes of community and hospital-acquired Acinetobacter baumannii bacteremia. *Journal of Microbiology, Immunology, and Infection = Wei Mian Yu Gan Ran Za Zhi*, *52*(5), 796–806. https://doi.org/10.1016/j.jmii.2019.03.004
- Diaz, P. (2016). Impact of Biofilm Infection and its Treatment. *DJ International Journal of Advances in Microbiology & Microbiological Research*, *1*, 7–13. https://doi.org/10.18831/djmicro.org/2016011002

- Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An increasing threat in hospitals: Multidrug-resistant Acinetobacter baumannii. *Nature Reviews Microbiology*, *5*(12), 939–951. https://doi.org/10.1038/nrmicro1789
- *Factors mediating Acinetobacter baumannii biofilm formation …* (n.d.). Www.Google.Com. Retrieved 27 April 2024, from https://www.google.com/imgres?imgurl=https://ars.els-cdn.com/content/image/1-s2.0-S2666517422000281-gr1.jpg&tbnid=hlral16ZiNSLgM&vet=1&imgrefurl=https://www.sciencedirect.com/science/article/pii/S2666517422000281&docid=1PrmYu-mmFREaM&w=659&h=413&itg=1&hl=en&source=sh/x/im/can/1&kgs=02c9c1b7af55a50c&shem=abme,trie&sfr=vfe
- Farzana, R., Swedberg, G., Giske, C. G., & Hasan, B. (2022). Molecular and genetic characterization of emerging carbapenemase-producing *Acinetobacter baumannii* strains from patients and hospital environments in Bangladesh. *Infection Prevention in Practice*, *4*(2), 100215. https://doi.org/10.1016/j.infpip.2022.100215
- Ferrara, A. M. (2006). Potentially multidrug-resistant non-fermentative Gram-negative pathogens causing nosocomial pneumonia. *International Journal of Antimicrobial Agents*, *27*(3), 183–195. https://doi.org/10.1016/j.ijantimicag.2005.11.005
- Friedland, I., Stinson, L., Ikaiddi, M., Harm, S., & Woods, G. L. (2003). Phenotypic antimicrobial resistance patterns in Pseudomonas aeruginosa and Acinetobacter: Results of a Multicenter Intensive Care Unit Surveillance Study, 1995-2000. *Diagnostic Microbiology and Infectious Disease*, *45*(4), 245–250. https://doi.org/10.1016/s0732-8893(02)00503-5
- Gedefie, A., Demsis, W., Ashagrie, M., Kassa, Y., Tesfaye, M., Tilahun, M., Bisetegn, H., & Sahle, Z. (2021). Acinetobacter baumannii Biofilm Formation and Its Role in Disease

Pathogenesis: A Review. *Infection and Drug Resistance*, *14*, 3711–3719. https://doi.org/10.2147/IDR.S332051

- Ghasemi, E., Ghalavand, Z., Goudarzi, H., Yeganeh, F., Hashemi, A., Dabiri, H., Mirsamadi, E. S., & Foroumand, M. (2018). Phenotypic and Genotypic Investigation of Biofilm Formation in Clinical and Environmental Isolates of Acinetobacter baumannii. *Archives of Clinical Infectious Diseases*, *13*(4), Article 4. https://doi.org/10.5812/archcid.12914
- Ghatak, S., Muthukumaran, R. B., & Nachimuthu, S. K. (2013). A Simple Method of Genomic DNA Extraction from Human Samples for PCR-RFLP Analysis. *Journal of Biomolecular Techniques : JBT*, *24*(4), 224–231. https://doi.org/10.7171/jbt.13-2404-001
- Gootz, T. D., & Marra, A. (2008). Acinetobacter baumannii: An emerging multidrug-resistant threat. *Expert Review of Anti-Infective Therapy*. https://www.tandfonline.com/doi/abs/10.1586/14787210.6.3.309
- Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., & Iqbal, M. (2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. *The Brazilian Journal of Infectious Diseases*, *15*(4), 305–311. https://doi.org/10.1016/S1413-8670(11)70197-0
- Høiby, N., Ciofu, O., Johansen, H. K., Song, Z., Moser, C., Jensen, P. Ø., Molin, S., Givskov, M., Tolker-Nielsen, T., & Bjarnsholt, T. (2011). The clinical impact of bacterial biofilms. *International Journal of Oral Science*, *3*(2), 55–65. https://doi.org/10.4248/IJOS11026
- Howard, A., O'Donoghue, M., Feeney, A., & Sleator, R. D. (2012). Acinetobacter baumannii. *Virulence*, *3*(3), 243–250. https://doi.org/10.4161/viru.19700
- Inchai, J., Pothirat, C., Bumroongkit, C., Limsukon, A., Khositsakulchai, W., & Liwsrisakun, C. (2015). Prognostic factors associated with mortality of drug-resistant Acinetobacter baumannii ventilator-associated pneumonia. *Journal of Intensive Care*, *3*, 9. https://doi.org/10.1186/s40560-015-0077-4

- Karaman, E., Çiçek, A. Ç., Şemen, V., & Şaban Beriş, F. (2024). Characterization of resistance genes and replicon typing in Carbapenem-resistant Klebsiella pneumoniae strains. *Annals of Clinical Microbiology and Antimicrobials*, *23*(1), Article 1. https://doi.org/10.1186/s12941-024-00672-9
- Kasperski, T., Romaniszyn, D., Jachowicz-Matczak, E., Pomorska-Wesołowska, M., Wójkowska-Mach, J., & Chmielarczyk, A. (2023). Extensive Drug Resistance of Strong Biofilm-Producing Acinetobacter baumannii Strains Isolated from Infections and Colonization Hospitalized Patients in Southern Poland. *Pathogens*, *12*(8), Article 8. https://doi.org/10.3390/pathogens12080975
- Krzyściak, P., Chmielarczyk, A., Pobiega, M., Romaniszyn, D., & Wójkowska-Mach, J. (2017). *Acinetobacter baumannii* isolated from hospital-acquired infection: Biofilm production and drug susceptibility. *APMIS*, *125*(11), 1017–1026. https://doi.org/10.1111/apm.12739
- Lai, C.-C., Wang, C.-Y., & Hsueh, P.-R. (2020). Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? *Journal of Microbiology, Immunology, and Infection*, *53*(4), 505–512. https://doi.org/10.1016/j.jmii.2020.05.013
- Liu, H., Wu, Y.-Q., Chen, L.-P., Gao, X., Huang, H.-N., Qiu, F.-L., & Wu, D.-C. (2016). Biofilm-Related Genes: Analyses in Multi-Antibiotic Resistant Acinetobacter Baumannii Isolates From Mainland China. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research*, *22*, 1801–1807. https://doi.org/10.12659/MSM.898959
- Lynch, J. P., Zhanel, G. G., & Clark, N. M. (2017). Infections Due to Acinetobacter baumannii in the ICU: Treatment Options. *Seminars in Respiratory and Critical Care Medicine*, *38*(3), 311–325. https://doi.org/10.1055/s-0037-1599225

- Ma, C., & McClean, S. (2021). Mapping Global Prevalence of Acinetobacter baumannii and Recent Vaccine Development to Tackle It. *Vaccines*, *9*(6), 570. https://doi.org/10.3390/vaccines9060570
- Mahmoudi Monfared, A., Rezaei, A., Poursina, F., & Faghri, J. (2019). Detection of Genes Involved in Biofilm Formation in MDR and XDR Acinetobacter baumannii Isolated from Human Clinical Specimens in Isfahan, Iran. *Archives of Clinical Infectious Diseases*, *In Press*(In Press). https://doi.org/10.5812/archcid.85766
- Metan, G., Alp, E., Aygen, B., & Sumerkan, B. (2007). Acinetobacter baumannii meningitis in post-neurosurgical patients: Clinical outcome and impact of carbapenem resistance. *The Journal of Antimicrobial Chemotherapy*, *60*(1), 197–199. https://doi.org/10.1093/jac/dkm181
- Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., & Rijal, G. (2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology*, *8*(3), 239–277. https://doi.org/10.3934/microbiol.2022019
- *Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance—PubMed*. (n.d.). Retrieved 29 June 2024, from https://pubmed.ncbi.nlm.nih.gov/21793988/
- Nesa, M., Anwar, S., & Saleh, A. A. (2018). Acinetobacter baumannii: Identification, Antibiotic Sensitivity and Biofilm Formation in Different Clinical Samples. *Bangladesh Journal of Medical Microbiology*, *12*(2), 4–9. https://doi.org/10.3329/bjmm.v12i2.51690
- Oncül, O., Keskin, O., Acar, H. V., Küçükardali, Y., Evrenkaya, R., Atasoyu, E. M., Top, C., Nalbant, S., Ozkan, S., Emekdaş, G., Cavuşlu, S., Us, M. H., Pahsa, A., & Gökben, M. (2002).

Hospital-acquired infections following the 1999 Marmara earthquake. *The Journal of Hospital Infection*, *51*(1), 47–51. https://doi.org/10.1053/jhin.2002.1205

- Pattnaik, D., Panda, S. S., Singh, N., Sahoo, S., Mohapatra, I., & Jena, J. (2019). Multidrug resistant, extensively drug resistant and pan drug resistant gram negative bacteria at a tertiary care centre in Bhubaneswar. *International Journal Of Community Medicine And Public Health*, *6*(2), 567–572. https://doi.org/10.18203/2394-6040.ijcmph20190170
- Perez, S., Innes, G. K., Walters, M. S., Mehr, J., Arias, J., Greeley, R., & Chew, D. (2020). Increase in Hospital-Acquired Carbapenem-Resistant Acinetobacter baumannii Infection and Colonization in an Acute Care Hospital During a Surge in COVID-19 Admissions—New Jersey, February–July 2020. *Morbidity and Mortality Weekly Report*, *69*(48), 1827–1831. https://doi.org/10.15585/mmwr.mm6948e1
- Poirel, L., & Nordmann, P. (2006). Carbapenem resistance in Acinetobacter baumannii: Mechanisms and epidemiology. *Clinical Microbiology and Infection*, *12*(9), 826–836. https://doi.org/10.1111/j.1469-0691.2006.01456.x
- Pour, N. K., Dusane, D. H., Dhakephalkar, P. K., Zamin, F. R., Zinjarde, S. S., & Chopade, B. A. (2011). Biofilm formation by Acinetobacter baumannii strains isolated from urinary tract infection and urinary catheters. *FEMS Immunology and Medical Microbiology*, *62*(3), 328–338. https://doi.org/10.1111/j.1574-695X.2011.00818.x
- Rafei, R., Dabboussi, F., Hamze, M., Eveillard, M., Lemarié, C., Mallat, H., Rolain, J.-M., Joly-Guillou, M.-L., & Kempf, M. (2014). First report of blaNDM-1-producing Acinetobacter baumannii isolated in Lebanon from civilians wounded during the Syrian war. *International Journal of Infectious Diseases: IJID: Official Publication of the International Society for Infectious Diseases*, *21*, 21–23. https://doi.org/10.1016/j.ijid.2014.01.004

- Ranjbar, R., & Farahani, A. (2019). Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in multidrug-resistant Acinetobacter baumannii isolated from burn wound infections in Iran. *Antimicrobial Resistance & Infection Control*, *8*(1), 172. https://doi.org/10.1186/s13756-019-0612-5
- Raut, S., Rijal, K. R., Khatiwada, S., Karna, S., Khanal, R., Adhikari, J., & Adhikari, B. (2020). Trend and Characteristics of Acinetobacter baumannii Infections in Patients Attending Universal College of Medical Sciences, Bhairahawa, Western Nepal: A Longitudinal Study of 2018. *Infection and Drug Resistance*, *13*, 1631–1641. https://doi.org/10.2147/IDR.S257851
- Rawat, N., Singh, F., Hirpurkar, S., SANNAT, C., & GADE, N. (2018). Detection and characterization of extended-spectrum beta-lactamase genes (bla TEM and bla SHV) among beta-lactam-resistant fecal coliforms of dairy cattle from Chhattisgarh, India. *TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES*, *42*, 503–511. https://doi.org/10.3906/vet-1706-77
- Roy, S., Chowdhury, G., Mukhopadhyay, A. K., Dutta, S., & Basu, S. (2022). Convergence of Biofilm Formation and Antibiotic Resistance in Acinetobacter baumannii Infection. *Frontiers in Medicine*, *9*, 793615. https://doi.org/10.3389/fmed.2022.793615
- Santajit, S., Bhoopong, P., Kong-Ngoen, T., Tunyong, W., Horpet, D., Paehoh-ele, W., Zahedeng, T., Pumirat, P., Sookrung, N., Hinthong, W., & Indrawattana, N. (2023). Phenotypic and Genotypic Investigation of Carbapenem-Resistant Acinetobacter baumannii in Maharaj Nakhon Si Thammarat Hospital, Thailand. *Antibiotics*, *12*(3), 580. https://doi.org/10.3390/antibiotics12030580
- Scott, P., Deye, G., Srinivasan, A., Murray, C., Moran, K., Hulten, E., Fishbain, J., Craft, D., Riddell, S., Lindler, L., Mancuso, J., Milstrey, E., Bautista, C. T., Patel, J., Ewell, A., Hamilton, T.,

Gaddy, C., Tenney, M., Christopher, G., … Petruccelli, B. (2007). An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *44*(12), 1577–1584. https://doi.org/10.1086/518170

- Shadan, A., Pathak, A., Ma, Y., Pathania, R., & Singh, R. P. (2023). Deciphering the virulence factors, regulation, and immune response to Acinetobacter baumannii infection. *Frontiers in Cellular and Infection Microbiology*, *13*. https://doi.org/10.3389/fcimb.2023.1053968
- Sharifipour, E., Shams, S., Esmkhani, M., Khodadadi, J., Fotouhi-Ardakani, R., Koohpaei, A., Doosti, Z., & EJ Golzari, S. (2020). Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. *BMC Infectious Diseases*, *20*, 646. https://doi.org/10.1186/s12879-020-05374-z
- Sung, J. Y. (2018). Molecular Characterization and Antimicrobial Susceptibility of Biofilm-forming <italic>Acinetobacter baumannii</italic> Clinical Isolates from Daejeon, Korea. *Korean Journal of Clinical Laboratory Science*, *50*(2), 100–109. https://doi.org/10.15324/kjcls.2018.50.2.100
- Tao, C., Kang, M., Chen, Z., Xie, Y., Fan, H., Qin, L., & Ma, Y. (2009). Microbiologic study of the pathogens isolated from wound culture among Wenchuan earthquake survivors. *Diagnostic Microbiology and Infectious Disease*, *63*(3), 268–270. https://doi.org/10.1016/j.diagmicrobio.2008.11.009
- Thummeepak, R., Kongthai, P., Leungtongkam, U., & Sitthisak, S. (2016). Distribution of virulence genes involved in biofilm formation in multi-drug resistant Acinetobacter baumannii clinical isolates. *International Microbiology: The Official Journal of the Spanish Society for Microbiology*, *19*(2), 121–129. https://doi.org/10.2436/20.1501.01.270

- Uddin, B. M. M., Akhand, F., Bari, S., Suchi, S. E., Saha, R., & Ahmed, M. U. (2021). Prevalence and Resistance Profile of Acinetobacter baumannii Isolated from Clinical Specimens at a Tertiary Care Hospital in Bangladesh. *Journal of National Institute of Neurosciences Bangladesh*, *7*(2), Article 2. https://doi.org/10.3329/jninb.v7i2.58117
- Vahhabi, A., Hasani, A., Rezaee, M. A., Baradaran, B., Hasani, A., Kafil, H. S., & Soltani, E. (2021). Carbapenem resistance in Acinetobacter baumannii clinical isolates from northwest Iran: High prevalence of OXA genes in sync. *Iranian Journal of Microbiology*, *13*(3), 282–293. https://doi.org/10.18502/ijm.v13i3.6388
- Valcek, A., Philippe, C., Whiteway, C., Robino, E., Nesporova, K., Bové, M., Coenye, T., De Pooter, T., De Coster, W., Strazisar, M., & Van der Henst, C. (n.d.). Phenotypic Characterization and Heterogeneity among Modern Clinical Isolates of Acinetobacter baumannii. *Microbiology Spectrum*, *11*(1), e03061-22. https://doi.org/10.1128/spectrum.03061-22
- Wisplinghoff, H., Bischoff, T., Tallent, S. M., Seifert, H., Wenzel, R. P., & Edmond, M. B. (2004). Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *39*(3), 309–317. https://doi.org/10.1086/421946
- Wisplinghoff, H., Paulus, T., Lugenheim, M., Stefanik, D., Higgins, P. G., Edmond, M. B., Wenzel, R. P., & Seifert, H. (2012). Nosocomial bloodstream infections due to Acinetobacter baumannii, Acinetobacter pittii and Acinetobacter nosocomialis in the United States. *The Journal of Infection*, *64*(3), 282–290. https://doi.org/10.1016/j.jinf.2011.12.008
- Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B., & Spellberg, B. (2017). Clinical and Pathophysiological Overview of Acinetobacter Infections: A Century of

Challenges. *Clinical Microbiology Reviews*, *30*(1), 409–447. https://doi.org/10.1128/CMR.00058-16

- Yang, C.-H., Su, P.-W., Moi, S.-H., & Chuang, L.-Y. (2019). Biofilm Formation in Acinetobacter Baumannii: Genotype-Phenotype Correlation. *Molecules*, *24*(10), 1849. https://doi.org/10.3390/molecules24101849
- Zeighami, H., Valadkhani, F., Shapouri, R., Samadi, E., & Haghi, F. (2019). Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. *BMC Infectious Diseases*, *19*(1), 629. https://doi.org/10.1186/s12879-019-4272-0