**Acinetobacter baumannii: An overview of emerging multidrug-resistant pathogen**

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**ABSTRACT**

The emergence of infections caused by *Acinetobacter baumannii*, a multidrug-resistant bacterium, has been a concern worldwide. This bacterium is an important hospital-acquired pathogen that causes several diseases, including ventilator-associated pneumonia, bloodstream infections, and meningitis. This study aimed to determine antibiotic-resistant mechanisms in the pathogenesis of *A. baumannii* and the alternative treatment strategies against it. The combined actions of the outer membrane protein A, formation of a biofilm on biotic and abiotic surfaces, phospholipases C and D, metal homeostatic system, lipopolysaccharides, and verotoxins are relevant for virulence and pathogenesis. *A. baumannii* resists to a broad-spectrum antibiotics by its mechanisms of resistance, such as β-lactamases, efflux pump, aminoglycoside modifying enzymes, permeability changes, and alternation of targets. In an attempt to overcome the resistance mechanisms, plant-derived compounds and a combination of the antibiotics and the plant phytocompounds have been focused. Nanoparticles synthesised with the plant extract have been studied extensively. Furthermore, we projected modern methods, including multi-omics analysis, to study insight into mechanisms of actions of antibiotics. The information suggested that the potential antibiotic mechanisms of *A. baumannii* could lead to an alternative treatment against *A. baumannii* infections.

**KEYWORDS:**

*Acinetobacter baumannii*, Multidrug resistance, Hospital-acquired infection, Advance diagnostic, Nanoparticles, and Plant-derived compounds

1. Introduction

*Acinetobacter baumannii* has been a human pathogen with increasing importance, since it causes a high number of infections and the occurrence of multidrug-resistant (MDR) strains. *Acinetobacter spp.* are characterised by being aerobic, non-fermentative, non-mobile, non-fastidious, catalase-positive, oxidative-negative, and Gram-negative cocci/bacilli. 1 This bacterium was first described in 1911, and having been isolated from the soil, it has been given several designations such as *Micrococcus calcoaceticus*, *Achromobacter*, *Alcaligenes*, *Bacterium anitratum*, *Moraxella glucidolytica*, *Neisseria winogradskyi*, *Alcaligenes haemolytodes*, *Mima polymorpha*, and *Moraxella bovifel*. Over the past few decades, the nomenclature of the genus *Acinetobacter* has been changed, and then, in 1974, it was described in Bergey’s Manual of Systematic Bacteriology, with one species only: *Acinetobacter calcoaceticus*. The complex *A. calcoaceticus-baumannii* includes four genospecies: genospecies 1, *A. calcoaceticus*; genospecies 2, *A. baumannii*; genospecies 3, *A. pittii*; and genospecies 13TU, *A. nosocomialis*. *A. baumannii* is the most important species in clinical settings due to nosocomial infections that are associated with the highest mortality rate. 1 habitat-wise, since they are ubiquitous, they are found everywhere, especially in wet/moist environments like ponds, waste water, water treatment plants, and soil/mud. 5 The environmental reservoirs like food and various types of livestock have served as an important source for resistance elements making their way from multiple environmental sources into the human population and changing into clinically relevant strains, often harbouring antibiotic resistance mechanisms, namely extended-spectrum-lactamases (ESBLs) and metallo-beta lactamases (MBL). 6,7 Usually, it is resistant to complete decolourisation and can deceive as Gram-positive cocci. It does not produce urease, indole, cytochrome oxidase, and citrate; however, it produces catalase enzyme. *A. baumannii* is able to grow at 44°C, as the only bacterium of this genus to grow on the commonly used media in the laboratory. 8 This article was accepted: 25 April 2022

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Once considered of low virulent activity, it has literally taken over most of the commonly used drugs and has made them impotent, and there has been a significant interest in this organism over the past few decades. It has been called ‘Iraqibacter’ as they have been isolated from individuals serving in the war in Iraq and Afghanistan. The bacteria have rapidly spread across the globe in many hospital settings, especially the intensive care units (ICUs), where it accounted for 20% of infections worldwide. Today, Acinetobacter infections have spread rapidly across the globe in the community. It can survive and thrive in diverse conditions of pH and temperature, in dry and moist surfaces that may account for their perseverance in the community-acquired infections like meningitis, bloodstream conditions, and on surgical tools, ventilators, catheters, and respirometers. As a result, they cause hospital- and community-acquired infections like meningitis, bloodstream infections, endocarditis, and wound and soft tissue infections. The WHO stated that one of the most resistant ESKAPE organisms (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter species) escaping the action of antibacterial drugs was A. baumannii and classified it as a top priority critical pathogen for antibiotic research and development. They can form biofilms on several abiotic surfaces that may account for their perseverance in the hospital environment, increasing the possibility of causing healthcare-associated infections and outbreaks. There are unknown virulence and resistance mechanisms developed by A. baumannii against the drugs counting with β-lactamase, low-porin expressions, lipopolysaccharides (LPS), alteration of target cells by mutations, iron-chelating systems, and capsular polysaccharides. Another significant factor that contributed to its spread was the failure to accurately diagnose this organism due to the similarities between species. Misidentification on several occasions by phenotypic and chemotaxonomic methods underrated the role of A. baumannii as a cause of nosocomial infection. In addition, the presence of a hospital environment fully supported a selective pressure for cloning of resistant properties of some antibiotics. The ability of A. baumannii to incorporate exogenous DNA, through horizontal genetic transfer (HGT), is one of the factors responsible for the multidrug resistance phenotype observed in several of clinical strains worldwide. With the advent of molecular techniques, such as mass spectrometry, ribotyping, multilocus sequence typing (MLST), RNA spacer fingerprinting, amplified fragment length polymorphism analysis, pulsed-field gel electrophoresis (PFGE), and matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF), identification of several virulence factors have been made easier.

This review article aims to focus on the virulence factors of A. baumannii, its pathogenesis, antimicrobial resistance, advanced technology for identification, and alternative treatment strategies, which provide information for the development and discovery of new antibiotics and for the determination of essential effective combination treatment to combat multidrug resistant infections.

2. Search Strategy

We searched full-text research and review articles on Acinetobacter baumannii through PubMed (Medline) databases and Google Scholar, published in English in the last 20 years, using the following keywords: ‘Acinetobacter baumannii’, ‘epidemiology’, ‘pathogenesis’, ‘antibiotic resistance’, ‘multi-omic study’, ‘in vitro studies’ ‘virulence’, and ‘treatment’. Exclusion criteria included irrelevant studies on A. baumannii (Figure 1).

3. Epidemiology

The epidemiology of A. baumannii infection is broad, which includes infections associated with hospital outbreaks, wars, natural disasters, and the community in tropical climates. Several previous research studies focused on the mechanisms of occurrence of MDR A. baumannii infection all around the globe, including Europe, North America, South America, China, Taiwan, Hongkong, Japan, Korea, and other parts of Asia and the Middle East. The outbreaks are commonly seen in critical care and burn units, with mechanically ventilated patients. An international report in ICUs showed that Acinetobacter infection rate was 19.2% in Asia, 17.1% in Eastern Europe, 14.8% in Africa, 13.8% in Central and South America, 5.6% in Western Europe, 4.4% in Oceania, and 3.7% in North America. It was found to be 15% in HIV-positive patients in South Africa and 13% in critical burn care units in Canada. Community-acquired pneumonia has been reported in the tropical climatic regions, mainly in Asia and Australia, during warm and humid months. A. baumannii was once also coined as ‘Iraqibacter’ because of its outbreak within military treatment facilities in Iraq war. UK and US military also detected an abundant number of multidrug-resistant A. baumannii calcoaceticus complex in military individuals injured during Iraq and Afghanistan war. A. baumannii was the most frequent isolated organism (32.5%) from the combat casualties in Iraq and Afghanistan battle victims with open tibia fractures. A. baumannii can cause outbreaks since it is highly resistant to antimicrobials and can overcome desiccation. It is noted that the ability of A. baumannii to form a biofilm is one of the major virulence factors to a large number of its clinical isolates.

The outbreaks of Acinetobacter have been attributed to source contamination, particularly contaminated respiratory and mechanical ventilators, and the cross-infection by the contaminated hands of healthcare workers caring for colonised or infected patients. The several risk factors associated with colonisation or infection by multidrug-resistant (MDR) A. baumannii are prior exposure to long-term antimicrobial therapy, mechanical ventilation, duration of hospital stay, the severity of disease, current surgery, and other invasive processes.

During 2016, the National Healthcare Safety Network (NHSN) of the United States reviewed the commonest drug-resistant organism involved in healthcare-associated infections where the Acinetobacter accounted for the following proportions among the most common Gram-negative isolates: ventilator-associated pneumonia (12.8%), central line-associated bloodstream infection (8.8%), catheter-associated UTIs (1.3%), and surgical site infection (1.3%). According to the prevalence study of infections in 2009, EPIC II (Extended Prevalence of Infection in Intensive Care) classified A. baumannii as the fifth most common pathogen in the community.
ICU in 75 countries.31 Furthermore, as reported by the international surveillance program (2009–2011), A. baumannii was the seventh most common pathogen isolated from ICU patients in the USA and European hospitals and ranked the eighth and seventh from non-ICU patients in the USA and European hospitals, respectively.40 The report of drug resistance in the USA (2011) showed that 63% of Acinetobacter spp. infections were caused by multi-drug resistance strains.47 The global rate of MDR A. baumannii was increased from 23% in 2004 to 63% in 2014.48 In the USA and Europe, from 2009–2011, the colistin-resistant A. baumannii was around 5% and 3%, respectively, whereas the worldwide prevalence of A. baumannii resistant to colistin and polymyxins B was only 0.9% and 0.8%, respectively.49,50

4. Pathogenesis

4.1. Virulence factors

The single virulence factors of A. baumannii are not clearly defined, and the joint action of multiple factors leads to the pathogenesis by adherence, biofilm formation, invasion, serum resistance, in vivo survival, and killing of host cells.51 Biofilm formation is one of the important factors that enhance its adherence to biotic and abiotic surfaces, including those of host tissues and medical devices.41 The production of biofilm-associated protein (BAP) gene is correlated to the formation and maturation of biofilms.42 The BAP enhances adherence to epithelial cells, and the inhibition of its production can control A. baumannii infection.43 The metallic homeostatic system, which is required for colonisation in different tissues is well defined in A. baumannii; among these, iron uptake system and zinc acquisition system play an important role in virulence.44,45 Another factor is that the K1 capsular polysaccharide prevents A. baumannii from phagocytosis by macrophages and facilitates its multiplication in fluid from human ascites and serum.46 Several other proteins, such as Omp38, RecA protein, phospholipases C, and phospholipases D, are estimated as probable virulence factors in A. baumannii as they lead to apoptosis of host cells, increase survival as a response to heat shock and desiccation, and enhance survival in human serum and epithelial cells invasion.47,48

Significantly, the other factors related to epithelial cells apoptosis caused by targeting the bacterial mitochondria is the outer membrane protein A', which is most abundantly present in A. baumannii.49 Once the A. baumannii enters the bloodstream, the lipopolysaccharides, an important component of cell envelop, may cause septic shock. A. baumannii also produces two antigenic types of verotoxins, vtx-1 and vtx-2, which enhance virulence by targeting the cell ribosome machinery and inhibiting protein synthesis.50,51

5. Clinical Relevance

A. baumannii can lead to several human infections, including ventilator-associated pneumonia, bacteremia, septicaemia, urinary tract infection, surgical site wound infection, and meningitis.52 The mortality rate ranging from 7.8% to 43% was seen in A. baumannii infections with higher levels in ICUs patients. Studies on morbidity reported that Acinetobacter pneumonia increases patients’ stay in ICU for several days.53

5.1. Hospital-associated pneumonia

Acinetobacter pneumonia is observed predominantly in ICU patients who are under mechanical ventilation, and however, sometimes it is not easy to distinguish between airway colonisation from true pneumonia. A. baumannii is the second commonest pathogen among Gram-negative bacteria causing hospital-associated pneumonia.32 The hospital-associated pneumonia caused by A. baumannii was around 3–5%, with a death rate of 30–75% being reported.54

5.2. Community-associated pneumonia

Community-associated Acinetobacter pneumonia shows sudden onset, which progresses rapidly, causing respiratory failure and hemodynamic instability, though the infection is rare.44,45 It has been reported in people who consume alcohol or in patients with chronic obstructive pulmonary disease from tropical areas of Asia and Australia during monsoon.44

5.3. Bloodstream infections

The vascular catheters and respiratory tract are the commonest sources for A. baumannii bacteremia, and the origin remains unknown in about 21–70% cases.55,56 About 1.5–2.4 % of the patients acquired infections nosocomially.57,58 The mortality rate of A. baumannii septicemia ranged 34–43.4% in critical care units and 16.3% in other units of the hospital.60 A. baumannii bloodstream infections are associated with various risk factors, including prolonged hospital and ICU stay, mechanical ventilation, surgery and other invasive procedures, wounds, burns, use of broad-spectrum antibiotics, and immunosuppression.57,61-62

5.4. Urinary tract infection

A. baumannii urinary tract infection is infrequent and accounts for only 1.6% cases.9 The setting of indwelling urinary catheters usually causes the colonisation of the urinary tract, leading to nosocomial urinary tract infections.55

5.5. Meningitis

Meningitis followed by neurosurgery induced by multidrug-resistant A. baumannii is a relevant issue.41 One study showed that about 2.1% of cases of meningitis post-craniotomy were caused by Acinetobacter.43 The certain risk factor associated with it includes surgery involving the brain and spinal cord, cerebrospinal fluid leakage, prior antibiotic treatment, and intracranial hemorrhage.44 Studies have shown that the mortality rate was about 20–30% and the survivors being left with severe neurologic deficits.55,56

5.6. Skin, soft tissue, and bone infection

The soft tissue infection progressing to osteomyelitis caused by contaminated surgical and traumatic wounds is seen in the case of A. baumannii infections.56 It rarely causes other skin infections such as cellulitis, folliculitis, skin abscesses, and necrotising fasciitis.55 The wound and soft tissue infections caused by multidrug-resistant A. baumannii are mainly recognised after war injuries. Among different isolated organisms, A. baumannii accounted for 32% of the war victims of combat casualties in Iraq and Afghanistan war.71

5.7. Other infections

Acinetobacter eye infection is mainly seen in contact lens wearers, which may lead to corneal ulcers, endophthalmitis, periorbital cellulitis, and traumatic infection.55,59 Some rarely
reported cases of A. baumannii infection are endocarditis, nosocomial sinusitis, and peritonitis.76-80

6. Laboratory Identification of A. baumannii

6.1. Conventional and molecular methods

A. baumannii is a pleomorphic coccobacillus bacterium that is 0.9-1.6 x 1.5-2.5 µm in size, which becomes spherical in the stationary phase of growth.81 It is a strict aerobe, Gram-negative, non-lactose fermenter, glucose oxidiser, catalase-positive, and oxidase-negative, which grows at 44°C, but practically these properties cannot confidently specify A. baumannii and could be easily misinterpreted with the other clinically relevant Acinetobacter species. The overnight colony morphology of A. baumannii on sheep blood agar at 37°C is light grey, circular, convex, entire, translucent, shiny, mucoid, and non-pigmented.

Automated methods like analytical profile index (API) kits, VITEK 2 system, and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI TOF MS) are currently used for the identification of different species.82 The 16S ribosomal DNA sequence comparison, amplified fragment length polymorphism (AFLP), and amplified 16S ribosomal DNA restriction analysis (ARDRA) are also used to identify Acinetobacter species in comparison with restriction enzyme-digested DNA fragment pattern on a gel.83 Amplification of recA and blaOXA-51-like gene using Real-time PCR is also used for identification of A. baumannii. At present, phylogenetic trees of rpoB gene or 7 housekeeping genes from multilocus sequence typing (MLST) are an indeed more reliable way to discriminate between species within Acb-complex that includes six species: A. calcoaceticus, A. baumannii, A. pittii, A. nosocomialis, A. seifertii, and A. djkshoorniae, as well as NIPH 542 and NIPH 817 that have no scientific names.84-86

6.2. Advance technology

6.2.1. Detection of Acinetobacter baumannii by nanoparticles

A. baumannii infections can cause serious damage to patients if not treated in a timely manner. Therefore, it is relevant to implement rapid analytical methods to detect A. baumannii to control its spread. DNA-based techniques as conventional methods have been employed for the identification of A. baumannii.87-90 However, these methods are complicated and require well-trained personnel. Currently, nanoparticle-based diagnostic procedures, such as fluorescence technique, colorimetric assays along with gold nanoparticles, and fluorescence nanoprobes, are used for detection of A. baumannii.91 As a result of the attraction and ease-of-functionalisation of magnetic nanoparticles, they are used very often to trap bacteria from complex clinical specimens, and those bacteria trapped by functional magnetic nanoparticles may be readily identified using MALDI-TOF mass spectrometry.92-94

Yi-Ling et al. reported on Fe3O4 and Al2O3 magnetic nanoparticles against A. baumannii, with values of M3237 and 54149, respectively.95 The bacteria trapped by functional nanoparticles were characterised using MALDI-TOF mass spectrometry, and the specificity and sensitivity of these nanoprobes against particular A. baumannii strains were evaluated.95 Similarly, Khalil et al. developed a nano-gold assay, which can colorimetrically identify and differentiate A. baumannii from other Gram-negative bacteria.96 Chan et al. reported that the gold nanoclusters encapsulated with lysosome in the presence of red photo-luminescence act as affinity probes to attract and accumulate infectious bacteria such as A. baumannii, Enterococcus faecalis, and Staphylococcus aureus.97 Chan et al. reported that MALDI-MS coupled with principal component analysis could identify bacteria in the conjugates. A fluorometric assay is used to demonstrate A. baumannii in the blood specimen using Zr-MOFs with methods such as functional coating for magnetic Fe3O4 nanoparticles to offer surface modification and as a carrier to fluorescein to create fluorescence indicators.98-100

6.2.2. Multi-omics analysis of A. baumannii

Infections caused by A. baumannii are a crucial cause of morbidity and mortality in hospital settings, and A. baumannii resists a wide spectrum of antibiotics used to treat the infections. Therefore, many researchers have focused on studying insight into the mechanisms of actions of the drugs against the pathogen.101-103 Multi-omics analysis, including proteomic, genomic, and transcriptomic analyses, is a powerful tool to shed light on the key expression of genes, metabolites, and proteins in the different metabolic pathways, as shown in Table 1. The method has been used to study the expression of genes and metabolites in exposure to antibiotics and nutrient-limited conditions.

In a study on proteomic analysis, Tiwari and team reported that the excess production of membrane proteins like ferric-acinetobactin, ferrienterochelin, ferric siderophore, and FhuE receptors were detected under iron-limited conditions.104 Besides, the interaction between FhuE receptor and siderophores was synthesised by A. baumannii as well as other bacteria in iron acquirement. Depending upon the immune status established by the host, the interaction between the siderophores and the corresponding receptors favours iron sequestration and bacterial survival. It has been concluded that the target-FhuE receptor inhibits siderophore-mediated iron acquirement in A. baumannii.105 It has been reported that a total of 65 unique periplasmic proteins of the pathogen were identified under exposure and un-exposure to imipenem; among these, the eight types of proteins were associated with protein fate in relation to antibiotic resistance, energy metabolism, and oxidative stress (Figure 2).106 In antibiotic resistance, four proteins were detected, which include GES-11, the carbapenemases OXA-23, the cephalosporinase AmpC, and the RND-type efflux pump AdeT. In protection against oxidative stress, ABUW_2868 encoding a heat shock protein was possibly found to be associated under upregulated imipenem-exposed bacteria.107

A combination of drugs is a powerful treatment to cure the infection caused by A. baumannii. A synergistic effect of colistin in combination with sulbactam against the organism has been reported. The combination was carried out by colistin through agitation of the levels of fatty acid and phospholipid at 1 hour. The biosynthesis of the bacterial cell wall was perturbed when A. baumannii was treated with sulbactam alone and the combination over 24 hours. Using metabolic analysis, expression of uridine diphosphate-N-
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<table>
<thead>
<tr>
<th>Type of Omics</th>
<th>Result</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Proteomic</td>
<td>- It was found that the outer membrane vesicles of antibiotic-sensitive strain consisted of 8 antibiotic resistance-conferring proteins. In contrarily, the vesicles of multidrug-resistant stain comprised 24 proteins of antibiotic resistance.</td>
<td>96</td>
</tr>
<tr>
<td>Proteomic</td>
<td>- It was shown that Type II secretion system secretome provides an advantage of the colonization to the pathogen multi-drug resistant strain rather than the reference strain used for biofilm formation.</td>
<td>97</td>
</tr>
<tr>
<td>Proteomic</td>
<td>- The over-expression of four membrane proteins including ferric-acinetobactin, ferrienterochelin, ferric siderophore, and Fhu-E receptors were detected under iron-limited conditions.</td>
<td>98</td>
</tr>
<tr>
<td>Proteomic</td>
<td>- The interaction between FhuE receptor and siderophores was produced by <em>A. baumannii</em> as well as other bacteria in iron acqirement.</td>
<td></td>
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<tr>
<td>Proteomic</td>
<td>- Under nutritional immunity established by the host, The interaction between the receptor and siderophores helps in iron sequestration and survival of <em>A. baumannii</em>.</td>
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<td>Proteomic</td>
<td>- FhuE receptor, as a target, was shown to inhibiting siderophore-mediated iron acqirement in <em>A. baumannii</em>.</td>
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<td>- A total of 65 unique periplasmic proteins were identified under exposure and un-exposure to imipenem.</td>
<td>99</td>
</tr>
<tr>
<td>Proteomic</td>
<td>- There are eight proteins involved in protein fate and response to antibiotic-resistance, energy metabolism, and oxidative stress.</td>
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</tr>
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<td>- In antibiotic-resistance, four proteins were detected which include GES-11, the carbapenemases OXA-23, the cephalosporinaseAmpC, and the RND-type efflux pump AdeT.</td>
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<tr>
<td>Metabolomic</td>
<td>- A synergistic effect of colistin in combination with sulbactam against <em>A. baumannii</em> has been reported.</td>
<td>100</td>
</tr>
<tr>
<td>Metabolomic</td>
<td>- The combination was carried out by colistin through agitation of the levels of fatty acid and phospholipid at 1 h.</td>
<td></td>
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<td>Metabolomic</td>
<td>- The biosynthesis of the bacterial cell wall was perturbed when <em>A. baumannii</em> was treated with sulbactam alone and the combination over 24 hrs.</td>
<td></td>
</tr>
<tr>
<td>Metabolomic</td>
<td>- Expression of uridine diphosphate-N-acetylglucosamine and uridine diphosphate-N-acetylmuramate involved in amino sugar metabolism were decreased when the pathogen was treated with the drug combination.</td>
<td>101</td>
</tr>
<tr>
<td>Metabolomic</td>
<td>- A synergistic effect of colistin in combination with doripenem against <em>A. baumannii</em> has been reported.</td>
<td></td>
</tr>
<tr>
<td>Metabolomic</td>
<td>- Perturbation of glycerophospholipids and fatty acids by colistin resulted in the disruption of <em>A. baumannii</em> outer membrane and cell wall.</td>
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<tr>
<td>Metabolomic</td>
<td>- Doripenem alone suppressed the expression of peptidoglycan biosynthesis metabolites at 4 h.</td>
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<tr>
<td>Metabolomic</td>
<td>- The combination of the drugs suppressed the expression of D-sedoheptulose 7-phosphate (nucleotid metabolism) and D-ribose 5-phosphate (pentose phosphate pathway).</td>
<td></td>
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<tr>
<td>Transcriptomic</td>
<td>- The upregulation of genes associated with transposable elements was detected when the bacteria were treated with antibiotic including amikacin, imipenem, and meropenem.</td>
<td>102</td>
</tr>
<tr>
<td>Transcriptomic</td>
<td>- In pan-drug resistant zstrains, overexpression of amino acid metabolism and membrane transporters has been reported.</td>
<td></td>
</tr>
<tr>
<td>Transcriptomic</td>
<td>- Antibiotic resistance genes were up-regulated in both antibiotic-resistant and -sensitive strains of <em>A. baumannii</em>.</td>
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</tr>
<tr>
<td>Transcriptomic</td>
<td>- Under tigecycline pressure, upregulation of efflux pumps including RND transporter permease subunit, EmrAB, MacB, and Tet resistance operon was detected in antibiotic-resistant <em>A. baumannii</em>, when compared with the sensitive strain.</td>
<td>103</td>
</tr>
<tr>
<td>Transcriptomic</td>
<td>- Genes related to benzene-containing compound metabolic process, translation, ribosomal structure, and biogenesis were found to be overexpress in the resistant strain treated with tigecycline.</td>
<td></td>
</tr>
<tr>
<td>Transcriptomic and</td>
<td>- Upregulation of ribosomal proteins and resistance pumps including MFS, RND, MATE, and ABC transporters were observed in <em>A. baumannii</em> treated with eravacycline.</td>
<td>104</td>
</tr>
<tr>
<td>proteomic</td>
<td>- In outer membrane vesicle, overexpression of ribosomal proteins, toluene tolerance protein, siderophore receptor, and peptidases was detected in multidrug-resistant <em>A. baumannii</em>.</td>
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Table II: Antimicrobial activity of plant-derived compounds against *Acinotorbacter baumannii*

<table>
<thead>
<tr>
<th>Product/Plant species</th>
<th>Examination procedure</th>
<th>Antibacterial activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norwogonin/Scutellaria baicalensis</td>
<td>MIC90 determination</td>
<td>MIC90 = 128 µg/mL</td>
<td>129</td>
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<tr>
<td>Terchebulin/Terminalia chebula</td>
<td>MIC90 determination</td>
<td>MIC90 = 500 µg/mL</td>
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</tr>
<tr>
<td>Ellagic acid/Terminalia chebula</td>
<td>MIC90 determination</td>
<td>67% inhibition at 250 µg/mL</td>
<td>129</td>
</tr>
<tr>
<td>Chebulagic acid/Terminalia chebula</td>
<td>MIC90 determination</td>
<td>60.39% inhibition at 62.5 µg/mL and 88% inhibition at 1,000 µg/mL</td>
<td>129</td>
</tr>
<tr>
<td>Chebulinic acid/Terminalia chebula</td>
<td>MIC90 determination</td>
<td>65% inhibition at 62.5 µg/mL</td>
<td>129</td>
</tr>
<tr>
<td>Corilagin/Terminalia chebula</td>
<td>MIC90 determination</td>
<td>56% inhibition at 15.625 µg/mL and 83% inhibition at 1,000 µg/mL</td>
<td>129</td>
</tr>
<tr>
<td>Norwogonin/Scutellaria baicalensis</td>
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Bio-AgNP: Biological silver nanoparticles, FICI: Fractional inhibitory concentration index, IZ: Inhibition zone, MBC: MIC: Minimum bactericidal concentration, Minimum inhibitory concentration, MID: Minimum inhibitory dose
Acinetobacter baumannii: An overview of emerging multidrug-resistant pathogen

Fig. 1: The overall flowchart of phases used to identify published articles included in this review.

Fig. 2: Periplasmic proteins identified in MDR A. baumannii strain A8707S cultured in the presence and absence of imipenem.
acetylglicosamine and uridine diphosphate-N-acetylmuramate involved in amino sugar metabolism was decreased when the pathogen was treated with the drug combination.\textsuperscript{100} Maifiah and the team reported the synergistic effects of colistin in combination with doripenem against \textit{A. baumannii}.\textsuperscript{101} The perturbation of glycerophospholipids and fatty acids by colistin resulted in the disruption of \textit{A. baumannii} outer membrane and cell wall. Doripenem alone suppressed the expression of peptidoglycan biosynthesis metabolites at 4 hours. The combination of the drugs suppressed the expression of D-sedoheptulose 7-phosphate (nucleotid metabolism) and D-ribose 5-phosphate (pentose phosphate pathway).\textsuperscript{101}

Study on transcriptomic analysis, Qin and team reported that upregulation of genes associated with transposable elements was detected when the bacteria were treated with antibiotics, including amikacin, imipenem, and meropenem.\textsuperscript{102} In pan-drug resistant \textit{A. baumannii} strains, overexpression of amino acid metabolism and membrane transporters has been reported. Antibiotic resistance genes were upregulated in both antibiotic-resistant and sensitive strains of \textit{A. baumannii}.\textsuperscript{103} Under tigecycline pressure, upregulation of efflux pumps, including RND transporter permease subunit, EmrAB, MacB, and Tet resistance operon, was detected in antibiotic-resistant \textit{A. baumannii}, compared with the sensitive strain. Genes linked to the metabolism of benzene-containing compounds, translation, ribosomal structure, and biogenesis were found to be overexpressed in the resistant strain treated with tigecycline.\textsuperscript{103} Integration of transcriptomic and proteomic analysis is a powerful tool to clarify the key expression of genes and proteins in the organism. Kesavan and team reported that the upregulation of ribosomal proteins and resistance pumps, including MFS, RND, MATE, and ABC transporters, was observed in \textit{A. baumannii} treated with eravacycline. In the outer membrane vesicle, overexpression of ribosomal proteins, toluene tolerance proteins, siderophore receptors, and peptidases was detected in multidrug-resistant \textit{A. baumannii}.\textsuperscript{104}

In summary, multi-omics analysis, including proteomic, genomic, and transcriptomic analyses, is a powerful tool to shed light on the key expression of genes, metabolites, and proteins in the different metabolic pathways. The analysis can be used to identify the antibacterial mode of action and the expression of the resistance mechanism of the drug against \textit{A. baumannii}.

7. Treatment

It is well known that carbapenems are the drug of choice against the infection caused by multidrug-resistant \textit{A. baumannii}. For \textit{A. baumannii} resistant to carbapenem, tigecycline and colistin are used.\textsuperscript{30} The global emergence of MDR, XDR, and PDR \textit{A. baumannii} and the paucity of newer antimicrobial compounds are a major challenge for the healthcare industries.\textsuperscript{105} For PDR \textit{A. baumannii}, combination therapies like carbapenem+ampicillin/sulbactam, carbapenem+colistin, colistin+rifampicin+suibactam, and tigecycline+rifampicin+ampicillin have been used worldwide. The combination therapies are costly, and their toxicity and adverse effects are strong.\textsuperscript{106}

7.1. Carbapenem resistance in \textit{A. baumannii}

Currently, most strains of \textit{A. baumannii} are highly resistant to broad-spectrum antibiotics used clinically. Several resistance mechanisms targeting different antibiotic classes are observed in \textit{A. baumannii}, such as production of β-lactamases, efflux pumps, aminoglycoside-modifying enzymes, permeability defects, and target sites alteration.\textsuperscript{107} Mostly these mechanisms target the different antibiotic classes; however, various mechanisms can also support the resistance to a single antibiotic class. The chief β-lactamases resistance
mechanism involving carbapenem-hydrolysing property is due to the presence of class D oxacillinases and class B metallo-
ß-lactamases (MBLs). In addition, the loss or mutation of CarO porins and alteration of penicillin-binding proteins also favour carbapenem resistance.108,109 The dissemination of multidrug resistance determinants in \textit{A. baumannii} is strongly due to plasmid conjugation and acquisition of transposons, which leads to mobilisation of a bunch of drug resistance genes to several antibiotic classes.110 Furthermore, the presence of insertion sequences also multiplies the antibiotic-resistant strains.111

The leading source of carbapenem resistance in \textit{A. baumannii} is due to the presence of class D (OXA) oxacillinase, a type of beta lactamase, which occurs naturally in \textit{A. baumannii} (OXA1/66 group). In most strains, the oxacillinase, OXA-51-like genes are expressed poorly, having less influence on susceptibility pattern to all beta-lactam antibiotics, including carbapenems. The expression and influence of these genes are also facilitated by the presence of insertion sequence ISAba1, when present upstream to the structural gene, further leading to the development of carbapenem resistance in \textit{A. baumannii}.\textsuperscript{112} Based on sequence homology, OXA carbapenemases are further grouped into the various clusters: OXA-23-like (includes OXA-27 and OXA-49), OXA-24/40-like (includes OXA-25, OXA-26, and OXA-40), and OXA-58.\textsuperscript{113} The OXA-23-like genes, which are most commonly seen in \textit{A. baumannii}, are mediated through both chromosome and plasmid. The OXA-23-like genes in \textit{A. baumannii} have been frequently observed since 1985, including the strains obtained from outbreaks in the UK, Asia, and South America. This strain exists in one multi-resistant clone, which is prevalent in the UK and identified as OXA 23 clone-1.\textsuperscript{114} Another oxacillinase group of resistance genes, OXA-24-like, which are also chromosomal or plasmid mediated, are less prevalent compared to OXA-23-like genes, with data mainly restricted to European countries and the United States.\textsuperscript{115} The expression of other genes like ambl class B metallo-beta lactamases (MBLs), such as IMP, VIM, and SIM-1, was also observed in \textit{A. baumannii}. The expression of these genes also confers a high level of resistance to most of the beta lactams, including carbapenems, but excluding aztreonam.\textsuperscript{116}

7.2. Drug metabolism

Carbapenem-resistant \textit{A. baumannii} strains are considered as a pathogen for causing life-threatening infections since no alternative therapy is available. Though the mechanisms leading to antibiotic resistance in \textit{A. baumannii} have been extensively studied, the general response to keep the viability of bacteria under antimicrobial exposure needs more investigation.\textsuperscript{117} A study based on periplasmic protein of MDR \textit{A. baumannii} strain AB7075 cultured in the presence and absence of imipenem reported that besides carbapenem, the periplasmic space also displays various other proteins with essential functions of the cell. In both types of culture conditions, a total of 65 periplasmic proteins were detected by proteomic approach, and out of this, eight proteins were associated with protein fate, resistance to antibiotics, energy breakdown, and reaction to oxidative stress. Among these proteins, some gene products like ABUW_1746 and ABUW_2363 presented the tetrapeptide repeat motif, which mediates the protein–protein interactions. These proteins expressed by the genes can regulate definite proteins and help to adapt well in altered environmental situations. The heat shock proteins coded by ABUW_2868 genes are possibly associated with defence against oxidative damage, which is seen upregulated in bacteria exposed to imipenem. Scribano et al. evidenced the first report on the content of the periplasmic proteins of a multidrug-resistant \textit{A. baumannii} strain and its susceptibility to imipenem, pointing towards the probable new targets to develop substitute antibiotics.\textsuperscript{118} The new antibacterial molecules can be designed with the knowledge of IMP upregulated proteins and their molecular functions, and it has been concluded that MDR \textit{A. baumannii} on stress exposure to IMP adapts various strategies to successfully cope with it.

ß-Lactamases, coded chromosomally, plays an important role in finding alternative and efficient therapy for treatment against multidrug-resistant \textit{Acinetobacter} spp. The occurrence of chromosome-mediated ß-lactamases, like class C \textit{Acinetobacter}-derived cephalosporinases and class D oxacillinases, and also the existence of plasmid-encoded class A ß-lactamases represent a therapeutic challenge in \textit{Acinetobacter} spp. The newly permitted ß-lactamase inhibitors such as avibactam and vaborbactam represent a range of gap in inhibition against OXA like ß-lactamase. The new, sensibly designed, diazabicyclooctene inhibitor ETX2514 adequately targets against all, class A, C, and D ß-lactamases.\textsuperscript{119} Barnes et al. showed that the sulbactam-ETX2514 combination has an extensive inhibitory range to target class D, A, and C ß-lactamases and also promising treatment options against infections induced by MDR \textit{Acinetobacter} spp.\textsuperscript{120} For instance, curcumin in combination with blue light is an effective photodynamic treatment (PDT), exerting antimicrobial operation. In one of the studies, Chang et al. explored the probable underlying mechanism to examine the protein carbonylation in response to the bactericidal action in the presence of oxidative stress when \textit{A. baumannii} resistant to imipenem was subjected to blue light assisted curcumin a shotgun proteomics approach has been implemented and afterwards, the bacterial proteins were extracted, 2,4-dinitrophenylhydrazine (DNPH) derivatised, and trypsin were digested.\textsuperscript{117} On searching the customised database, the carbonylated proteins were documented, and the analysis of the peptides was conducted using LC-nano ESI ion trap mass spectrometry. After utilising the investigation of gene ontology, annotation and the STRING protein association network for the 70 identified proteins, the commonest was the protein related to the membrane, translation, and oxidative stress response. Various proteins, which are associated in interpretation of \textit{A. baumannii}, were described to be carbonylated targets. These proteins incorporate the lengthening factor Tu and P, two ribosomal proteins, and ribosome discharging factor. A maximum number of these interpretation-associated proteins in bacteria has been documented in past studies based on the exploration of the target macromolecules in microorganisms under oxidative pressure.\textsuperscript{118}

Several micronutrients are required for the survival of \textit{A. baumannii} inside the host. Among these micronutrients, the bio-availability of iron is limited by the nutritional immunity
created by the host, and because of this, *A. baumannii* needs to develop a mechanism to uptake iron while causing infections. Research by Tiwari et al. attempted to recognise membrane proteins associated with the iron sequestration process of *A. baumannii* with the use of two-dimensional electrophoresis and liquid chromatography with tandem mass. The distinguished iron-directed layer protein (IRMP) of *A. baumannii* was utilised during communication studies with various siderophores, and inhibitor against *A. baumannii* was also designed focusing on this IRMP212. The four membrane proteins were overexpressed in the membrane proteomic results, which include FhuE receptor, ferric-acinetobactin receptor, ferrientochelin receptor, and ferric siderophore receptor, under iron-constrained condition. Iron-managed layer proteins like FhuE receptor cause the bacteria to obligate during difficult situations inside the host. A great association has been observed between the siderophores produced by *A. baumannii* and the FhuE receptor. Similar results also demonstrated that FhuE receptor has an association with siderophores delivered by bacteria other than *A. baumannii*. This connection between the FhuE receptor and siderophores supports iron sequestration and bacterial survival under a nutritionally invulnerable environment. Therefore, it gets basic to locate a possible FhuE receptor-inhibitor through which the survival of *A. baumannii* within the host is suppressed. In-silico screening and molecular mechanics studies recognised ZINC03794794 and ZINC01530652 as major structure inhibitors against the FhuE receptor of *A. baumannii*. The structured inhibitors are tentatively approved for their bactericidal action against *A. baumannii*. Thus, a structured inhibitor affects the iron uptake mechanism of Acinetobacter, and therefore, it might be favourable in the prevention of infections caused by *A. baumannii* by constraining nutrient accessibility. Additionally, a study involving an animal model is to be performed further to explore the utilisation of FhuE receptor inhibitor and to validate its function.

8. Alternative strategies for MDR *A. baumannii*

With rising antibiotic resistance and treatment difficulties, many studies have focused on alternative drugs and phytomedicine. Combined actions of antibiotics and active components of plant extracts have been studied mostly as an alternative strategy. Many studies stated that the synergistic action of plant active components and the antibiotics could play a role to combat drug resistance and increase bacterial susceptibility. Along with the screening of herbal compounds, a nanomaterial-based approach has been tried to find suitable alternative agents to MDR *A. baumannii*. The nanoparticles having low molecular weight were potentially effective against most bacteria causing human infections. Silver nanoparticles have shown antimicrobial activity against a wide array of microbes, including *A. baumannii*, probably caused by their several bactericidal mechanisms. The effectiveness of synergistic action of nanoparticles and plant active components against bacterial inhibition was seen high compared to its independent action. The study also demonstrated a synergistic effect of imipenem and silver nanoparticles against *A. baumannii* planktonic cells as well as biofilms.

8.1. Use of natural products and nanoparticles

Many plant active compounds (Table II) are being used worldwide as traditional remedies against several antibiotic-resistant bacteria, including carbapenem-resistant *A. baumannii*. For example, flavones, tannins, and phenolic compounds are demonstrated to have inhibitory activity against Acinetobacter. Miyasaka et al. reported that six compounds, including ellagic acid extracted from *Rosa* sp., norwogonin extracted from *Scutellaria baicalensis*, and chebulagic acid, chebulinic acid, corilagin, and terchebulin extracted from *Terminalia chebula*, had the maximum antimicrobial effect against *A. baumannii* in vitro. Norwogonin (5,6,7-trihydroxyflavone) from *Scutellaria baicalensis* was identified as the most potent compound with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 128 μg/mL and 256 μg/mL, respectively, against clinical isolates of *A. baumannii*. Other herbal compounds, including ellagic acid (67% inhibition at 250 μg/mL), chebulinic acid (65% inhibition at 62.5 μg/mL), chebulagic acid (60.39% and 88% inhibition at 62.5 μg/mL and 1,000 μg/mL, respectively), and corilagin (56% and 83% inhibition at 15.625 μg/mL and 1,000 μg/mL, respectively) exhibited lower antimicrobial activity. Furthermore, several plant-derived phenolic compounds are reported in the medical literature to increase the strength of synthetic antibiotics against *A. baumannii*. For instance, the in vitro activity of several antibiotics, including novobiocin, coumarinycin, chlorobiocin, rifampicin, and fusidic acid, has been increased against MDR *A. baumannii* in the presence of ellagic and tannic acids. Synergy was also observed between the epigallocatechin-3-gallate (EGCG), a purified polyphenol from green tea leaves, and topical mafenide acetate (Sulfamylon) against a clinical isolate of MDR *A. baumannii* in vitro. Another study showed that the MIC of aminoglycosides (e.g., gentamicin and kanamycin) in combination with oleanc acid (a pentacyclic triterpenoid compound) decreased to one-fourth of the MIC alone in *A. baumannii*. Moreover, the fractional inhibitory concentration index (FICI) values of both aminoglycosides in combination with oleanc acid were indicative of synergism. In contrast, Miyasaka et al. observed no synergy effect between anti-Gram-negative antibiotics and norwogonin.
Nanoparticles have received great attention in recent years to combat antimicrobial resistance in microbial pathogens. Several types of nanoparticles, such as silver, gold, zinc, chitosan, platinum, iron, copper, and carbon nanotubes, have been evaluated for their antimicrobial activity in combination with essential oils. One study assessed the antimicrobial activity of tree tea oil nanoeumulsion (nanoTTO) against different microbial pathogens associated with pneumonia, including A. baumannii. The nanoTTOs showed strong antibacterial effects in A. baumannii ATCC19606 with the MIC of 3.52 mg TTO/mL. Furthermore, this nanoeumulsion notably decreased the lung injury of pneumonia induced by A. baumannii in the rat model, indicating its relatively high in vivo anti-A. baumannii effect, which is vital for the treatment of bacterial pneumonia. In another study, Origanum vulgare essential oil (OEO) and the biologically synthesised silver nanoparticle (bio-AgNP) showed a bactericidal effect in low concentration against all bacterial strains resistant to multi-drugs tested, with MBC values of 0.298 mg/mL and 125 μM, respectively, for multidrug-resistant to carbapenem-resistant A. baumannii isolate.

Besides, the combination of OEO and bio-AgNP resulted in significantly lower MICs compared to individual treatment, where the two compounds together led to additive antibacterial activity against A. baumannii. Taken together, the promising results of synergistic and additive interactions are a milestone that facilitates the combination of nanoparticles and antimicrobial compounds derived from plants as antimicrobial agents to be applied in certain industries (e.g., cosmetics, food, and pharmaceuticals) and healthcare facilities for the control and treatment of various infections or disinfection of hospitals to combat pathogens resistant to several multi-drugs, particularly A. baumannii.

The synergistic action of nanoparticles and antibiotics could be a promising treatment option to combat bacterial resistance in future. Moreover, nanoparticles have the property to deliver antibiotics to the infected cells and also decrease the dose and toxicity of antibiotics. The synergistic bactericidal action of antibiotics and silver nanoparticles at low concentrations against different bacteria including A. baumannii has been reported. Though the plant extract and metal-based nanoparticles, alone or in synergy with antibiotics, have shown their bactericidal activity against A. baumannii and other bacteria in vitro, to confirm this as a novel treatment against various bacterial infections, without causing severe damage to the human cells, there should be a randomised control study. The nanomaterial causes huge cell damage in vitro, which is not suitable for direct application to human cells without proper dosage recommendation based on clinical trials. The appropriate and effective dosage that is suitable for use in human infections needs to be studied thoroughly, and more clinical trials are needed before the application of nanoparticle-based treatment in patients.

9. Conclusion

The clinical significance of A. baumannii in the past one and half decades has been increased by its isolation in ICU patients causing several infections, high morbidity, and mortality, its resistant-acquiring mechanisms, and its emergence as a prominent nosocomial pathogen challenging the current antibiotic era. To combat dissemination of this MDR bug, strict hospital aseptic procedures and appropriate antimicrobial stewardship policies are highly recommended. The prompt diagnosis of A. baumannii infections to overcome serious damage to the patients is a sole priority. The MALDI-TOF mass spectrometry and nanoparticle-based diagnostic procedures involving fluorescence technique, colorimetric assays, and fluorescence nanoprobes are used as advanced diagnostic tools. The antibiotics and phytochemicals in combination or synergy with silver or gold nanoparticles showed a promising result to overcome this MDR challenge in future with further intensive research (Figure 3).

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