KNOWLEDGE GAPS OF *ACINETOBACTER BAUMANNII* TREATMENT

By

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Abstract

Background

The incidence of healthcare-associated infections (HAIs) caused by antimicrobial-resistant pathogens is accelerating, leading to an increase in morbidity and mortality throughout the world. *Acinetobacter baumannii* (*A. baumannii*), a gram-negative, aerobic bacteria that infects blood, urinary tract, lungs, and wounds, has developed resistance to the most commonly utilized antibiotics to treat severe infections, Carbapenems. *A. baumannii* infections are a growing threat within the hospital environment, and treating rapidly evolving antibiotic resistance is a high priority.

Objectives

The goal of this thesis is to better understand the concerns and treatment options for A. baumannii. The following objectives were implemented to achieve this goal: identify knowledge gaps in antibiotic resistance mechanisms and treatment associated with *A. baumannii* infections and understand *A. baumannii* in hospitals by gathering personal experiences of professionals addressing exposure with the bacteria.

Methods/Adjectives

Primary literature was reviewed to create an extensive annotated bibliography. Knowledge gaps in treatment associated with *A. baumannii* were identified. Health professionals were surveyed to help bridge the gaps in *A. baumannii* antibiotic resistance and practiced treatments.

Results

The most prominent knowledge gaps include alternate approach anti-virulence strategies, application methods for Bacteriophage therapy, treatment for Carbapenem-Resistant *A. baumannii* (CRAB) infections, clinical and microbiological characteristics of Colistin resistance, and quantitative measurements for environmental and activity factors of *A. baumannii*. Survey responses proposed infection control interventions, and the need for advancements in the utilization of bacteriophage therapy.

Discussion

There are various methods for prospective treatments and infection control interventions. D-glutamate is identified as a potential vaccine candidate. Modes of bacteriophage administration and the creation of phage libraries are needed. The benefits of combination therapy on CRAB remains unclear, and clinicians must monitor emerging resistance to colistin. It is unknown how the environment and various sanitation methods impact the presence and quantity.

Conclusion

The main finding of this research is to identify the best current course of action for the treatment of antibiotic-resistant *A. baumannii* infections. In doing so, the need for future studies can be identified. As a result, there could be an effective treatment protocol, a national surveillance system for growing resistance, and a new means of infection prevention control of healthcare-associated Infections associated with *A. baumannii.*

Keywords: antibiotic resistance, bacteriophage therapy, healthcare-associated infections, multidrug-resistant organisms, knowledge gaps, *Acinetobacter baumannii*, treatment, infection prevention control

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Literature Review

Introduction

The incidence of healthcare-associated infections (HAIs) caused by antimicrobial-resistant pathogens is accelerating, leading to an increase in morbidity and mortality. *Acinetobacter baumannii* (*A. baumannii*) infections are a growing threat within the hospital environment. *A. baumannii* has developed resistance to the one of the most utilized antibiotics, carbapenems. To overcome this problem, knowledge of antibiotic resistance and prospective methods of treatment are important. This review summarizes current studies on the virulence factors and resistance mechanisms of *A. baumannii* pathogenesis. Fundamental gaps in knowledge are identified for antibiotic resistance mechanisms and treatment associated with *A. baumannii* infections. Prospective treatment options and future studies for multidrug-resistant (MDR) *A. baumannii* infections are proposed.

Goal

To identify knowledge gaps in antibiotic resistance mechanisms and treatment associated with *A. baumannii* infections and understand *A. baumannii* in hospitals by gathering personal experiences of professionals addressing exposure with the bacteria.

Objectives

1. Identifying knowledge gaps in *A. baumannii* research through a scoping literature review
2. Survey targeting epidemiologists, infectious disease specialists, and microbiologists.

Background

Antibiotic Resistance

Antibiotic resistance threatens the ability of modern medicine. Antibiotic resistance is the act of bacteria mutating in response to the overuse of antibiotics; thus, making it resistant to certain medications (World Health Organization, 2020). Antimicrobial resistance is a naturally occurring process that has occurred throughout history as a response to natural antibiotics. This occurs when microorganisms are exposed to antibiotics or antifungals, and then the exposed microorganisms regrow with the resistant genetics (CDC, 2019). The current increase in antibiotic resistance is attributed to the overuse and overprescription of pharmaceuticals, and a decrease in the production of new medications (Schooley et al., 2017). As antibiotic resistance can happen to anyone, the cost of burden is extremely high when the efficacy of first-line antibiotics depletes. Consequently, more expensive medicines are administered resulting in a longer duration of hospital stay, and an overall increase in healthcare costs (World Health Organization, 2020). Furthermore, new resistance mechanisms are constantly developing and spreading across the globe. Doctors are finding it difficult to treat common infectious diseases, such as pneumonia, tuberculosis, and gonorrhea (World Health Organization, 2020). In addition, medical procedures - such as organ transplantations, chemotherapy, and surgeries - have become much more dangerous without effective antibiotics (World Health Organization, 2020). As a result, the rate of healthcare associated infections (HAIs) has increased, resulting in significant illness and death (CDC, 2019).

Healthcare Associated Infections

Healthcare associated infections (HAIs) are any infection resulting from complications of healthcare settings including up to 30 days post-discharge (CDC, 2021a). The Centers for Disease Control and Prevention (CDC) reports that 1 in 31 hospitalized patients acquire an HAI (CDC, 2021a). HAIs occur from multidrug-resistant organisms (MDRO) like *Clostridium difficile*,

methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococci* (VRE) (Al-Tawfiq and Tambyah, 2014). MDROs have been found throughout hospital environments including on high-touched surfaces in hospitalized patient rooms (Facciolà et al, 2019). These surfaces include bed bars, bedside tables, bathroom grab bars, and nurse taps (Facciolà et al, 2019). MDROs are able to persist for hours to months in hospital settings as circulation is favored by healthcare workers (Facciolà et al, 2019). Facciolà et al. found that "non-classical" surfaces such as healthcare workers' mobile phones and personal computers, oxygen humidifiers, and the protective lead garments of operating rooms contributed to the spread of HAIs (2019).

HAIs are a major complication in occupational health. In the United States, HAIs occur in two million patients a year, with 99,000 deaths (Al-Tawfiq and Tambyah, 2014). This cost of burden is about $33 billion per patient (Al-Tawfiq and Tambyah, 2014). HAIs can be attributed to an increase in age and complexity of patients, the use of invasive devices, and inappropriate use of antimicrobial therapy (Al-Tawfiq and Tambyah, 2014). Common invasive devices in association with HAIs include: central-line associated bloodstream infections (CLABSI), catheter-associated urinary tract infections (CAUTI), ventilator-associated pneumonia (VAP), and surgical site infections (SSI) (Al-Tawfiq and Tambyah, 2014). HAIs lead to higher antimicrobial resistant rates, an extended hospital stay, extra financial burden, long-term disability, and preventable deaths (Ahmed et al., 2021).

Multidrug-Resistant *Acinetobacter baumannii*

An emerging MDRO is *Acinetobacter baumannii*. *A. baumannii* is a Gram-negative, aerobic bacterium, commonly found in the environment like soil and water (Abdul-Mutakabbir et al., 2021). Its oxidative properties allow it to colonize humans easily as it is responsible for opportunistic infections of the skin, bloodstream, urinary tract, and soft tissues (Lee et al., 2017). Multidrug-resistant (MDR) *A. baumannii* has spread to civilian hospitals by cross-infection of

injured military patients, earning it the nickname “Iraqibacter” (Lee et al., 2017). Numerous studies indicated that the severity of underlying disease, broad-spectrum antimicrobial use, prolonged hospitalization, intensive care unit (ICU) stays, the presence of invasive devices, and prolonged mechanical ventilation were risk factors for acquisition of multi-drug resistant *A. baumannii* strains (Ibrahim, 2019).

A. baumannii is now one of the highest priority threats to antibiotic resistance and HAIs as the nosocomial pathogen has increased resistance to hospital setting disinfectants and antibiotic therapy (Abdul-Mutakabbir et al., 2021). Globally, 45% of clinical isolates are multidrug-resistant (Harding et al., 2018). In regions throughout Latin America and the Middle East, MDR rates of *A. baumannii* are up to 70% (Harding et al., 2018). In the United States and Europe, *A. baumannii* infections account for 2% of all HAIs (Harding et al., 2018). A CDC report, “Antibiotic Resistance Threats in the United States, 2019”, highlighted carbapenem-resistant *A. baumannii* (CRAB) as an urgent threat that caused 8,500 infections and 700 deaths in the United States in 2017 (CDC, 2021b). *A. baumannii* is of particular concern to the CDC as MDR *A. baumannii* infections are difficult to treat with available antibiotics like fluoroquinoline, ampicillin/sulbactam, and trimethoprim/sulfamethoxazole (CDC, 2021b). Another concern is that *A. baumannii* can readily contaminate a patient’s environment. During health facility outbreaks, *A. baumannii* has been found on bed rails, pillows, mattresses, tables, and ventilators (Farrow et al., 2020). Clinical isolates have shown that *A. baumannii* can survive on dry surfaces for at least 20 days (Farrow et al., 2020). Some strains have demonstrated the ability to survive over 3 months (Farrow et al., 2020).

In addition, the frequency of community-acquired *A. baumannii* infections has increased as genomic and phenotypic analyses have identified several virulence factors (Lee et al., 2017). These include: outer membrane proteins, phospholipases, proteases, lipopolysaccharides (LPS), capsular polysaccharides, protein secretion systems, and iron-chelating systems (Lee et

al., 2017). Furthermore, *A. baumannii* resistance mechanisms include: enzymatic degradation of drugs, target modifications, multidrug efflux pumps, and permeability defects (Lee et al., 2017).

Virulence Factors

Porins

Porins are outer membrane proteins which modulate cellular permeability (Lee et al., 2017). OmpA is one of the most abundant β-barrel porins in the outer membrane. In *A. baumannii*, OmpA is a very well-characterized virulence factor (Lee et al., 2017). For example, OmpA facilitates surface motility and biofilm formations, and regulates biogenesis of outer membrane vesicles (Lee et al., 2017). In addition, mouse models have identified the Omp22 porin as a safe antigen for developing vaccines against *A. baumannii* infections (Lee et al., 2017). Active and passive immunizations with the Omp22 porin have been found to increase the survival rates of mice, suppress bacterial burdens in organs and blood, and reduce the serum levels of inflammatory cytokines (Lee et al., 2017).

Penicillin-Binding Protein

The penicillin-binding protein (PBP) 7/8 is a virulence factor in *A. baumannii* (Lee et al., 2017). It was found that the loss of the PBP7/8 affects the peptidoglycan structure, which may affect susceptibility to host defense factors (Lee et al., 2017).

Biofilm

Biofilm formation plays an important role in immune evasion by *A. baumannii*. Pili is needed for adherence and biofilm formation on abiotic surfaces (Lee et al., 2017). Lee et al. found that the imipenem treatment of imipenem-resistant *A. baumannii* isolates induced the

expression of genes needed to synthesize the type IV pili (2017). Therefore, the ability to overproduce pili results in a biological advantage for *A. baumannii* in the presence of biofilms.

Phospholipase

Phospholipase is a lipolytic enzyme essential for phospholipid metabolism (Lee et al, 2017). Phospholipases are an important virulence factor in *A. baumannii* pathogenesis due to host cell invasion (Lee et al., 2017). In a mice model of pneumonia, it was found that disrupting two of an *A. baumannii* strain's phospholipases C (PLCs) gene resulted in reduced resistance to human serum, decreased capacity for invading epithelial cells, and decreased virulence (Lee et al., 2017).

Outer-Membrane Vesicles (OMVs)

Outer-membrane vesicles (OMVs) are vesicles secreted by the outer membranes of various Gram-negative pathogenic bacteria (Lee et al., 2017). They are recognized as delivery vehicles for bacterial effectors to host cells (Lee et al., 2017). Many *A. baumannii* strains secrete OMVs containing various virulence factors, including OmpA, proteases, and phospholipases (Lee et al., 2017). Through lipid rafts, these vesicles deliver bacterial effectors to host cells, resulting in cytotoxicity (Lee et al., 2017). Therefore, an *A. baumannii* strain producing more OMVs induces a stronger innate immune response and is thus more cytotoxic in comparison to *A. baumannii* strains with fewer OMVs (Lee et al., 2017). OMVs have been tested as an acellular vaccine (Lee et al., 2017). A mouse model of disseminated sepsis produced positive results when it concluded that a vaccination with *A. baumannii* ATCC 19607 strain OMVs protected mice against other clinical isolates (Lee et al., 2017). Furthermore, active and passive immunizations with OMVs in the pneumonia model decreased bacterial burden, inflammatory cell infiltration, and inflammatory cytokine accumulation (Lee et al., 2017).

Capsular Polysaccharides and Lipopolysaccharides

Many isolates from patients with *A. baumannii* infections express surface capsular polysaccharides (Lee et al., 2017). A conserved gene cluster, the K locus, determines the production of these capsular polysaccharides (Lee et al., 2017). Capsulars polysaccharides are a target for protective antibody-based interventions as mutations in the pglC or pglL gene, found on capsular polysaccharides, result in abnormal biofilm structures (Lee et al., 2017). These mutants have lower resistance to peptide antibiotics, which induces the hyperproduction of capsular polysaccharides in the presence of an antibiotic (Lee et al., 2017). In a mouse model of systemic infection it was found that transcriptional increase in K locus gene expression led to increased capsule production after antibiotic exposure (Lee et al., 2017).

Protein Secretion Systems

The type II secretion system (T2SS) is a multi-protein, secretion complex found in *A. baumannii* (Lee et al., 2017). There are numerous MDR *A. baumannii* strains with large, self-transmissible plasmids which carry negative regulators for the secretion system (Lee et al., 2017). These multi-protein, secretion complexes have become silent in plasmid-containing, antibiotic-resistant cells (Lee et al., 2017). Furthermore, those in plasmid-losing cells have become susceptible to antibiotics, suggesting a molecular switch between secretion systems and antibiotic resistance (Lee et al., 2017).

Metal Acquisition System

Most aerobic bacteria have a high-affinity for iron, and thus produce a siderophore, a low molecular weight compound (Lee et al., 2017). The best-characterized *A. baumannii* siderophore is acinetobactin, which features an oxazoline ring derived from threonine (Lee et al., 2017). Iron acquisition functions play a critical role in *A. baumannii* virulence as iron

starvations increase the production of phospholipase C (PLC), thus increasing its hemolytic activity (Lee et al., 2017).

In addition, zinc is known to inhibit bacterial growth (Lee et al., 2017). *A. baumannii* can use a zinc acquisition system (ZnuABC) to starve the znuB mutant strain, which contributes to the pathogenesis of *A. baumannii* pulmonary infections (Lee et al., 2017).

Lastly, there is a connection between metal starvation and metabolic stress. In order to resist the antimicrobial activities of calprotectin, Mn starvation is induced, which increases the transcription of a Natural Resistance-Associated Macrophage Protein (NRAMP), and a urea carboxylase (Lee et al., 2017). *A. baumannii* has the ability to utilize urea as a sole nitrogen source (Lee et al., 2017).

Resistance Mechanisms

β-Lactamases

A major antibiotic resistance mechanism of *A. baumannii* is the inactivation of β-lactams by the four molecular classes of β-lactamases (A, B, C and D) (Lee et al, 2017). In fact, *A. baumannii* has natural competence to incorporate exogenous deoxyribonucleic acid (DNA), implying frequent gene transfer (Lee et al., 2017).

Aminoglycosdie-Modifying Enzymes

A. baumannii is resistant to aminoglycosides due to its possession of aminoglycoside-modifying enzymes. These enzymes can be acetyltransferase, adenylyltransferase, or phosphotransferases (Lee et al., 2017). Studies from China and Greece both identified MDR *A. baumannii* strains carrying aminoglycoside-modifying enzymes, indicating their high prevalence in *A. baumannii* (Lee et al., 2017).

Alteration of Target Sites

It is possible for alterations in antibiotic target sites to cause antibiotic resistance (Lee et al., 2017). For example, the overexpression of altered penicillin-binding proteins (PBP) with a low affinity for imipenem, gave rise to imipenem resistance (Lee et al., 2017). In addition, modifications or the loss of lipopolysaccharides (LPS) have displayed a decrease in the susceptibility of *A. baumannii* to antibiotics like colistin (Lee et al., 2017).

Efflux Pumps

Efflux pumps are associated with resistance against antibiotics such as imipenem and tigecycline (Lee et al., 2017). The antimicrobial resistance of *A. baumannii* is related to four categories of efflux pumps: resistance-nodulation-division superfamily, multidrug and toxic compound extrusion family, major facilitator superfamily, and multidrug resistance family transporters (Lee et al., 2017). In low-dose antimicrobial therapy, the overexpression of the AdeFGH efflux pump increases the synthesis and transport of autoinducer molecules (Lee et al., 2017). Autoinducer molecules induce biofilm formation, which is a strong ability in clinical isolates of *A. baumannii* (Lee et al., 2017). Therefore, a link can be concluded between low-dose antimicrobial therapy, and increased biofilm infections from *A. baumannii*.

Permeability Defects

A change in envelope permeability can influence antibiotic resistance. For example, porins form channels that allow transport of molecules across the outer membrane and play a significant role in *A. baumannii* virulence (Lee et al., 2017). Besides outer membrane proteins, envelope components, such as LPS and peptidoglycans, also affect antibiotic resistance of *A. baumannii*. Loss or modification of LPS decreases membrane integrity and increases colistin resistance in *A. baumannii*.

Carbapenem-Resistant *Acinetobacter baumannii* Infections

A. baumannii has developed resistance to one of the most readily available and commonly used antibiotics, carbapenems (Ahmed et al., 2021). As a result, growing antibiotic resistance as seen in CRAB infections is the most common difficult-to-treat form of antibiotic resistance in the United States (Abdul-Mutakabbir et al., 2021).

Clonal lineages - Clonal Complex (CC) 1, 2, and 3 - account for the majority of *A. baumannii* infections (Abdul-Mutakabbir et al., 2021). In the United States, CC2 accounts for 75% of all CRAB infections (Abdul-Mutakabbir et al., 2021). In addition, individual sequence types are associated with varying antibiotic resistant genes and susceptibility patterns (Abdul-Mutakabbir et al., 2021). The Clonal Complexes' multi-locus sequence types (ST) are defined as ST1, ST2, and ST3 (Abdul-Mutakabbir et al., 2021). In a genomic epidemiology study of isolates collected from 2017-2018, amikacin susceptibility increased in isolates from ST208, ST281, and ST499 lineages (Abdul-Mutakabbir et al., 2021). Another alarming resistance trend is that the rate of ampicillin-sulbactam resistance increased from 49% to 80% in isolates of ST122 and ST208, and largely in ST281 (Abdul-Mutakabbir et al., 2021). These trends showcase that reliance on these antibiotic combination therapies is not efficient in treating *A. baumannii* infections (Abdul-Mutakabbir et al., 2021).

The preferred treatment of CRAB infections has not been identified. Therefore, in vitro efficacy, host factors, and pharmacokinetic-pharmacodynamic data is crucial to treatment (Abdul-Mutakabbir et al., 2021). As strains of CRAB infections vary by region, treatment must be region specific and dependent on local epidemiology (Abdul-Mutakabbir et al., 2021).

Treatments

Sulbactam

In order to prevent further resistance, β-lactam therapy remains the first-line treatment for susceptible *A. baumannii* strains (Abdul-Mutakabbir et al., 2021). Sulbactam is a β-lactamase inhibitor with an affinity for the penicillin-binding proteins (PBPs) of *A. baumannii* (Abdul-Mutakabbir et al., 2021). Sulbactam is able to retain activity against some strains of OXA-23 isolates, which are the carbapenemase enzymes responsible for CRAB infections (Abdul-Mutakabbir et al., 2021). So far, combination therapy of ampicillin with sulbactam is effective for treating MDR *A. baumannii* bloodstream infections (Abdul-Mutakabbir et al., 2021). This was concluded based on ampicillin-sulbactam combination therapy being tested against multi-locus sequence types (ST) of *A. baumannii* clonal lineages. It was found that ampicillin with sulbactam non-susceptibility increased from 49% to 80% in the clinical isolate of ST281 (Abdul-Mutakabbir et al., 2021). In addition, combination therapy with sulbactam, ampicillin and carbapenem is effective for treating CRAB skin and soft tissue infections but not effective in ventilator-associated pneumonia *A. baumannii* infections (Lee et al., 2017). However, some studies have shown that doses of more than 6g per day of sulbactam are effective against CRAB infections, specifically in ventilator-associated pneumonia (Abdul-Mutakabbir et al., 2021).

Cefiderocol

Bassetti et al. assessed 95 hospitals in 16 countries throughout North America, South America, Europe, and Asia (Bassetti et al., 2020). Patients enrolled were aged 18 years or older admitted to hospital with nosocomial pneumonia, bloodstream infections or sepsis, or complicated urinary tract infections (UTI), and with evidence of a carbapenem-resistant Gram-negative pathogen (Bassetti et al., 2020). It was found that more deaths occurred in the

cefiderocol group, primarily in the patient subset with *Acinetobacter spp* infections. Collectively, the findings from this study support cefiderocol as an option for the treatment of carbapenem-resistant infections in patients with limited treatment options (Bassetti et al., 2020). However, it concludes that cefiderocol cannot be used as treatment for infections associated with *Acinetobacter* species (Bassetti et al., 2020).

Tetracyclines

The tetracycline class of antibiotics are a promising form of treatment as they have in vitro activity against more than 60% of CRAB isolates (Abdul-Mutakabbir et al., 2021). Of this percentage, eravacycline is the most potent, followed by tigecycline, minocycline, and tetracycline (Abdul-Mutakabbir et al., 2021). It is important to note that only tigecycline has reported clinical experience against CRAB infections (Abdul-Mutakabbir et al., 2021).

Approximately 20% of *A. baumannii* isolates were not susceptible to minocycline due to the TetB efflux pump (Lee et al., 2017). This poses a problem as combined therapies of minocycline and colistin are not synergistic in *A. baumannii* isolates with the tetB gene (Lee et al., 2017). Furthermore, there is a lack of target attainment with minocycline administered as 200 mg IV over 12 hours IV against CRAB isolates (Abdul-Mutakabbir et al., 2021). Scientists thus believe that minocyclines have retained activity against CRAB, even when susceptibility to other forms of tetracyclines is lost (Abdul-Mutakabbir et al., 2021).

Tigecycline exhibits bacteriostatic activity by binding to the 30S ribosomal subunit, making it active against *A. baumannii* infections (Lee et al., 2017). In order to test Tigecyclines capabilities, a study of 266 patients with MDR *A. baumannii* infections was conducted. The results show that tigecycline-based therapy was not more effective than non-tigecycline-based therapies (Lee et al., 2017). It was found that tigecycline resistance is associated with the overexpression of efflux pumps like AdeABC (Lee et al., 2017). Therefore, tigecycline can only

be used in limited cases for treating *A. baumannii* infections as the presence of efflux pumps determine its susceptibility (Lee et al., 2017).

Polymyxins (Colistin and Polymyxin B)

Polymyxins are a group of polycationic peptide antibiotics which exhibit potent efficacy against most Gram-negative bacteria (Lee et al., 2017). Polymyxin B and E (colistin) are the only variations used clinically. Colistin seems to be the only effective antimicrobial agent as the rate of colistin resistance in MDR *A. baumannii* isolates is 10.4% (Lee et al., 2017). Colistin has a significantly lower rate than rifampicin resistance (47.8%) or tigecycline resistance (45.5%) (Lee et al., 2017). Only a few polymyxins related antibiotic options are available to treat MDR *A. baumannii* infections which includes combination therapies of: colistin/imipenem, colistin/meropenem, colistin/rifampicin, colistin/tigecycline, colistin/sulbactam, colistin/teicoplanin, and imipenem/sulbactam (Lee et al., 2017).

Unfortunately, the outbreak of colistin-resistant *A. baumannii* strains has increased worldwide. Elham and Fawzia conducted a study of 142 patients with 136 *Acinetobacter* isolates, 73% of which were *A. baumannii* (2019). It was found that there were 8.5% of colistin resistant isolates (Elham and Fawzia, 2019). All patients had been previously given colistin antibiotics, and 50% were on mechanical ventilation and a central venous catheter (Elham and Fawzia, 2019). The emergence of colistin resistant *A. baumannii* (Col-R-Ab) strains are the result of structure changes in lipo-polysaccharida (LPS) (Ibrahim, 2019). Mutations in *lpxA/D/C* and *pmrA/B* genes result in a downward regulation and thus causes modifications of lipid A biosynthesis (Ibrahim, 2019). Mechanisms of colistin resistance include loss of LPS by the PmrAB two-component system (Lee et al., 2017).

Studies were conducted to evaluate polymyxin B combinations with various carbapenems such as meropenem, doripenem, and imipenem (Lee et al., 2017). Increasing the dose intensity of polymyxin B has been found to amplify polymyxin B resistance in *A. baumannii*

(Lee et al., 2017). Therefore, polymyxin B combination therapies seem to be the most promising options for minimizing resistance.

Non-Antibiotic Therapies: Phage and Others

Bacteriophages are bacterial viruses which invade bacterial cells resulting in the disruption of bacterial metabolism, causing the bacteria to lyse (Sulakvelidze et al., 2001). Prior to the discovery and widespread use of antibiotics, the administration of bacteriophages was thought to be a method for infection treatment (Sulakvelidze et al., 2001). As the United States and Western Europe began to utilize antibiotics, phages continued to be utilized in the former Soviet Union and Eastern Europe (Sulakvelidze et al., 2001). Phage studies were extensively published in non-English journals, primarily Russian, Georgian, or Polish (Sulakvelidze et al., 2001). As a result, the western scientific community continued their widespread use of antibiotics as data on phage therapy was not readily available to them (Sulakvelidze et al., 2001).

Phages have been reported to be more effective than antibiotics in treating certain infections in humans and experimentally infected animals. For example, in one study, *Staphylococcus aureus* phages were used to treat patients having purulent disease of the lungs and pleura (Sulakvelidze et al., 2001). Complete recovery was observed in 82% of the patients in the phage-treated group as opposed to 64% of the patients in the antibiotic-treated group (Sulakvelidze et al., 2001). In the group receiving phage therapy intravenously, the recovery rate was 95%, while the recovery rate for all 223 phage-treated patients was 82% (Sulakvelidze et al., 2001). Additionally, Schooley et al. reports a method used to produce a personalized bacteriophage-based therapeutic treatment for a 68-year-old diabetic patient with necrotizing pancreatitis complicated by an MDR *A. baumannii* infection (2017). Two laboratories identified nine different bacteriophages with lytic activity for an *A. baumannii* isolate from the patient (Schooley et al., 2017). These bacteriophages were administered intravenously and

percutaneously into the abscess cavities (Schooley et al., 2017). The administration of these bacteriophages was associated with reversal of the patient's downward clinical trajectory, clearance of the *A. baumannii* infection, and a return to health (Schooley et al., 2017).

Additionally, a study by Wienhold et al., reaffirms the potential of phage therapy to treat MDR *A. baumannii* infections (2021). The study tested the efficacy of purified lytic phage, vB_AbaM_Acibel004, against the MDR *A. baumannii* isolate RUH 2037 in immunocompetent mouse models and a human lung tissue model (Wienhold et al., 2021). The results show that a single application of a lytic bacteriophage reduced the viability of the isolate under ex vivo and in vivo conditions (Wienhold et al., 2021). The outcome of these cases suggests methods for the production of bacteriophage therapies and the need for the use of bacteriophages for MDR bacterial infections to be warranted.

Novel Therapeutic Agents

Lysins are bacteriophage-encoded peptidoglycan hydrolases (Chu et al., 2022). There has been success in the novel phage lysin Abp013 against MDR strains of *A. baumannii* (Chu et al., 2022). In fact, Abp013 was able to tolerate the presence of human serum by up to 10%, and was able to access and kill the bacterial cells residing in the biofilm (Chu et al., 2022). Overall, Abp013 was able to display host selectivity. Host selectivity opens the possibility for efficient swapping of the binding or catalytic domain with other lysins, antimicrobial components, or fusion with antimicrobial peptides (Chu et al., 2022).

Artilysins

A bacteriophage-encoded endolysin is a lytic enzyme that degrades the cell wall of bacterial hosts (Lee et al., 2017). Endolysins have been engineered to penetrate the outer membranes of Gram-negative pathogens via outer membrane-destabilizing peptides (Lee et al., 2017). These engineered endolysins are called "artilysins". Artilysins show highly effective

antimicrobial activity against *A. baumannii* (Lee et al., 2017). Upon contact with the artilysin Art-175, instantaneous killing of *A. baumannii* could be visualized after immobilization of the bacteria in the bacterial cell (Defraine et al., 2016). The cells were then observed to be killed through osmotic lysis after peptidoglycan degradation (Defraine et al., 2016). Under selection pressure there was no development of resistance to Art-175 (Defraine et al., 2016). The only known current downside to treating *A. baumannii* infections with artilysins is their short half-life serum and their high production costs (Lee et al., 2017).

Aside from promising results of bacteriophage therapy, the pharmacokinetics of therapeutic phage preparation must be identified (Sulakvelidze et al., 2001). It is also important for scientists to get a better understanding of bacterial activity as subsequent studies revealed that not all phages replicate similarly and that there are important differences in the replication cycles of lytic and lysogenic phages (Sulakvelidze et al., 2001). Furthermore, a study by Gordillo-Altamirano et al. tested two phages with strong lytic activity against clinical isolates of *A. baumannii* (2021). Researchers observed the emergence of phage-resistant mutants (Gordillo-Altamirano et al., 2021). Additional research is needed in order to obtain pharmacological data, including full-scale toxicological studies, of lytic phages before their administration in the United States (Sulakvelidze et al., 2001).

Methods

Literature Review

This research was conducted by a scoping literature review. The PubMed database was utilized to find primary literature. Search terms included “Multidrug-resistant *A. baumannii* and treatment” “Healthcare-Associated Infections”, and “*A. baumannii* and Bacteriophage Therapy”. An annotated bibliography was created utilizing 30 papers from scholarly journals. The literature sources were then reviewed to identify knowledge gaps in antibiotic resistant mechanisms and treatment. Fundamental knowledge gaps in *A. baumannii* treatment were identified and ranked.

Based upon the literature review, the top five most important knowledge gaps identified were: various alternate approaches to anti-virulence strategies, application methods for bacteriophage therapy, treatment for carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections, clinical and microbiological mechanisms of colistin resistance, and quantitative measurements for environmental and activity factors of *A. baumannii*. The survey was based upon these five knowledge gaps.

Survey

Health professionals were surveyed to help bridge the gaps in *A. baumannii* antibiotic resistance and practiced treatments. A set of questions (Appendix) was devised with the goal of establishing prospective infection control treatment and methods. The survey was created via Qualtrics, and was aimed to target public health professionals, infectious disease specialists, epidemiologists, pharmacologists, microbiologists, infection preventionists, and multidrug-resistant experts. Inclusion criteria for the survey was based on occupation, and participants being over the age of 18. The survey recipients were selected via recommendation, and through occupational faculty databases. The survey gained approval through the University of Arizona eIRB as STUDY00002567. The Qualtrics survey link was disseminated via email.

The email describes the contents of the study and what to expect. Participants were told that by taking the survey (provided via link) they are allowing their responses to be used for research purposes. Thirteen individuals were contacted, and only three were willing to participate. All participants were de-identified for this thesis.

See the Appendix for the questions asked. Questions 4-8 were only available if the participant answered “yes” to question 3. If the participant answered “no” to question 3 then they were redirected to question 9.

Results

Knowledge Gaps

Based upon the literature review, the following knowledge gaps were identified: anti-virulence strategies, bacteriophage therapy, treatment for CRAB infections, colistin resistance, quantitative measurements of environmental and activity factors, healthcare worker activity, disinfectant resistance, effectiveness of antibiotics and developing novel antibiotics, national collaboration discrepancies, and desiccation resistance.

Anti-Virulence Strategy

Instead of focusing on central growth pathways, anti-virulence is an alternative approach focusing on the interference of bacterial virulence factors. Anti-virulence strategies are important as it offers scientists additional paths in understanding the mechanism for treatment. Researchers have identified various anti-virulence strategies with potential to treat *A. baumannii* infections. Key focus areas include expanding our understanding of: Bacteriophage therapy, metabolic interference strategy, antimicrobial peptide therapy, and vaccine candidates (Lee et al., 2017).

Bacteriophage Therapy

Bacteriophage therapy can be utilized as an alternative to antibiotics when bacteria develop resistance (Schooley et al., 2017). Currently, there is no known optimal route, dosage form, dose or duration of therapy for bacteriophages (Schooley et al., 2017). As mode of administration is unknown, bacteriophage therapy is unable to progress as a mainstream treatment option.

Treatment for CRAB Infections

As of right now, all scientific studies on CRAB are regionally specific based on local epidemiology. Scientists have still not identified the most ideal combination of drugs for treatment (Lee et al., 2017). A focus must be on combination therapy as the benefits remain unclear (Abdul-Mutakabbir et al., 2021).

Colistin Resistance

It was found that there was an increase of Colistin resistance among clinical isolates of *A. baumanni* causing serious infections especially in critically ill patients (Elham and Fawzia, 2019). Clinicians must remain alert regarding the prospect of colistin resistance across MDR bacteria. Data on the clinical and microbiological characteristics of Colistin-resistant *A. baumannii* infections remain scarce (Elham and Fawzia, 2019).

Quantitative Measurements of Environmental and Activity Factors

Surfaces, air, and indoor structure including ventilation systems have all been shown to act as reservoirs for pathogens, and in some cases, these pathogens can persist for months in a hospital environment (Hiwar et al., 2021). IAQ parameters such as temperature, relative humidity, CO2 level, particle mass concentration, and particle size are important for understanding the presence of HAIs (Hiwar et al., 2021). Currently, there is a major knowledge

gap of environmental and activity factors affecting the presence and quantity of *A. baumannii* in the occupational health sector (Hiwar et al., 2021).

Healthcare Worker Activity

Currently, emerging resistance of *A. baumannii* strains is a serious issue for patients in the intensive care unit (ICU). The observation of healthcare worker activity would provide an alternative approach for real-time monitoring of the healthcare environment (Cristina et al., 2013). In terms of indoor air quality, foot traffic would influence both the generation of microorganisms and their deposition rate onto surfaces in the hospital environment (Cristina et al., 2013). The observation of healthcare worker activity would bridge the gap in the understanding of spatial and temporal fluctuation of *A. baumannii* burden in hospitals (Cristina et al., 2013).

Disinfectant Resistance

The full underlying molecular mechanisms of the virulence strategy of *A. baumannii* vs various disinfectants is needed for the occupational health sector as studies show *A. baumannii* with an increasing resistance to disinfectants like chlorhexidine and ethanol (Harding et al., 2018).

The Effectiveness of Antibiotics and Developing Novel Antibiotics

Novel, designed strategies and screening-based approaches are required to discover new classes of antibiotics (Chu et al., 2022)

National Collaboration Discrepancies

Facility and regional differences in resistance rates contribute to the lack of containment interventions and the ability to contain microbial threats (Mutair et al., 2021). There is a need for

linking resistance data to community and hospital antimicrobial use patterns and infection prevention interventions (Mutair et al., 2021).

Desiccation Resistance

Occupational health facilities have prolonged periods of desiccation and routine disinfection regimes (Harding et al., 2018). Clinical isolates of *A. baumannii* have shown desiccation resistance, meaning the pathogen can remain viable under dry conditions (Harding et al., 2018). Desiccation resistance is multifactorial and not yet defined for *A. baumannii* (Harding et al., 2018). All that is concluded is that viability depends on the conditions of water limitations for *A. baumannii* (Harding et al., 2018).

Survey Responses

This survey was sent to 13 individuals with backgrounds in epidemiology, pharmacology, infectious diseases, and public health. We received responses from three participants, all professors at universities. The participants specialize in pharmacology, epidemiology, and environmental health. All participants answered “no” to having an occupation clinically focused in infectious disease which omitted questions 4-8 from their available question bank.

Q9. What infection control practices would you suggest to limit the spread of *A. baumannii*, particularly in the healthcare environment?

Participants had different approaches to infection control practices. One participant suggested “Follow a data driven environmental cleaning protocol for all terminal and daily room cleaning”. The next participant suggested that *A. baumannii* must be made into a reportable infection. The last participant answered that adherence to medication is the most crucial to infection control practices.

Q9a. What innovative sanitation methods can be achieved?

In this response, two of the three participants provided potential methods. The first participant suggested the incorporation of phage applications. The second participant suggested the use of automated cleaning technology via hydrogen peroxide, fogging, and UVC robots.

Q9b. What interventions can be implemented to avoid cross-transmission involving healthcare workers?

Two of the three participants were unsure of an answer. The last participant suggested the utilization of “Molecular surveillance in near real time”.

Q10. How can early detection be achieved with *A. baumannii* infections?

Two of the three participants were unsure of an answer. One participant provided the response of, “make it a reportable infection”.

Q11. There have been some studies on Biofilm formation in opsonophagocytosis assays being a potential vaccine target for *A. baumannii*. Glycan based vaccines are difficult due to variability of glycan structures. Nonetheless, inhibitors of the glycan synthesis pathway might be a valuable therapy for treatment. Should glycan synthesis be considered as a pathway for treatment?

Of the participants, 33% were unsure, and 66.6% said that glycan synthesis should be considered as a pathway for treatment. A participant followed their response by saying, “There is no reason to not explore all avenues, despite likely rapid resistance”.

Q12a. Bacteriophage Therapy has shown success in few patients, and requires special clearance under the FDA. Currently, the FDA only authorizes bacteriophage use in food products and certain cleaning products. Of the following, steps would make you comfortable

leading to Bacteriophage Therapy being offered as a routine treatment option for *A. baumannii* infections?

-FDA approval (Phase 1-4)

-10 successful clinical trials

-A risk assessment for safety, efficacy, and optimal use

-Creation of readily available phage libraries

-Improving bacterial diagnostic technologies

Sixty-six percent of participants believed that FDA approval and a risk assessment would make them feel comfortable in utilizing bacteriophage therapy. Thirty-three percent agreed but would also like to see the addition of 10 successful clinical trials, and the creation of readily available phage libraries. No participant selected “improving bacterial diagnostic technologies”.

Q12b. Why is phage therapy modes of administration (optimal route, dosage form, dose, or duration of therapy) needed? How can these knowledge gaps be achieved?

Of the participants 66.6% answered that they were unsure. The third participant believes that modes of administration are needed for bacteriophage therapy, but there is also a need for phage libraries for *A. baumannii* and possibly genetically engineered phage cocktails. The participant believes this would be useful as *A. baumannii* is associated with multiple types of infection, including sepsis and pneumonia.

Q12c. Once bacteriophage therapy is initiated, what kind of methodologies can be implemented for monitoring the emergence of bacteriophage resistance?

Thirty-three percent of participants answered that genotyping can be implemented for monitoring the emergence of bacteriophage resistance. Meanwhile, 66.6% of participants were unsure.

Discussion

The goal was to identify knowledge gaps in antibiotic resistance mechanisms and treatment associated with *A. baumannii* infections and understand *A. baumannii* in hospitals by gathering personal experiences of professionals addressing exposure with the bacteria. The findings show various methods for prospective treatments and infection control interventions.

Anti-Virulence Strategies

Based upon research into anti-virulence strategies, *A. baumannii* mutant auxotrophic for d-glutamate tested as a promising live attenuated vaccine candidate. This is given due to its inability to synthesize mature peptidoglycan. More research is needed to test the efficacy of this vaccine candidate. This can be achieved by the utilization of modern clinical isolates, like AB5075, for understanding mechanism survival in this era. In addition, surveyed participants agreed that vaccines via glycan synthesis pathways should be tested as a prospective method of treatment.

Bacteriophage Therapy

The review found that bacteriophage therapy has shown success rates as an alternative to antibiotics in few clinical trials. In order for this treatment to progress as an alternative for antibiotics, scientists must study modes of administration to identify a national protocol. Once a protocol is established, it is important to monitor growing resistance via genotyping surveillance. As phages can be found in soil, sewage, and water, there will always be a new phage utilizing different receptors to combat bacteriophage-resistant *A. baumannii* (UC Health, n.d.). Lastly, the survey concluded that the use of phage libraries could greatly expedite this process, and allow for the widespread use of bacteriophage therapy as the patient's bacterial infection can be matched and purified within days (UC Health, n.d.).

Treatment for CRAB Infections

Treatment for Carbapenem-resistant *A. baumannii* (CRAB) is extremely difficult and requires alternative methods of treatment. Nationwide antimicrobial infection prevention programs might help to improve our knowledge of resistance patterns and develop a treatment protocol for decreasing the infection burden. While there are about 25 strains existing in different locations, it is important to create a baseline treatment for all cases of *A. baumannii* (Lee et al., 2017). In order to establish a more successful treatment, clinicians must understand the activity of different antimicrobials in combination.

Colistin Resistance

Colistin, also known as polymyxin E, is an antibiotic medication used as a last-resort treatment for multidrug-resistant Gram-negative infections (Elham and Fawzia, 2019). It was found that there was an increase of Colistin resistance among clinical isolates of *A. baumanni* causing serious infections especially in critically ill patients. Mechanisms of acquired resistance for Colistin must be monitored upon administration of Colistin to patients.

Quantitative Measurements of Environmental and Activity Factors

Numerous trials collecting quantitative measurements, whilst accounting for seasonal and geographic factors, would produce *A. baumannii* data for constituency and comparability in different locations across the world. Seasonal and geographic factors must be reported quantitatively based on temperature and relative humidity (Hiwar et al., 2021). It is critical to obtain more active and passing air samplings in occupational health environments contaminated with *A. baumannii.* In doing so, scientists must intensely perform multiple samples over time instead of snapshot air samplings (Hiwar et al., 2021). These results could improve methods of treatment, and allow for global cooperation in infection control efforts.

Furthermore, survey results demonstrate that ‘No-touch” methods like UV-C light and hydrogen peroxide vapors show promise in reducing HAIS by improving terminal room disinfection (Weber et al., 2013). These innovative sanitation methods could also reduce the bioburden on hospital surfaces (Weber et al., 2013).

Conclusion

Fundamental gaps in knowledge hinder action against antimicrobial resistant *A. baumannii*. It is important to promote widespread knowledge of *Acinetobacter baumannii*. This would make the bacterium more easily identified in the occupational health sector. Limitations of research pose an even bigger obstacle as there is a need for an established national intervention for MDR *A. baumannii* infections. The investigation of vaccine candidates, and bacteriophage therapy is strongly encouraged.

Furthermore, a national surveillance system would efficiently monitor growing resistance among regional strains. In addition, there is a need for testing sanitation methods, and conducting passive and active air samplings in hospitals. These studies could result in new means of infection prevention control for HAIs associated with *A. baumannii*. Overall, the creation of new therapeutic alternatives and limiting the use of broad-spectrum antibiotics is critical. As this is a growing threat, global collaboration is essential to the development of antimicrobial solutions.

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Appendix

Survey Questions

1. What is your full name?
2. What is your occupation?
3. Is your occupation clinically focused on infectious disease?
4. In treating Carbapenem-resistant Gram-negative bacteria the tide is turning with clinical trials, like CREDIBLE-CR and durlobactam-sulbactam, utilizing a pathogen-focused rather than an infection-site approach, despite significantly more challenging design, costs, and execution. Do you believe the pathogen-focused approach or infection-site approach is the future of *Acinetobacter Baumannii* treatments?
5. When treating Carbapenem-Resistant *A. baumannii* (CRAB) infections, which empiric therapy do you believe should be selected first, combination therapy or monotherapy?
6. Environmental and activity factors affecting the presence and quantity of airborne microorganisms such as seasonal and geographic factors based on temperature and relative humidity would showcase constituency and comparability in different locations across the world. How best is this to be achieved?
    a. Would quantitative measurements such as ambient air temperature, ambient relative humidity, CO2, and airborne particles be useful data in relation to HAI from *A. baumannii*?
7. How can a nationwide antimicrobial infection prevention program for *A. baumannii* help to improve our knowledge of resistance patterns and develop a treatment protocol for decreasing the infection burden?
    a. How can national collaboration to tackle facility and regional differences in *A. baumannii* infection rates be achieved?

8. How important is maintaining the effectiveness of antibiotics and developing novel antibiotics to the control of *A. baumannii* infections?
9. What infection control practices would you suggest to limit the spread of *A. baumannii*, particularly in the healthcare environment?
    a. What innovative sanitation methods can be achieved?
    b. What interventions can be implemented to avoid cross-transmission involving healthcare workers?
10. How can early detection be achieved with *A. baumannii* infections?
11. There have been some studies on Biofilm formation in opsonophagocytosis assays being a potential vaccine target for *A. baumannii*. Glycan based vaccines are difficult due to variability of glycan structures. Nonetheless, inhibitors of the glycan synthesis pathway might be a valuable therapy for treatment. Should glycan synthesis be considered as a pathway for treatment?
12. Bacteriophage Therapy has shown success in few patients, and requires special clearance under the FDA. Currently, the FDA only authorizes bacteriophage use in food products and certain cleaning products.
    a. What steps would make you comfortable leading to Bacteriophage Therapy being offered as a routine treatment option for *A. baumannii* infections?
        i. FDA approval (Phase 1-4)
        ii. 10 successful clinical trials
        iii. A risk assessment for safety, efficacy, and optimal use
        iv. Creation of readily available phage libraries
        v. Improving bacterial diagnostic technologies
    b. Why is phage therapy modes of administration (optimal route, dosage form, dose, or duration of therapy) needed? How can these knowledge gaps be achieved?

c. Once bacteriophage therapy is initiated, what kind of methodologies can be implemented for monitoring the emergence of bacteriophage resistance?