Modern Approaches to Treating Hospital Acquired Infections: An Overview of Current Treatments using Antibiotics and New Therapies Centered Around Photo-activated Porphyrins.

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2024

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial fulfillment of the requirements for graduation from the Honors Program of Liberty University.

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Abstract

Annually, hospital-acquired infections (HAIs) affect hundreds of thousands of people in the U.S. and impose a great economic burden. The current problem is further exacerbated due to the failure of traditional treatment strategies considering the rise in antimicrobial resistance rates. Besides the fact that antimicrobial resistances complicates clinical treatment strategies, patients are also more vulnerable to secondary infections and complications from prolonged antibiotic use. As a result, research investigating novel treatment strategies for bacterial infections has recently increased. A strategy using porphyrin-based compounds is showing promise. Porphyrins and their derivatives have exhibited bactericidal effects against Gram-positive bacteria by the destruction of cell walls. The mechanism of action of porphyrin-based compounds against Gram-negative bacteria is still under investigation. Given the current research and proposed mechanism of action, further research into the effectiveness of these compounds against Gram-positive and negative bacteria is still warranted.

Introduction

Every year, upwards of 633,300 patients in the United States contract a hospital-acquired infection (HAI) which is defined as an infection that develops during the treatment of another condition while at a medical facility (Agency for Healthcare Research and Quality [AHRQ], 2019; U.S. Department of Health and Human Services, 2021). It has been noted that 1 in 31 patients seen in acute care settings and 1 in 43 residing in long-term care facilities contract HAIs annually (CDC, 2022) resulting in roughly 72,000 deaths (CDC, 2023). It has also been noted that a single case of a HAI can cost the U.S. healthcare systems upwards of $50,000 (Burchum & Rosenthal, 2021). Annually, HAIs place a burden of $24.8 billion on healthcare providers as insurance companies will not pay for the cost as HAIs are typically considered to arise from negligence regarding infection control procedures (CDC, 2023). The 10% mortality rate and the fiscal burden on U.S. healthcare systems warrant research efforts for the elucidation and characterization of improved methods for the prevention and effective treatment of these infections.

There are six major classes of HAIs: central line bloodstream infections (CLABSI), surgical site infections (SSI), catheter-associated urinary tract infections (CAUTI), ventilator-associated pneumonia (VAP), *Clostridium difficile* (*C. diff.*), and methicillin-resistant *Staphylococcus aureus* (MRSA) (CDC, 2014). As these infections are caused by bacterial agents, an antibiotic regimen is generally the most common and effective treatment. However, antibiotic overuse and misuse pose the risk of promoting antimicrobial resistance and an environment that fosters the development of secondary HAIs (Mullish, 2018). The probability of these complications occurring rises with the long-term use of broad-spectrum, non-selective antibiotics

for severe infections (Guenezan *et al.*, 2018). Also, the causative agents of HAIs have exhibited a greater rate of antimicrobial resistance than the infectious agents that are acquired from the community (Michels *et al.*, 2021). In light of these problems, current research has been focused on developing new treatment methods that have selective antimicrobial action that will not lead to antibiotic resistance.

Common Causative Agents of Bacterial HAIs

Staphylococcus aureus

Staphylococcus aureus (*S. aureus)* is a Gram-positive bacterium that forms spherical-shaped, smooth-surfaced colonies that are yellow/white in appearance due to their production of carotenoids. When grown in media, they are typically arranged in clusters that resemble grapes. The individual bacterium is usually 0.5 -1.0 μm in diameter with a thick cell wall, distinct cytoplasmic membrane, and amorphic cytoplasm. As an aerobe and facultative anaerobe (uses both aerobic and anaerobic respiration to produce ATP), they can thrive in a variety of environments, which enhances their survivability and pervasiveness (Gnanamani *et al.*, 2016).

These bacteria also produce several virulence factors: 4 hemolysins (α, β, γ, and Δ), coagulase, a polysaccharide microcapsule, protein A, Panton-Valentine leucocidin (PVL), α-toxin, chemotaxis-inhibitory protein of *S. aureus* (CHIPS), and microbial surface components recognizing adhesive matrix molecules (MSCRAMM). The hemolysins allow *S. aureus* to lyse erythrocytes and scavenge iron from hemoglobin. The production of coagulase allows these bacteria to convert fibrinogen to fibrin – initiating the clotting cascade in the host. The formation of a thrombus provides the bacteria with an adhesion point and a means of protection from host immune cells (McAdow *et al.*, 2012).

MSCRAMM are cell surface proteins that are vital for the bacteria to adhere to host tissue. These proteins consist of multiple domains with one anchored in the peptidoglycan of the cell wall (Foster, 2019). The characteristic domains are two subdomains that consist of IgG-like folds (Foster, 2019). *S. aureus* has seven compounds categorized as MSCRAMM (ClfA, ClfB, SdrE, Bbp, FnBPA, FnBPB, and Cna) (Foster, 2019). Each protein has different ligands aiding in differential attachment (Foster, 2019). The polysaccharide microcapsule helps the bacterium evade phagocytosis as it prevents complement binding by neutrophils (Mohamed *et al.*, 2019). Protein A prevents opsonization by the immune system. PVL and α-toxin are toxins that work to initiate pore formation in the cellular membrane of host cells. The compromised cellular membrane will lead to apoptosis – resulting in the release of substrates that can be scavenged by the bacteria. CHIPS is an extracellular protein that prevents chemotaxis of neutrophils and monocytes to the bacteria. Lastly, Eap binds to components of the extracellular matrix and augments intercellular adhesion between host cells (Gnanamani *et al.*, 2016).

In addition to the virulence factors mentioned, multiple strains of *S. aureus* are resistant to antibiotics. It is believed that the clinical use of antibiotics has contributed to the rise in the occurrence of multi-drug-resistant *Staphylococcus aureus*, and specifically methicillin-resistant *S. aureus* (MRSA), infections (Vestergaard *et al.*, 2019). MRSA differs from other strains of *S. aureus* because of novel mutations in various genes on the staphylococcal cassette chromosome and on R plasmids that are acquired via horizontal gene transfer (Vestergaard *et al.*, 2019). Resistance to the different classes of antibiotics is typically associated with a specific mutation or gene transfer event. Resistance to beta-lactams is mediated by the acquisition of the *blaZ* gene. This gene codes for beta-lactamase which inactivates penicillin by cleaving the beta-lactam ring.

MRSA develops from the horizontal transfer of *mecA* which encodes for PBP2a (a transpeptidase). This protein exhibits a low affinity for beta-lactam antibiotics, greatly decreasing its efficacy. *vanA* operons have led to vancomycin-resistant *S. aureus* as these bacteria synthesize D-ala-D-lactate which cannot bind vancomycin (Vestergaard *et al.*, 2019). The prevalence rate of these bacteria is lower than that of MRSA (Vestergaard *et al.*, 2019). These mutations and acquisitions have led to strains of *S. aureus* that are extremely difficult to treat and lead to chronic colonization (Gnanamani *et al.*, 2016).

Coagulase-negative *Staphylococci*

Coagulase-negative staphylococci (CoNS) cannot produce coagulase making them less pervasive, virulent, and more detectable by the host's immune system. It was previously believed that these bacteria are not pathogenic because they are commensals of human skin (Grice & Segre, 2011). However, it is now known that these organisms can cause pathogenesis under specific conditions such as breaches of cutaneous barriers (Argemi *et al.*, 2019).

Despite exhibiting fewer virulence factors than their coagulase-producing counterparts, CoNSs still exhibit many virulence factors such as adhesions, biofilm, hemolysins/siderophores, exoenzymes, and superantigens. Adhesions are vital for the bacteria to be able to anchor to host cells. Biofilm production protects the bacteria from the host's immune system. As an extracellular matrix primarily composed of polysaccharides, it protects bacteria by restricting the access of antibiotics and phagocytes (Lichtenberg *et al.*, 2023). Hemolysins lyse host erythrocytes allowing siderophores to scavenge iron from hemoglobin (Vieira de Souza *et al.*, 2019). This iron aids in DNA replication, transcription, and energy production via cellular respiration (Caza & Kronstad, 2013). Various exoenzymes aid in anchoring, breakdown of host

cells, and modulation of the immune response. Superantigens stimulate the nonspecific proliferation of T cells leading to shock-like symptoms in the host (Schlievert, 1993). This overactivation leads to a downregulation of the immune system thus decreasing its efficacy (Schlievert, 1993).

Escherichia coli

Escherichia coli (*E. coli*) is a Gram-negative, non-sporulating, rod-shaped bacterium. It ranges in size from 1 x 0.4 μm -3 x 0.7 μm. As a non-sporulating bacterium, it does not spread easily. However, the presence of a bundle of flagella makes this bacterium motile. It is a facultative anaerobe that thrives in oxygen-poor environments as it relies on lactose fermentation for energy production. These characteristics indicate why *E. coli* typically colonizes the gastrointestinal (GI) tracts of humans and animals. When colonized, they typically form large, thick, greyish-white, moist colonies. The colonies can either have a rough or smooth appearance. The cell walls of these bacteria consist of an outer membrane, 1-2 layers of peptidoglycan, and the cellular membrane (Basavaraju & Gunashree, 2021).

Though *E. coli* is native to the digestive tract, pathogenesis is not typically seen as a result of intrinsic *E. coli*. However, pathogenesis can arise when extrinsic *E. coli* from other animals is ingested due to food or soil contamination or when the horizontal gene transfer of virulence-factor-encoding genes occurs. Virulence factor genes code for proteins aiding in adhesion (*bfp, eae, fimA*), biofilm production (*Eha, Saa, NleB*), invasion of GI tract wall (*pic, pet sigA*), and iron scavenging (*elyA* & *hlyA*). Pathogenesis arises as the extrinsic *E. coli* expressing various virulence factors competes with the natural microbiota of the intestines (Lehmacher *et al.*, 1998; Pakbin *et al.*, 2021).

Clostridium difficile

Clostridium difficile (*C. diff.*) is a Gram-positive, anaerobic bacterium that is normally found in the intestines of humans. As a sporulator, it spreads easily during times of high stress (low-nutrient states). These spores settle and germinate as nutrient conditions improve, which leads to the pathogenic state (Zhu *et al.*, 2018). It has been found that some *C. diff.* produces exosporium that will either loosely or tightly surround the endospores (Malyshev & Baillie, 2020). The exosporium acts as a semipermeable membrane that protects the bacterium from toxic metabolites and antibiotics (Stewart, 2015). *C. diff.* is an anaerobic bacterium, and because of this, the oxygen-poor GI tract is a great environment for this species to thrive (Malyshev & Baillie, 2020; Zhu *et al.*, 2018). In a healthy GI tract, competition for resources keeps the levels of each bacteria species that constitute the microbiota in a normalized range. Disruption of the balance of normal gut flora can result in *C. diff.* pathogenesis (Malyshev & Baillie, 2020).

C. diff. produces two main toxins during the late log and stationary phases of growth-TcdA and TcdB (Zhu *et al.*, 2018). These toxins function by inactivating GTP-binding proteins (Rho, Rac, and Cdc42) as they have glucosyltransferase activity. This inhibits intracellular signaling and cellular reproduction and causes cytoskeleton reorganization, specifically actin condensation, leading to apoptosis (Mosaddeghzadeh & Ahmadian, 2021; Voth & Ballard, 2005). It has been noted that TcdA can initiate disease independently while TcdB is reliant on the presence of TcdA (Voth & Ballard, 2005). Studies have shown that these toxins are hazardous to heart, lung, and liver function [these effects are further discussed under the analysis of the HAI] (Zhu *et al.*, 2018).

Pseudomonas aeruginosa

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative, rod-shaped bacterium that ranges in size from 1x 0.1 μm - 5 x 0.5 μm It is a facultative aerobe and is highly motile as it possesses both pili and flagella. These allow the bacterium to perform swimming, twitching, and swarming motions. These appendages aid the bacterium in binding to epithelial cells (pili) and mucin (flagella). Bacteria of this species that lack pili and flagella have exhibited lower virulence (Diggle & Whiteley, 2019). Likewise, the outer membrane of this strain exhibits high concentrations of LPS, which contribute to the negative charge of the outer membrane and increase its structural integrity (Diggle & Whiteley, 2019).

P. aeruginosa has many special characteristics that make it a very potent pathogen. It possesses efflux pumps and produces biofilm, pyocyanin, pyoverdine/pyochelin, proteases, exotoxin A, and hydrogen cyanide. The efflux pumps make this bacterium especially difficult to treat as they increase the rate of multi-drug resistance (Lorusso *et al.*, 2022). There are 6 different classes of efflux pumps. They function by capturing the antibiotic and using the proton motive force to expel it from the cell. This expulsion prevents the antibiotic from binding to its intended target (Lorusso *et al.*, 2022). The biofilm structure and function are analogous to CoNS (Diggle & Whiteley, 2019). Pyocyanin is an aromatic exotoxin associated with proinflammatory and free-radical action leading to apoptosis of both host cells and other bacteria (Hall *et al.*, 2016). Mediating the levels of other bacteria and the integrity of host cells provides more substrate for energy production and cellular reproduction. The cellular products pyoverdine and pyochelin allow iron scavenging in low-concentration iron environments making them fit for

stressful environments. The ability to survive in unsuitable environments lends to the opportunistic nature of these bacteria (Diggle & Whiteley, 2019).

Klebsiella pneumoniae

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative, non-motile, rod-shaped bacterium that is reliant on iron for replication. This iron is scavenged by the virulence factor, siderophores, as they have a higher affinity for iron than transferrin leading to iron-leeching (Riwu *et al.*, 2022). Likewise, the production of a capsule provides it with protection from environmental disinfectants and antibiotics increasing the pervasiveness of this bacterium (Lenchenko *et al.*, 2020). It is a facultative anaerobe that is typically found in mucosal passages (Chang *et al.*, 2021). This colonization does not directly lead to pathogenesis as this bacterium can colonize the nasal passages and GI tract without causing disease. However, these bacteria are opportunistic as they are resistant to β-lactam antibiotics. This organism can cause liver abscesses, bacteremia, meningitis, endophthalmitis, and necrotizing fasciitis (Chang *et al.*, 2021; Riwu *et al.*, 2022).

As an opportunistic bacterium, *K. pneumoniae* possesses many virulence factors that aid its pathogenesis. As mentioned above, the capsule protects the bacterium. Different strains differ in the type of or presence of a capsule. There have been 78 different isolated capsule types from *K. pneumoniae*, with six being implicated in disease (K1, K2, K54, K57, K20, & K5) (Riwu *et al.*, 2022). Each capsule type has been found to offer a differing level of protection, and a capsule is necessary for pathogenesis to occur (Riwu *et al.,* 2022). Similarly, exopolysaccharides increase the viscosity of this bacterium in mucus lending to easier movement through the body. Another virulence factor is Lipid A. This molecule is a constituent part of the LPS that comprise

the outer membrane (Riwu *et al.*, 2022). Lipid A is important for pathogenesis as it inhibits the bactericidal effects of antimicrobial peptides. It also functions as an antigen that prevents tagging by the immune system (Riwu *et al.,* 2022). Additionally, fimbriae and pili serve as adhesins, which allow the bacterium to anchor to host cells. In *K. pneumoniae,* there are 3 different types of fimbriae: 1, 3, and *K. pneumoniae* carbapenemase. Type 1 fimbriae are important for the establishment of infection in the urinary tract (Riwu et al, 2022). Type 3 fimbriae also aid in adhesion and have been implicated in infections associated with indwelling devices (Murphy *et al*., 2013). Both type 1 and 3 are important for the development of biofilms (Riwu *et al*., 2022).

Enterococcus faecalis **and** ***Enterococcus faecium***

Enterococci are Gram-positive, facultative anaerobic bacteria that grow best in a basic environment when the pH is ~9.6 (Manero & Blanch, 1999). It has been found that *Entercocci* can migrate across the intact intestinal mucosa, increasing its pervasiveness and increasing the difficulty of treatment (Jett *et al.*, 1994). Likewise, enterococci have shown a high level of antibiotic resistance due to the easy incorporation of plasmids and transposons from surrounding bacteria via horizontal gene transfer (Vergis *et al.*, 2002) (Jett *et al.*, 1994). This rapid rate of mutation limits the efficacy of various treatments and increases its infectability.

These bacteria also exhibit a wide array of virulence factors. Gelatinase is an enzyme that hydrolyzes gelatin, collagen, hemoglobin, and other peptides. By breaking down these compounds, they negatively modulate the functioning of host cells by reducing intracellular adhesion and signaling, as well as nutrient/waste transport. Similarly, they produce hemolysins that lysis erythrocytes (Vergis *et al.*, 2002). Further, pheromone production prompts the production of aggregation substance proteins creating cell-cell adhesion (Jett *et al.*, 1994). These

bacteria also produce surface carbohydrates and fibronectin-binding moieties which increase adherence to host tissues while enterococcal surface proteins aid in the production of biofilm (Hendrickx *et al.*, 2009; Vergis *et al.*, 2002). Enterococci also produce the exotoxin cytolysin, which, in higher concentrations is connected to increased mortality (Vergis *et al.*, 2002). The main difference between *E. faecalis* and *E. faecium* are their susceptibility to antibiotics. *E. faecalis* is susceptible to ampicillin, vancomycin, and penicillin while many strains of *E. faecium* are not, making it harder to treat (Quiloan *et al.*, 2012).

Classes of Antibiotics Used to Treat HAIs and their Mechanisms of Action

Beta-lactams

β-Lactams (cephalosporins, carbapenems, monobactams, & penicillins) are a class of antibiotics that consists of a β-lactam (4-membered amide) ring which exhibits high torsional strain leading to characteristically high reactivity (Kim *et al.*, 2023). The early members of this class are narrow spectrum; however, recent antibiotic developments have led to the creation of broad-spectrum β-lactams. It has been shown that this class of antibiotics exhibits antimicrobial action through the dysregulation of cell wall formation and maintenance (Cho *et al.*, 2014).

β-Lactams work by binding and inactivating low molecular weight penicillin-binding proteins (PBPs), specifically class A PBPs (aPBPs). aPBPs exhibit glycosyltransferase and transpeptidase activity making them critical for peptidoglycan production as they facilitate polymerization and crosslinking. Without proper functioning of PBPs, cell wall synthesis is compromised increasing the chances of lysis. Similarly, β-lactams cause dysregulation in the coordination between peptidoglycan synthases and hydrolases. This disruption leads to elevated hydrolase activity and decreased synthase activity causing a breakdown of polymerized

peptidoglycan. This dysregulation also increases the cellular need for ATP leading to a reduction in available bacterial energy substrate resources. These events in conjunction lead to cell lysis (Cho *et al.*, 2014).

Glycopeptides

Glycopeptides (ex. Vancomycin) are narrow-spectrum antibiotics used for the treatment of Gram-positive bacterial infections. They structurally consist of three to four fused cyclic heptapeptide cores that are typically glycosylated with occasional fatty acid side chain modifications. There are four different subclasses of glycopeptides that vary by location of substituents (Butler *et al.*, 2014). The functional groups present in these compounds facilitate its binding to peptidoglycan precursors of the cell wall (Zeng *et al.*, 2016). Due to limited oral bioavailability, most of these antibiotics are administered intravenously (Zeng *et al.,* 2016).

The mechanism of action of glycopeptides is similar to that of β-lactams. As Gram-positive bacterial walls consist of thick layers of peptidoglycan, these antibiotics work by one, sequestering cell wall precursors, two, preventing cross-linking between peptidoglycan moieties, and three, preventing the incorporation of amino acids into the peptidoglycan monomers. By sequestering the precursors, further modification and incorporation of these monomers is inhibited. Similarly, the strength of the cell wall of Gram-positive bacteria comes from the cross-linking between varying peptidoglycan layers. The inhibition of this process leads to a weaker, more fragile cell wall. This weakening is also seen with the inhibition of transpeptidation. Gram-positive bacteria rely on amino acids for the binding of peptidoglycan moieties. Without amino acids, there will be no intralayer binding of peptidoglycan resulting in holes in the membrane. In all, the antimicrobial effects are a result of the dysregulation in the production and modification

of the cell wall, leaving the bacterium vulnerable to cell lysis. However, levels of resistance to this class of antibiotic are increasing due to horizontal gene transfer of resistant plasmids (Zeng *et al.*, 2016).

Cyclic Lipopeptides

Cyclic lipopeptides (ex. Daptomycin) are narrow-spectrum antibiotics used for the treatment of Gram-positive bacterial infections (Schneider *et al.*, 2014) (Bionda *et al.*, 2013). The general structure of these antibiotics is a macrocyclic ring composed of a short oligopeptide linked to a fatty acid tail. The amino group and fatty acid make this class of antibiotics amphiphilic. However, it is the various amino acids found within the oligopeptide that make this class of antibiotics toxic (Schneider *et al.*, 2014).

These antibiotics work by targeting the cell membrane and the cell wall. To work, it must be inserted into the various membranes. As it is amphiphilic, insertion happens readily when facilitated by elevated serum calcium levels. Once incorporated into the cell wall/membrane, it interacts with various enzymes, RNA, and DNA and increases the permeability of the membrane. Specifically, it prevents the synthesis of UDP-MurNAc-pentapeptide (a peptidoglycan precursor). It also causes an efflux of magnesium and ATP causing decreased survival and replication fitness. In all, this class of antibiotics works by inhibiting the synthesis of the cell wall/membrane, scavenging vital resources, and limiting the fitness of the bacterium (Bionda *et al.*, 2013).

Fluoroquinolones

Fluoroquinolones (ex. ciprofloxacin and levofloxacin) are a class of broad-spectrum antibiotics whose mechanism of action is preventing DNA replication. It was elucidated that this class of antibiotics inhibits the action of topoisomerase IV (in Gram-positive bacterium) and DNA gyrase (in Gram-negative bacterium). These proteins work to relieve supercoiling in the DNA to allow for the docking of polymerases. This is accomplished by introducing small breaks between the deoxyribose moieties of the DNA strands. These breaks cause relaxation of the specific section of DNA before it is reannealed to the rest of the strand (Redgrave *et al.*, 2014).

In the presence of fluoroquinolone, the topoisomerase or gyrase is bound along with the DNA via a water-metal ion bridge. This bridge is formed between the oxygen of the amide group of the fluoroquinolone and the hydroxyl group in the serine or other acidic residues of topoisomerase IV or gyrase. This bond is mediated by the presence of magnesium ions. As this bond is covalent in nature, the DNA is left cleaved and unable to reanneal. This damage causes the initiation of the SOS response and, ultimately, cell death (Redgrave *et al.*, 2014).

Aminoglycosides

Aminoglycosides (ex. Amikacin and Streptomycin) are broad-spectrum antibiotics that are active against aerobic bacteria. They structurally consist of amino sugars connected to an aminocyclitol ring (Germovsek *et al.*, 2016). With the ascribed functional groups, these antibiotics show elevated reactivity and specificity for nucleic acids. This targeting leads to inhibition of post-transcriptional modifications and translation (Kotra *et al.*, 2000).

It is noted that aminoglycosides are reactive to ribozymes, tRNA, and splicing introns (Kotra *et al.*, 2000). The inhibition of these molecules prevents post-transcriptional modification and translation. Specifically with ribozymes, aminoglycosides have been noted to bind to the A site of the bacterial ribosome. This binding prevents the proper initiation of protein synthesis as it promotes an artificial reading of codons leading to incorrect incorporation of amino acids. It is also suggested that A-site binding might impact the translocation of the growing amino acid chain from the A site to the P site (Kotra *et al.*, 2000). Ultimately, this stunts the production of primary proteins. Without the synthesis of essential proteins, other vital cellular processes will cease, leading to cell death.

Tetracyclines

The tetracyclines (ex. Methacycline & Doxycycline) are a class of broad-spectrum antibiotics commonly used to treat skin and respiratory tract infections (Chopra & Roberts, 2001). These antibiotics consist of a system of four linear fused cyclic carbonyl rings. The differences between the various antibiotics arise from the functional groups associated with each ring. These functional groups lend different reactivity and selectivity (Chopra & Roberts, 2001). Antimicrobial activity is due to the binding of the antibiotic to the 30S ribosomal subunit. The binding of the 30S subunit prevents the binding of t-RNA to the A-site. Ultimately, protein synthesis is inhibited (Chopra & Roberts, 2001). Resistance to tetracyclines is due to a base substitution of cytosine to guanine which prevents the binding of the antibiotic. This mutation is accomplished by the horizontal gene transfer of mobile plasmids or the mutation/ transposition of genes. This point mutation allows the ribosome to continue functioning in the presence of this antibiotic. (Chopra & Roberts, 2001)

Lincosamides

Lincosamides (ex. Clindamycin) is a class of broad-spectrum antibiotics that are preferentially active against Gram-positive bacteria. Its core structure consists of an amino acid (prophylhygric acid) and sugar moieties (methylthio-lincosamide) (Morar *et al.*, 2009) (Spížek & Řezanka, 2017). Some synthetic versions consist of different functional group modifications (Clindamycin is halogenated). The mechanism of action is the inhibition of protein because of the sugar moiety binding to the 23S rRNA of the 50S subunit (Morar *et al.*, 2009). 23S rRNA has been implicated in the binding of tRNA and the peptidyl transferase activity of the 50S subunit (Bocchetta *et al.*, 1998). By binding to this rRNA, amino acid chain elongation is inhibited preventing the synthesis of primary proteins. This ultimately prevents the synthesis of essential proteins which leads to bacterial death (Morar *et al.*, 2009). Regarding resistance, there are noted mutations in the genes encoding methyltransferases (which target 23S rRNA). This mutation prevents the binding of methylthio-lincosamide, preventing the disruption of protein synthesis (Spížek & Řezanka, 2017).

Nitroimidazoles

Nitroimidazoles (ex. Metronidazole) are nitro-heterocyclic antibiotics that are used to treat anaerobic infections. Structurally, they consist of an imidazole ring with a nitro group substituent. The nitrogen creates resonance around the ring and the nitro group increases the reactivity of the compound. It is this reactivity that confers antimicrobial properties (Ang *et al.*, 2017).

The mechanism of action for this class of antibiotics is radical initiation via the nitro group. During redox, which is facilitated by anaerobic conditions, the nitro group is made into a

radical anion. The nitrate byproduct is then converted into an imidazole radical due to the stability of an aromatic ring radical (Ang *et al.*, 2017). The high reactivity of this radical causes DNA oxidation/degradation, RNA degradation, and the degradation of intracellular proteins. Regarding DNA, the radical causes breakage in the DNA strands that cannot be repaired. This breakage halts transcription and translation preventing the synthesis of proteins. As the bacteria is now unable to synthesize new proteins to replace the denatured ones, cellular processes will cease. These deleterious effects lead to cell death. However, resistance to nitroimidazoles has been noted to occur with downregulated reduction enzyme production (Lamp *et al.*, 1999).

Sulfonamides

Sulfonamides (ex. Sulfamethoxazole) are broad-spectrum antibiotics that are classified as metabolic antagonists. They consist of either a central $-SO_2NH_s$ or $-SO_2NH-$ attached to one or multiple 5- or 6-membered heterocyclic rings. The mechanism of action for this antibiotic is as a functional antagonist of p-aminobenzoic acid (PABA). PABA is required for folic acid production as it activates dihydropteroate synthase (Ovung & Bhattacharya, 2021). This synthase is responsible for the addition of p-aminobenzoic acid to dihydropterin pyrophosphate- creating a necessary precursor in folic acid synthesis (Hevener *et al.*, 2011). Because folic acid is required for DNA synthesis, these competitive antagonists halt folic acid synthesis and subsequently DNA replication. It is important to note that sulfonamides are not bactericidal but rather bacteriostatic. However, by limiting the replication of the bacteria, the host's immune system has adequate time to tag and phagocytize the bacteria (Ovung & Bhattacharyya, 2021).

Diaminopyrimidines

The diaminopyrimidines (ex. Trimethoprim) are a class of broad-spectrum antibiotics that consist of pyrimidines and amino groups (Wróbel *et al*., 2019). They are classified as metabolic antagonists as they target folic acid synthesis by inhibiting covalent bond formation between the nitrogen of the amino groups and the carboxylate group of dihydrofolate reductase. This enzyme is responsible for the reduction of dihydrofolate to tetrahydrofolate (Wróbel *et al*., 2019). By halting folate synthesis, DNA replication cannot occur. Like sulfonamides, diaminopyrimidines are bacteriostatic (Wróbel *et al*., 2019). Resistance to this class of antibiotics has been seen due to an acquired mutation in the gene encoding for dihydrofolate reductase (Gleckman *et al.*, 1981). This mutation prevents diaminopyrimidines from binding to dihydrofolate reductase, thus preventing its inhibitory effects (Gleckman *et al.,* 1981).

The Most Prevalent HAIs

CAUTI

Catheter-associated urinary tract infections (CAUTIs) are infections that arise within 48 hours of catheter placement into the urinary bladder (Werneburg, 2022). Associated symptoms are fever, suprapubic tenderness, and delirium (Blodgett *et al.*, 2015). CAUTIs associated with long-term indwelling catheters can be the result of a polymicrobial infection that cannot be treated by a single antibiotic (Gaston *et al.*, 2020). In the United States, 1 million cases occur annually with over 13,000 fatalities (Podkovik *et al.*, 2019; Werneburg, 2022). CAUTIs account for 40% of all HAIs annually in the U.S. (Blodgett *et al.*, 2015). It has been estimated that CAUTIs cost the U.S. healthcare system between $115 million and $1.82 billion annually (Werneburg, 2022). This expanded range accounts for more complex cases, the need for

extended antibiotic-based treatment, and treatment complications (secondary infections) (Werneburg, 2022).

The six most prominent causative agents of CAUTIs are *E. coli*, Enterococcus, *K. pneumoniae*, *S. aureus, Proteus mirabilis,* and *P. aeruginosa* (Werneburg, 2022). Broad-spectrum antibiotics are typically prescribed until culturing is completed. Amoxicillin and an aminoglycoside is a common treatment method. Other similar options are a second-generation cephalosporin with an aminoglycoside, or a third-generation cephalosporin given intravenously (IV). The first two options can be administered outside of a medical facility making them more financially accessible. The third option is typically reserved for cases that do not respond well to initial oral treatments and/or for a progressing case. Due to the route of administration, longer periods of acute care are needed. Typically, a 7-day course of antibiotics is prescribed for infections that respond well to treatment. For those that do not respond well, a 10-14-day antibiotic course is common (Werenburg, 2022). This use of multiple antibiotics at once can leave a patient open to secondary infections (especially *C. diff* – this will be discussed below).

CLABSI

Central-line associated bloodstream infections (CLABSIs) are infections that arise from the site of an inserted central line within 48 hours of insertions (Alshahrani *et al.*, 2023). Central lines are vital sites of venous access for those needing to receive large volumes of fluids or chemotherapy. It is the direct access to vasculature that allows for pathogenic microbes to enter the bloodstream (Patel *et al.*, 2019). 250,000 CLABSI cases occur annually in the United States, and the associated mortality rate is 12-25% (Han *et al.*, 2010). Likewise, CLABSIs have an incidence rate of 0.5-5 per 1000 catheter days and an odds ratio of 1.028-5.52 (the range of the

odds ratio is due in part to the indwelling period of the central line). As of 2010, CLABSIs were estimated to cost the U.S. healthcare system $32,000 per case – this is equivalent to $45,299 as of 2023 (inflation adjustment made using CPI inflation calculator provided by the Bureau of Labor Statistics). In all, this represents a loss of over $11 billion to the U.S. healthcare system annually.

The five most common causes of CLABSIs are Enterobacteriaceae, *S. aureus*, Coagulase-negative *Staphylococci*, Enterococcus, and *P. aeruginosa*. The most common cause of CLABSIs is Enterobacteriaceae- it is implicated in 23-31% of cases. The second most prominent bacterial agent is *S. aureus*- it is linked to 16% of cases (Alshahrani *et al.*, 2023). It is common for patients to be placed on a 7-14- day antibiotic regimen. The antibiotic prescribed is dependent on the bacterial agent. Ampicillin (a beta-lactam) is often used to treat Enterococcus (Sandoe *et al.*, 2002). Fluoroquinolones (specifically ciprofloxacin or levofloxacin) are often used to treat *P. aeruginosa* (Zakhour *et al.* 2022). However, a 2-4-week antibiotic regimen of vancomycin is commonly used to treat *S. aureus* (especially MRSA infections) (Liu *et al.*, 2011). Patients who begin to present symptoms of bacteremia or fail to demonstrate clinical improvement within 72 hours of beginning an antibiotic regimen, may be prescribed a different antibiotic for up to 6 weeks (Han *et al.*, 2010). If the infection is not controlled by these antibiotics, patients can face complications such as sepsis, organ failure, and endocarditis (Alshahrani *et al.*, 2023) (Han *et al.*, 2010).

SSI

Surgical site infections (SSIs) arise at the site of incisions and affect the surrounding tissues/organs. These infections can develop between 48 hours to 1 year following

surgery/prosthesis introduction. They are especially common in the prostheses used in joint replacements as they harbor bacteria. Annually, SSIs account for 21.8% of all HAIs in the U.S. (Anderson *et al.*, 2008). It is estimated that 2-5% of all patients who have surgery will develop an SSI. As of 2008, the mortality rate was 3%. However, this number is decreasing with improved infection prevention methods (Anderson *et al.*, 2008). These infections are classified in 3 main ways: superficial incisional, deep incisional, and organ/space. Superficial incisional is characterized by the epidermis surrounding the incision harboring infection. Deep incisional is characterized by the underlying dermis being infected. Organ/space is characterized by the infection compromising the organ, fascia, or the surrounding body cavity. The severity of the infection increases proportionally to the number of layers that are affected (Barry, 2020).

SSIs tend to be more complicated than the other 5 HAIs. This is due to a multitude of different causative agents that vary based on the location of the surgery. 30 different bacteria (17 Gram-positive and 13 Gram-negative) have been cultured from infected incisional sites. The most common are *S. aureus*, CoNS, and enterococci (specifically *Enterococcus faecium* and *Enterococcus faecalis*) (Bamberger & Boyd, 2005; Barry, 2020). Treatment of SSIs consists of draining purulence, debridement of the infected area, and an antibiotic regimen. For simple, superficial incisional infections, a 5–7-day antibiotic regimen is common, but with deep incisional infections and bacteremia, a 2–4-week antibiotic regimen is typical. The common antibiotics used to treat SSIs are cephalexin, clindamycin, linezolid, vancomycin, and daptomycin (Bamberger & Boyd, 2005). In all, these treatments cost the U.S. healthcare system, on average, $20,785 per patient, resulting in a loss of $700 million annually (Barry, 2020). The

treatment of these infections also increases the risk of secondary infections (Barry, 2020; Liu & Dickter, 2020; Mohsen et al, 2020).

VAP

Ventilator-acquired pneumonia (VAP) is an infection of the lungs that develops 48+ hours after intubation. This infection poses a great risk of mortality as it further compromises already damaged lungs. These infections have an estimated 24-76% mortality rate. The range accounts for comorbidities and the varying diagnoses that lead to ventilation. In the United States, VAP occurs in 8-38% of patients who are mechanically ventilated. Complications of VAP are sepsis/septic shock, acute respiratory distress syndrome, and atelectasis (Chi *et al.*, 2012) (Othman & Abdelazim, 2017). VAP also compromises the immune system, leaving the patient highly susceptible to other opportunistic infections. The development of a secondary infection increases the duration of ventilation and stay in the ICU (Othman & Abdelazim, 2017).

VAP is caused by both Gram-negative and Gram-positive bacteria. The most common Gram-negative bacteria are *P. aeruginosa, E. coli, K. pneumoniae,* and Acinetobacter. The most common cause of VAP is *S. aureus*. The most common treatment is a 7-day antibiotic regimen (Metersky *et al.*, 2023). For Gram-positive bacteria presenting with MRSA activity, vancomycin or oxazolidinones is recommended. For Gram-negative bacteria with antipseudomonal activity, piperacillin-tazobactam or cefepime is recommended. Also, for similar bacteria that do not show resistance to beta-lactams, ciprofloxacin or levofloxacin is recommended (Kalil *et al.*, 2016). In all, the cost of these treatments on average in 2012 was $39,828 per patient (Kollef *et al.*, 2012). The adjusted rate is now an average of $54,193 (this value was calculated using the CPI Inflation Calculator provided by the Bureau of Labor Statistics).

Clostridium difficile

C. diff. is a Gram-positive, anaerobic bacillus that is a resident flora of the GI tract. However, its levels are mediated by competition for cholate with other bacteria. As the mediating bacteria are killed by antibiotics, *C. diff.* is left with an optimal growing environment. With the outgrowth of *C. diff.*, the remaining living bacteria are killed by *C. diff.* exotoxins. These toxins also affect the host cells. TcdA and TcdB have been implicated in severe systemic effects in the human body (Bella *et al.,* 2016). TcdB has been linked to cardiac muscle damage, reduced heart rate, reduced blood profusion, deformation of the cardiac atrium and ventricles, and reduced cardiomyocyte viability (Bella *et al.,* 2016). TcdA and TcdB in conjunction have been linked to acute kidney injury, increased apoptosis of nephritic cells, reduced glomerular filtration rate, and apoptosis of cerebellar neurons (Bella *et al.,* 2016). In summation, *C. diff.* exotoxins negatively impact the functioning of the heart, kidneys, and GI tract (Bella *et al.*, 2016). Specifically, *C. diff.* can cause pseudomembranous colitis in the GI tract which has been linked to increased morbidity and mortality and longer hospitalizations (Surawicz & McFarland, 1999).

Hospital-acquired *C. diff.* infections develop in approximately 235,700 Americans annually, as of 2017 (Guh *et al.*, 2020). These infections have an associated mortality rate of 5.7-6.9% within the first 30 days of infection. Due to the mortality rate increasing over the previous years, *C. diff.* poses a serious risk to patients (Guh *et al.*, 2020). *C. diff.* infections typically present with the symptom of watery diarrhea. This diarrhea is unique as it has a unique characteristic smell that has been likened to rotting meet and a sickly sweetness (Cleveland Clinic, 2023). Likewise, this diarrhea is hard to control, and is usually profuse (Bella *et al.*, 2016). The presence of watery diarrhea poses a serious risk to the patient as dehydration is

common (Lurienne *et al.*, 2020). Likewise, *C. diff.* infections have been noted to increase the rate of depression in patients (Lurienne *et al.*, 2020). The rates of depression increase with the number of *C. diff.* reoccurrences (Lurienne *et al.*, 2020). This continues to be a growing problem as patients are 40-60% more likely to have a second reoccurrence once one reoccurrence happens (Lurienne *et al.*, 2020).

For treatments, the most common one is an antibiotic course of metronidazole or vancomycin accompanied with fluids and electrolytes (Lurienne *et al.*, 2020) (Steele *et al.*, 2015). However, in extreme cases, a fecal transplant to reintroduce healthy bacteria may be necessary (Bella *et al.*, 2016). Treatment usually lasts 10-14 days for mild to moderate infections but can take 3-4 weeks for severe cases. Also, reoccurrences can occur for months or years following the initial infection. In total, these treatments cost the U. S. healthcare system upwards of $4.8 billion annually (Lessa *et al.*, 2015).

MRSA

The rise of MRSA coincides with the discovery of antibiotics. MRSA is a specific group of *S. aureus* that has become resistant to methicillin and other beta-lactams due to a mutation (via point mutation or horizontal gene transfer) in the penicillin-binding proteins (Fukunaga *et al.*, 2016) (Lukhundi & Zhnag, 2018). This mutation creates a bacterial strain that is extremely difficult to treat. In the U.S. 1.3% of the population are colonized with MRSA and roughly 2 million people are infected with MRSA every year (Fukunaga *et al.*, 2016). This equates to an incidence of 31.8 per 100,000 people. Accordingly, MRSA accounts for roughly 23,000 deaths annually in the U.S. (Hassoun *et al.*, 2017) (Fukunaga *et al.*, 2016).

For the treatment of MRSA, a 7-14-day antibiotic regimen of vancomycin or daptomycin is common for those with community-acquired MRSA. This time frame can increase for those who acquired MRSA in a healthcare setting (Choo & Chambers, 2016). A 4-6-week antibiotic regimen is typical for those with complex infections (Liu *et al.*, 2011). Those colonized, however, may be on antibiotics for upwards of 3+ months (Hassoun *et al.*, 2017). If left untreated, MRSA can cause endocarditis, osteomyelitis, meningitis, septic arthritis, pneumonia, and brain abscesses (Liu *et al.*, 2011). In all, these treatments cost the U.S. healthcare system roughly $55 billion annually (Fukunaga *et al.*, 2016).

The Need for New Therapies

HAIs pose a serious fiscal problem to U.S. healthcare with an annual cost upwards of $24.8 billion. This number, however, fails to account for the monetary loss patients incur due to extended periods of absence from work and various hospital bills not covered by insurance. The problems caused by HAIs, though, extend far beyond monetary means. Recently, the effects of HAIs on mental health and the effects of high antibiotic usage on antimicrobial resistance have been recent avenues of research (Lurienne *et al.*, 2020).

HAI research has sought to explain the pathophysiology, merits of treatments, and the epidemiology of these infections (Currie *et al.,* 2018). Little attention has been directed to research on the effects of HAIs on the mental health of patients. Research that has been completed on this topic has focused on *C. diff*. This focus is due in part to the symptoms of the infection being very noticeable to both patients and others. With this awareness, researchers sought to understand the level of social isolation that a diagnosis of an HAI brings. It was described that the effects of HAIs on mental health were proportional to the physical symptoms

the patient experienced: the more severe the physical symptoms, the more severe the mental symptoms (Currie *et al.,* 2018). Likewise, many patients expressed feelings of being "dirty" and were ashamed of their diagnosis (Currie *et al.,* 2018). These feelings led them to distance themselves from their social support systems due to societal norms making them fearful of contaminating others (Currie *et al.*, 2018). As research on this subject is expanded, a deeper understanding of the impacts of HAIs on mental health will be gained. This knowledge can help shape treatment for patients to meet both their physical and mental health needs.

Another, and vastly important, area of research is on the prevalence and impact of antibiotic resistance on the prevention, treatment, and control of HAIs. Antibiotic resistance is a relatively new phenomenon that has been increasing in prominence since the beginning of the widespread use of penicillin in the 1940s. Since then, and even with the development of new antibiotics, antibiotic resistance has continued to be a growing problem. It is believed that for almost every available antibiotic, there is at least one resistant microbe (Ventola, 2015). Because of this, research to find/develop new antibiotics continues to be a field of interest.

With increasing antibiotic resistance, treatment of HAIs becomes increasingly difficult. As different antibiotics and treatment times are utilized to address these problems, further problems arise. The increased use of antibiotics is leading to greater rates of antibiotic resistance. This trend applies to both community-acquired and hospital-acquired infections. This results in decreased efficacy of current antibiotics on the market (National Foundation for Infectious Diseases, 2023). Similarly, it has been discovered that antibiotic regimens for extended periods of time support the developmental outgrowth of *C. diff.* in the gastrointestinal tract of humans (Shah *et al.*, 2021). The development of a *C. diff.* infection during treatment of another HAI can

lead to serious complications. Usually, treatment requires changing antibiotics which further inhibits the regrowth of the healthy microbiota (Shah *et al.*, 2021). This change further reduces the fitness of the host's immune system (Lurienne *et al.*, 2020). It is for these reasons that new treatment methods for bacterial HAIs need to be developed. A promising avenue for this may be porphyrins.

What are Porphyrins?

Porphyrins are a class of planar organic molecules that consist of 4 pyrrole groups bridged by four α-carbons. These carbons connect the pyrrole groups via methine bridges. These molecules are large and aromatic consisting of an 18π electron system (Giovannetti, 2012). This conjugated system provides porphyrins with its characteristic stability and reactivity as electrons diffuse around the system leading to high resonance. Similarly, this resonance provides porphyrins with the unique ability to create both cationic and anionic radicals. These radicals are intermediates in the reactions of peroxidases, catalases, and cytochrome P450 (Giovannetti, 2012) (Hift, 2020). This stability and reactivity are only enhanced by the introduction of metallic ions that bind due to the inward-facing nitrogen (Ishimizu *et al.*, 2019).

In nature, porphyrins are found in both plants and animals. In plants, they are found within chlorophyll. Porphyrins make up cytochrome P450 which harnesses the photon energy necessary for the initiation of photosynthesis. Without porphyrins, plants would be unable to synthesize sugar. Porphyrins play a different, yet just as important, role in humans. Found within red blood cells, porphyrins constitute heme groups. Four heme groups make up one hemoglobin molecule. These porphyrins, along with their iron cores, are vital for the transport of oxygen and carbon dioxide throughout the body. Without this functionality, there would be an increase in the

production of ROSs as oxygen is the last electron receptor in the electron transport chain. The reduction of oxygen to water subsequently prevents the development of these radicals (Yang *et al.*, 2019).

Important Chemistry

Porphyrins exhibit a high level of conjugation which accounts for the characteristic resonance. This conjugation also accounts for the characteristic UVvis spectrum produced by porphyrins. These UV-vis will have a Soret band (absorbance of 400 nm) and Q-bands (varying absorbances from 500-600 nm). As all these absorbance wavelengths fall within the visible light spectrum (400-700 nm). These absorbances are why porphyrins are vividly colored when placed in visible light or ultraviolet light. Similarly, porphyrins have exhibited luminescence, paramagnetism, photoconduction, and semiconduction (Ishimizu *et al.*, 2019). These traits are the result of their high degree of resonance stability. As mentioned above, these molecules are vital for carbon fixation in photosynthesis and the binding and transport of oxygen through the human body. Likewise, porphyrins are a major functional group in vitamin B12 which aids in nerve health and conduction (Green *et al.*, 2017).

Bactericidal Effects

Research into the bactericidal effects of porphyrins has been a growing field. It was elucidated that porphyrins, and especially photo-activated porphyrins, exhibit bactericidal effects against Gram-positive bacteria (Silhavy *et al.*, 2010). The research on the use of this technology on Gram-negative bacteria has been slower, but advancements have been made (Silhavy *et al.*, 2010). The reason for the difference in the levels of bactericidal efficacy for these two groups depends on the structure of their cell walls. Gram-negative bacteria possess an outer membrane

that is highly selectively permeable. Limited permeability decreases the rate at which treatments can interact with the bacteria, especially the peptidoglycan. Gram-positive bacteria, however, possess a thick layer of peptidoglycan that characteristically has a higher level of permeability (Silhavy *et al.*, 2010).

For Gram-positive bacteria, it has been elucidated that porphyrins selectively bind to the cellular membrane- porphyrins readily bind to proteins, DNA, and membranes (Malik *et al.*, 1989). This process is pH-dependent and happens optimally at pH 6.8. This value is vastly lower than that of the regular human body (especially that of circulating blood). The need for this lowered pH poses a challenge for the use of this treatment. However, as the proximal small intestine exhibits an average pH of 6.6, this treatment could prove useful in treating bacterial infections of the GI tract. Research suggested that photo-activated porphyrins exhibited a lethality rate of over 99.99% to these pathogens (Evans *et al.*, 1988) (Malik *et al.*, 1989).

The lethality of photo-activated porphyrins is due to the production of reactive oxygen species (ROS). As photon energy is absorbed, the porphyrin enters an excited triplet state. When in an aqueous state, this energy is transferred to the oxygen in water, creating a reactive oxygen species. Some of the most common ROSs are hydroxyl, biomolecules, and superoxide radicals. These ROSs have been found to interact with ion pumps of the electron transport chain (Malik *et al.*, 1989). By disrupting the structure and functionality of these pumps, the production of ATP decreases and ultimately leads to the death of the bacteria. Likewise, ROSs have been implicated in DNA damage. As this damage is unable to be fixed, apoptosis occurs, and bactericidal effects are seen. Similarly, porphyrins have been observed to disrupt the development of the cell wall and cellular membranes (Malik *et al.*, 1989). By disrupting membranes, bacteria (which mostly

exist in a hypotonic environment) can no longer control the influx of water into the cell. With a marked influx of water, the increase and inability to control turgor pressure ultimately leads to lysis. It is important to note that photo-activated porphyrins are not effective against Gram-positive bacteria during their lag phase. However, this should not pose a great clinical setback as most infections are not diagnosed until bacterial counts are high enough for detection (Malik *et al.*, 1989).

For Gram-negative bacteria, it has been deducted that for a photo-activated porphyrin to exhibit bactericidal effects it must be positively charged (Sulek *et al.*, 2020). By possessing a positive charge, the porphyrin can electrostatically adhere to the negatively charged outer membrane. This adherence aids in the penetration of these porphyrins through the outer membrane and eventually through the deeper peptidoglycan and cytoplasmic membrane. It has also been suggested that the substitution of a halogen in the center of the porphyrin might create a viable option as they aid in the creation of radicals due to their extremely high electronegativity (Sulek *et al.*, 2020). Once adherence occurs, the mechanism of action is the same for Gram-positive bacteria (Malik *et al.*, 1989).

Avenues for Future Research

This new technology could combat the increasing virulence and antibiotic resistance exhibited by HAIs today. With the development of this new treatment, it is believed that various photo-activated porphyrins could contribute to the elimination of the problem of antibiotic resistance. It is currently believed that resistance to porphyrins is not highly probable as the mechanism of action involves radicals and causes the disruption of various cellular processes (Cieplik *et al.*, 2018). However, for porphyrins to truly become the clinically established

treatment for bacterial infections, significant research still needs to be conducted. The current research is only in its beginning stages. Optimal methods of delivery, whether they could be photoactivated inside of the human body (and what damage that might cause to the host's tissues), whether non-photoactivated porphyrins can exhibit the same bactericidal effects, how to ensure target specificity, and how to increase the efficacy of bactericidal effects against Gram-negative bacteria are warranted areas of research. Once these areas of inquiry are addressed, porphyrin-based therapies may begin a new age in the treatment of bacterial infections.

Conclusion

In all, HAIs continue to pose a serious risk to the health of patients both physically and mentally while placing a great strain on the economy. The current treatments used clinically continue to exhibit decreasing efficacy as bacterial strains continue to mutate to increase resistance. Likewise, current treatments can lead to serious complications such as secondary infections due to the need for increasingly potent antibiotics. Because of this, research into new treatment methods is of ever-increasing importance. Over the last few decades, research into the bactericidal effects of porphyrins, their derivatives, and the photo-activation of porphyrins has been of increasing interest. It has been found that these molecules are quite effective against Gram-positive bacteria but have less efficacy against Gram-negative bacteria. However, research into how to increase the bactericidal effects against Gram-negative bacteria has been increasing and showing promising growth. Before this technology can be introduced in a clinical setting, many more questions regarding safety, viability, and delivery must be addressed, but this could be a promising avenue for discovery.

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