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ESKAPE Pathogens: The clinical Prevalence and Molecular Mechanisms of Antibiotic Resistance

Anusha Attre
anusha.attre@uconn.edu

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Faculty Advisor: Dr. Patricia Rossi, Molecular and Cell Biology

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Abstract

The ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are the leading cause of all nosocomial, or healthcare-associated (HAI), infections (Navidinia, 2016). The purpose of this research study is to determine the burden of ESKAPE infections on healthcare and study the antibiotic resistance in these high-risk pathogens to provide direction for researchers to develop new antimicrobial innovations to reduce ESKAPE infectivity and improve patient outcomes. To study the burden of ESKAPE infections, this review analyzes the current statistics explaining the clinical prevalence of each pathogen in causing HAIs. Additionally, each pathogen is investigated to determine the health risks and factors that make certain communities more susceptible to infection. To study the mechanisms of resistance, numerous studies across molecular biology are utilized to provide a comprehensive report of existing and emerging resistance patterns.

Introduction

Antimicrobial resistance (AMR) has been declared "one of the top 10 global health threats facing humanity" by the World Health Organization (WHO) (Ghosh, 2021). AMR occurs due to adaptations in the bacteria, viruses, fungi, and parasites that cause illnesses. These adaptations cause the treatments currently available to be less effective or not effective at all to stop an infection (World Health Organization, 2021). Pathogens with antimicrobial resistance are emerging rapidly in clinical settings. The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) are the leading cause of all nosocomial, or healthcare-associated (HAI), infections (Navidinia, 2016). The Centers for Disease Control and Prevention estimates that in the United States alone, more than 2.8 million antibiotic-resistant infections occur yearly. The annual cost to treat these infections is over $4.6 billion in America (Nelson et al., 2021). From a global public health perspective, the growing prevalence of infectious diseases is overwhelming the healthcare industry and increasing the risk of severe or deadly infections in human patients. Prolonged infections lead to longer inpatient admissions and extensive medication regimens which are long-term burdens for patients.

The purpose of this research study is to determine the burden of ESKAPE infections on healthcare. Additionally, studying the molecular mechanisms of antibiotic resistance in these high-risk pathogens will provide direction for pharmaceutical researchers to develop new antimicrobial innovations to reduce ESKAPE infectivity and improve patient outcomes. For this literature review, an interdisciplinary analysis utilizing public health, medical, biological, and statistical concepts is required.

Clinical Prevalence

ESKAPE pathogens are uniquely characterized by the pathogenic nature of the bacteria and characteristics of antimicrobial resistance. In the Arbune 2021 retrospective study of ESKAPE prevalence in a Romanian hospital from 2015-2020, researchers collected 4293 isolates from patients with nosocomial bacterial infections. *S. aureus* was the ESKAPE pathogen with the highest prevalence with 26% of the samples testing positive. In decreasing order of prevalence, *K. pneumoniae* (9.55%), *P. aeruginosa* (8.78%), and *Enterococcus* species (3.55%) were found. *Enterobacter* and *Acinetobacter* species were not found in significant quantities (Arbune et al., 2021).

Enterococcus Species

E. faecium and *Enterococcus faecalis* are Gram-positive opportunistic pathogens which can cause HAIs. *E. faecium* and *E. faecalis* typically inhabit the gastrointestinal tract in humans and under normal conditions are nonpathogenic. Sequencing data has classified the *E. faecium* population into two clades, clade A and clade B, where HAIs are associated with clade A (Zhou et al., 2020).

Of the 148 strains of *Enterococcus* in the Arbune 2021 study, only samples collected from 2019 were identified at the species level. In the 26 specific cases that were identified, two were *E. faecium* and 24 were *E. faecalis.* Ciprofloxacin (CIP) sensitivity was low in all samples. Of the *E. faecium* samples, linezolid and vancomycin were effective (Arbune et al., 2021).

Staphylococcus aureus

S. aureus is commonly part of the skin microbiota and can be well isolated from the nostrils and other moist environments. *S. aureus* takes advantage of wounds and lesions to cause infections. *S. aureus* can present as an acute or chronic infection. Chronic infections are exacerbated by the ability of the pathogen to create biofilms in post-operative recovery (Pendleton et al., 2014). Biofilm-associated infections are generally not susceptible to antimicrobial treatments because of the protective exocellular matrix and subpopulations of dormant persistent cultures of cells leading to chronic infections. A large majority of *S. aureus* infections are additionally intensified by the pathogen's ability to secrete hyaluronidase and collagenase, which are two enzymes that cause tissue deterioration in the epidermis and dermis of infected patients (Pendleton et al., 2014). Many different types of collagenase exist and are considered a virulence factor as they aid in the breakdown of peptide bonds in collagen in connective tissues. This increases the penetration of pathogens and subsequent movement to establish infection within the tissue, and provides nutrients and host factors for sustained pathogenic presence. In roughly 25% of isolates, *S. aureus* utilizes exotoxin TSST-1, which causes gastroenteritis (Pendleton et al., 2014).

In the Arbune study, *S. aureus* was identified as 89% of all Staphylococcal isolates. While the *S. aureus* isolates had several effective antibiotics against them, the Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were not as susceptible as the non Methicillin-resistant *S. aureus* strains.

Klebsiella pneumoniae

K. pneumoniae is a Gram-negative *Enterobacteriaceae* that can be found in the flora of the mouth, skin, and intestines in humans. It is non-motile, encapsulated, and a facultative anaerobe. *Klebsiella* species were identified in roughly 5% of bacteremia diagnosis from a study in the UK (Pendleton et al., 2014). Fimbrial adhesins and a thick capsule physically prevent the immune cells of the host from phagocytosing the pathogen, known as antiphagocytic protection. When pathogens can evade the natural defense of the host's immune system, the typical limits for pathogen growth and spread are not capable of slowing or stopping infection, which leads to more aggressive infectivity.

In the 2021 Arbune study, *K. pneumoniae* susceptibility was decreased against sulfamethoxazole-trimethoprim, ciprofloxacin, gentamicin, meropenem, and beta-lactamines.

Acinetobacter baumannii

A. baumannii is a Gram-negative, rod-shaped opportunistic pathogen commonly seen in clinical settings including intensive care units and surgical wards (Pendleton et al., 2014). Interestingly, during the Iraq War, "Iraqibacter" or *A. baumannii* became a nosocomial infection in US military healthcare treatment facilities. It continued to be a source of infections for those who served in the Afghanistan War as well. The *A. baumannii* drug resistance strains have moved to civilian clinical spaces as infected military personnel were moved to these different healthcare institutions for their care. Other *Acinetobacter* species are typically isolated from soil samples, but *A. baumannii* is primarily isolated from clinical settings (Antunes et al., 2014). These particular clinical settings overuse antibiotics to permit resistance to be generated. *A. baumannii* is highly adapted to thrive in clinical environments by transfer of resistance genes.

In the Arbune study, *A. baumannii* was identified in 19 samples collected from skin infections, urine and blood cultures, airway secretions, and catheter sites. Resistance against cephalosporins, quinolones, and aminoglycosides was noted.

Pseudomonas aeruginosa

P. aeruginosa, a Gram-negative facultative anaerobe, can thrive in mucosa. The opportunistic pathogen is most commonly identified in patients diagnosed with cystic fibrosis, cancers, or burn victims (Pendleton et al., 2014). *P. aeruginosa* produces multiple secondary metabolites, polymers, and can use various carbon sources and electron acceptors for energy leading to widespread presence in clinical settings and resistance to antibiotics. As it can survive on abiotic and biotic surfaces in biofilms, *P. aeruginosa* is common as a HAI including ventilator-associated pneumonia, central line-associated bloodstream infection, urinary catheter-related infection, and surgical/transplantation infections. Biofilms that form within the respiratory tract of cystic fibrosis patients are harder to treat due to the more embedded nature of the biofilms in the excess mucus and cilia of the lungs. The antibiotic concentration to kill the bacteria within these biofilms is 1,000 times the strength of the minimum inhibitory concentration (Gnanadhas et al., 2015).

In the Arbune study, 204 strains of *P. aeruginosa* were found from primarily skin infections. The Multidrug resistant (MDR) rate was 29.1%. The collected strains lacked complete susceptibility to all of the tested antibiotics (amoxicillin clavulanate, cefuroxime, cefotaxime, ceftazidime, cefepime, piperacillin- tazobactam, ertapenem, meropenem, ciprofloxacin, gentamicin, and sulfamethoxazole-trimethoprim).

Enterobacter species

The *Enterobacter* genus consists of 22 species of opportunistic pathogens, all of which are facultative anaerobes. *Enterobacter* species are also found as commensal bacteria in the gut microbiota. The most common causes of nosocomial infections are *Enterobacter aerogenes, E. cloacae,* and *E. hormaechei.* These pathogens commonly infect patients in intensive care units because these immunocompromised patients are at higher risk due to prolonged recent use of antimicrobial treatment and use of invasive procedures with medical devices. Biofilms can also survive on these medical devices and within the patient, making these bacteria excellent colonizers (Davin-Regli, et al., 2019).

Health Risks and Factors

Each pathogen species has unique risk factors and impacts populations differently. HAIs primarily fall into the following categories: central line-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections, and ventilator-associated pneumonia (Centers for Disease Control and Prevention, 2014).

Enterococcus species cause life-threatening infections in various sites, including the bloodstream, surgical sites, and urinary tract infections. In 2017, there were 54,500 cases in clinical settings and 5,400 deaths in the United States (Arroyo Pulgar, 2019). Immunocompromised patients face heightened likelihood of an adverse infection because their immune systems are weakened and unable to fend off aggressive infections that require immune intervention (Pendleton et al., 2014).

S. aureus is typically found in the normal skin microbiota of humans. Most *S. aureus* infections are found in superficial locations, including skin lesions and wounds (Arroyo Pulgar, 2019). Specifically, communities of patients with postoperative lesions and wounds are at a higher risk to develop a serious *S. aureus* infection. Typically, the mucus membranes and skin form physical barriers to prevent infection. However, if lesions or wounds exist, pathogens can access the deeper tissues and thrive from the abundant nutrients available in the new environment to form infections. Surgical site infections are common causes of further complications, and readmission to the hospitals with increased morbidity and mortality. Therefore, patients with postoperative lesions at highest risk for developing nosocomial *S. aureus* infections.

K. pneumoniae impacts the young, the old, and immunocompromised individuals most commonly (Pendleton et al., 2014). *K. pneumoniae* is naturally found in the human gastrointestinal system. Patients receiving care in clinical settings, especially individuals who require medical devices as interventions, face an increased risk of developing an infection. This is because the medical devices act as mediums to allow the pathogen to access more susceptible locations of the body that lack protective epithelial tissue (Centers for Disease Control and Prevention, 2010).

A. baumannii impacts patients who use antibiotics prior to infection. Typically, the pathogen exhibits more resistance in respiratory infections in comparison to the infections found in the bloodstream, urinary tracts, or skin lesions (Ellis et al., 2015).

P. aeruginosa impacts immunocompromised individuals most severely. Individuals with chronic lung diseases including asthma, cystic fibrosis, and chronic obstructive pulmonary disease are at the highest risk of severe infection (Arroyo Pulgar, 2019).

Enterobacter species can infect the circulatory, respiratory, and urinary systems, making those with relevant preexisting conditions such as diabetes, cancer malignancy, and lupus more susceptible (Arroyo Pulgar, 2019) (Pendleton et al., 2014). Immunocompromised patients are most at risk for chronic *Enterobacter*-caused infections.

Molecular Mechanisms of Resistance

AMR naturally occurs as bacteria evolve. Resistance first arose with antibiotics intrinsically, without the requirement of direct antibiotic treatment. These mutations against antimicrobial susceptibility are encouraged when antibiotics are applied, and the only remaining bacteria are those with the resistance traits. Misuse of antibiotics in modern times occurs both in clinical and non-clinical settings. Incorrect prescribing (dosing and duration-decision making) is a large issue in clinical settings (Homes et al., 2015; Ayobami et al., 2022). Overprescribing of antibiotics increases the risk of adverse effects and more frequent reinfections due to a decrease of the natural flora in the body. People also often do not complete their antibiotic course of medicine once they are feeling better which can lead to development of antibiotic resistance. In addition, antibiotics are sometimes prescribed when infections are viral, which is an ineffective course of treatment for any infection that is not bacterial.

Other larger-scale antibiotic misuse occurs in the agricultural sector, where antibiotics cause increased growth promotion for better yields. Economically, utilizing antibiotics in agriculture allows farmers to make larger profits in less time as they do not need to wait for livestock to mature as long. Excess antibiotic use can collect within the farming system in soil, run-off, and sewage, which contains naturally-occurring microorganisms. Once these microorganisms develop resistance against the antibiotics contaminating the environment, the

microorganisms and residual antibiotics are able to spread outside of the confined agricultural setting.

The WHO collaborative project, One Health, provides an explanation about the relationship between human health and our physical environment. The One Health initiative explains that the overuse and abuse of antibiotics in human medicine, agriculture, and our environment have all contributed to the AMR public health crisis (McEwen and Collignon, 2018). One Health focuses on the impact of humans, animals, plants, wildlife, and the environment on antimicrobial overuse and antimicrobial resistance. Some examples include studies on large-scale, low-dose antibiotic feeding of animals with common antimicrobials in commercialized agriculture where the target consumers are humans. Additional studies within the initiative focus on the importance of how good hygiene, infection control, drinking water, sanitation, and the prevention of pollution from industrial, residential, and farm waste can help control antimicrobial resistance.

Broadly, antibiotic resistance can occur by mutation or horizontal gene-transfer (HGT). Mutations leading to AMR typically occur in three classes of genes: genes that encode the targets of the antibiotic, genes that encode their transporters, and genes that encode the regulators that repress the expression of transporters. The genetic resistome includes the mobile genetic elements and other relevant antibiotic resistance genes in pathogens which pathogenic bacteria can acquire via horizontal gene transfer (HGT) (Wright, 2007). The HGT mechanisms of conjugation, transformation, and transduction lead to spread of antibiotic resistance nonspecifically between non-pathogenic species to pathogenic species. This allows bacteria to develop resistance traits without needing to be exposed directly to the antimicrobial treatment itself. Conjugation is the transfer of DNA through a multi-step process requiring cell to cell

contact via cell surface pili or adhesins. In the transformation process, bacteria can uptake and express extracellular DNA fragments. In transduction, the bacteriophages can transfer genes to bacterial pathogens. For horizontal gene transfer, the idea that the genes for antibiotic resistance must come from the environmental microorganisms that produce antibiotics is shown to be false with the examples of two known genetic resistances coming from *Shewanella algae* and *Kluyvera* which are not antibiotic producers.

Resistance can be grouped functionally into mechanisms that modify the target of an antibiotic or those that modify the concentration of the antibiotic. Antibiotic concentration can be reduced by impeding entry of the antibiotic, extruding the antibiotic through efflux pumps, or changing the structure of the antibiotic by antibiotic-inactivating enzymes or mutations in the enzyme that activates a pre-antibiotic. (Martinez, 2014). Mechanisms of drug resistance fall into several broad categories: drug inactivation and alteration, modification of drug binding sites, changes in the permeability of the cell, and biofilm formation (Santajit and Nitaya, 2016).

With drug inactivation and alteration, bacteria can synthesize enzymes that inactivate antibiotics by altering the structure of the drug. The most prevalent example is the production of β-lactamases. These enzymes hydrolyze the β-lactam rings within the structure of the β-lactam antibiotic class. The most common classification system for β-lactamases is the Ambler system, which groups β-lactamases by amino acid sequence (Hall and Barlow, 2005). β-lactams include penicillins, cephalosporins, monobactams, and carbapenems. Extended spectrum β-lactamases (ESBLs) are a significant type of β-lactamase that falls in the molecular classification of Ambler class A enzymes, with serine active-sites. ESBLs include CTX-M β-lactamases, which have been identified in ESKAPE pathogens. The enzyme responsible for this particular ESBL phenotype not affecting ceftazidime was named as CTX-M-1 in reference to its preferential hydrolytic

activity against cefotaxime. Imipenemase (IMP) metallo-β-lactamases (MBLs) were found in *P. aeruginosa, K. pneumoniae, A. baumannii,* and *Enterobacter cloacae*, whereas Verona integron encoded metallo-β-lactamases (VIM-type) enzymes were *in P. aeruginosa* and *A. baumannii*. MBLs are characterized by the necessity of zinc as a cofactor (Canton et al., 2012). Carbapenemases such as KPC-1 (found in *K. pneumoniae*) causes resistance to imipenem, meropenem, amoxicillin/clavulanate, piperacillin/tazobactam, ceftazidime, aztreonam, and ceftriaxone (Santajit and Nitaya, 2016).

The modification of drug binding sites is another key mechanism of resistance exhibited by the ESKAPEs. Penicillin-binding proteins (PBPs) are proteins used in the synthesis of the peptidoglycan component of bacterial cell walls. Mutations in PBP structure prevent effective binding of penicillin to the bacterial cell wall with mutated PBP, so penicillins are not effective as an antimicrobial agent in PBP-mutated bacteria. Mutations cause a lower affinity for β-lactams including methicillin that prevents antimicrobial activity on the bacteria. Some pathogens are able to alter the peptidoglycan cross-link target so that D-Ala-D-Ala can alter to D-Ala-D-Lac or D-Ala-D-Ser. This increases resistance to glycopeptides such as vancomycin and teicoplanin, and this alteration is completed when the gene cluster *Van-A* through *Van-G* is impacted (Santajit and Nitaya, 2016). Like β-lactams, glycopeptides also kill bacteria by inhibiting cell wall synthesis. However, unlike β-lactams, vancomycin and teicoplanin do not directly interact with PBPs. Instead, glycopeptides bind to the terminal D-alanine-D-alanine (D-Ala-D-Ala) of peptidoglycan, to prevent PBP-mediated cross-linking, to stop cell wall synthesis and lead to bacterial death. However, when pathogens are able to alter their peptidoglycan cross-link target so that D-Ala-D-Ala becomes D-Ala-D-Lac or D-Ala-D-Ser, there is increased

resistance to glycopeptides such as vancomycin and teicoplanin and survival of bacteria in the presence of these antibiotics.

Increasingly, researchers are focusing on the ability of bacteria to reduce the relative intracellular drug accumulation. Efflux pumps are of interest in bacteria as the transmembrane pump is able to decrease the concentration of an antibiotic within the cell of a pathogen, reducing the susceptibility of the bacteria. Efflux pumps are used to pump toxins, heavy metals, and metabolites out of the cell. Evolutions have allowed efflux pumps to also pump antibiotics out of the cell either specifically by electrostatic interactions or generically using size cutoff. Many efflux pumps that use ATP or a chemical gradient as an energy source to transport the antibiotics out of the cell against the concentration gradient. The five super families of efflux pumps are the ATP-binding cassette (ABC) family, the small multidrug resistance family, the major facilitator superfamily, the resistance-nodulation-division (RND) family, and the multidrug and toxic compound extrusion family (Santajit and Nitaya, 2016). Virtually all microorganisms have conserved genetic sequences that encode efflux pumps. In Gram-negative bacteria, complex multi-layered RND family pumps are used. Efflux pumps remove a broad spectrum of antibiotics including fluoroquinolones, β-lactams, tetracycline and linezolid.

Porins are proteins that span across the outer membranes of Gram-negative bacteria that create channels through which antibiotics can enter or exit the cell. Porins are large enough in size to act as a channel to allow for passive diffusion of specific molecules. The molecule of focus for each porin is typically specified by the size of the porin. Some, extremely selective porins, are smaller than typical porins and can favor anions or cations for diffusion based on the amino acids lining the channel (Choi and Lee, 2019). Reduction in the number of porins decreases the influx of antimicrobial agents into bacteria allowing them to develop resistance

against different antimicrobials. In many of the ESKAPEs, the reduction in the number of porins and mutations that impact the porin size create a resistance against a variety of antibiotics. MDR *K. pneumoniae* also utilizes porin reduction to prevent susceptibility to β-lactams (Santajit and Nitaya, 2016).

Biofilm formation can occur on biotic or abiotic surfaces. These microbial communities allow microorganisms to interact with the environment and can extend the longevity of bacterial risk. Biofilms thrive through the process of adhesion, growth and maturation, and detachment. This process protects pathogens from harmful conditions. When biofilms form, the microorganisms can withstand the typically lethal fluctuations of pH, oxygen availability, and temperature (Santajit and Nitaya, 2016). The physical protection provided to the pathogens by biofilms coupled with uptake of resistance genes by HGT within the biofilm causes failure of antibiotic treatments. The only way to remove pathogenic biofilms often includes removal of implants and prosthetic biomaterials from the patients.

Enterococcus Species

Enterococcus species exhibit ampicillin and vancomycin resistance. Nearly all *E. faecium* nosocomial infections exhibit β-lactam antibiotic resistance (Pendleton et al., 2014). There are six types of vancomycin resistant *Enterococcus* (VRE), Van-A, Van-B, Van-C, Van-D, Van-E, and Van-G. Van-A is most common and shows highest levels of resistance against glycopeptide antibiotics due to the altering of the terminal sequence of cell wall precursors to decrease the binding affinity of the glycopeptide to the cell wall (Pendleton et al., 2014). Enterococcal surface protein (ESP) positive strains create thicker abiotic and biotic biofilms which restricts the effectiveness of antimicrobial agents on the pathogen (Pendleton et al., 2014). *E. faecium* and *E.*

faecalis, are able to alter the peptidoglycan cross-link target so that glycopeptide antibiotics are ineffective against the pathogens (Santajit and Nitaya, 2016).

Staphylococcus aureus

Excessive use of penicillin for traditional infections created a positive pressure to maintain and enhance resistance against β-lactams in *Staphylococcus* isolates. MRSA currently accounts for 25% of *S. aureus* collections. Typically, glycopeptide antibiotics including vancomycin and teicoplanin are first prescribed, but now there is developing vancomycin-intermediate and vancomycin-resistant *S. aureus*, known as VISA and VRSA respectively. VISA is characterized by the increased cell wall thickness due to upregulation of related genes (*glmS, vraR/S, sgtB, murZ,* and *PBP4*), which reduces or prevents vancomycin functionality (Cui et al., 2021). VISA is therefore difficult to treat and requires novel or uncommon antimicrobial therapies to treat (Pendleton et al., 2014). VRSA has a unique set of resistance genes making it difficult to treat: this pathogen has *mec-A,* a gene which encodes an alternative PBP, and *van-A* resistances (Wielders et al., 2002). In *S. aureus*, the penicillin binding protein, PBP2a, prevents antimicrobial activity on the bacteria. *S. aureus* is also capable of forming biofilms (Santajit and Nitaya, 2016).

Klebsiella pneumoniae

More β-lactamases have been found in *K. pneumoniae* recently, which are enzymes that alter the structure of β-lactam antibiotics. Carbapenem-resistant *K. pneumoniae* (CRKP), with resistance encoded by blaKPC, is clinically prevalent as carbapenems are typically used to treat chronic infections. The proportion of CRKP infections in clinical settings is directly correlated to

the rise of the prevalence of the enzyme, New Delhi metallo-β-lactamase-1 (NDM-1) which has carbapenem neutralizing activity (Pendleton et al., 2014; Moellering, 2010). CTX-M β-lactamases have been identified in *K. pneumoniae.* Efflux pumps have been found in *K. pneumoniae* isolates related to HAIs. Additionally, *K. pneumoniae* can form biofilms (Santajit and Nitaya, 2016).

Acinetobacter baumannii

This pathogen can be highly infectious and cause increased rates of illness due to effortless cross contamination as *A. baumannii* has a long survival time on hands and high-touch surfaces in clinical settings, and can form biofilms. Carbapenemase-producing *A. baumannii* strains carry IMP metallo-β-lactamases and oxacillinase serine β-lactamases. The AMR against colistin and imipenem makes the typical antibiotic treatments ineffective (Pendleton et al., 2014). CTX-M β-lactamases have been identified in *A. baumannii.* An MDR phenotype occurs when there is an overexpression of RND-type efflux pumps. The susceptibility to fluoroquinolones, β-lactams, tetracyclines, and aminoglycosides is lost (Santajit and Nitaya, 2016).

Pseudomonas aeruginosa

P. aeruginosa commonly is resistant to imipenem due to a combination of chromosomal AmpC production and porin change (Pendleton et al., 2014). AmpC enzymes are encoded within the chromosomal data within *P. aeruginosa* and act as β-lactamases (Jacoby et al., 2009). This pathogen also produces extended spectrum β-lactamases (ESBL) (Chouchani et al., 2011). CTX-M β-lactamases have been identified in *P. aeruginosa.* The bacteria also exhibits multiple efflux pumps, including four RND-type MDR pumps which causes resistance against carbapenems,

fluoroquinolones, and aminoglycosides. Additionally, the species is capable of forming biofilms (Santajit and Nitaya, 2016).

Enterobacter species

Many *Enterobacter* variants utilize ESBLs and carbapenemases, including Verona integron-borne metallo- β-lactamase (VIM), oxacillin hydrolyzing enzymes (OXA), metallo-β-lactamase-1, and *K. pneumoniae* carbapenemase (KPC) (Arnold et al., 2011, Evans and Amyes, 2014). Only tigecycline and colistin are effective against highly resistant *Enterobacter* species (cloverbio_webadmin, 2022). CTX-M β-lactamases have been identified in *Enterobacter* species. Additionally, efflux pumps are present in HAI-causing *Enterobacter aerogenes* along with porin reduction (Santajit and Nitaya, 2016).

Conclusion

With both an economic burden and a negative impact on global public health, combatting the ESKAPEs requires continuous interdisciplinary studies. While this review utilizes research across the fields of public health, medicine, molecular biology, and statistics, limitations of the current research naturally exist. The ESKAPEs are constantly evolving and mutating to adapt to their environment and overcome the pressures of the antimicrobial therapeutic agents. As new therapeutic agents have to be discovered and strategic approaches must be applied, the data from the current research will soon be outdated. As it is not possible to compare all emerging therapies, combination therapies, and alternative therapeutic approaches, this analysis is not fully comprehensive in nature.

The few remaining antibiotics that have not been overused need to be kept to minimal use and rationed carefully. Across the healthcare system internationally, medical providers must be more cautious when prescribing these medications. Additionally, patients are sometimes non-compliant and do not complete their full antibiotic therapy if they feel better, which allows antibiotic resistance to develop due to low antimicrobial pressure on the microorganisms. Healthcare providers must ensure the patient is adequately educated on medication management and the necessity of completing a treatment course. However, non-compliance is not always due to an educational issue; instead it can be due to patients experiencing side effects that can lead to non-compliance. There are systemic barriers that limit ideal patient care: limited transportation/excessive traveling to different areas, complex medication management between acute and chronic conditions, and underlying comorbidities that may interfere with treatment options. Additionally, knowledge of endemic susceptibility patterns and surveillance are relevant to ensure antimicrobial stewardship. It is important for a medical provider to utilize an optimal holistic approach and understand their patients unique circumstances while prescribing antibiotic therapy for their specific infection.

As Levy and Marshall stated in the 2004 article, “Antibacterial resistance worldwide: causes, challenges and responses” antibiotic resistance has been exacerbated due to “--the legacy of past decades of antimicrobial use and misuse.” The article, ahead of its time, appropriately declares this antimicrobial resistance as a global public health threat, where the entire population is at risk due to an increasing presence of drug-resistant pathogens.

Ongoing research and pharmaceutical objectives must focus on creating new therapies to combat the ever-evolving pathogens. This review thoroughly describes the clinical prevalence and burden of the ESKAPE pathogens. By reviewing and analyzing the mechanisms of

antimicrobial resistance, this literature review provides directions for researchers to develop new antimicrobial innovations to reduce ESKAPE infectivity and improve patient outcomes.

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