INTRODUCTION:

In the contemporary landscape of healthcare, the emergence and proliferation of antimicrobial resistance present an unparalleled challenge. Within this arena, a group of six pathogens, known by the acronym ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species), have assumed a position of particular prominence. Their ability to 'escape' the effects of antibiotics has not only compromised our ability to treat infections effectively but has also amplified concerns for global public health. This report represents an exhaustive exploration of the dynamics of these ESKAPE pathogens. Over the years, they have evolved, displaying fluctuating prevalence, shifting resistance patterns, and posing a growing threat to patients and healthcare systems worldwide. Our mission herein is to delve deep into their epidemiological trajectories, analyze their resistance profiles against commonly used antibiotics, and employ machine learning to forecast their future behaviors. By doing so, we hope to provide valuable insights into this escalating global health concern and offer actionable strategies to mitigate the impending catastrophe."

OBJECTIVES:

1. To understand the Prevalence, Geographical spread, and Epidemiology of organisms, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter species* (ESKAPE) from a global dataset from 2004 to 2021.
2. To analyze the anti-microbial sensitivity and resistance trends in these ESKAPE isolates for commonly used Antibiotics.
3. To predict future antimicrobial resistance trends using Machine learning models for each of the ESKAPE pathogens.
4.

MATERIALS AND METHODS:]

The study data was collected electronically from the Vivli AMR Register and analyzed specimen types, isolate species, country of collection, and antimicrobial susceptibility results for a range of antibiotics. The focus was on curating the global antimicrobial susceptibility information of the ESKAPE pathogens. Subsequently, the collected data was analyzed to identify resistance patterns for different antibiotics and their distribution across various geographical regions.

Regression models were employed to predict future antimicrobial resistance, using an open-source R programming package-AMR package, (Berends et al. 2021). The package uses a logistic regression model trained on a dataset of clinical microbiology data annotated with AMR information to predict AMR probabilities. The code used for the analysis is accessible on the GitHub repository available at https://github.com/srikanth7892/AMR-Prediction-Visualization-R

RESULTS AND DISCUSSION:

Distribution of samples and the number of isolates

A dataset containing over 8 lakh isolates from 82 countries across the globe was analyzed for ESKAPE isolates. The antibiotic susceptibility results of 4,34,714 ESKAPE isolates were investigated, among which, 16,661 were *Enterococcus faecium,* 1,47,921 were *Staphylococcus aureus,* 88,712 were Klebsiella pneumoniae, 38,036 isolates were *Acinetobacter baumannii,* 94,460 were *Pseudomonas aeruginosa,* and 48,041 were *Enterobacter species.* The majority of the isolates were derived from the Respiratory (n = 1,25,489), Skin (n =79202), Blood (n= 72780), and Urinary and Reproductive tract (n=58204). The specimen-wise distribution of each organism is detailed in Table 1.

Table 1 Specimen-wise distribution of ESKAPE pathogens from 2004-2021 (uploaded on https://doi.org/10.5281/zenodo.8215568)

Figure 1: Global distribution of ESKAPE pathogens sample size (uploaded on https://doi.org/10.5281/zenodo.8215788)

Prevalence of ESKAPE pathogens and their distribution across the Globe

The prevalence of all six ESKAPE pathogens increased over the time period. Figure. 2 shows the prevalence of ESKAPE pathogens across the globe from 2005 to 2020, *Staphylococcus aureus* was highly prevalent with over 15,000 isolates seen between the years 2010 to 2015 globally, The prevalence of *Klebsiella pneumoniae* and *Pseudomonas aeruginos*a has also increased by reaching a peak of 7,500 isolates each during the same time period. These findings suggest that the infections caused due to ESKAPE pathogens are a growing problem in the world currently. The majority number of isolates were from the United States, followed by China and Europe. The distribution of ESKAPE pathogen isolates is spread across 82 different countries around the globe in the given dataset as shown in Figure 1.

To understand the impact of multidrug resistance (MDR) on these pathogens, we focused on examining the percentage of MDR isolates among the total number of isolates for each pathogen. Our findings revealed an alarming percentage of *Acinetobacter baumanii* 61%, *Enterococcus faecium* 34%, and *Klebsiella pneumoniae* 31% are multi-drug resistant indicating that these strains have rendered most of the useful antibiotics ineffective resulting in increased mortality and morbidity. The data highlights the need for research for the development of alternate therapies.

Figure 3: Distribution of MDR isolates count among total isolates of ESKAPE pathogens (uploaded on https://doi.org/10.5281/zenodo.8215790)

Comprehensive Antibiotic Resistance Profiling of ESKAPE Pathogens

The most commonly used Antibiotics to treat the ESKAPE group of pathogens were shortlisted from previous surveillance studies reported in the literature (De Oliveira et al. 2020), They were examined for

their antibiotic sensitivity and resistance profile and studied to predict future resistance trends. The antibiotics shortlisted are a) Vancomycin, Ampicillin, and Linezolid for *E. faecium*. b) Oxacillin, Clindamycin, and Erythromycin for *S. aureus*. c) Meropenem and Imipenem for *K. pneumoniae*, A. baumanii, and *P. aeruginosa*. d) Ceftriaxone, Cefepime, Meropenem, and Imipenem for Enterobacter species.

Vancomycin-resistant *Enterococcus faecium* (VRE)

Enterococcus faecium is a significant cause of hospital-acquired infections and increased resistance to vancomycin and ampicillin is a major threat. Ampicillin showed the highest resistance rate (90%, n=14,460/16661). Vancomycin resistance was the second most prevalent (30%, n=5000/16661). This shows that in a worldwide setting, the prevalence of ampicillin resistance for *Enterococcus faecium* is higher than the prevalence of vancomycin resistance in the given dataset.

Figure 4: Antibiotic sensitivity and resistance pattern of Enterococcus faecium (uploaded on https://doi.org/10.5281/zenodo.8215796)

The number of *E. faecium* isolates resistant to Vancomycin is observed to be at a peak of 55% in the year 2005 and gradually decreasing to 25% by the year 2020. However, the predicted antibiotic resistance for Vancomycin-resistant Enterococci (VRE) over the next ten years shows a promising trend, with a gradual decline from 25 percent to 15 percent between 2020 and 2030.

Figure 5: Theoretical Prediction of Vancomycin Resistance in *Enterococcus faecium* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215648)

The resistance to Ampicillin remains high throughout the years (above 80%) and it is observed to have reached a peak of close to 90% following the year 2020, and the predicted resistance seems to follow the same pattern and indicates to remain at the peak of 90% even in the following years to come.

Figure 6: Theoretical Prediction of Ampicillin Resistance in *Enterococcus faecium* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215651)

Methicillin-resistant *Staphylococcus aureus* (MRSA)

S. aureus has developed resistance to a number of antibiotics. The three most commonly used antibiotics are oxacillin, erythromycin, and clindamycin. When we analyzed the dataset provided, we found that Oxacillin resistance was the most common case (50.75%, n=45,000/88,712) followed by Erythromycin (48.31%, n=42,870/88,712).

Figure 7: Antibiotic sensitivity and resistance pattern of Staphylococcus Aureus (uploaded on https://doi.org/10.5281/zenodo.8215664)

The highest number of isolates resistant to Oxacillin (90%) was observed during the period from 2004 to 2005 globally, which was followed by a gradual decline in Oxacillin resistance by the end of the year 2020.

In the predicted resistance trend from 2020 to 2030 and beyond, a similar declining trend is observed, reaching nearly 5% in the future.

Figure 8: Theoretical Prediction of Oxacillin Resistance in *Staphylococcus aureus* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215684)

The highest number of isolates resistant to Oxacillin (90%) was observed during the period from 2004 to 2005 globally, which was followed by a gradual decline in Oxacillin resistance by the end of the year 2020. In the predicted resistance trend from 2020 to 2030 and beyond, a similar declining trend is observed, reaching nearly 5% in the future.

Figure 9: Theoretical Prediction of Erythromycin Resistance in *Staphylococcus aureus* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215689)

In the case of Erythromycin resistance, The resistance indicates a descending trend globally with 65% of the isolates being resistant to Erythromycin in the initial period of 2004-2005 and gradually decreasing to 40% by the end of the year 2020, the predicted resistance indicates that Erythromycin resistance could fall to 20% following the years after 2030.

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP)

Carbapenem resistance in *Klebsiella pneumoniae* was evaluated for Meropenem and Imipenem, There is an upsurge in the resistance trends for Meropenem and Imipenem gradually throughout the years, where the resistant isolates were close to 5% and 10% in the time period between 2004-2006 for Imipenem and Meropenem respectively, and we have observed that the resistance has increased up to 20% in the recent years following 2020 in both these Antibiotics. The predicted resistance trends indicate an uplift of up to 60% and 50% in the following 10 years from now.

Figure 10: Antibiotic sensitivity and resistance pattern of *Klebsiella pneumoniae* (uploaded on https://doi.org/10.5281/zenodo.8215694)

Figure 11: Theoretical Prediction of Meropenem Resistance in *Klebsiella pneumoniae* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215696)

Figure 12: Theoretical Prediction of Imipenem Resistance in *Klebsiella Pneumoniae* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215702)

Carbapenem-resistant *Acinetobacter baumanii* (CRAB)

Carbapenem resistance in *Acinetobacter baumannii* was evaluated for Meropenem and Imipenem. There has been a sharp upsurge in the resistance trends for both antibiotics over the years. In the early period of observation, the resistant isolates were relatively low (17% and 20% for Meropenem and Imipenem respectively) but after 2020, there has been a significant increase in resistance (70% for both Meropenem and Imipenem). The predicted resistance trends for the following 10 years indicate a further substantial uplift in resistance levels reaching 90% in both the drugs following the year 2030.

Fig13: Antibiotic sensitivity and resistance pattern of *Acinetobacter Baumannii* (uploaded on https://doi.org/10.5281/zenodo.8215706)
Figure 14: Theoretical Prediction of Imipenem Resistance in *Acinetobacter Baumannii* 2020-2030 (uploaded on https://doi.org/10.5281/zenodo.8215710)
Figure 15: Theoretical prediction of Meropenem resistance in *Acinetobacter baumanii* (uploaded on https://doi.org/10.5281/zenodo.8215720)

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA)

Carbapenem resistance in *Pseudomonas aeruginosa* was assessed for Meropenem and Imipenem. A pronounced surge in resistance trends has been observed for Imipenem over time. Initially, the occurrence of resistant isolates was relatively limited to 15-20% during the time period 2004 to 2010, but subsequent to 2020, a notable increase in Imipenem resistance has been observed (30%). The projected Imipenem resistance trends for the upcoming decade indicate a further increase of up to 35%, however in the case of Meropenem there has been a plateau of a gradual decrease in resistance from 30% in 2005 to 20% following the year 2020. The predicted Meropenem resistance trends indicate a further decline to 15% in the following decade.

Figure 16: Antibiotic sensitivity and resistance pattern of *Pseudomonas aeruginosa* (uploaded on https://doi.org/10.5281/zenodo.8215726)
Figure 17: Theoretical Prediction of Imipenem Resistance in *Pseudomonas aeruginosa* (uploaded on https://doi.org/10.5281/zenodo.8215734)
Figure 18: Theoretical Prediction of Meropenem Resistance in *Pseudomonas aeruginosa* (uploaded on https://doi.org/10.5281/zenodo.8215738)

Enterobacter species

Resistance trends of *Enterobacter species* were evaluated for Carbapenems, third and fourth-generation Cephalosporins. Over time, there has been a noticeable increase in Cefepime resistance levels from 5% to 18% from the period 2004 to 2020 respectively, The projected resistance stands at 25% following the 2030 decade. However, in the case of Ceftriaxone, predictions indicate a static trend in the following decade as well (35%). Initially, resistance rates were relatively low for both Meropenem and Imipenem respectively, but a slight increase can be noticed in both cases. For Meropenem, Predictions indicate that this current trend may persist in the coming decade. However, for Imipenem the projected resistance rate is estimated to spike up to 25% in the following years after 2030.

Figure 19: Antibiotic sensitivity and resistance pattern of *Enterobacter spp* (uploaded on https://doi.org/10.5281/zenodo.8215744)

Figure 20: Theoretical resistance prediction of Cefepime (https://doi.org/10.5281/zenodo.8215748)
Figure 21: Theoretical resistance prediction of Ceftriaxone (https://doi.org/10.5281/zenodo.8215758)

Figure 22: Theoretical resistance prediction of Meropenem (https://doi.org/10.5281/zenodo.8215764)

Figure 23: Theoretical resistance prediction of Imipenem (https://doi.org/10.5281/zenodo.8215771)

DISCUSSION:

The increasing resistance among ESKAPE pathogens poses a severe threat to public health, leading to more infections, higher mortality rates, and increased healthcare costs. These bacteria have become resistant to crucial antibiotics, including last-resort drugs like carbapenems, glycopeptides, and polymyxins, due to genetic mutations and the uptake of mobile genetic elements. The World Health Organization has recognized the urgency of this issue and allocated resources for research. Our analysis aims to predict the threat posed by these organisms. *Staphylococcus aureus* showed the highest prevalence between 2010 and 2020, followed by *Klebsiella pneumonia*e and *Pseudomonas aeruginosa*. *Acinetobacter baumanii* exhibited the highest multidrug-resistant (MDR) rate of 61%, while *Enterococcus faecium* and *Klebsiella pneumoniae* had 34% and 31% MDR rates, respectively. High MDR rates in these pathogens raise concerns for public health and clinical management. Resistance trends for each pathogen varied from 2005 to 2020, with predictions suggesting an alarming increase in antimicrobial resistance for *Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa* against specific antibiotics. *Enterococcus faecium* showed a decline in vancomycin resistance but persistent high ampicillin resistance. *Staphylococcus aureus* demonstrated promising decreases in oxacillin and erythromycin resistance. *Pseudomonas aeruginos*a exhibited a gradual increase in imipenem resistance but a decline in meropenem resistance. *Enterobacter species* showed concerning rises in cefepime and imipenem resistance. Our predictive analysis underscores the urgent need for targeted interventions and ongoing surveillance to combat antimicrobial resistance effectively. Continuous updates to predictive models are essential for improved accuracy and real-world applicability. Implementing strategies to address specific resistance patterns for each pathogen is crucial to preserve the effectiveness of available antibiotics and ensure better patient outcomes in the future.

Impact of the Work:

This work provides global insights into the distribution and prevalence of ESKAPE pathogens, enabling effective interventions against multi-drug resistant isolates and informing optimized treatment strategies for better clinical management, thereby preparing for future antimicrobial resistance challenges and safeguarding public health.