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REVIEW

A comprehensive review on genomics, systems biology and structural biology approaches for combating antimicrobial resistance in ESKAPE pathogens: computational tools and recent advancements

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Abstract

In recent decades, antimicrobial resistance has been augmented as a global concern to public health owing to the global spread of multidrug-resistant strains from different ESKAPE pathogens. This alarming trend and the lack of new antibiotics with novel modes of action in the pipeline necessitate the development of non-antibiotic ways to treat illnesses caused by these isolates. In molecular biology, computational approaches have become crucial tools, particularly in one of the most challenging areas of multidrug resistance. The rapid advancements in bioinformatics have led to a plethora of computational approaches involving genomics, systems biology, and structural biology currently gaining momentum among molecular biologists since they can be useful and provide valuable information on the complex mechanisms of AMR research in ESKAPE pathogens. These computational approaches would be helpful in elucidating the AMR mechanisms, identifying important hub genes/proteins, and their promising targets together with their interactions with important drug targets, which is a crucial step in drug discovery. Therefore, the present review aims to provide holistic information on currently employed bioinformatic tools and their application in the discovery of multifunctional novel therapeutic drugs to combat the current problem of AMR in ESKAPE pathogens. The review also summarizes the recent advancement in the AMR research in ESKAPE pathogens utilizing the *in silico* approaches.

Keywords Bioinformatics · Computational Biology · Drug Discovery · Drug Resistance · Gene Prediction · Therapeutic target

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Introduction

Hospital-acquired infections (HAI) caused by bacterial pathogens have emerged as a world health hazard and challenge to physicians due to their adaptation to antimicrobial resistance (AMR) (Chandler 2019). Conventional therapeutic drugs have largely improved the landscape of therapeutics. However, AMR in the microbial population remains a major problem in treating human infections, leading to the failure of conventional therapies. World Health Organisation (WHO) has recognized AMR as a global concern because of its recurrence in the emerging isolates predominantly caused by indiscriminate use of antibiotics. According to Global Antimicrobial Resistance and Use Surveillance System (GLASS), fluoroquinolones resistance has increased dramatically worldwide (70–80%) in *Escherichia coli* and *Klebsiella pneumoniae*, leaving carbapenems and colistin as the last resort. However, in Europe, Carbapenem-Resistant

K. pneumoniae (CR-Kp) is the fastest-growing AMR threat, with a rising human mortality rate of 30–70% (Waddington et al. 2022). Recent reports have also suggested the acquisition of colistin resistance among extensive drug-resistant (XDR) enteric pathogens (El-Sayed Ahmed et al. 2020). According to GLASS, Methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* are developing resistance (12.11% and 36.0%, respectively) to third-generation cephalosporins antibiotics. Center for Disease Control and Prevention (CDC) has predicted that *Acinetobacter baumannii* and *Enterobacteriaceae* are the global threats, and cause pneumonia infection in the bloodstream (Enfield et al. 2014; Gupta et al. 2019).

Infectious Disease Society of America (IDSA) named these bacterial groups as 'ESKAPE' pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) (Tiwari 2019). These pathogens become adaptable through time, and the overuse of antibiotics in clinical practices has resulted in resistance to antimicrobial drugs. These pathogens can transfer the AMR genes by gene mutation, extrachromosomal (plasmid-mediated), horizontal gene transfer (HGT) via conjugation or transformation, transposons, and integrons (Sun et al. 2019). Now-a-days, the synthesis of antimicrobial peptides (AMPs) gains an attention and has been considered as an alternative to the conventional therapies. They can easily interact with the microbial cell wall peptidoglycan through penetration or dissolving the biofilms (Bhattacharjya and Straus 2020; Dash and Bhattacharjya 2021; Bhattacharjya et al. 2022). Although AMPs may serve as promising leads against drug resistant microbes, only a few AMPs are in advanced clinical trials because of the development of a plethora of AMR in ESKAPE pathogens. This includes reduced cellular uptake of the drug, or enhanced efflux from cells, changed expression of drug targets, inactivating mutations of the targets, and others, resulting in fewer treatment options for patients and hence, increased morbidity and mortality (Goler-Baron and Assaraf 2011; Tolios et al. 2020).

To date, many research studies have unveiled countless putative AMR therapies. However, only a few of them have reached actual validation. In this context, bioinformatics research has immensely advanced over the last few years due to the high-throughput approaches introduced for promoting different aspects of contemporary biology. Currently, computational approaches serve different levels of micro-and macromolecular interactions responsible for the survival and development of numerous cellular systems (Vakser and Deeds 2019). With the dawn of genomics pipelines, specialized gene predictions, annotations, and variants calling for whole genomes and metagenomes, secondary analyses such as plasmid profiling have provided broad insights into the biomolecular framework of a living system (Su et al. 2019). The newly booming systems biology approach elucidates biomolecular cross-talks and their functional associations to shed light on the significant genes/proteins that can be interpreted as controlling hub biomolecules in the interaction networks (Sigurdsson et al. 2012). Tremendous improvements and immense applications of structural biology methods have encouraged scientists from all over the world to reach a new height in pharmaceutical research for solving a wide range of biomedical problems (Pandurangan et al. 2017; Ndagi et al. 2020). The hazardous complications concerning elevating levels of AMR in ESKAPE pathogens have compelled the bioinformaticians to scrutinize several possibilities for mitigating the dilemma.

The present review aims to provide up-to-date information on different computational approaches, encompassing genomics, systems biology, and structural biology, relevant to AMR research in ESKAPE pathogens. Further, in this study, we have compiled a comprehensive collection of various databases, servers, and tools to identify hub genes or proteins, the optimization of drug-target interactions, and, the use of these computational approaches to pave the way toward the discovery of novel drugs with multifunctional characteristics. This propels the finding of novel therapeutic possibilities against drug resistance in ESKAPE pathogens. This review could serve as a guide and provide insight to readers and medical researchers about the possibility and progression in designing potent therapeutic strategies for overcoming the current research gaps and problems associated with AMR in evolving ESKAPE pathogens.

Important ESKAPE pathogens

Enterococcus faecium

E. faecium is a Gram-positive coccus that causes severe nosocomial infections in humans and other immunocompromised patients (Bhatia et al. 2021). The resistance to antibiotics such as penicillin and vancomycin has been a significant concern worldwide. Glycopeptides are the most preferred choice for treating Enterococcal-mediated infections; however, their increasing resistance to antibiotics, viz., penicillin, tetracycline, vancomycin, and erythromycin has been a matter of global concern (Cauwerts et al. 2007; Pfaller et al. 2019).

Staphylococcus aureus

S. aureus is a Gram-positive coccus and is associated with different parts of the normal skin, especially the nose and perineum of humans and animals. This pathogen is typically

harmful only in immunocompromised individuals. The infections caused by this species were treated with the well-known antibiotic penicillin; however, the repetitive use of this antibiotic could lead to the development of β-lactamase resistance. MRSA, an emerging strain of this pathogen, has evolved resistance against β-lactam antibiotics. Some other strains have also been identified as resistant to Vancomycin and are termed as Vancomycin-Resistant *S. aureus* (VRSA) (Loomba et al. 2010; McGuinness et al. 2017). The MRSA, coupled with the emergence of VRSA, has become a significant concern in the healthcare system.

Klebsiella pneumoniae

K. pneumoniae is an opportunistic Gram-negative bacterium that affects immunocompromised individuals and is associated with high morbidity and mortality due to the rare treatment options. *K. Pneumonia* reportedly involves extra-intestinal infections that includes urinary tract infections (UTIs), pneumonia, surgical wound infections and septicemia (Navon-Venezia et al. 2017; Ali et al. 2022). Besides this, *K. pneumoniae* shows intrinsic resistance toward ampicillin due to the presence of *SHV-1* penicillinase in its chromosome. In contrast, resistance to other drugs may occur through chromosomal mutations or through the acquisition of AMR genes by HGT, especially through large conjugative plasmids (Wyres and Holt 2018).

Acinetobacter baumannii

A. baumannii is a Gram-negative pathogen and primary agent of nosocomial infections, including bacteremia, pneumonia, meningitis, and urinary tract infections in immune-compromised patients. This pathogen has been identified as one of the six most notorious microorganisms by the IDSA (Shafiee et al. 2021). Currently, owing to its unparalleled capacity to acquire foreign resistance mechanisms and prolonged survival in healthcare environments, large proportions of the *A. baumannii* strains are Multi Drug Resistant (MDR) and display high resistance to nearly all known antibiotics (Luo et al. 2015).

Pseudomonas aeruginosa

P. aeruginosa is a motile Gram-negative bacterium that affects patients, especially those who recovered from post-surgery or during their admission in Intensive Care Unit (Hilliam et al. 2020). In addition, a number of intrinsic (genetically encoded in the core genome) and acquired resistance (rises from the gain of resistance genes from other microorganisms) mechanisms exist within the *P. aeruginosa* population. Further, this pathogen possesses a whole armamentarium of virulence factors, including toxins, adhesins, siderophores, and others (Strateva and Mitov 2011). Sometimes the cooperative interaction between antibiotic resistance and virulence may lead to the development of 'high-risk' clones, which are widely disseminated and challenging to treat (Horna and Ruiz 2021).

Enterobacter species

Enterobacter spp. is motile Gram-negative bacilli belonging to the Enterobacterales order. Major nosocomial pathogens include *E. cloacae*, *E. hormaechei*, and *E. aerogenes* and they cause lung, soft tissue, intra-abdominal cavity, and urinary tract infection (Davin-Regli et al. 2019). The complication in treatment regimens has emerged due to increasing drug-resistant strains. *E. aerogenes* show resistance to third-generation cephalosporins by releasing β-lactamases and hydrolyzing the β-lactam ring. *E. cloacae* imparts resistance to aminoglycoside gentamicin by acquiring genetic elements, i.e., integrons conferring AMR genes (Davin-Regli and PagÃ¨s 2015).

Mechanism of Antibiotic Resistance attained by ESKAPE pathogens

The pathogens acquire resistance through various mechanisms, such as inhibition of the drug influx, modification of the drug target, enzymatic inactivation and alteration of the drug, activation of drug efflux pump resulting in rapid extrusion of drugs (Santajit and Indrawattana 2016). The well-known AMR mechanisms in ESKAPE pathogens have been depicted in Fig. 1.

In silico approaches for the study of AMR in ESKAPE pathogens

Modern *in silico* approaches have made the drug target identification process more accessible than the conventional model (Basu et al. 2021b). There are various *in silico* approaches involved in genomics, systems biology and structural analysis and they are schematically represented in Fig. 2. These approaches play a significant role in identifying AMR genes, optimizing drug targets and screening lead compounds to identify those that could help to combat the resistance.

Understanding pathogenic transmission is essential to manage the outbreak that can be inferred through genotyping methods. Genome studies, including whole-genome analysis, transcriptomics studies, plasmid profiling, metagenomics and phylogenetic analysis, provide insights into the

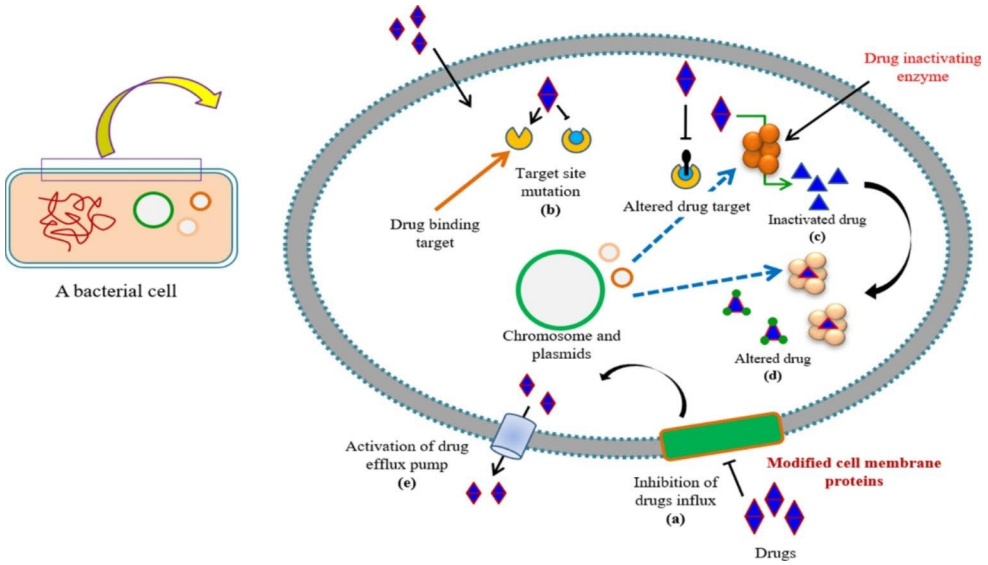

Fig. 1 A schematic representation of the AMR mechanisms in ESKAPE pathogens. Bacteria may resist the action of drugs by (a) Inhibition of the drug influx (cell membrane modification), (b) modification of the drug target site, (c) inactivation of drug-by-drug inactivating enzymes, (d) drug alteration, and (e) activation of drug efflux pump resulting in rapid extrusion of drugs

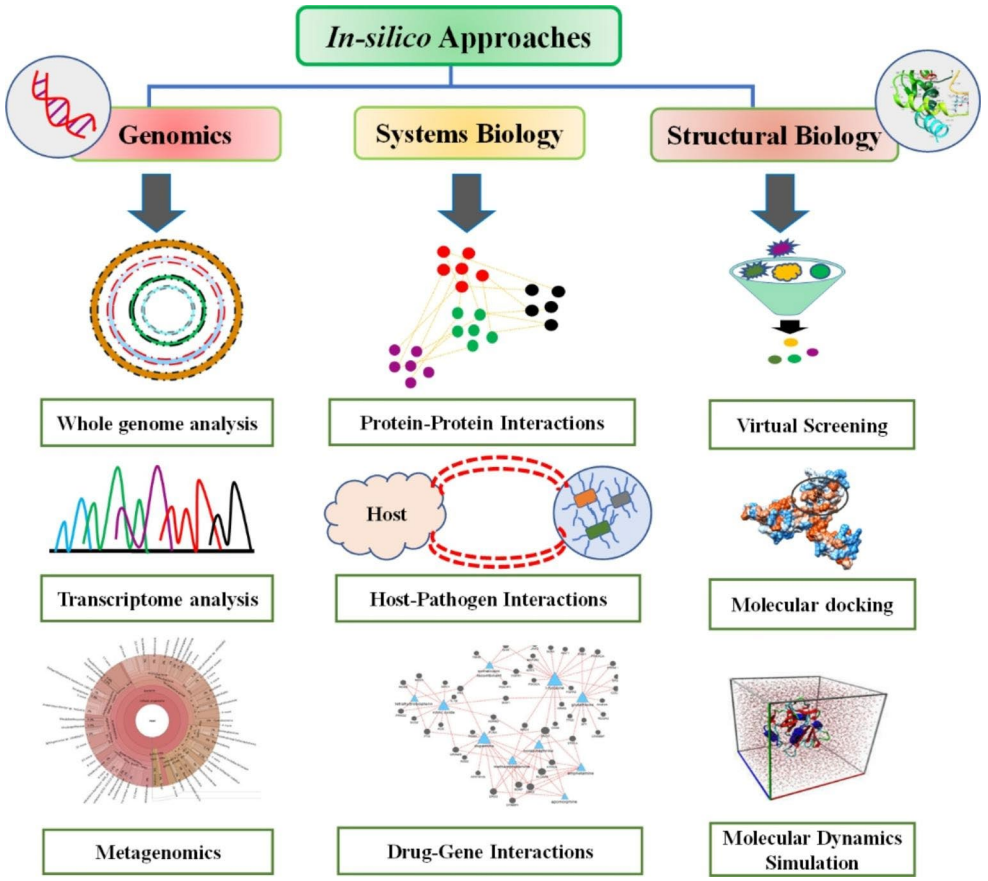

Fig. 2 A diagrammatic sketch of the different *in silico* approaches studied under genomics, systems biology, and structural biology that can help to understand the antimicrobial resistance in ESKAPE pathogens

transmission, virulence and AMR (Quainoo et al. 2017). Systems biology helps to identify hub genes with their interacting partners that have a critical role at the molecular level in causing resistance, whereas structural biology plays a pivotal role in drug discoveries and could combat the resistance (Ndagi et al. 2020). Different *in silico* approaches to identify AMR genes and their function have been discussed below.

Genomics approaches to identify AMR genes in ESKAPE pathogens

The genomics approaches have revolutionized the identification of specific novel AMR genes in various ESKAPE pathogens (Hosen et al. 2014). Different bioinformatics databases and tools are shown in Fig. 3, which facilitates a comparative genomics approach to prioritize and identify specific AMR determinants. The genomics approach applies two criteria for drug target identification, i.e., "essentiality" and "selectivity" (Hossain et al. 2017). These AMR genes are involved in unique metabolic pathways which would help to design novel drugs and therapeutic components to combat the problem of resistance.

Whole Genome Sequencing (WGS) analysis is a high throughput technology that identifies AMR determinants and aids in developing novel antibiotics by elucidating various factors for resistance. It determines the genetic variants such as Single Nucleotide Polymorphism (SNPs), frameshift mutations, and copy number variation (CNVs) in the altered genome (Crofts et al. 2017; Hunt et al. 2017; Shi et al. 2019). WGS is used to acknowledge the infection and helps to diagnose the cause of resistance (Köser et al. 2014). In order to predict the factors responsible for phenotypic resistance in pathogens, different computational approaches, such as read-based (Genefinder), de Bruijn graph-based (Mykrobe), and BLAST-based (Typewriter) are used. The WGS study's implication has helped AMR surveillance by consuming less time and cost. Wareth et al. (2021) have identified a broad range of AMR genes such as bla_{OXA-51}-like, bla_{OXA-23}, bla_{ADC-25}, bla_{ADC-73}, bla_{TEM-1} and bla_{NDM-1}, which mediates resistance against β-lactam antibiotics. Another set of genes which shows resistance against aminoglycosides are *ant(3")-IIa*, *aph(3")-Ib*, *armA*, *aph(6)-Id* and *aph(3')-Ia.* The gene *catB8* has been identified to promote resistance against phenicol's, *tet.B* and *tet.39* against tetracyclines, and *sul.1* and *sul.2* against sulfonamides. Certain genes such as *mphE*, *msrE* and *abaF* have been reported to play a significant role in bacterial resistance to macrolides and lincosamide. The presence of *tetM* and *tetL* genes in *Enterococcus* strains show resistance against tetracycline, *erm*B against erythromycin, and *vanA*, *vanB*, and *vanM* shows resistance against vancomycin (Lee et al. 2020; Cui et al. 2020).

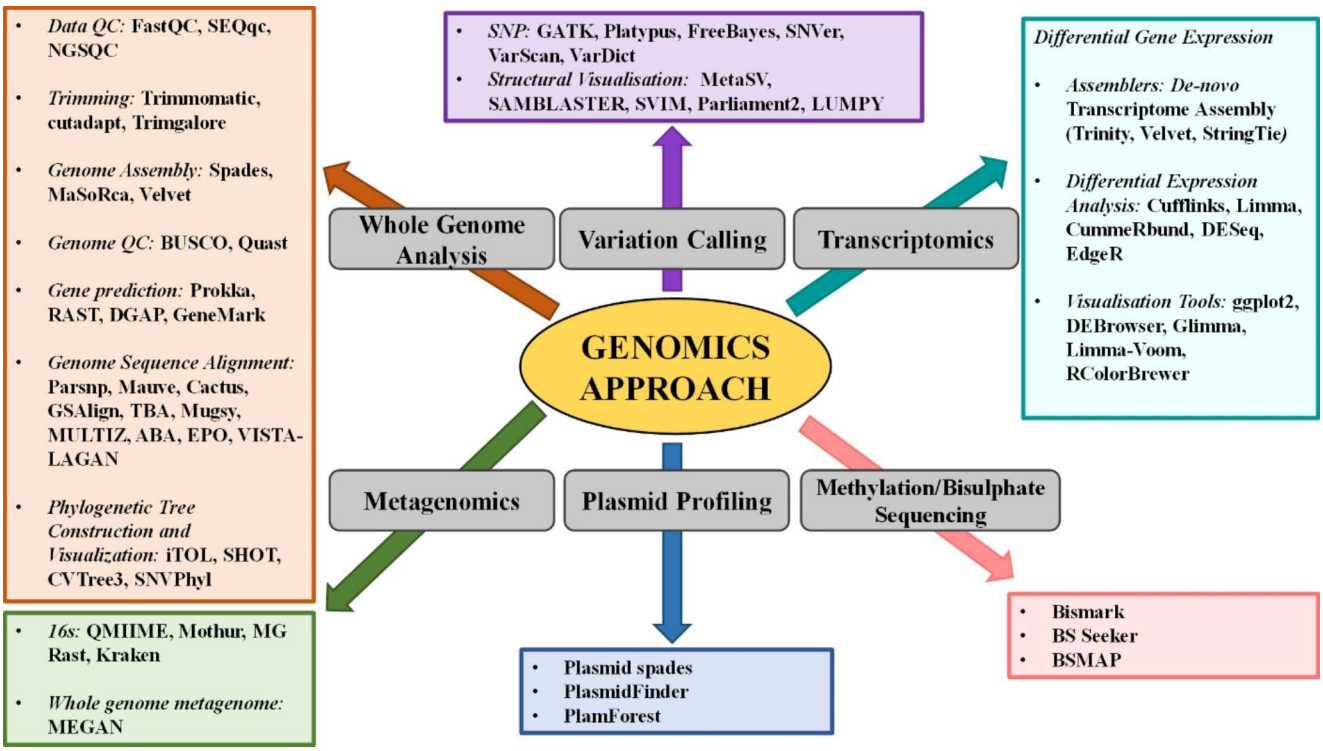

Fig. 3 Commonly used bioinformatics tools for Genomics studies. Whole Genome Analysis, Variant Calling, Transcriptomics analysis, Metagenomics, Methylation/Bisulphate sequencing and Plasmid Profiling can be utilised to predict and analyze AMR genes in ESKAPE Pathogens

In *S. aureus* several genes, including *blaZ* against penicillin, *mecA* against methicillin, *msrA* against erythromycin, *ermA, ermB, ermC*, and *ermT* against erythromycin and clindamycin, *tetK, tetL*, and *tetM* against tetracycline, *vanA* against vancomycin; and *fusA* against fusidic acid have been reported to be responsible for the development of antimicrobial resistance (Gordon et al. 2014). Likewise, in *K. pneumoniae*, there are many genes (viz., *mcr-1*, bla_{CTX-M}, bla_{OKP}, bla_{OXA}, bla_{SHV}, bla_{TEM}, bla_{LEN}, *aac(3')-Ia*, *aac-(3')-IIe*, *aac(6')-Ib-cr*, *aac-(3')-IId, aadA4, aadA5, aadA1a*, *aadA16*, *aph(6')-Id*, *aph(3')-Ia*, *qnrB*, and *qnrS)* have been studied and reported to cause resistance against colistin (Stoesser et al. 2013; Schürch and van Schaik 2017). In *A. baumannii*, the co-existence of bla_{OXA-23}, bla_{OXA-66}, bla_{ADC-25}, and *armA* genes have been reported to be responsible for the development of multidrug resistance against carbapenems (Rao et al. 2020; Hwang et al. 2021); *gyrA* and *parC* show resistance against fluoroquinolones (Lean et al. 2015). *EnvZ* (osmolarity sensor histidine kinase) and phosphate regulon response regulator are associated with a two-component system by which *E. coli* show resistance against carbapenems. While, *FtsZ* gene is involved in the β-lactam resistance pathway in *S. aureus* and is sensitive to β-lactam antibiotics.

Transcriptomics analysis includes the RNA-sequencing of the bacterial gene expression that has the potential to fill the gap of antimicrobial resistance. The transcriptomic data links the genotypic and phenotypic results, which are important in understanding the resistance mechanism. This analysis also helps to reveal the putative gene effect that contributes to antimicrobial resistance and identifies cases where non-coding regulatory RNA promotes bacterial resistance. In *P.aeruginosa*, the transcriptome analysis identifies the key genes which show the resistance against aminoglycosides, β-lactam, and fluoroquinolone antibiotics (Khaledi et al. 2016). Another study states that the mutation in a two-component system (*amgRS* and *pmrAB*) shows colistin resistance (Schniederjans et al. 2017). In the case of *K. pneumoniae*, $bla_{NDM^{-1}}$ is mainly responsible for carbapenem resistance (Low et al. 2018). It has been reported that the downregulation of *SprX* might lead to increased resistance in *S. aureus* (Eyraud et al. 2014).

Metagenomics analysis provides insights and elucidates a relationship between AMR and the microbiome based on a microbial community's structural and functional diversities. The advantage of metagenome analysis is that it covers a more significant number of species at a time and identifies the potential AMR determinants together with functional gene analysis. It employs two approaches for analysis: a function-driven approach that screens for an expressed trait and a sequence-driven approach for particular DNA sequences (Schloss and Handelsman 2003). Metagenomic data analysis starts from raw data sequencing, quality control, and obtaining high-quality reads. The reads are assembled using reference-based or *de novo*, for which different tools are available, represented in Fig. 3.

The contigs or scaffolds generated are further used for gene prediction. A list of gene sequences is collected and annotated, followed by putative gene function identification. Depending on the results, the taxonomical analysis is performed. The pipeline integrates many bioinformatics algorithms that address different aspects of complete metagenomic analysis. The downstream analysis identifies the antibiotic resistance genes and plasmids through gene network analysis. A study by (Matamoros-Recio et al. 2021) proved that the metagenome sequences from the sample and Comprehensive Antibiotic Resistance Database could be screened to find the antibiotic resistance gene. The analysis found that 259 ARGs are included in the database that contributes to antibiotic resistance. Yadav and Kapley (2019) carried out a metagenomic analysis study to monitor the AMR genes in *E. coli* and found a mobile genetic element, P-1 in plasmid pKJK5 that causes resistance. In addition, IncP-1beta (multi-resistance plasmid) and pB8 are important in spreading AMR through horizontal gene transfer.

Plasmid profiling is another important genomics approach that acknowledges the problem of resistance in bacterial pathogens by exchanging the AMR genes through HGT. The accessory genes responsible for the development of virulence and antibiotic resistance have clinically relevant traits. The plasmid profiling of these genes could be used to understand their molecular epidemiology. It has been illustrated that IncFIB plasmid is the most prevalent in *E. coli* and *K. pneumoniae*. Genes such as *TEM-1B*, *aph*, *sul*, *tetA*, and *dfr* are present in the plasmid showed resistance against penicillins, aminoglycosides, sulfonamides, tetracycline, and trimethoprim. In Norwegian isolates of *E. coli* and *K. pneumoniae*, ARGs viz., *aph(3")-Ib*, *aph(6)-Id*, *sul1*, *sul2*, *tet(D)*, and *qnrS1* have been reported to be the most abundant plasmid-mediated resistance; plasmid with bla_{CTX-M} gene and its interaction may be responsible for the development of resistance in these pathogens (Khezri et al. 2021). Plasmid types such as IncHI1B(pNDM-MAR), IncFIB(pQil), IncFIA, IncFII(K), IncR, IncFII(pRSB107), IncFIB(Mar), ColKP3 and ColpVC were found in *K. pneumoniae* strain from south India that were found to be involved in resistance. IncFIA, IncFII, IncFIB, Col (BS512), IncL1, IncX3 and IncH were mainly present in other isolates that showed resistance. In *S. aureus*, seven different plasmid groups viz., *rep*5, pSAS1 (*rep*7), pDLK1 (*rep*10), pUB110 (*rep*US12), Saa6159 (*rep*16), pKH12 (*rep*21), and pSA1308 (*rep*21) has been addressed for resistance. The *E. coli* plasmid

IncHI1B(R27) with bla_{NDM-1} and $bla_{CTX-M-15}$ sites and *K. pneumoniae* plasmids IncHI1B (pNDM-MAR) with $bla_{CTX-M-15}$ site promotes resistance against β-lactamases. The bla_{TEM-1B} shows a relationship between the genotypic and phenotypic expression of AMR that could further help to improve the strategies for better control of AMR dissemination (Ragupathi et al. 2019).

The genomics approaches adhere to profound surveillance to identify antimicrobial resistance with a greater fidelity that could refine the pathogenic risk. The amalgamation of the genomics approach with systems biology can help to deduce AMR networks and their functional analysis. The genomics approach shares a synergic area of interest with the systems biology field of network deduction and biological pathway analysis. Once the differentially expressed AMR genes are identified, their functional partners and mechanism of action can be better comprehended using the systems biology approach discussed below.

Systems Biology approach to identify hub gene/protein contributing towards AMR in ESKAPE pathogens

Systems biology-based networks are essential in pathological studies to decipher the disease-causing genes and their associated functional partners (Grimes et al. 2019). The approach mainly focuses on the coordinated systems-level interactions and they are crucial for the underlying mechanisms in various infectious diseases. The approach using gene interaction network (GIN) studies have efficiently explored the AMR mechanisms by identifying the potential target genes that can be targeted using a novel or repurposed drug (Debroy et al. 2020).

Systems biology approaches can be applied to decipher various insights about pathogenic genes (Miryala and Ramaiah 2019). The interaction data curated from various databases can be inferred to understand the direct functional interactors of pathogenic genes. Moreover, the indirect interactors essential for the progression of infection can be correlated using the interaction data. The constructed interactome can be better studied using various analyses such as clustering, topological parameter (TPA), functional enrichment (FEA) and pathway analyses (Naha et al. 2020). Clustering analysis aids in identifying stable gene clusters with a high probability of performing similar functions. Identifying such clusters is often important, as confining a set of genes from the interactome increases their specificity. Once the important pathogen-specific clusters are identified, their functional role can be validated using FEA. It consists of three main gene ontology terms, namely Biological Process (BP), Molecular Functions (MF) and Cellular Component (CC). The identification of enriched BP, MF and CC helps to easily comprehend various pathogen-associated genes' functional status (Ashok et al. 2021).

Additionally, pathway analyses project the predominantly enhanced biological or signaling pathways critical for organism-induced infection. Another key insight that can be retrieved using TPA is the significance of each gene in the constructed interactome. This can be achieved by computing the various topological matrices and centrality parameters. Different parameters signify the genes importance and connectivity in the interactome. Network biology studies can be based on different biological entities depending on the biomolecule of interest such as genes, proteins, small molecules, and regulatory molecules. Therefore, different kinds of interactome can be constructed such as GIN, Protein-protein interaction (PPI), Gene Regulatory Network (GRN), Gene-co-expression Network (GCN), Drug-Gene interaction network (DGIN) and Host-Pathogen Interaction Network (HPIN) (Miryala et al. 2018).

PPI studies on antimicrobial-resistant genes have recognized different mechanisms in ESKAPE pathogens. Based on a PPI study, in *S. aureus*, the following genes have been identified to play a role in AMR resistance and they are *NorA, aacA-aphD* (*aac6ie*), *aad9ib* (*ant*), *aadd* (*knt*), *baca* (*uppP*), *bl2a_pc* (*blaZ*), *ble, ermA*, SAV0052 (*ermb*), *ermc, fosB, mecA* (*mecI*), *mecR* (*mecr1*), *mepA, msrA1, qacA, vraR* (*str*), *tet38* and *tetM* (Anitha et al. 2016). A similar study in *K. pneumoniae* observed the role of *SHV-11* gene in drug-resistance and functional partner genes *gyrA, parC, glsA, osmE, yjhA, yhdT, rimL, pepB, KPN_00437, KPN_01875* (Miryala et al. 2020). In the case of *A. baumannii*, it has been reported that the presence of *dfrA1* and *dfrA10* genes confers resistance against trimethoprim (Anitha et al. 2014). The genes *adeA*, *adeB* and *adeC* are major components of adeABC efflux-pump and have been predicted to cause resistance in *A. baumannii*. The AMR genes associated with *P. aeruginosa* (*arnA, cat, gyrA, str, fabG, parC, parE, phoP, phoQ*, *pmrApmrB*) have also been identified to show resistance through gene network study. Genes *gyrA* and *parC* were also shown to be enriched through gene ontology terms that infer their role in *P. aeruginosa* (Miryala et al. 2019).

Intracellular pathogen requires an understanding of molecular-level interactions between the host and the pathogens, which are critical for their mechanism of infection. This interaction also indicates the probable drug-resistance mechanism in pathogens against antimicrobial therapeutics (Nourani et al. 2015; Basu et al. 2021a). The HPI network study in *E. faecium* has inscribed the role of hub genes *asrR* and *vanA* promoting AMR by associating with resistance to oxidative stress and membrane vesicle, which shows an increase in the survivability within the host (Lebreton et al. 2012; Wagner et al. 2018). In *K.*

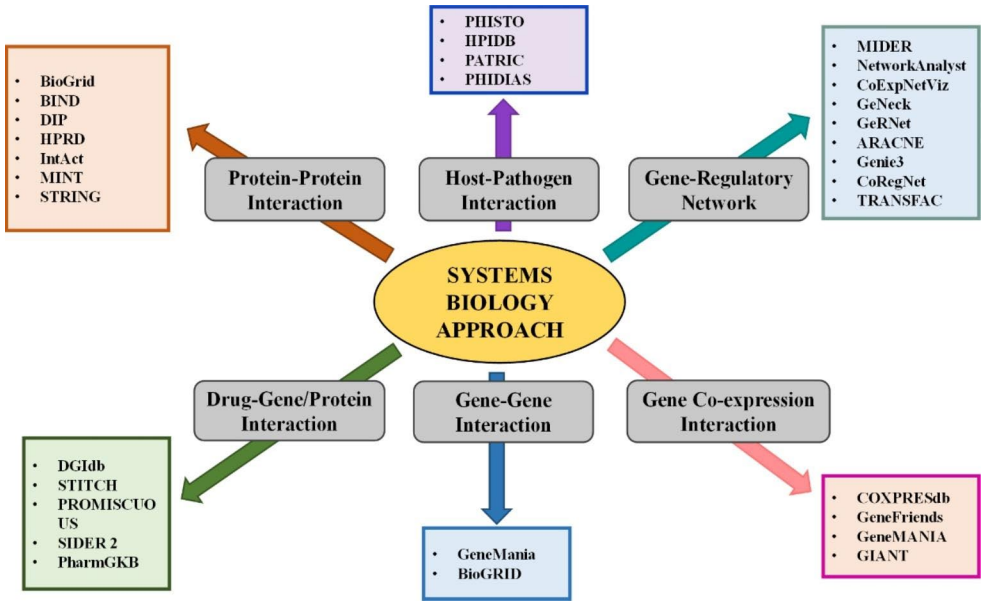

Fig. 4 An outline of tools and databases in Systems biology approach. Protein-Protein Interaction (PPI), Host-Pathogen Interaction (HPI), Gene-Regulatory Network (GRN), Drug-Gene/Protein Interaction (DGI), Gene-Gene Interaction (GGI), Gene Co-expression Interaction studies can aid to elucidate biomolecular level cross-talks that are involved in AMR mechanisms

pneumoniae, *mgrB* mutation resists polymyxin by governing PhoPQ (Kidd et al. 2017), while AcrAB efflux pump is another virulence factor that resists the defense and promotes virulence in *K. pneumoniae* (Padilla et al. 2010). The antibiotic resistance genes specific to biofilm in *P. aeruginosa* are *ndvB*, *PA1875–1877* and *tssC1 (*Zhang et al. 2013) and *Esp* protein in *S. aureus* (Sugimoto et al. 2013) have been studied, respectively. The interaction between host and bacterial protein could either lead to tolerance or resistance in the host. Thus, these interactions may lead to bacteria colonization, causing drug insensitivity (Casadevall and Pirofski 2000). The experimental methods to check the interaction between thousands of human proteins and pathogen proteins are expensive and time-consuming. So, systems biology approaches can be a better-suited means of predicting the putative interactions that can be further validated using experimental techniques. PPI also helps to address the important pathways that help bacteria survive and cause pathogenic virulence in the host system (Miryala and Ramaiah 2022). The various tools that can be utilized for the different analyses are depicted in Fig. 4.

Systems biology approaches to identify targets are easy, time-saving and cost-effective. Instead of the conventional trial and error method, which consumes time and money, systems biology approaches using network studies can efficiently identify functional targets playing a crucial role in infectious diseases. The identified target can be structurally validated using various structural biology approaches discussed below.

Structural Biology approach for target optimization and drug discovery to combat AMR in ESKAPE pathogens

Structural assessment and dynamicity of proteins, peptides and chemical compounds can be substantial tasks in computational biology that are being reliably dealt with the existing structural biology methods (Edwards and Cottage 2003). Preliminary data for structural evaluations can be collected from text-mining, literature-based databases and tools, and independent analyses in genomics and systems biology. The various servers and tools that are being commonly employed in the major aspects involved in the structural biology approaches have been represented in Fig. 5.

Macromolecular structural predictions or modeling amalgamated with step-wise optimizations and validations have become vital sectors of contemporary bioinformatics. Protein optimization or refinement involves generating an improved 3D-structure of the protein with abundant Ramachandran favoured regions and a low number of Ramachandran outliers and poor rotamers (Sobolev et al. 2020). For this, energy minimization could be performed to obtain the protein conformation with the least internal energy, which corresponds to a stable protein configuration. Further, secondary structural predictions are being performed to determine the regions of α-helices, β-sheets and random coils in the protein (Ma et al. 2018). Secondary and tertiary structural validations include evaluation of the local and gross errors, number of misfolds, disorderness, solvent accessibility and

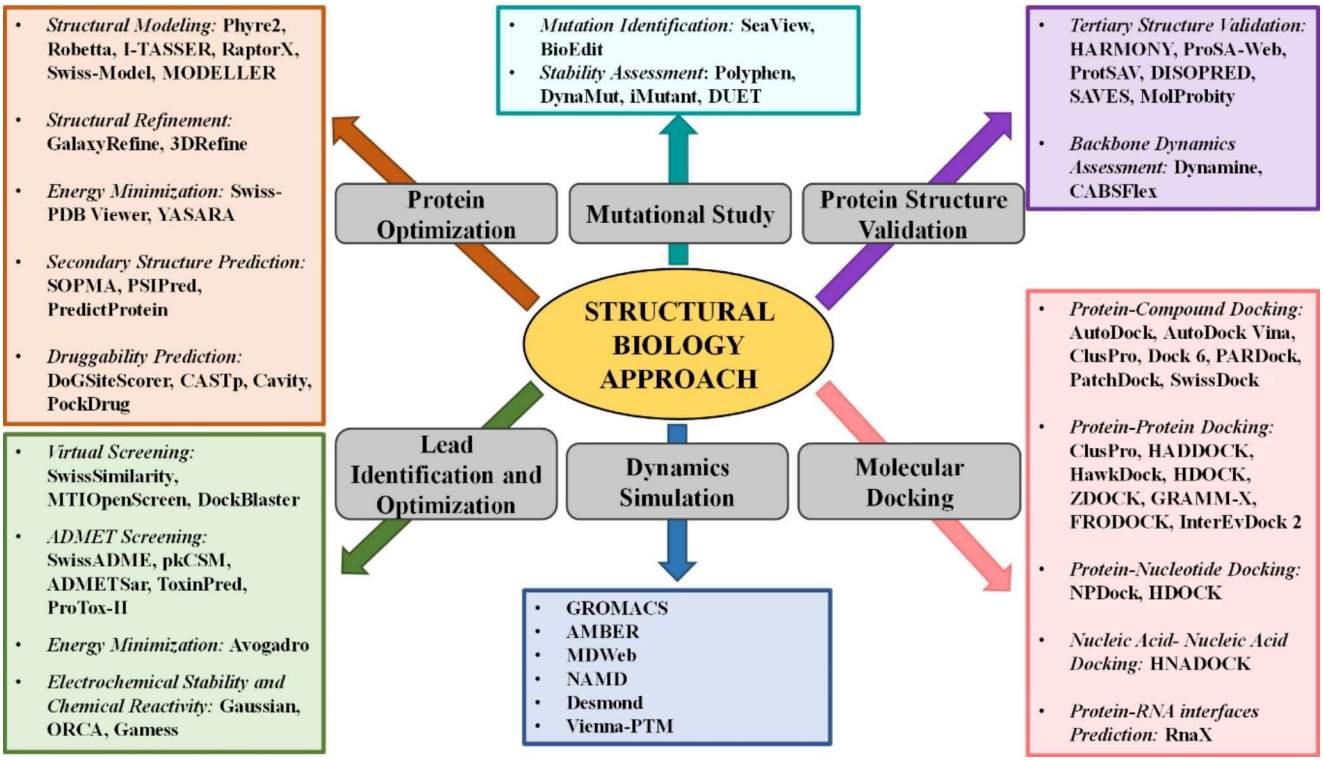

Fig. 5 Important tools and databases commonly utilized in Structural biology approach. Protein Optimization and Validation, Mutational Study, Lead Identification and Optimization, Dynamics Simulation and Molecular Docking are vital aspects for designing therapeutic strategies against drug-resistant ESKAPE pathogens

thermal stability, and the backbone flexibility in the protein (Kesheri et al. 2015). In order to understand the functionality of a protein, active site identification and validation play an important role in studying the structure-function or structure-activity relationships (SAR) (Guha 2013). The stability analyses encompass the protein's residual fluctuations and compactness that can be assessed through dynamics simulations (Justino et al. 2021). The prediction of drug-binding sites or pockets (druggability) in the protein structure must be executed to establish a protein as a therapeutic target.

The study of protein mutations is an important aspect of SAR studies, especially in the context of AMR evaluation in bacteria, since mutations can be responsible for transforming a non-AMR protein into an AMR protein and vice versa. Hence, a mutation's functional outcome (gain or loss of function) is being assessed within the protein of interest in the emerging isolates (Singh et al. 2021; Shankar et al. 2021). The emergence of mutations in the conventional AMR proteins may result in the ineffective binding of standard drugs and has resulted in the investigation of alternative drug targets in many ESKAPE pathogens (Saha and Sarkar 2021).

On the other hand, ligand identification through virtual screening and prediction of its pharmacokinetic/ pharmacodynamics (PK/PD) profile has been widely performed as preliminary analyses in subsequent drug discovery (Gallo 2010). Ligands with favourable 'Absorption, Distribution, Metabolism, Excretion and Toxicity' (ADMET) profiles and high LD_{50} values need to be shortlisted for being considered safe for administration (Wu et al. 2020). Ligand optimizations, electrochemical stability, and reactivity profiles have greatly facilitated research revolving around drug design and repurposing.

The intermolecular interactions viz., protein-drug, protein-protein, and protein-nucleic acid accompanied by dynamics simulations have been proven tremendously beneficial in modern-day targeted therapy (Basu et al. 2020). Molecular docking has introspected the understanding of the interaction between the ligands and pathogen proteins by predicting their binding affinity through the estimated binding energy or cumulative docking scores (Takaya et al. 2008; Dar and Mir 2017). The intermolecular interaction profiles encompassing direct Hydrogen bonds, van der Waals forces and other non-canonical interactions in the protein-ligand complexes are being extensively studied (Varghese et al. 2022). Molecular dynamics simulations of the docked complexes indicate their stability profiles and strengthen the notion of the proposed therapeutic option (Miryala et al. 2021, 2022; Karthika et al. 2021). The protein-ligand complex studies have boosted the therapeutics

development to combat the current problem (Naha et al. 2021).

Computer-aided drug design has been used to identify ZINC48942, which binds to the 'Scm' receptor that is involved in infection caused by *E. faecium* (Rasheed et al. 2021). Drugs such as ofloxacin, furazolidone and roflumilast affinity against the 'FmtA' active site is well studied in *S. aureus* and have a role in methicillin resistance (Dalal et al. 2021). Through virtual screening 1,3,4-oxadiazole (Naaz et al. 2021), 2-phenylquinolines (Sabatini et al. 2013), and CID 44,330,438 (Zárate et al. 2019) have been identified as potent inhibitors of NorA efflux pump that can combat AMR. It has been shown that BBN149 acts as the putative inhibitor of ArnT inhibitor in *K. pneumoniae* and *P. aeruginosa* (Ghirga et al. 2020). ZINC21811621, ZINC93091917 and ZINC19488569 have been identified as potent inhibitors against CTX-M-15 in *K. pneumoniae* (Farhadi et al. 2018). ZINC12670903, ZINC17465965, ZINC11681166 and ZINC13099024 against AmpC/ β-lactamases in *P. aeruginosa* (Farmer et al. 2010). In *A. baumannii*, *BfmR* is mainly involved in biofilm formation and, through structural analysis, compounds such as Calystegine B3,7,7 A-Diepialexine and Alpha-Methylnoradrenaline could bind to its active site (Lokhande et al. 2022). Another study pertaining to a similar approach has addressed ZINC4085364 as the potent inhibitor of $bla_{\mathrm{VEB-1}}$ in *E. coli* and *P. aeruginosa* (Messaoudi et al. 2013).

In order to combat multidrug resistance, the use of AMPs is one of the most efficient alternatives to the conventional approach. AMPs with therapeutic and antimicrobial activity against Gram-positive and Gram-negative bacteria are isolated from bacteria. (Mwangi et al. 2019). AMPs can be potentially useful in treating drug-resistant bacteria (Bhattacharjya and Straus 2020). Some AMPs such as Lactoferricin B and LL-37 belonging to both anionic and cationic peptide fragments have been reported to be effective against *K. pneumoniae, S. aureus*, and *Enterobacter spp*. Other sets of AMPs such as Parkerin, Ranalexin, BM Moricin, Melittin, and Circulin-A target *P. aeruginosa* and *S. aureus.* A study on Lactoferricin B-Mutant (M4) shows that this class of AMP have good binding high affinity towards SHV1, OXB48, NDM1, and AmpC, with the highest affinity for NDM1 (Basu et al. 2022). Polymyxin, another effective peptide with antimicrobial properties shows its effectiveness against *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* (Vaara 2019). Thanatin is another AMP that can be used against both Gram-positive and Gram-negative bacteria (Dash and Bhattacharjya 2021).

Depending on the structures, it has been reported that different AMPs having a different modes of action against bacterial pathogens depending on the structuere have been reported (Bhattacharjya et al. 2022). *In silico* approach can be implicated in designing and evaluating the antimicrobial activity of AMPs. Different databases such as Anti-microbial Peptide Database (APD3) and PhytAMP database have a collection of AMPs and are screened according to different classes such as anionic, cationic, cysteine-rich, and anionic/cationic peptide fragments. The efficacy of the AMPs can be enhanced by altering the template sequence (Duval et al. 2009). The enhancements of the altered/mutant AMPs can be assessed by iAMPred web tool, whereas HLP and CellPPD can be used to evaluate the physiochemical property of the parent AMPs to set a standard. For antigenic and toxicity prediction of AMPs, antigenic peptide tool and ToxinPred servers are being used. The 3D structures of the AMPs can be well optimised and modeled using servers like PEP-FOLD-3 and Swiss-Model servers, while the model can be validated by MolProbity tool. On the other hand, different protein structures of the ESKAPE pathogens can be retrieved and optimised by different tools mentioned in Fig. 5. The docking of AMPs with bacterial proteins can be performed through ClusPro2.0 and HPEP-DOCK servers. The simulation of the peptides-protein complex with superior binding profiles can be performed by CABS-dock server. Further, the stability of the complex can be determined using the MDWeb server (Basu et al. 2022).

Future directions

In silico approach not only facilitates the identification of novel resistance genes or proteins but also helps to enhance therapeutic options. As genome and protein sequencing are easily accessible and relentlessly performed in the present scenario, a staggering amount of high-throughput data has been generated and continues to date. The robust model of predicting the resistant genes helps to regulate the resistance at the local and global levels to monitor within countries. Determining precise resistant genes will help to facilitate the personalized approach to drive the current treatment regimens to the next level. Furthermore, Artificial Intelligence and Machine Learning algorithms can be applied to omics-based studies to establish advancements in medical informatics. The currently employed computational approaches emphasize enriching the therapeutic options and identifying the potential drug candidates that could combat the emerging and re-emerging AMR in the ESKAPE pathogens. Structural biology methods involving the prediction and optimization of therapeutic drug targets and potential lead molecules can be a promising approach to mitigate the dilemma of AMR. In antibiotic research, the early finding of prospective therapeutic targets and lead compounds is extremely desirable in order to reduce the research duration and experimental expenses. The review hopes for an enriching future of novel

drug discovery against the ESKAPE pathogens and establishing a healthy collaboration between computational and experimental researchers.

Conclusions

The recent advancements in gaining knowledge of AMR in ESKAPE pathogens have been supported through different bioinformatics services in genomics, systems and structural biology. Numerous computational servers and tools have been designed and are currently being utilized globally to address the problem of creeping AMR in the recent ESKAPE isolates. The genomics approach provides a fast and affordable way of analyzing the outbreak of resistance and monitoring the AMR profiles in the emerging ESKAPE strains globally. Systems biology approaches have helped us identify the AMR genes and their interacting partners to better understand the AMR mechanisms in the pathogens. Structural biology methods have tremendously aided in investigating several alternative therapeutic options against the detected drug resistance. This review covers different aspects of the bioinformatics approaches which can be utilized to formulate effective research strategies for understanding the molecular mechanisms of drug resistance in ESKAPE pathogens. These approaches play a pivotal role in accelerating and enhancing drug discovery to combat bacterial drug resistance.

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Declarations

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