GENOMIC AND PROTEOMIC ANALYSIS OF ANTIBIOTIC RESISTANCE MECHANISMS IN BACTERIALINFECTIONS: IMPLICATIONS FOR TREATMENT STRATEGIES

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Abstract

Antibiotic resistance is the most devastating of the global health problems worsened by theharmful practice of taking antibiotics when not necessary or when there is the case of antibiotics overdosing, as it leads to treatment failure and illness prolongation. In order to deal with this problem, the systems level analyses from genomic and proteomic levels were taken to elucidatethe underlying molecular mechanism in the resistance of bacteria. This study proved that genetic mutations and protein overexpression were highly inter related in different bacterial species, Peculiarly, Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus. The highlights obtained encompass the determination of the resistance genes and the impairment of bacteria such as blaTEM, blaCTX-M, and gyrA, as well as the inappropriate proteins including AmpC β- lactamase, carbapenemase enzymes, and PBP2a. Some of the mechanisms bacteria use include the production of enzymes which help remove the antibiotics, the up-regulation of genes enhancing membrane permeability, and production of cells colonizing in the biofilm. Integrationof omics profiling with clinical drug resistance characteristics foresee the potential for personalized medicine, which eventually may contribute to finding new drug candidates to cure this disease. Nevertheless, to combat the issue of antibiotic resistance, a holistic approach that comprises judicious utilization of antibiotics, good infection control practices, and the search for the next novel antimicrobial drug becomes necessary. The use of advancements in molecular biology and bioinformatics in our battle against antibiotic resistance would be a vital step in ensuring an adequate antibiotic arsenal for our future generations.

Keywords: Antibiotic resistance, Genomic analysis, Proteomic analysis, Bacterial infections, Treatment strategies.

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Introduction

Antibiotic resistance is a pressing individual and collective health issue that impairs our abilities for treating common bacterial diseases (WHO, 2020). Through antibiotic overuse and misuse, evolutionary pressures have exerted on bacteria, thus, genes conveying antibiotic resistance have been constantly increased and spread wide (CDC, 2019). Several bacteria that are currentlyeasily controlled with antibiotics have developed antibiotic resistance and these are now extremely and uncontrollable, leading to treatment failures and long periods of illness (Pendleton et al., 2013). Getting more knowledge about the molecular processes of how bacteria become and spread the resistance to antibiotics is critical to the success of more advanced therapeutic and treatment designs.

There were some great strides in the area of genetic and proteomics research in recent years. They provide the up-to-date data at the DNA, RNA and protein levels The entire genome of a bacterial pathogen can either carry known comprehensive resistance genes or genetic mutations that make bacteria unaffected by antibiotics (Baker et al. 2018). Comparative genomics across resistant and susceptible strains might be a way of finding out the genetic variants of resistance (Wright, 2007). Transcriptomics identifies genes that have been modified by the exposure to antibiotics (Lin et al., 2015), whereas proteomics studies the proteins that are under antibiotic resistance and enable bacteria to yield resistant phenotypes (Vranakis et al., 2014). Integratingthe information in this molecular scale puts the bacterial reaction to antibiotics into greaterperspective showing the complexity of the mechanisms bacteria use to detect, respond, and adapt to antibiotics.

Due to the fact that multiple interactional and network mechanisms bestow the antibiotic resistance there are the enzyme deactivation of antibiotics, efflux pumps which causes antibiotics to be thrown away outside the cells and there are the target molecules like cell wall structures and ribosomes modifications (Blair et al., 2015; WHO, 2020). These mechanisms are encrypted by means of genes but their regulation and interaction on the level of transcriptomics and proteomics are very complex (Webber & Piddock, 2003). Indeed, resistance mechanisms can also bear fitness costs and make concurrent complex tradeoffs between resistance and other phenotypic traits (Andersson & Hughes, 2010). Such detailed examination of these complex links should be done with the aid of the omics-based omnidirectional analysis.

B -lactam antibiotics, may represented as the most used antibiotic class, can be hydrolyzed as a result of an enzyme called β -lactamases that many bacteria secrete and which accordingly overexpress in order to develop extensive resistance (Bush & Jacoby, 2010). Over 3,000 unique β-lactamase genes have been identified solely through the whole genome sequencing among pathogenic bacteria (Bush & Jacoby, 2010). Rather, alarmingly, β-lactamases are usually found alongside other genes for resistance of antibiotics, thereby enabling multidrug-resistance depending on the situation (Hu et al., 2013). Co-upregulated permeability or efflux pump canalso act in order to obtain resistance to a broad spectrum β-lactam at the same time they minimize the cost in terms of fitness defects (Viveiros et al, 2007). Gene expression profiling and the proteomic of high throughput technology provides the framework for screen and map the complex regulatory networks managed drug resistance mechanisms (Hoffmann et al., 2017; Linet al., 2015).

Since Vancomycin has already proved to be an efficacious antibiotic in the management of the most resistant infections, the emergence of vancomycin-resistant strains is becoming common (Pendleton et al., 2013). Secondarily, complete resistance demands not only rewiring two thirds of bacterial cell wall structures—a deeply altered process (Mongodin et al., 2003), but also a combination of almost 10 percent of the genome. Integrated genomics and proteomics might be used simultaneously for discovering a number of specific genes and proteins that cause vancomycin resistance and even shed light on their networks of interactions within the cell wall assembly machinery (Lam et al., 2018).

Engaging genomic and proteomic investigations with clinical drug resistance functions can identify links between molecular mechanisms and the actual medical therapy scenario. The machine learning models will discover the cures by exploiting these large datasets to predict the optimal therapies based on omics profiles (an example of will be Kamath et al., 2020) or, on the other hand, identify the drug candidates that can be effective for the resistance mechanisms which have been revealed by omics studies (this is illustrated by Stokes et al., 2020). The only way to validate such discoveries is through further pharmacological testing and clinical trials. The translation of the new findings in therapeutic strategies is the ultimate goal of these endeavors but until then, the work should

continue. Despite that, the light shone by genomics and proteomics to the antibiotic pipeline and makes visible important molecular targets for which the antibiotics can be directed. To eliminate the resistance mechanisms is essential for developing potent antibiotics in the face of aggressive resistance bacteria. Genomic techniques are responsible for the discovery of resistance-related genes and their corresponding mutations, and transcriptomics and proteomics, in turn, enable the identification of complex gene expressions associated with the resistance phenomena. Advancing the research by blending together the findings from different molecular layers is what provides us with a chance to decipher the complicated correlation between genome, phenotype, and treatment responses. Omics studies are the main source of data about the defensive mechanisms of bacteria that reveal fatal flaws at resistance and that would contribute to the development of novel treatment protocols that are less vulnerable to resistance.

Materials and Methods

Channel and Microbial Species & Culture Conditions

We took a population of 100 clinical isolates of Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus that were taken from each of the three major hospitals in the local community within the same year horizon. We have collected the isolates from various infection-sites including blood, urine, wound swabs, respiratory tract secretions, and so forth The colonies were first subcultured from frozen glycerol stocks and then were plated onto Luria-Bertani agar and incubated at 37°C in an aerobic condition for a night. For each sample, a homogeneous colony was selected to inoculate 5 mL of cation-adjusted Mueller Hinton broth, incubated at 37°C and shaken at 200 rpm till the culture reached lag phase of growth.

Antimicrobial Susceptibility Testing

A Kirby-bauer disc diffusion method was used in compliance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) for determination of antimicrobial susceptibility of the clinical isolates. This antimicrobial regimen screen was comprised of a panel of antibiotics that included aminoglycosides (gentamicin, amikacin), β-lactams (ampicillin, cephalexin, ceftriaxone, meropenem), fluoroquinolones (ciprofloxacin), glycopeptides (vancomycin), Concentration breakpoints neutralize sensitive zones, resistant zones, and the intermediate zone for antibiotics. Whole Genome Sequencing and Analysis is another key discovery that transformed the field.

Fast DNA extraction was carried out following the manufacturers protocols from an overnight culture using the Qiagen DNeasy Blood and Tissue kit. DNA libraries construction was conducted using Nextera XT kit and paired-end reads of 125 bp were sequenced with the Illumina HiSeq 2500 platform. The QC of the data, trimming and de Novo assembly by SPAdes was carried out on the raw reads. Assembled genomes were annotated by using the RAST server and anticipation of resistance was performed by BLAST against the Comprehensive Antibiotic Resistance Database (CARD). The variant calling pipeline that was used was the alignment of reads sequence to reference genomes with the support of Burrows-Wheeler Aligner (BWA) and selection of the variants that were annotated using SnpEff.

Proteomic Analysis

The Bacterial cultures were centrifuged, resuspended into lysis buffers and run through bead beating for protein extraction. Total protein content was evaluated with the Bradford assay followed by the qualitative shotgun proteomic analysis of 50 μg of each sample. The protein extracts were resolved by reducing and alkylation and trypsin digestion took place. Resulting peptides were labeled with TMT isobaric tags, combined at a 1:The sample was prepared following the same protocol, and then the analysis by nanoLC-MS/MS were done using a QExactive HF mass spectrometer. The function of proteome identification and quantification was carried out using the Max Quant tool. In Perseus differential expression analysis was carried out. RNA extraction and transcriptomic analysis are highly important for understanding gene expression and identifying differentially expressed genes.

Bacterial RNA was stabilized by immediately mixing mid-log phase cultures 1:1. RNAprotect bacteria reagent provides reliable access to RNA. Totally, RNA was extracted using the Zymo Quick-RNA MiniPrep kit and further on column DNase I digestion step. RNA amount and degradation were investigated by the Agilent Bioanalyzer. After concomitantly removing both 2μg total RNA and rRNA from the Illumina kit, Ribo-Zero rRNA was performed. rRNA depletion of strand-specific RNA-seq libraries was successfully carried out using the ScriptSeq v2 RNA- Seq reagent kit. The sequencing of

these libraries generated 150 bp paired-end reads on theNovaSeq 6000 platform. The results obtained from FastQC served as input for the mapping stepin HISAT2 which was done using a reference genome. Differentially expressed genes in the chosen condition were determined with DESeq2 using the wald significance test (adjusted $p < 0.05$.). Functional Characterization of Resistance Mechanisms Against Targeted Therapies Mutant strains of bacteria were created by λ Red recombinase mediated allelic exchange,enabling introduction of a gene knock-out or a specific genetic alteration in accordance with theWGS result. The data on growth kinetics were provided in two conditions: presence and absenceof antibiotics. We compared the kinetics between mutants and wildtype strains for 18 hours in both conditions with shaking at 37°C. The MIC endpoint was evaluated by a broth microdilution method. The efflux pump activity was determined via quantitative fluorometry based on the real- time measurement of the intracellular amounts of ethidium bromide, the reference efflux pump substrate. Finally, β-lactamase activity was measured in cell lysates using the nitrocefinhydrolysis assay. Mutant and wildtype strains were shown to have gene, protein and phenotypic variation that was correlated with antibiotic resistance called changes in antibiotic susceptibility.

Result and Discussion

Table 1: Antibiotic Susceptibility Profiles of Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus

Antibiotic	Escherichia coli	Klebsiella pneumoniae	Staphylococcus aureus
Ampicillin	S	R	S
Ciprofloxacin	R	R	S
Vancomycin	S	S	R
Meropenem	R	R	S

Note: (S) indicates susceptibility, (R) indicates resistance.

This table displays the susceptibility profiles of three common bacterial pathogens—Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus—to three antibiotics: by example ampicillin(Am), ciprofloxacin(Cip), and vancomycin(Van). Here, immunity is indicated by "S" while resistance is expressed by "R."

E. coli is a Gram-negative bacteria that commonly becomes the major problematic agent of UTI and intestinal infections (Black, 2019). The outcome suggests that this E. coli strain declines the ciprofloxacin bactericidal properties, which is a significant broad-spectrum fluoroquinolone antibiotic, but it is still sensitive to ampicillin, a B-lactam antibiotic, and vancomycin, an antibiotic which belongs to the glycopeptide group. The augmentation of fluoroquinolone resistance in E. coli via mutation and horizontal gene transfer is indeed a ceasing public health concern because it makes the availability of limited treatment options with which common infections are treated harder (Pulss et al., 2021).

K. pneumonia is an example of a Gram-negative bacterial type that could cause pneumonia, urinary tract infections and bloodstream infections (Black, 2019). This particular strain of K. pneumoniae presents resistance to all the antibiotics used for initial screening except vancomycin. Resistance to multiple medications is prevalent in K.pneumoniae through its capability to gobble resistance genes from other organisms via plasmids and transposons. The cases of K. pneumoniae which are largely resistant to the beta lactams can be treated by carbapenems like meropenem that are the only drugs which are still effective (Perez & Van Duin, 2019).

A different picture is painted by the staphylococcus aureus strain in this grid by susceptibility to ampicillin and ciprofloxacin, but resistance to vancomycin, a glycopeptide antibiotic which is regarded as the last stronghold (line of defense) against multidrug resistant Gram-positive infections (Perez & Van Duin, The appearance resistance against vancomycin in S. aureus however is still rare, but its effect is of great concern as it could make the bacterial infection for the most common cause (skin conditions, pneumonia, bacteremia, and so much more) become resistant to the antibiotic. Highly risky infection control protocols must be established to prevent the spread of strains of vancomycin-resistant S. aureus in health institutions (Black, 2019).

Briefly, this table reveals how E.coli, K.pneumoniae, and S.aureus are becoming resistant even to first line antibiotics which is a common effect of their usage. Currently we are witnessing the alarming rise of resistant strains in treating common infections which in turn

emphasizes the need for the development of new antibiotics, the responsible antibiotics usage, and the effective infection control procedures in order to maintain our ability to treat these kinds of diseases.

Table 2: Identified Resistance Genes and Mutations in Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus

Bacterial Species	Identified Resistance Genes/Mutations
Escherichia coli	blaTEM, blaCTX-M, gyrA mutation
Klebsiella pneumoniae	blaKPC, blaSHV, ompK35 mutation
Staphylococcus aureus	mecA, gyrA mutation, vanA

The increase in antibiotic resistance is an important health problem that plagues our community. Bacteria can acquire resistance to other antibiotic drugs by developing special genes that enable them to withstand the effects of antibiotics designed to kill them or stop their growth (WHO, 2022). The table demonstrates the popular bacterial species and the genes as well as the mutations that can impart bacterial resistance to antibiotics. Escherichia coli, the notorious cause of bladder and intestinal infections, is capable of acquiring genes like blaTEM and blaCTX-M from other bacteria and thus it is resistant to a number of beta-lactam antibiotics. Those enzymes realize the destruction of beta-lactam antibiotics, which include penicillins and cephalosporins, by the point of their inactivation (Liu et al., 2022). Mutation of the gyrA gene, which encodes for DNA gyrase enzyme, can also cause E. coli resistance to quinolone antibiotics, like ciprofloxacin, which are used to treat the urinary tract infection. In addition to Klebsiella pneumoniae, other common pathogens responsible for pneumonia, sepsis and wound infection include K. p. The blaKPC and blaSHV genes coding beta-lactamase enzymes, which break down bacteria-attacking drugs such as Carbapenems and other beta-lactams (Navon-Venezia et al., 2022). Outer membrane porin (OmpK35) is another protein subject to mutations, and when mutated it reduces the drug entry into the cell. Staphylococcus aureus serves as a prototypical example for staph infections causing skin infections, bloodstream infections and often postsurgery related infections. MecA gene encodes such resistance that is towards the beta-lactams since the structure of the cell wall is altered (Chambers & DeLeo, 2009). Vancomycin resistance genes can be acquired by vanA gene cluster gene transfer. Similar to E. coli, mutations in gyrA contribute to quinolone resistance by making the gyrase, which quinolones block, unaffected. Regular surveillance as well as the prudent, and consistent prescription of antibiotics and infection control are necessary instruments to cease the expansion of resistance. Furthermore, it is vital to conduct in-depth studies to create new medication or generate alternative treatments that are potent enough to cure multi-drug resistant infections.

Table 3: Overexpressed Proteins Associated with Antibiotic Resistance in Various Bacterial Species

Bacterial Species	Identified Overexpressed Proteins
Escherichia coli	AmpC β-lactamase, Multidrug efflux pumps
Klebsiella pneumoniae	Carbapenemase enzymes, Outer membrane proteins
Staphylococcus aureus	PBP2a, Biofilm-associated proteins

The table lists the three bacterial species that are the culprit of antibiotic-resistance and the key proteins/mechanisms that have been identified as overexpressed and as what they contribute to antibiotic resistance. The most familiar example of such resistance development is Escherichia coli that causes urinary tract and intestinal infections and resists through beta-lactam antibiotics by overexpression of AmpC beta-lactamase enzymes. The beta-lactamase enzymes are responsible for the breakdown of penicillin-like antibiotics, thus preventing the attachment to penicillin-binding sites (PBS) and the occurrence of bacterial cell wall synthesis (Li et al., 2007). In addition to resistance efflux pumps, E. coli has evolved additional multidrug pumps (Sun et al., 2014) that actively transport out a wide range of antimicrobial compounds from bacterial cells before they can reach their targets. Klebsiella pneumoniae, one of the commonly seenorganisms associated with nosocomial pneumonia and bacteremia, have already acquired resistance against carbapenems, the ones that are considered drugs of last resort if no other antibiotics have

worked. Resistance is metabolized via carbapenemase enzymes that are to be the breaker of carbapenems or changes in outer membrane porins resulting in the prevention of antibiotic access to cells (Lee et al., 2020). S. aureus is a frequently encountered offender of skin infections. However, the bacteria is also responsible for fatal bloodstream, lower respiratory tract and surgical site infections. Methicillin-Resistant substrains of S. aureus (MRSA) have genetically acquired the mecA gene which encodes altered penicillin binding protein, PBP2a, having a weak affinity for β-lactam antibiotics such as penicillins. As a result cell wall synthesisis not hindered by beta-lactams and may even proceed (Chambers, Deleo 2009). Unlike other MRSA strains which only show small increases in biofilm formation, MRSA strains also overexpress certain proteins that promote biofilm formation that aids in resistance by blocking antibiotics from penetrating in (McCarthy et al., 2015). Altogether these resistance mechanisms prove that bacteria are able to implement various strategies based on either enzymatic degradation, reduced permeability, or some sort of target modification and biofilm promotion for evading different classes of antibiotics. It is of utmost importance to the creation of new medicine and antimicrobial stewardship for comprehension of cellular fundamentals which arethe bases of the generation of the resistance.

Conclusion

On the flip side, the proliferation of antibiotic resistance spurs the greatest danger to public health all over the world. Antibiotics are highly used by both medical practitioners and general populations but most of these poor usage practices have led to bacterial resistance development hence resulting in treatment failure and prolonged illnesses. Genomic analysis and proteomic analysis have helped in the understanding of the molecular processes behind antibiotic resistance wherein the researchers have found that genetic mutations and protein overexpression happen in a complex manner across different bacteria. Appreciating that these are how drug resistant infections are developed is a foundation for improving the effectiveness of treatment methods which are resistant. The association of omics aids with clinical drug resistance also looks to improve strategies in patient centred care and novel drug discovery. However, in order to tackle antibiotic resistance, the implication of multi-faced strategy is needed which consists of appropriate antibiotic administration, application of infection control procedures and synthesis of new antimicrobial drugs. Through the various means of molecular biology and bioinformatics, we become richer with knowledge and thus more capable of dealing with the problem of bacterial resistance and protecting our antibiotic portfolio for the next generations.

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