Chapter

Molecular Tools for the Study of Resistance to Disinfectants

Samantha Mc Carlie, Gunther Staats, Bernadette Belter, Boudine Van Der Walt and Robert Bragg

Abstract

Disinfectants, antiseptics, and sanitizers are crucial for hygiene standards and disease control, as recently emphasized by the SARS-CoV-2 (COVID-19) pandemic. With the foreshadowing of antibiotic resistance, new cutting-edge technologies and innovative methodology need to be applied to prevent the latest emerging antimicrobial resistance crisis, resistance to disinfectants. Disinfectant resistance is a relatively novel field of study, and although some molecular mechanisms have been elucidated, little is known about complex mechanisms, cross-resistance with antibiotics, and the existence of resistance biomarkers. Fortunately, great advances have been made in the field of sequencing technology and bioinformatics. Although there are many limitations to this technology, various "omics" approaches to disinfectant resistance will be crucial in directing environment-specific disinfection programs. In addition, the vast amounts of data generated by sequencing technologies can be applied by artificial intelligence (AI) models to identify key disinfectant resistance markers and in the surveillance of disinfectant resistance genes. A combination of these approaches will be crucial in identifying new disinfectant resistance mechanisms, in monitoring resistant populations, and in identifying cellular targets for new disinfectant formulations. These molecular tools will be vital in the battle against disinfectant resistance, the latest development in the antimicrobial resistance crisis.

Keywords: biomarkers, antimicrobial resistance, biocide resistance, omics, artificial intelligence

1. Introduction

The SARS-CoV-2 (COVID-19) pandemic has highlighted our reliance on disinfectants, antiseptics, and sanitizers. These products are used extensively in the agricultural, food, and beverage industries, as well as in veterinary and medical environments. Disinfectants play a crucial role in biosecurity as a preventative measure in hygiene and disease control. A great deal of research has been carried out on antimicrobial resistance (AMR) and antibiotic resistance in particular, however little is known about resistance to disinfectants. Disinfectant resistance is a relatively novel field of study; however, this phenomenon is emerging at a troubling rate.

Quaternary ammonium compounds (QACs) are one of the most widely-used disinfectants globally and are the best studied in terms of disinfectant resistance. In general, the underlying basis of resistance is a decrease in the intracellular concentration of the disinfectant within the microbial cell [1]. Resistance mechanisms may include changes in cell membrane structures, biofilm formation, efflux pumps, enzymatic activity and metabolism, and degradation of these compounds [1, 2]. These properties may be selected for and proliferate under stress conditions (such as QAC exposure), and some may be transferred on mobile genetic elements to other organisms (also to/from other species) [3]. Nonspecific resistance mechanisms, such as multidrug efflux pumps, can result in cross-resistance to several antimicrobials, including resistance to antibiotics, disinfectants, and antiseptics simultaneously [3, 4].

Although advances have been made in the study of disinfectant resistance, the literature is vastly less than that on antibiotic resistance. One main difference between antibiotics and disinfectants is that antibiotics often have one or two cellular targets, whereas disinfectants have multiple cellular targets to bring about the microbicidal effect [1, 5]. As a result, the antimicrobial effect of disinfectants is much more complex and so are the resistance mechanisms. Therefore, it may become crucial to use various "omics" methods in the study of disinfectant resistance that can reveal the full extent of what is happening inside a cell. This includes whole-genome sequencing, metagenomics, transcriptomics, proteome analysis, and metabolome research. This technology has become crucial in the study of disinfectant resistance and may be applied to discover new antimicrobial compounds.

In addition, to sequencing technology, recent advances in artificial intelligence (AI) have resulted in models that can trace and predict antimicrobial resistance patterns [6]. AI models together with disinfectant resistance biomarkers will be integral in the tracking of resistant populations and directing disinfection programs. Although extensive work is still required to develop these techniques and the database they rely on, these pose a promising alternative to studying the rapidly emerging disinfectant resistance crisis.

2. Mechanisms of disinfectant resistance

The biocidal activity of QACs has been attributed to their cationic charge interacting with the anionic charge cell wall of microorganisms and diffuse binding to the cytoplasmic membrane resulting in the formation of an electrostatic bond [5, 7, 8]. QACs cause damage by disrupting the cell membrane, distorting the permeability of the cell wall, loss of osmoregulation, disrupting the flow of nutrients into the cell, leakage of intracellular molecules, protein denaturation, and degradation of nucleic acids; ultimately resulting in cell death [8, 9].

While QACs and their active concentrations vary based on target organisms, they are typically utilized in concentrations below 1000 ppm. Nevertheless, the inappropriate use of disinfectants exposing microbes to sub-lethal concentrations can facilitate tolerance, reduced susceptibility, and resistance to these compounds [10, 11]. Furthermore, the release of QACs in run-off into the environment from food, healthcare, and animal production industries further poses a risk by exposing potential pathogens to sub-inhibitory concentrations of QACs. Bacterial resistance to biocides may develop by several mechanisms.

2.1 Cell membrane/wall alterations

While many studies have examined the development and spread of antibiotic resistance, the rise of disinfectant resistance further threatens biosecurity. While it has been shown that QACs act primarily by disrupting the cellular membranes, some microorganisms have intrinsic resistance provided by their phenotypic and physiological characteristics that challenge the penetration of the QACs [1, 2]. The phenotypic traits that facilitate inherent resistance to QACs often involve sophisticated membrane lipid permeability barriers, reducing the penetration of these compounds. The unique outer membrane, rich in lipopolysaccharides (LPS), phospholipids, and lipoproteins, that surrounds the cellular membrane of Gram-negative bacteria, makes them less susceptible than Gram-positive bacteria [10]. In addition, slime layers and cell walls rich in complex lipid molecules may confer tolerance to QACs based on physiological traits [1].

Apart from intrinsic resistance, reduced susceptibility to QACs can be induced over time by exposure to sub-inhibitory concentrations [12]. This change can be in the form of acquired resistance through the reduction of membrane permeability by changes in the fatty acid and phospholipid composition, and LPS [13, 14]. These alterations result in the cellular membrane becoming more negatively charged and hydrophobic, limiting the diffusion of QACs into the cell via the membrane [2]. Another mechanism to avoid QAC penetration involves density reduction, changes in the composition of porins, and protein composition of the outer membrane [15].

2.2 Biofilms

Exposure to QACs at sub-lethal concentrations enhances biofilm formation [16]. The physiological adaptation of certain bacteria to biofilms aids in their survival as these cells embed in the biofilm polysaccharide matrix and form part of microenvironments [16]. Within the biofilm, any antimicrobial treatment is hindered due to a lack of cell penetration and lower intracellular inhibitory concentrations [10, 16].

2.3 Efflux pumps

Inherent resistance to QACs may also result from the basic activity of broad-spectrum chromosomally encoded efflux pumps [7, 17]. These are transmembrane proteins that may provide resistance to numerous antimicrobial agents, including antibiotics and QACs [17]. While their main physiological purpose includes the transport of natural substances, it has been found that the resistance nodulation division (RND) family, the major facilitator (MF) superfamily, the small multidrug-resistance (SMR) family, and the multidrug and toxic compound extrusion (MATE) family of efflux pumps can expel antimicrobials from cells [7, 17, 18].

QAC resistance mediated through the action of efflux pumps has received considerable attention due to its genetic origin, ability to confer co-resistance to both antibiotics and other antimicrobials, and the ability to be transferred across microbial species via horizontal gene transfer (HGT) [17, 18]. QAC resistance may be induced by the overexpression of these pumps following exposure to QACs [12]. QAC resistance genes for efflux pumps can also be acquired, such as *qacE*, *qacF*, *qacG*, *qacH*, *qacI*, *qacJ*, and *qacZ*, which form part of the SMR efflux family [2, 18]. These

QAC resistance genes have mainly been found on mobile genetic elements, including transposons, plasmids, integrons, and integrative and conjugative elements (ICEs) allowing for HGT [3, 19].

2.4 Degradation and metabolism of QACs

Various studies have suggested an alternative fate of QACs that includes, degradation and metabolism [20, 21]. Some microorganisms have demonstrated the ability to degrade QACs under aerobic conditions, as a result of exposure to a range of sub-inhibitory concentrations [22]. QAC degradation has been found in various microbial communities, where microbes have been able to utilize QACs, (benzalkonium chloride (BC) and dodecyl dimethylammonium chloride (DDAC)) as their sole carbon and energy source [20, 22, 23].

2.5 Mobile genetic elements

Apart from intrinsic resistance mechanisms, the acquisition of mobile genetic elements (MGEs) is an important method for the attainment of resistance genes. Multidrug-resistant microorganisms develop via the acquisition of resistance determinants that exist in the global microbial gene pool [24]. The selective pressure of QACs may enhance the transfer of MGEs, such as ICEs, plasmids, insertion sequences, integrons, and transposons. Furthermore, the movement of these elements facilitates HGT and leads to the rise of antimicrobial resistance as a result of the acquisition and spread of resistance genes [25].

The exposure of bacteria to disinfectants may result in nonoptimal gene expression, which potentially reduces susceptibility through altered gene expression and mutations [26]. Hence, any microbe can possess resistance genes and while these may not always be expressed, they may also be constitutively expressed or induced, resulting in optimal expression in response to environmental changes.

2.6 Linking antibiotic and disinfectant resistance

The mobilization of resistance genes through intracellular mechanisms allows multiple resistance genes to cluster together, forming a single genetic unit. This means that bacteria may acquire resistance to multiple compounds (including disinfectants and antibiotics) simultaneously through one conjugation event [24, 27, 28]. This type of co-resistance to disinfectants and antibiotics has already been observed in multiple bacterial groups and co-resistance plasmids with similar structural arrangements have been isolated from various groups of unrelated bacteria [27, 29, 30].

Multidrug efflux pumps play important roles in linking antibiotic and disinfectant resistance, as they are effective against quaternary ammonium compounds along with various antibiotics. Additionally, disinfectant resistance genes (*qac*) are often found alongside antibiotic resistance genes on plasmids, further increasing the significance and complexity of resistance [30–32].

3. Innovative and cutting-edge solutions to counter disinfectant resistance

3.1 Biomarkers: a new way to track disinfectant resistance

The infectious disease poses a great threat to global healthcare systems and is estimated to cause annual mortality of over 17 million worldwide according to the

World Health Organization [33]. This estimate can be expected to rise due to AMR becoming more prevalent. Antimicrobial resistance arises from the overuse and abuse of antibiotics and other antimicrobial chemicals [2, 34]. The ability to accurately diagnose infections will guide clinicians to select the correct treatment strategy while maintaining sensible use of antimicrobials to prevent further proliferation of AMR. Moreover, scheduled surveying of the microbial population and AMR status of the population within a given setting can provide vital information to attempt to control resistance development.

To appropriately respond to AMR either in a clinical sense or as a public health threat, rapid detection of multidrug-resistant (MDR) isolates will be required. Conventional susceptibility testing, such as agar dilution, disk diffusion, gradient diffusion, or broth macrodilution techniques, has the limitation of only providing results 24 h after bacterial isolation [35]. To overcome this limitation the combination of biological marker indicators, cost-effective and time-efficient techniques could provide the solution.

Biological markers or biomarkers are indicators of a particular disease state or other physiological states of an organism. These indicators can include genes, proteins, genetic variations, and/or differences in metabolic expression [36]. The presence or absence of certain biomarker indicators can provide insight into the physiological state of the cell. Recent developments in biomarker identification and research have focused on antibiotic resistance and virulence biomarkers [37]. However, some techniques and workflows allow for elucidation of novel resistance mechanisms, which could be used to expand the currently small pool of disinfectant biomarkers [38].

A functional metagenomic workflow was used to rapidly identify and validate AMR biomarkers from clinical isolates for three antibiotics, namely tobramycin, ciprofloxacin, and trimethoprim-sulfamethoxazole [38]. This technique was functionally verified for known AMR biomarkers but of substantial interest would be the applicability to elucidate potentially novel resistance mechanisms. While the focus of AMR remains on antibiotics due to the connectivity associated with patient utility and infection, disinfectant resistance is an emerging issue and needs to be approached in the same manner.

Currently, one of the key tools that can be used to combat AMR is rapid detection and diagnostic evaluation of infections. Rapid elucidation of the AMR status of infections can improve the antimicrobial treatment prescribed and affect the outcome of patients. Machine learning approaches have successfully used genomic and transcriptomic data to distinguish and provide antibiotic resistance capabilities for the bacterial pathogen *Pseudomonas aeruginosa* [39]. Genetic features, such as gene expression, gene presence/absence, and single nucleotide polymorphisms (SNPs), were analyzed to categorize the isolates as susceptible or resistant. Interestingly, the resistance predictions were heavily dependent on the specific antimicrobial investigated. However, contrasting prediction criteria are present in other bacterial families, such as the Enterobacteriaceae, where the presence of resistance-conferring genes is sufficient for susceptibility predictions [39].

Another rapid technique for the detection of MDR isolates that have been implemented in clinical microbiology laboratories is matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS). This technique is used to either replace or assist conventional phenotypic identification for many bacterial isolates [40]. MADLI-TOF MS can rapidly provide the simultaneous detection of multiple resistance characteristic peaks in the spectra generated when

the identification of a specific strain occurs. Although the detection of the peaks responsible for resistance is feasible, the identification of the protein responsible for the peak is lacking [41]. Even though the proteins responsible for resistance cannot be identified, the presence of resistance can be identified, this could aid in the potential of MADLI-TOF MS to detect resistance presence or absence as potential biomarker candidates.

In the case of the detection of disinfectant resistance biomarkers, the need for urgency and rapid results is not as pressing as with antibiotic resistance markers present in hospitalized patients. The concept of tracking disinfectant resistance using biomarkers does not require as much speed, because rapid treatment is not of utmost importance. However, being able to elucidate what disinfectant resistance is present is necessary to maintain sanitary surfaces within hospitals, food production, or animal husbandry facilities. Once the disinfectant resistance biomarkers have been detected, follow-up procedures, such as susceptibility testing, can be conducted to appropriately correlate the MADLI-TOF MS peaks with distinctive minimum inhibitory concentration results [41].

3.2 Expanded surveillance of disinfectant resistance

With the growing threat of antimicrobial resistance and emerging pathogens, the need for effective surveillance and tracking of the spread of antimicrobial resistance and possible determinants is a new avenue for possible disease prevention [42]. Resistant determinants include both resistance genes and mutations that provide microbes with the ability to resist the effects, typically biocidal, of antimicrobials or other drugs. As with antibiotic resistance, disinfectant resistance may also be intrinsic or acquired via HGT on plasmids or other mobile genetic elements [25, 42].

Continued misuse and overuse of current essential antibiotics have resulted in less effective therapeutic options and a push into a post-antibiotic era [34, 43, 44]. While much research is underway in developing novel antimicrobials, there is also a need to establish effective strategies and preventative measures to reduce antimicrobial resistance. As disinfection is the main form of biosecurity in many human and animal environments, this has quickly become a concern as these treatments are becoming less reliable [45].

Some advances in molecular typing methods and the availability of genome information for various microorganisms provide tools and insights for further understanding molecular epidemiology, genetic content, and the spread of antimicrobial resistance [19, 46]. Hegstad and coworkers [19] examined mobile genetic elements and their contribution to the resistant Enterococcus species and described specific resistance and virulence determinants. They saw certain genes, transposons, and plasmids may confer certain phenotypic characteristics such as resistance to specific antibiotics. In addition, the development of simplistic typing methods allowed for an assessment of the relative contributions of microbes, such as enterococci, to the spread of defined resistance phenotypes [19]. These typing methods also allowed elucidation of the potential risk for the transfer of conjugative elements to other bacteria genera. In some cases, R-plasmids transferred antibiotic resistance without the selection pressure present. It was also suggested that genetically manipulated CRISPR interference mechanisms may be useful in limiting the spread of antibiotic-resistant enterococci [19].

Investigation of potential pathogens requires the knowledge of pre-established resistance determinants such as antibiotic resistance genes (β-lactam) or disinfectant

resistance genes (*qacH*, *qacA*, *qacB*, and *qacC*) [32]. With the use of these known resistance determinants, microbes can be isolated from areas such as those observed in healthcare, food, and animal production industries, and genomic screening of the isolates can be done to elucidate their susceptibility to antibiotics or disinfectants. Such screening was done by Zmantar and coworkers [47] through the examination of *Staphylococcus aureus* associated with dental infections to monitor the epidemiology and spread of the multi-drug resistant staphylococci. In this study, *S. aureus* strains were isolated from bloodstream infections and identified using specific primers, and minimal inhibitory concentrations of BAC and antibiotics were determined. Approximately 50% of the isolated strains were resistant to BAC and harbored efflux-mediated resistance genes [47]. The *qacA/B* and other *qac* genes were found following molecular analysis, these are typically found on pSK1 plasmids and could be why such a high proportion of this population exhibited resistance to BAC [48, 49].

The tracking of resistance determinants provides surveillance of potential pathogens and resistance genes, however, the prevention of the spread of these determinants is a major concern as the control of microbes in many environments is difficult [50]. It may be possible to enforce proper control and sterilization or disinfection procedures in controlled environments such as healthcare and food production industries. Through the screening of nosocomial pathogens in hospitals the possible resistance profile of these pathogens may be elucidated, and help to tailor individual antimicrobial strategies, including changes in terms of QAC concentrations or the use of alternative disinfectants [50].

4. "Omics" methods to study disinfectant resistance

The omics revolution stems from recent significant advances in sequencing technology and bioinformatics. From the beginning of the central dogma, whole-genome sequencing can identify which genes are present in a cell or population. Thereafter, transcriptomics can be used to study how these genes are transcribed and under what conditions. Furthermore, proteomics can be used to reveal which proteins have matured, if any post-translational modifications have occurred, and how the protein profile of a cell can change due to environmental factors. Lastly, metabolomics has the power to elucidate the impact of a changing proteome on metabolic pathways, and which metabolic pathways are key in a microbial response to antimicrobials.

Pan-genome analysis and metagenomics are powerful tools that can reveal any resistance determinants present in the genome of a microorganism. Together with bioinformatics, this approach can be applied to determine what is present in a resistant strain that is absent in a related susceptible strain and therefore infers the cause of the resistance phenotype. By comparison of susceptible against resistant strains, complex resistance mechanisms can be elucidated, and the additive effect of hundreds of genes throughout the genome can be revealed. Bland and coworkers [51] used whole-genome sequencing to gain insight into sanitizer tolerance amongst *Listeria monocytogenes* isolates exhibiting different resistance profiles. The genomic relatedness of the isolates was analyzed, and the origin and dissemination of these populations were tracked throughout the facility where they were isolates [51]. In addition, genetic elements were found associated with decreased susceptibility to QAC-based disinfectants, this included bcrABC efflux cassette and four *Listeria* pathogenicity islands (LIPI-4, [51]). This application of whole-genome sequencing and data mining shows that this methodology can be used to reveal known disinfectant resistance determinants and be applied in the surveillance and tracking of persistent resistance

populations. Metagenomics in this approach can reveal the impact of a bacterial community and population dynamics that lead to the development of resistant populations [46].

An additional advantage of whole-genome sequencing is an analysis of full genome sequences and the comparison of the role of the core genome and accessory genome in the resistance phenotype. A great deal can be revealed by focusing on acquired resistance determinants; however, recently it has been discovered that the core genome can play a much greater role than initially anticipated [52]. Gallagher and coworkers [52] used whole-genome and transposon sequencing to reveal that elements of the core genome of *A. baumanii* play an instrumental role in the extreme antibiotic resistance phenotype and not the accessory elements. Research into resistance to disinfectants has focused mainly on accessory genome elements that are acquired through horizontal gene transfer. This has led to the discovery of efflux pumps (*smr* and *qac* genes) harbored on mobile genetic elements and numerous plasmids responsible for resistance to disinfectants [53–55]. However, the core genome may present an equally important untapped refuge for disinfectant resistance determinants. Future work should include a combination of these strategies, the comparison of susceptible and resistant strains together with metagenomics and pan-genome sequencing to elucidate novel molecular mechanisms of resistance in both the accessory and core genomes of bacteria eliciting resistance to disinfectants and antiseptics.

Whole-genome sequencing has played an integral role in identifying genetic elements responsible for decreased susceptibility to antimicrobials. However, simply because a resistance gene is present does not mean it plays a role in the resistance phenotype. Gene activation and regulation of expression can be crucial in resistance to an antimicrobial [12, 56]. Transcriptomics has the power to link the genotype of resistance to the resultant phenotype exhibited. This can be particularly useful when genotypic data needs to be linked to a phenotype, or when a resistance phenotype is exhibited but no obvious antimicrobial resistance genes can be found.

A few methods to analyze transcriptomics are by using Microarray, Real-Time PCR, and RNA-Sequencing technology. Microarray and real-time PCR can show differential expression in response to antimicrobial treatment. However, certain genes need to be targeted, and therefore the mechanisms and gene sequence needs to be known and characterized. Whereas, RNA-Sequencing requires no prior knowledge, and allows for a full view of the core and accessory genomes. In this way, novel mechanisms of resistance can be found more easily, and differential expression can be seen genome-wide.

The transcriptomic analysis allows for the numeration of differentially expressed genes and quantitative data on what fold change is the down or upregulation of expression. This allows for a generation of a complete differential expression profile for thousands of genes for an isolate when exposed to certain environmental conditions. This means that a "screenshot" of gene expression can be taken at a certain time under specific conditions. This gene expression profile can then be compared at the same time under different conditions or under the same conditions but over time. As an application of this information, entire networks and metabolic pathways of hundreds of genes can be characterized. In addition, the impact of complex interconnected pathways can be mapped, and regulatory circuits can be revealed. This is an important application when a resistant isolate does not harbor any attributable resistance genes, the resistance phenotype could be elicited by overexpression of non-resistant "housekeeping" genes or those yet to be annotated as resistance genes [57].

Kim and coworkers [4, 12] combined a genomic and transcriptomic approach to study bacterial resistance to the disinfectant benzalkonium chloride (BC). In a population exposed to BC long-term, RNA-Sequencing revealed an upregulation in efflux pump genes, down-regulation of porins, and a reduced growth rate [12]. In addition, mutations in the *pmrB* genes and upregulation of spermidine synthase genes affected the charge on the cell membrane of bacteria in the resistant population, resulting in a hindrance of BC uptake [12]. These mutations and differential expression, identified by a combination of genomic and transcriptomic methods, work synergistically to reduce the intracellular concentration of BC. The use of both methods revealed a complex multifaceted approach used by bacterial populations to reduce susceptibility to disinfectants. Due to this discovery, the mutation in the *pmrB* gene could be an attractive biomarker for BC resistance moving forward.

Although transcriptomics can give insight and detail into gene function and regulation, a great deal can change after expression. Resistance profiles can be affected during the protein synthesis stage in the form of post-translational modifications, protein maturation, and combinatory effects. Proteomics can also reveal intercellular changes due to antimicrobial treatment. A protein profile includes any extracellular proteins and those that are secreted to form part of the intercellular microenvironment. Proteomics can be studied by Liquid chromatography-mass spectrometry (LC-MS) and two-dimensional difference in gel electrophoresis (DIGE) and generates an overall protein profile of the cell [46]. This includes both qualitative and quantitative data so not only can we see which proteins are present but in what number. Similarly, to transcriptomics, this protein profile can be generated to reveal protein changes in different conditions or can be monitored over time. Proteomics is best used in combination with real-time PCR to determine if regulation of protein expression occurs at the level of transcription, translation, or post-translational modification [46]. This information can tell us how the proteome changes in response to different antimicrobials and what cellular structures are affected. Zhang and coworkers [58] used proteomic analysis to gain insight into the effect of environmental conditions on an increased tolerance of *P. aeruginosa* to monochloramine disinfection in drinking water. The proteomic profile was compared under different environmental conditions and revealed that stress conditions (starvation and low temperatures) significantly aided in tolerance to monochloramine disinfection [58]. This decrease in susceptibility occurred by triggering oxidative stress defense, dormancy, osmotic stress response, and the stringent response; these responses have been shown to play a vital role in reduced susceptibility to disinfectants [58]. Therefore, proteomic signatures can be generated including protein networks for different antimicrobials, and thus provide insight into how resistance to these antimicrobials develops. Additionally, protein profiles can be used to identify antimicrobial targets and therefore in the design of new antimicrobials [46].

Finally, the metabolome is a new area of study in terms of antimicrobial resistance. Metabolomics reveals a profile of metabolites in a system at a specific time under certain conditions. As with proteomics, this is not limited to the cellular metabolome but includes the intercellular metabolome as well (secreted metabolites). The metabolome can be studied through LC-MS and Nuclear Magnetic Resonance (NMR) [46]. Metabolomics is integral in identifying which metabolic pathways are affected by which antimicrobials, to create a metabolic profile for certain antimicrobial treatments. Metabolomic profiles can be created for individual antimicrobials or antimicrobials used in combination. This will provide insight into the mode of action

for antimicrobials, any combinatory effects and reveal how resistance mechanisms develop. In turn, metabolomics can also create a metabolic profile of resistant organisms under different stress conditions over time and identify important metabolic pathways in resistance. Lin and coworkers [59] used comparative metabolomics on susceptible and MDR *Escherichia coli* strains to identify and characterize 273 differing metabolites between the susceptible and resistant strains. Bioinformatics analyses revealed that the resistant strains all had enriched biosynthesis of amino acids, biosynthesis of phenylpropanoids, and purine metabolism while the susceptible strains did not [59]. This study represents the first step in the prediction and characterization of metabolic pathways crucial in multidrug-resistance profiles in bacteria.

The "omics" methods mentioned generate immense amounts of data. In particular, transcriptomics, proteomics, and metabolomics provide a screenshot of what is happening within a cell at a certain time under certain conditions. These methods can also be used to study how the response of an organism changes over time to a certain antimicrobial, or how the response of the microbe changes between different antimicrobials. However, these methods are all limited by what can be done with the data generated.

5. Artificial intelligence for the prediction of antimicrobial resistance profiles and directing of antimicrobial treatment

Artificial intelligence (AI) has been used to predict antimicrobial resistance profiles based on sequence data. This is done using several models including random forests, naïve Bayes, decision trees, artificial neural networks, and support vector machines [6]. Whole-genome sequencing is a technology that is readily available and becoming more cost-effective year on year, in turn, a massive amount of genomic data now exists. This genomic information has been stored on various databases (NCBI, GenBank, etc.) and is widely available. Artificial intelligence models use this data to identify multiple biomarkers of resistance, in turn, these biomarkers allow for the generation of a predicted resistance profile. This is done by searching the genome for the presence of resistance determinants and labeling them as biomarkers for associated predicted resistance phenotypes.

The Naïve Bayes method has been used to identify resistance determinants and build a resistance profile of biomarkers [60]. In addition, this method has been used in to determine the probability of effective antibiotic treatment when not targeted to a specific pathogen [61]. The support vector machines model has been used to label susceptible or resistant isolates, when applied to *E. coli* for antibiotic resistance it correctly predicted the susceptibility profile with a 95% accuracy [62]. This model can be applied to antimicrobial resistance surveillance and tracking as well as directing treatment for clinical pathogens [6]. Random forest is an algorithm that can direct antibiotic combinations to find synergistic properties and lower total dosage given over time to patients [6]. The decision tree model has been used to estimate the impact of antimicrobial resistance and strategize a proportional response for the allocation of medical resources [63, 64]. This model has also been applied to direct antibiotic use to shorten treatment time [65, 66]. Finally, artificial neural network models have been used to identify new antimicrobial compounds and methods to modify existing antimicrobials to increase effectiveness [6, 67].

The resultant resistance profile generated can determine which genes, mutations, and resistance mechanisms exist and therefore which antimicrobials will be least likely to be effective [61, 62]. In turn, this resistance profile can be used to determine

which resistance biomarkers are not present and therefore which antimicrobials the isolate is most likely susceptible [62]. This generates a list of potential antimicrobials that will be effective against a particular pathogen based on its genomic characteristics and biomarkers present in the genome [60].

This technology can be used for an individual isolate, an infection caused by multiple microbes (as part of a biofilm) or an environmental population, as the total DNA can be extracted, and pan-genome metagenomics is used to screen multiple genomes for biomarkers [62]. This may be of importance when analyzing population dynamics in environments like hospitals where multiple MDR isolates could be harbored together, each exhibiting a different resistance profile. These techniques can be used to determine which antimicrobial will be effective against all microbes present in the population to ensure that one or two do not persist and give rise to a new resistant population.

The resistance profile for an isolate can be used to direct individual targeted antimicrobial treatment. Currently, this method is still in development and being directed mainly to antibiotic treatment of persistent infections [62]. However, this technology has a wide range of applications including chemical treatment of microorganisms by disinfection. Disinfectant resistance is emerging at an alarming rate and some molecular mechanisms of resistance to disinfectants have been discovered [3]. If the molecular resistance mechanisms are known, these resistance genes or mutations that give rise to the resistance phenotype can be flagged as biomarkers of disinfectant resistance. A resistance profile can be generated to determine which active ingredient is most likely to be effective, based on a lack of resistance biomarkers for that specific compound. From there, chemical treatment by certain disinfectants can be recommended based on their active ingredient. Molecular mechanisms of resistance to quaternary ammonium compound (QAC) disinfectants are well characterized. The *qac* gene family plays a direct role in resistance to QAC-based disinfectants as well as smr efflux pump genes [3]. These genes can be flagged as potential resistance markers for QAC-based disinfectants.

This technology can also be applied to direct day-to-day antibiotic treatment by predicting which drug combinations will give the best treatment against a certain pathogen [6]. AI models have been used to elucidate which drug combinations could work synergistically to amplify the antibiotic effect, minimize patient side effects, and prevent the development of antibiotic resistance [6, 65, 66]. This methodology can be applied to antimicrobial chemical treatments (such as disinfectants), which can be applied in combination to create a synergistic effect and prevent the development of resistance to disinfectants. For example, the presence of *smr* efflux pump genes in a microbial population can bring about resistance to a variety of antimicrobial compounds [53]. However, this effect can be negated by the addition of efflux pump inhibitors [68, 69]. This information can be used to direct the treatment of a resistant population by adding efflux pump inhibitors to be used in combination synergistically with disinfectants.

6. Measures to counter resistant organisms once identified

Alternative control methods have been suggested for many years to overcome MDR bacteria, including bacteriocins, essential oils, bacteriophage therapies, nanotherapeutics, antibodies, and more recently quorum sensing inhibitors [44, 70]. Many of these methods currently suffer from their inability to be stand-alone replacements

for antibiotics due to their infancy. Additionally, these alternative methods will most likely be classified as supplementary options in addition to traditional antibiotic or antimicrobial treatments. Suggesting that combination therapy could be the best way forward to combat the global threat of MDR.

Combination therapy involves the coadministration of antibiotics/antimicrobial agents with other chemicals that lack antimicrobial properties or different antibiotics/antimicrobials with differing modes of action [71, 72]. The advantage of combination therapy is that further resistance development can be hindered but the susceptibility of MDR bacteria to treatment can also be restored [73]. The success of combination therapy is shadowed by theoretical predictions that can cause unexpected administration results. The interaction between the different chemicals administered with one another and/or the environment could be antagonistic rather than synergistic, resulting in failure of combinational treatment [74]. Emphasizing that solutions to MDR bacteria are not simple but rather require a great degree of complexity to ensure that the trend of resistance spread does not continue.

There are a plethora of resistance mechanisms present in bacteria, some of the most prevalent include low outer membrane permeability, production of degradation enzymes, efflux pumps, and target modification [75]. Efflux pumps possibly provide the most versatile mechanism to both provide resistance to a broad range of antimicrobial compounds while simultaneously providing additional characteristics, such as increased virulence [76]. Multidrug transporters are efflux pumps with the capability to recognize a wide variety of dissimilar substrates and these types of transporters are often key in MDR bacteria [76].

Since the discovery of MDR caused by efflux pumps the development of efflux pump inhibitors (EPIs) as a strategy to combat this resistance has been considered. The combination of antibiotics/antimicrobials and EPIs could allow the return of certain antibiotics/antimicrobial chemicals that have lost functionality in clinical practice. Additionally, the spectrum of usable compounds could be broadened by the addition of EPIs to allow antimicrobials to be able to adequately target Gram-positive and more naturally resistant Gram-negative bacteria [77]. The inhibition of efflux pumps can have dual purposes, of increasing the susceptibility to some of the antimicrobial substrates which would normally be resisted and potentially some attenuation of the virulence that is connected to efflux pump expression.

Many EPIs have been elucidated, some having specific inhibitory activity against select efflux pumps and others having broader inhibitory effects [77]. Some examples of EPIs include verapamil, reserpine, phenylalanine-arginine beta-naphthylamide (PAβN), 1-(1-naphthylmethyl)-piperazine (NMP), and carbonyl cyanide m-chlorophenyl hydrazone (CCCP). The inhibition of efflux pumps that have substrates of high clinical significance appears to be a very attractive approach. However, this approach will only prove productive for bacterial populations with prevalent efflux-mediated resistance. EPI use has been shown to reduce the frequency of emergence of bacterial strains with clinically relevant levels of resistance to certain antibacterial chemicals [78]. However, the introduction of EPIs as a viable form of combination therapy suffers from several downfalls, which prevent its immediate introduction into clinical settings. When considering EPIs that function to inhibit efflux by specific interaction with the efflux protein via competitive inhibition. Some substrates of the same efflux pump might have different binding sites that are not inhibited by the competitive binding inhibition of the EPI. Highlighting the complexity of interactions between antibacterial compounds and efflux pumps, and how unique combinations of antimicrobials and EPIs will have to be identified to provide desired inhibition effects [78, 79].

EPIs with broad inhibitory capabilities, such as CCCP, inhibit efflux by targeting the energy production of the cell [80]. In both nonspecific and specific inhibition, the end result is to increase the susceptibility of bacterial isolates to antibacterial compounds by increasing intracellular accumulation. The major downfall of EPIs is related to the stringent requirements that these chemicals need to obtain to be successfully classified and used. These compounds must not have inherent antibacterial properties, which could potentiate the problem of MDR bacteria, and must be selective for bacterial efflux pumps, without interaction with eukaryotic efflux systems. Additionally, they must fulfill certain pharmacological characteristics, most notably non-toxicity, and must be economically feasible at commercial production levels [81]. To date, no EPIs have reached clinical use, as none can successfully meet all the requirements to be regarded safe for combination therapy in humans. However, the utility of EPIs could be beneficial for studying the prevalence and contribution that efflux plays in acquired and intrinsic resistance to antibacterial compounds within clinical bacterial isolates to allow better understating of efflux-mediated MDR in the clinical setting. Increased study into alternatives to antibiotics has put a spotlight on treatment options, such as EPIs, further research will be required to get EPIs into clinical use as a combinational therapy option. EPIs can also be used in combination with disinfectants and antiseptics where efflux pumps contribute to reduced susceptibility of these compounds. The synergistic use of EPIs together with antimicrobials may be a crucial step in the fight against AMR in the near future.

7. Conclusions

The antibiotic resistance crisis is a foreshadowing of an equally troubling phenomenon, resistance to disinfectants. Although we have elucidated molecular mechanisms of disinfectant resistance there is still a great deal we do not understand. Complex mechanisms of disinfectant resistance are poorly characterized, little to no research has been done on disinfectant resistance biomarkers and surveillance of disinfectant resistant populations is not a priority. Our current disinfectants need to be safeguarded and the search for new disinfectant formulas must become a priority. Advances in sequencing technologies in the form of omics, biomarkers, and AI will be key in the battle against emerging disinfectant resistance and will go far in characterizing synergistic treatment options. Disinfectants are heavily relied on for hygiene purposes and infection control in the agricultural, food and beverage industries, and so designing a disinfection program that is effective is vital. Additionally, characterizing disinfectant resistance profiles in environments such as the food industry and medical environments will be key to effective control ever changing persistent resistant populations. Advances in sequencing and antimicrobial resistance research have never been so important, as this methodology can be applied to disinfectant resistance to avoid another worldwide resistance crisis.

Acknowledgements

The authors would like to acknowledge the National Research Foundation (NRF) of South Africa for funding and support.

Conflict of interest

The authors declare no conflict of interest.

IntechOpen

Author details

Samantha Mc Carlie, Gunther Staats, Bernadette Belter, Boudine Van Der Walt and Robert Bragg*
University of the Free State, Bloemfontein, South Africa

*Address all correspondence to: braggrr@ufs.ac.za

IntechOpen

References

[1] McDonnell G, Russell A. Antiseptics and disinfectants: Activity , action, and resistance. Clinical Microbiology Reviews. 1999;**12**(1):147-179

[2] Bragg R, Jansen A, Coetzee M, van der Westhuizen W. Bacterial resistance to quaternary ammonium compounds (QAC) disinfectants. Advances in Experimental Medicine and Biology. 2014;**808**(1):1-13. DOI: 10.1007/978-81-322-1774-9

[3] Mc Carlie S, Boucher C, Bragg R. Molecular basis of bacterial disinfectant resistance. Drug Resistance Updates. 2020;**48**:100672. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31830738

[4] Kim M, Weigand MR, Oh S, Hatt JK, Krishnan R, Tezel U, et al. Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. Applied and Environmental Microbiology. 2018;**84**(17):e01201-18. DOI: 10.1128/AEM.01201-18

[5] Yoshimatsu T, Hiyama KI. Mechanism of the action of didecyldimethylammonium chloride (DDAC) against *Escherichia coli* and morphological changes of the cells. Biocontrol Science. 2007;**12**(3):93-99. Available from: http://joi.jlc.jst.go.jp/JST.Journalarchive/bio1996/12.93?from=CrossRef

[6] Lv J, Deng S, Zhang L. A review of artificial intelligence applications for antimicrobial resistance. Biosafety and Health. 2021;**3**(1):22-31

[7] Jansen A, Boucher CE, Coetsee E, Kock J, van Wyk P, Swart H, et al. The influence of didecyldimethylammonium chloride on the morphology and elemental composition of *Staphylococcus aureus* as determined by NanoSAM. Scientific Research and Essays. 2013;**8**(3):152-160

[8] Gerba CP. Quaternary ammonium biocides: Efficacy in application. Applied and Environmental Microbiology. 2015;**81**(2):464-469. DOI: 10.1128/AEM.02633-14

[9] Denyer SP, Stewart GSAB. Mechanisms of action of disinfectants. International Biodeterioration & Biodegradation. 1998;**41**(3-4):261-268. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0964830598000237

[10] Tezel U, Pavlostathis SG. Quaternary ammonium disinfectants: Microbial adaptation, degradation and ecology. Current Opinion in Biotechnology. 2015;**33**:296-304. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0958166915000622

[11] Tezel U, Pavlostathis SG. Role of quaternary ammonium compounds on antimicrobial resistance in the environment. In: Keen P, Mantforts M, editors. Antimicrobial Resistance in the Environment. 1st ed. Hoboken, NJ: Wiley-Blackwell; 2012. pp. 349-387

[12] Kim M, Hatt JK, Weigand MR, Krishnan R, Pavlostathis SG, Konstantinidis KT. Genomic and transcriptomic insights into how bacteria withstand high concentrations of benzalkonium chloride biocides. Applied and Environmental Microbiology. 2018;**84**(12):1-15

[13] Boeris PS, Liffourrena AS, Salvano MA, Lucchesi GI. Physiological role of phosphatidylcholine in the *pseudomonas putida* a ATCC 12633 response

to tetradecyltrimethylammonium bromide and aluminium. Letters in Applied Microbiology. 2009;**49**(4):491-496. DOI: 10.1111/j.1472-765X.2009.02699.x

[14] Poursat BAJ, van Spanning RJM, de Voogt P, Parsons JR. Implications of microbial adaptation for the assessment of environmental persistence of chemicals. Critical Reviews in Environmental Science and Technology. 2019;**49**(23):2220-2255. DOI: 10.1080/10643389.2019.1607687

[15] Ishikawa S, Matsumura Y, Yoshizako F, Tsuchido T. Characterization of a cationic surfactant-resistant mutant isolated spontaneously from *Escherichia coli*. Journal of Applied Microbiology. 2002;**92**(2):261-268. DOI: 10.1046/j.1365-2672.2002.01526.x

[16] Pagedar A, Singh J, Batish VK. Adaptation to benzalkonium chloride and ciprofloxacin affects biofilm formation potential, efflux pump and haemolysin activity of *Escherichia coli* of dairy origin. The Journal of Dairy Research. 2012;**79**(4):383-389. Available from: https://www.cambridge.org/core/product/identifier/S0022029912000295/type/journal_article

[17] Poole K. Efflux pumps as antimicrobial resistance mechanisms. Annals of Medicine. 2007;**39**(3):162-176. DOI: 10.1080/07853890701195262

[18] Braga T, Marujo P, Pomba C, Lopes M. Involvement and dissemination of the enterococcal small multidrug resistance transporter QacZ in resistance to quaternary ammonium compounds. The Journal of Antimicrobial Chemotherapy. 2011;**66**:283-286

[19] Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. Clinical Microbiology and Infection. 2010;**16**(6):541-554. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20569265

[20] Dean-Raymond D, Alexander M. Bacterial metabolism of quaternary ammonium compounds. Applied and Environmental Microbiology. 1977;**33**(5):1037-1041. Available from: https://aem.asm.org/content/33/5/1037

[21] van Ginkel CG, van Dijk JB, Kroon AG. Metabolism of hexadecyltrimethylammonium chloride in *pseudomonas* strain B1. Applied and Environmental Microbiology. 1992;**58**(9):3083-3087. Available from: https://aem.asm.org/content/58/9/3083

[22] van Ginkel CG. Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms. Biodegradation. 1996;**7**(2):151-164. DOI: 10.1007/s002030050390

[23] Patrauchan MA, Oriel PJ. Degradation of benzyldimethylalkylammonium chloride by *Aeromonas hydrophila* sp. K. Journal of Applied Microbiology. 2003;**94**(2):266-272. DOI: 10.1046/j.1365-2672.2003.01829.x

[24] Frost LS, Leplae R, Summers AO, Toussaint A. Mobile genetic elements: The agents of open source evolution. Nature Reviews. Microbiology. 2005;**3**(9):722-732. Available from: http://www.nature.com/articles/nrmicro1235

[25] Van Der Meer JR. Environmental pollution promotes selection of microbial degradation pathways. Frontiers in Ecology and the Environment. 2006;**4**(1):35-42

[26] Palmer AC, Chait R, Kishony R. Nonoptimal gene expression creates latent potential for antibiotic resistance.

Csaba P, editor. Molecular Biology and Evolution 2018;**34**(11):2669-2684. 10.1093/molbev/msy163/5085501

[27] Barlow M. What Antimicrobial Resistance Has Taught Us About Horizontal Gene Transfer. Berlin/Heidelberg, Germany: Springer; 2009. pp. 397-411. DOI: 10.1007/978-1-60327-853-9_23

[28] Carattoli A. Plasmids and the spread of resistance. International Journal of Medical Microbiology. 2013;**303**(6-7):298-304. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1438422113000167

[29] Poirel L, Cattoir V, Nordmann P. Is plasmid-mediated quinolone resistance a clinically significant problem? Clinical Microbiology and Infection. 2008;**14**(4):295-297. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X14627993

[30] Russel A. Plamids and bacterial resistance to biocides. Journal of Applied Microbiology. 1997;**83**(2):155-165

[31] Langsrud S, Sundheim G, Brogman-Stahsen R. Intrinsic and acquired resistance to quaternary ammonium compounds in food-related *pseudomonas* spp. Journal of Applied Microbiology. 2003;**95**:874-882

[32] Jaglic Z, Cervinkova D. Genetic basis of resistance to quaternary ammonium compounds & the *qac* genes and their role: A review. Veterinární Medicína (Praha). 2012;**57**(6):275-281. Available from: http://www.agriculturejournals.cz/web/vetmed.htm?volume=57&firstPage=275&type=publishedArticle

[33] Bloom DE, Cadarette D. Infectious disease threats in the twenty-first century: Strengthening the global response. Frontiers in Immunology. 2019;**28**:10. DOI: 10.3389/fimmu.2019.00549/full

[34] Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance—The need for global solutions. The Lancet Infectious Diseases. 2013;**13**(12):1057-1098. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1473309913703189

[35] Hindler JA, Humphries RM. Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant gram-negative *bacilli*. Journal of Clinical Microbiology. 2013;**51**(6):1678-1684. DOI: 10.1128/JCM.03385-12

[36] Huss R. Chapter 19 Biomarkers. Translational Regenerative Medicine. Amsterdam, Netherlands: Elsevier Inc.; 2015. p. 235-241

[37] Russo TA, Olson R, Fang C-T, Stoesser N, Miller M, MacDonald U, et al. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from Classical *K. pneumoniae*. Journal of Clinical Microbiology. 2018;**56**(9):e00776-18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29925642

[38] Volz C, Ramoni J, Beisken S, Galata V, Keller A, Plum A, et al. Clinical resistome screening of 1,110 *Escherichia coli* isolates efficiently recovers diagnostically relevant antibiotic resistance biomarkers and potential novel resistance mechanisms. Frontiers in Microbiology. 2019;**13**:10. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2019.01671/full

[39] Khaledi A, Weimann A, Schniederjans M, Asgari E, Kuo T, Oliver A, et al. Predicting antimicrobial resistance in *Pseudomonas aeruginosa* with machine learning-enabled molecular diagnostics. EMBO Molecular Medicine. 2020;**12**(3):e10264. DOI: 10.15252/emmm.201910264

[40] Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clinical Microbiology and Infection. 2010;**16**(11):1614-1619. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X1460553X

[41] Burckhardt I, Zimmermann S. Susceptibility testing of bacteria using Maldi-Tof mass spectrometry. Frontiers in Microbiology. 2018;**6**:9. DOI: 10.3389/fmicb.2018.01744/full

[42] Meade E, Slattery MA, Garvey M. Biocidal resistance in clinically relevant microbial species: A major public health risk. Pathogens. 2021;**10**(5):598. Available from: https://www.mdpi.com/2076-0817/10/5/598

[43] Allen HK, Trachsel J, Looft T, Casey TA. Finding alternatives to antibiotics. Annals of the New York Academy of Sciences. 2014;**1323**(1):91-100. DOI: 10.1111/nyas.12468

[44] Bragg RR, Meyburgh CM, Lee JY, Coetzee M. Potential treatment options in a post-antibiotic era. In: Advances in Experimental Medicine and Biology. Vol. 1052. Singapore: Springer; 2018. pp. 51-61. DOI: 10.1007/978-981-10-7572-8_5

[45] Hulme PE. One biosecurity: A unified concept to integrate human, animal, plant, and environmental health. Emerging Topics in Life Sciences. 2020;**4**(5):539-549. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33111945

[46] Chernov VM, Chernova OA, Mouzykantov AA, Lopukhov LL, Aminov RI. Omics of antimicrobials and antimicrobial resistance. Expert Opinion on Drug Discovery. 2019;**14**(5):455-468. DOI: 10.1080/17460441.2019.1588880

[47] Zmantar T, Kouidhi B, Hentati H, Bakhrouf A. Detection of disinfectant and antibiotic resistance genes in *Staphylococcus aureus* isolated from the oral cavity of Tunisian children. Annales de Microbiologie. 20122;**62**(1):123-128. DOI: 10.1007/s13213-011-0236-3

[48] Littlejohn TG, DiBerardino D, Messerotti LJ, Spiers SJ, Skurray RA. Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. Gene. 1991;**101**(1):59-66. Available from: http://linkinghub.elsevier.com/retrieve/pii/037811199190224Y

[49] Noguchi N. Antiseptic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiology Letters. 1999;**172**(2):247-253. DOI: 10.1016/S0378-1097(99)00041-5

[50] Liu S, Wang M, Zheng L, Guan W. Antimicrobial resistance profiles of nosocomial pathogens in regional China: A brief report from two tertiary hospitals in China. Medical Science Monitor. 2018;**24**:8602-8607. Available from: https://www.medscimonit.com/abstract/index/idArt/911229

[51] Bland RN, Johnson JD, Waite-Cusic JG, Weisberg AJ, Riutta ER, Chang JH, et al. Application of whole genome sequencing to understand diversity and presence of genes associated with sanitizer tolerance in listeria monocytogenes from produce handling sources. Foods (Basel, Switzerland). 2021;**10**(10):2454. Available from: http://www.ncbi.nlm.nih.gov/pubmed/34681501

[52] Gallagher LA, Lee SA, Manoil C. Importance of Core genome functions for an extreme antibiotic resistance trait. mBio. 2017;**8**(6):e01655-17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29233894

[53] Bjorland J, Sunde M, Waage S. Plasmid-borne *smr* gene causes resistance to quaternary ammonium compounds in bovine *Staphylococcus aureus*. Journal of Clinical Microbiology. 2001:3999-4004

[54] Heir E, Sundheim G, Holck A. The *qacG* gene on plasmid pST94 confers resistance to quaternary ammonium compounds in *staphylococci* isolated from the food industry. Journal of Applied Microbiology. 1999;**86**:378-388

[55] Bjorland J, Steinum T, Sunde M, Waage S, Heir E. Novel plasmid borne gene *qacJ* mediates resistance to quaternary ammonium compounds in equine *Staphylococcus aureus, Staphylococcus simulans,* and *Staphylococcus intermedius*. Antimicrobial Agents and Chemotherapy. 2003;**47**:3046-3052

[56] Khaledi A, Schniederjans M, Pohl S, Rainer R, Bodenhofer U, Xia B, et al. Transcriptome profiling of antimicrobial resistance in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2016;**60**(8):4722-4733

[57] Tansirichaiya S, Reynolds LJ, Cristarella G, Wong LC, Rosendahl K, Roberts AP. Reduced susceptibility to antiseptics is conferred by heterologous housekeeping genes. Microbial Drug Resistance. 2018;**24**(2):105-112. DOI: 10.1089/mdr.2017.0105

[58] Zhang S-P, Feng H-Z, Wang Q, Quan S-W, Yu X-Q, Tao X, et al. Proteomic analysis reveals the mechanism of different environmental stress-induced tolerance of *Pseudomonas aeruginosa* to monochloramine disinfection. Journal of Hazardous Materials. 2021;**417**:126082. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304389421010463

[59] Lin Y, Li W, Sun L, Lin Z, Jiang Y, Ling Y, et al. Comparative metabolomics shows the metabolic profiles fluctuate in multi-drug resistant *Escherichia coli* strains. Journal of Proteomics. 2019;**207**:103468. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1874391919302404

[60] Rezaei-hachesu P, Samad-Soltani T, Yaghoubi S, GhaziSaeedi M, Mirnia K, Masoumi-Asl H, et al. The design and evaluation of an antimicrobial resistance surveillance system for neonatal intensive care units in Iran. International Journal of Medical Informatics. 2018;**115**:24-34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1386505618304106

[61] Choisy M, Van Cuong N, Bao TD, Kiet BT, Hien BV, Thu HV, et al. Assessing antimicrobial misuse in small-scale chicken farms in Vietnam from an observational study. BMC Veterinary Research. 2019;**15**(1):206. DOI: 10.1186/s12917-019-1947-0

[62] Her H-L, Wu Y-W. A pan-genome-based machine learning approach for predicting antimicrobial resistance activities of the *Escherichia coli* strains. Bioinformatics. 2018;**34**(13):i89-i95. Available from: https://academic.oup.com/bioinformatics/article/34/13/i89/5045729

[63] Naylor NR, Zhu N, Hulscher M, Holmes A, Ahmad R, Robotham JV. Is antimicrobial stewardship cost-effective? A narrative review of the evidence. Clinical Microbiology and Infection. 2017;**23**(11):806-811. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X17303300

[64] Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, et al. Estimating the burden of antimicrobial resistance: A systematic literature review. Antimicrobial Resistance and Infection

Control. 2018;7(1):58. DOI: 10.1186/s13756-018-0336-y

[65] Voermans AM, Mewes JC, Broyles MR, Steuten LMG. Cost-effectiveness analysis of a procalcitonin-guided decision algorithm for antibiotic stewardship using real-world U.S. hospital data. OMICS: A Journal of Integrative Biology. 2019;**23**(10):508-515. DOI: 10.1089/omi.2019.0113

[66] Schuetz P, Amin DN, Greenwald JL. Role of procalcitonin in managing adult patients with respiratory tract infections. Chest. 2012;**141**(4):1063-1073. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0012369212602311

[67] Stokes JM, Yang K, Swanson K, Jin W, Cubillos-Ruiz A, Donghia NM, et al. A deep learning approach to antibiotic discovery. Cell. 2020;**180**(4):688-702.e13. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867420301021

[68] Wang Y, Mowla R, Guo L, Ogunniyi AD, Rahman T, De Barros Lopes MA, et al. Evaluation of a series of 2-napthamide derivatives as inhibitors of the drug efflux pump AcrB for the reversal of antimicrobial resistance. Bioorganic & Medicinal Chemistry Letters. 2017;**27**(4):733-739. DOI: 10.1016/j.bmcl.2017.01.042

[69] Lowrence RC, Subramaniapillai SG, Ulaganathan V, Nagarajan S. Tackling drug resistance with efflux pump inhibitors: From bacteria to cancerous cells. Critical Reviews in Microbiology. 2019;**45**(3):334-353. DOI: 10.1080/1040841X.2019.1607248

[70] Zaidi S, Misba L, Khan AU. Nano-therapeutics: A revolution in infection control in post antibiotic era. Nanomedicine Nanotechnology, Biology and Medicine. 2017;**13**(7):2281-2301. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1549963417301235

[71] Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clinical Microbiology Reviews. 2012;**25**(3):450-470. DOI: 10.1128/CMR.05041-11

[72] Paul M, Lador A, Leibovici L. Beta lactam antibiotic monotherapy versus beta lactam- aminoglycoside antibiotic combination therapy for sepsis (review). The Cochrane Library. 2014;**1**:1-147

[73] Mouton JW. Combination therapy as a tool to prevent emergence of bacterial resistance. Infection. 1999;**27**(S2):S24-S28. DOI: 10.1007/BF02561666

[74] Brochado AR, Telzerow A, Bobonis J, Banzhaf M, Mateus A, Selkrig J, et al. Species-specific activity of antibacterial drug combinations. Nature. 2018;**559**(7713):259-263. Available from: http://www.nature.com/articles/s41586-018-0278-9

[75] Nikaido H. Multidrug Resistance in Bacteria. Annual Review of Biochemistry. 2009;**78**(1):119-146. DOI: 10.1146/annurev.biochem.78.082907.145923

[76] Alcalde-Rico M, Hernando-Amado S, Blanco P, Martínez JL. Multidrug efflux pumps at the crossroad between antibiotic resistance and bacterial virulence. Frontiers in Microbiology. 2016;**21**:7. DOI: 10.3389/fmicb.2016.01483/abstract

[77] Lomovskaya O, Watkins W. Inhibition of efflux pumps as a novel approach to combat drug resistance in bacteria. Journal of Molecular Microbiology and Biotechnology. 2001;**3**(2):225-236. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/11321578

[78] Lomovskaya O, Bostian KA. Practical applications and feasibility of efflux pump inhibitors in the clinic—A vision for applied use. Biochemical Pharmacology. 2006;**71**(7):910-918. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006295205008154

[79] Dreier J, Ruggerone P. Interaction of antibacterial compounds with RND efflux pumps in *Pseudomonas aeruginosa*. Frontiers in Microbiology. 2015;**6**:1-21

[80] Mallea M, Chevalier J, Bornet C, Eyraud A, Davin-Regli A, Bollet C, et al. Porin alteration and active efflux: Two in vivo drug resistance strategies used by *Enterobacter aerogenes*. Microbiology. 1998;**144**(11):3003-3009. DOI: 10.1099/00221287-144-11-3003

[81] Bhardwaj AK, Mohanty P. Bacterial efflux pumps involved in multidrug resistance and their inhibitors: Rejuvinating the antimicrobial chemotherapy. Recent Patents on Anti-Infective Drug Discovery. 2012;**7**(1):73-89. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1574-891X&volume=7&issue=1&spage=73