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One Health Perspective on

Antimicrobial Resistance and Some Strategies for its Mitigation

Edited by
Hari Mohan Saxena
Simeon Fogue Kouam

Centre for Science and Technology of the Non-Aligned and Other Developing Countries (NAM S&T Centre)

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ABOUT THE BOOK

Antimicrobial Resistance (AMR) is a natural process by which microorganisms (bacteria, viruses, parasites, fungi and other pathogens) develop resistance to the drugs used to fight against them. The abuse and misuse of antibiotics and other antimicrobial drugs favours the development and spread of resistant micro organisms, and generates the need for alternative treatments effective against such pathogens.

AMR also concerns other non-bacterial diseases. Resistance of the malarial parasite to the antimalarial drugs chloroquine and sulfadoxine-pyrethamine is widespread in most countries where malaria is endemic. Similarly, resistance is also an increasing concern in the treatment of HIV infection due to rapid increase in the availability of antiretroviral therapy in recent years.

Fighting resistant pathogens requires responsible action including: (1) strengthening the current systems for tracking and monitoring antimicrobial resistance; (2) ensuring access to quality-assured essential drugs and promoting only the rational use of antibiotics in both humans and animals; (3) improving the prevention and control of infections; (4) promoting research, innovation and development of new tools (antibiotics and vaccines etc).

The book includes 15 scientific and technical papers contributed by experts and professionals from 7 different countries namely: India, Indonesia, Mongolia, Nepal, Nigeria, South Africa and Sri Lanka highlighting one health perspective on antimicrobial resistance and put forward the best possible strategies for its mitigation in the NAM and other developing countries.

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Foreword

Antimicrobial Resistance (AMR) occurs naturally over time when microorganisms (such as bacteria, fungi, viruses, and parasites) go through genetic changes when exposed to antimicrobial drugs. Microorganisms that develop antimicrobial resistance are sometimes referred to as "superbugs". As a result, the medicines become ineffective and infections persist in the body.

Antimicrobial resistant-microbes are found in people, animals, food and the environment (in water, soil and air). They can transmit between humans and animals; which includes from food of animal origin, and from a person to another. New resistance mechanisms are emerging and spreading globally, threatening the ability of antimicrobial agents to treat common infectious diseases, resulting in prolonged illness, disability, and death. Without effective antimicrobials for prevention and treatment of infections, even medical procedures have very high risk. Antimicrobial resistance is even putting the gains of the Millennium Development Goals at risk while endangering the achievement of the Sustainable Development Goals of 2030.

In order to discuss and deliberate on the current global, national and regional status of "Antimicrobial Resistance", the Centre for Science and Technology of the Non-Aligned and Other Developing Countries (NAM S&T Centre) had organised a three-day dynamic International Training Workshop on 'Antimicrobial Resistance and Strategies for its Mitigation' in collaboration with Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana, during 10–13 July, 2018 in Ludhiana (Punjab), India with the objective to evolve strategies for mitigation of microbial resistance in humans and animals to antimicrobial agents and foster international, regional and sub-regional cooperation and collaboration in its effective implementation. It also aimed to focus the attention of the non-aligned

and other developing on the global problem of ever increasing resistance of microorganisms to various antimicrobial agents.

As a follow up, the NAM S&T Centre has brought out a book titled "One Health Perspective on Antimicrobial Resistance and Some Strategies for its Mitigation" which comprises 15 papers contributed by the participants of the Ludhiana Workshop and other experts in this field. The volume has been edited by Dr. Hari Mohan Saxena, DRI-cum-Dean PGS, Bihar Animal Sciences University (BASU), Patna, India and Dr. Simeon Fogue Kouam, Professor and Natural Product Chemist, Department of Chemistry, Higher Teacher Training College, University of Yaounde I, Cameroon. The papers included in this book highlight the current status and the extent of antimicrobial resistance in humans and animals; focusing on evolving new and effective mitigation strategies for combating antimicrobial resistance in pathogenic microbes.

I congratulate the NAM S&T Centre for bringing out this valuable publication. It will be of great value to microbiologists, immunologists, virologists, biotechnologists, biochemists and other healthcare professionals working in the academic and research institutions, government departments and ministries and other relevant industries alike.

I wish the very best for all future endeavours of the NAM S&T Centre towards enhancing South-South S&T Cooperation amongst the NAM and other developing countries.

Prof Mahmoud M. Sakr
President
Academy of Scientific Research & Technology
ASRT

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Introduction

Antimicrobial Resistance (AMR) is one of the most serious global public health concerns and profoundly threatening our ability to treat a wide range of infectious diseases caused by bacteria, parasites, viruses and fungi. It is occurring everywhere in the world and undermining many global advances in health and medicine. It is not only threatening the very core of modern medicine but also the sustainability of an effective public health response to the enduring threat from the ever-increasing range of infections. Systematic misuse and overuse of the antimicrobial drugs have put every nation at risk.

Addressing the rising threat of antimicrobial resistance requires a holistic and multi-sectoral approach referred to as '**One Health**' because antimicrobials used to treat various infectious diseases in animals may be the same or similar to those used for humans as well. Resistant bacteria arising in humans, animals or the environment may spread from one to the other, and from one country to another as AMR does not recognize geographic limitations and human-animal borders.

Alert to this crisis, the World Health Assembly held in May 2015 adopted a global action plan to tackle antimicrobial resistance, including antibiotic resistance; the most urgent drug resistance trend.

The global action plan ensures, for as long as possible, the continuity of successful treatment and prevention of infectious diseases with effective and safe medicines that are quality-assured, used in a responsible way, and accessible to all who need them. The plan outlines: to improve awareness and understanding of antimicrobial resistance, to strengthen knowledge through surveillance and research, to reduce the incidence of infection, to optimize the use of antimicrobial agents, develop the economic situation for sustainable investment that takes account of the needs of all countries, and increase investment in new R&D programmes on drug development, new medicines, diagnostic tools, vaccines and other related interventions.

In order to focus the attention of the *non-aligned and other developing countries* on this ever-increasing global problem of antimicrobial resistance, the *Centre for Science and Technology of the Non-Aligned and Other Developing Countries (NAM S&T Centre), New Delhi*, in

partnership with the *Guru Angad Dev Veterinary & Animal Sciences University (GADVASU), Ludhiana* organised an International Training Workshop on '**Antimicrobial Resistance and Strategies for its Mitigation**' during 10–13 July, 2018 in Ludhiana (Punjab), India. The three-day Workshop brought together 53 researchers and scientists from 15 countries namely: Afghanistan, Egypt, Indonesia, Iran, Kenya, Malaysia, Mauritius, Myanmar, Nepal, Nigeria, Palestine, South Africa, Sri Lanka and Zambia and the host country India with an objective to identify the current status of antimicrobial resistance in NAM and other developing countries, specifically focusing to discuss antimicrobial resistance in animal and human pathogens; particularly the zoonotic infections, nosocomial and other chronic infections, reasons for vaccine failures and consequently evolve and implement strategies for its mitigation.

As a follow up of the Ludhiana Workshop, the present book titled "**One Health Perspective on Antimicrobial Resistance and Some Strategies for its Mitigation**" is being published by the NAM S&T Centre which includes 15 scientific papers contributed by the participants and other professionals and experts from seven developing countries namely: India, Indonesia, Mongolia, Nepal, Nigeria, South Africa and Sri Lanka.

I highly appreciate the time and efforts put in place by the Editors of this book, Dr. Hari Mohan Saxena, DRI-cum-Dean (PGS), Bihar Animal Sciences University (BASU), Patna, India and Dr. Simeon Fogue Kouam, Professor and Natural Product Chemist, Department of Chemistry, Higher Teacher Training College, University of Yaounde I, Cameroon, for the technical editing of the manuscripts.

I take this opportunity to express my gratitude to Prof. Mahmoud M. Sakr, President, The Academy of Scientific Research and Technology (ASRT), Cairo, Egypt for writing the "Foreword" for this book in spite of his very busy schedule.

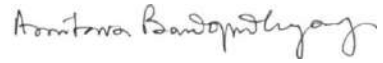
I also acknowledge the interest and valuable efforts of the entire team of the NAM S&T Centre, and especially thank Mr. M. Bandyopadhyay, Senior Adviser for his guidance and supervision; and Ms. Jasmeet Kaur, Programme Officer; Ms. Nidhi Utreja, Research Associate; and Mr. Pankaj Buttan, Data Processing Manager for compiling and proof reading of the manuscripts, liaising with

authors of the papers, designing the cover page, formatting and taking all the necessary steps in giving a shape to this book.

A special thanks to Prof. Arun P. Kulshreshtha, Former Director-General, NAM S&T Centre, for his interest and support in organising this important and valuable event.

I am also thankful to Mr. Sharad Gupta, Publishing Consultant and Mr. Jagdish Singh from the Publication Department, Allied Publishers, New Delhi, for their significant efforts in bringing out this valuable publication.

I am sure this book will be of great significance and immense use to all those associated with the multifaceted phenomenon of anti-microbial resistance.



(**Amitava Bandopadhyay**, ***Ph.D.***)
Director General
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Preface

Antimicrobial Resistance (AMR) is a major global public health issue. AMR is responsible for approximately 700,000 deaths each year, and is expected to cause one death every three seconds worldwide by 2050. The development of bacterial resistance to one or several antimicrobials is associated with the over-use of antimicrobials in human and veterinary medicine. Antimicrobials have played an important role in controlling bacterial infectious diseases in both humans and animals. In livestock, antimicrobials are used mainly for the treatment and prevention of diseases. The worldwide consumption of antimicrobials in animal-food production has been reported at around 57 million kg. Antimicrobials are not fully metabolized when administered to either humans or livestock. Upto 90% of many of the antibiotics used in livestock are excreted in urine or feces. Bacteria residing in the gastrointestinal tract may become resistant to these antibiotics and, once released into the environment, they may transfer Antimicrobial Resistance Genes (ARGs) to other bacteria including potential human pathogens. Furthermore, residual antibiotics may enter the environment through run-off from manure, where they may select for antimicrobial resistant bacteria. For almost every livestock-associated bacterial pathogen, resistance to at least one antimicrobial from each antimicrobial class has been reported. Antimicrobials, antimicrobial resistant bacteria and antimicrobial resistance genes are frequently detected in Sewage Treatment Plants (STPs) and as a result these can act as a potential hotspot for antibiotic resistance, where ARGs spread among bacteria via horizontal gene transfer. These biological pollutants are also released into the environment in STP effluent.

Antimicrobial Resistance is a global public health threat, and in the United States alone at least 23,000 people die each year due to resistant bacterial infections. It is also a 'One Health' issue because AMR emergence in bacteria from humans, animals, or the environment can impact the health of the others. As such, it is critical to identify and characterize emerging AMR threats in different parts of the world so that integrated control policies may be developed. It is essential that data from animal pathogens collected by veterinary diagnostic

laboratories be incorporated into AMR surveillance activities as part of the One Health framework. These data, from bacterial pathogens of clinically ill animals, are an important addition to other surveillance programs that look at bacteria from healthy farm animals, foods and ill humans. Including veterinary pathogens in AMR surveillance will directly assist the veterinary profession treating our companion animals and will indirectly enhance our understanding of the epidemiology of AMR. The data from such studies can also be used to develop Antimicrobial Use (AMU) guidelines to educate veterinarians on the principles of good antimicrobial stewardship in their daily practice. Since the health of humans and animals are intricately linked, this data source is one of the critical components of One Health surveillance.

Medical doctors and veterinarians should emphasize the prudent use of antimicrobials. Antibiotics should be strictly used only when required. In addition to the conventional prevention strategies, special emphasis is required in the use of alternative prevention strategies, including vaccines, prebiotics, probiotics, and herbal drugs, to improve the production performance and health status of livestock. In beef production, antimicrobials are important to maintain or improve animal health and increase productivity. Although the development of AMR is a complex multifactorial process, use of potent broad-spectrum antimicrobials is a key factor for its development. The use of bacteriophage-based products, vaccines as well as other infection prevention and control approaches are promising alternatives to antimicrobials. One Health approaches to optimization of AMU have been suggested as measures for prolonging the therapeutic life of available antimicrobial drugs. A collective action towards promoting the prudent/judicious use of antimicrobials and search for alternative therapies is being advocated on a global scale.

Considering the global importance of the problem of ever increasing Antimicrobial Resistance, the Centre for Science and Technology of the Non-Aligned and Other Developing Countries (NAM S&T Centre). New Delhi and Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, took the initiative to organize an International Training Workshop on 'Antimicrobial Resistance and Strategies for its Mitigation' in July, 2018 at Ludhiana (Punjab), India. Medical doctors, veterinarians and scientists from 15 non-aligned and other developing countries participated in the

Workshop and presented their findings on the prevailing scenario of AMR in their regions and shared some of their experiences with various strategies for its mitigation. It was decided to make these presentations available globally for the benefit of other scientists and researchers. The present book is a collection of selected articles on the topic contributed by the participants. It is hoped that this compilation will be useful and foster international and interdisciplinary collaborations for further research and action to combat the menace of AMR among pathogens of humans and livestock.

Hari Mohan Saxena

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Contents

Bacterial Resistance to Disinfectants: A Review of Biosecurity, Resistance Mechanisms and the Use of Sequencing Technology

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ABSTRACT: *Antibiotic resistance is becoming one of the biggest life-threatening challenges of our time. Bacterial infections present in hospitals and agriculture are becoming unresponsive to many of the antibiotics currently in use, marking the start of a post-antibiotic era. One of the other lines of defence against bacterial growth and infections are disinfectants and improved biosecurity. This review looks at the emergence of disinfectant resistance as well as the mechanisms identified thus far. Currently, the transmembrane efflux pump is the most widespread mechanism of resistance to disinfectants, lowering the intracellular concentration of antimicrobial agents. This resistance mechanism is effective against both antibiotics and disinfectants in some multi-resistant strains, leading to cross-resistance. Resistance to disinfectants could prove difficult to prevent. However, resistance to disinfectants could be slowed down until other options for control of bacterial diseases can be developed and applied on an industrial scale. New sequencing technologies have vastly improved identification and the study of disinfectant resistance mechanisms. This is mainly due to the identification of resistance genes, their mechanisms and bacterial characterisation.*

Keywords: Disinfectant Resistance, Biosecurity, Resistance Mechanisms, Cross-resistance, QAC Resistance Genes, Next Generation Sequencing.

1. INTRODUCTION

Bacterial resistance to antibiotics has become a significant issue. The resistance to antibiotics is disseminating rapidly due to the lack of proper biosecurity measures from the hospital environment to the food and agricultural industry (Hawkey and Jones, 2009, Bragg *et al.*, 2014, Spellberg and Gilbert, 2014, Ventola, 2015). Currently, the best viable protection we have left against bacteria is disinfectants (McDonnell and Russell 1999), however this makes it only more troubling that disinfectant resistance is also emerging at an alarming speed (Templeton *et al.*, 2009). In order to combat emerging resistance to disinfectants, we need to understand the mechanisms of resistance. Once the mechanisms are identified, possible solutions can be discussed (Khan *et al.*, 2016). If biosecurity is implemented properly, it can slow down the rate of developing resistance to disinfectants (Gross, 2013). As widespread resistance to disinfectants is highly likely, it is imperative that the other viable options for the control of bacterial growth need to be researched. The purpose of this paper is to consider bacterial resistance, particularly to disinfectants, to discuss the mechanisms of resistance, as well as sequencing technology to detect and monitor bacterial resistance to disinfectants.

2. THE SIGNIFICANCE OF BACTERIAL RESISTANCE TO ANTIBIOTICS AND BIOSECURITY

Antibiotics are used worldwide in many environments such as hospitals, agriculture, dentistry and veterinary practices. Antibiotics and disinfectants play an integral role in infection control and quality standards (Rutala, 1995, McDonnell and Russell, 1999). The average human life expectancy before antibiotics, in the early 20th Century, was 47 years in the United States of America, whereas currently it is 78.8 years (CDC 2016). This is largely due to the use of antibiotics to treat bacterial diseases such as cholera and typhoid fever which were devastating at the time (Adedeji, 2016). The rapid emergence of bacterial resistance to antibiotics is a significant threat to industry and life as we know it. The term 'resistant' can be used to describe an organism that is insusceptible to a concentration of disinfectant used in practice, or that is not inactivated or inhibited by a concentration

that would otherwise inactivate the majority of strains of that organism (Russell 1987). Resistance to antibiotics is nothing new as bacteria such as *Streptomyces griseus* and yeasts like *Penicillium notatum* have been producing the antibiotics *Streptomycin* and *Penicillin*, respectively, for millions of years (Zaffiri *et al.,* 2012). In response to this, bacteria in the affected population develop the ability of resistance to these antibiotics. The use of any antimicrobial including antibiotics creates selective pressure allowing for the development of resistance in the affected population (Canton *et al.,* 2015).

The modern era of antibiotics started in 1928 when Sir Alexander Fleming discovered penicillin (Gould and Bal, 2013, Gross, 2013, Sengupta *et al.,* 2013). Thereafter more antibiotics and derivatives were developed, such as additional β-lactams and vancomycin. However, within 8 years of commercial use of these antibiotics, resistance was detected and these antibiotics no longer worked in some cases (Sengupta *et al.,* 2013, Spellberg and Gilbert 2014). The prevalence of antibiotic resistance is not limited to the medical environment. The agricultural industry uses antibiotics extensively on livestock, often irresponsibly which leads to accelerated rate of the emergence of resistance (Bragg *et al.,* 2014). Once antibiotic resistance has emerged, it easily spreads over vast areas in short amounts of time (Hawkey and Jones 2009, Davis *et al.,* 2015). Resistance genes such as the blaCTX-M family of ***Extended-Spectrum β-Lactamase (ESBL)*** genes have spread globally within 30 years in the dairy industry (Canton *et al.,* 2012, D'Andrea *et al.,* 2013). Antibiotic resistance like this is never isolated, but finds a way to weave into almost every industry. The presence of resistant strains reported in dairy cows may not be isolated to the dairy industry only. A study has shown that these resistance genes may be able to find their way into the food industry through the manure used to fertilise crop fields (Udikovic-Kolic *et al.,* 2014).

Currently there are little to no novel antibiotics produced as the pharmaceutical industry has very little financial incentive for their production (Allen *et al.,* 2014). Alternatives to antibiotics are numerous. Vaccines work to prevent both viral and bacterial infections by stimulating specific immune protection. Yet, there is limited cross-protection from pathogenic strains and it is costly to administer to an animal or human population (van Panhuis *et al.,* 2013). The mode of action of immune response in both animals and humans makes it very

difficult to consistently obtain 100% protection with any bacterial vaccine. New generation vaccines and reverse vaccinology could improve the current levels of protection offered by first generation vaccines. However, many of these new generation vaccines are still in the developmental process. Alternatively, phage therapy could be applied for infectious disease treatment as they are target-specific and can be synergistic with antibiotics (Allen *et al.,* 2014). In addition, multiple bacteriophages can be used to hinder the development of resistance and studies have shown the efficacy of topical applications (Brussow 2007). However, there is potential for resistance development and the high levels of specificity of bacteriophages hampers administration against multiple subspecies (Johnson *et al.,* 2008, Chan *et al.,* 2013). Alternative treatment options such as bacteriocins, antimicrobial peptides and using competitive bacteria are possible but all of these methods are still in development, with their own safety concerns (Fox and Blommel 2009, Cotter *et al.,* 2013, Allen *et al.,* 2014). The 'post-antibiotic era' is upon us, with the only other viable option to be used on a large scale for industry being disinfectants. At the moment, good biosecurity and disinfection may be our only hope in a post-antibiotic era (McDonnell and Russell 1999).

3. RESISTANCE TO DISINFECTANTS AND THE IMPACT OF LACK OF PROPER BIOSECURITY MEASURES

The worldwide emergence of resistance is accelerated by the abuse and improper use of disinfectants (Bragg *et al.,* 2014). This provides the opportunity for the acquisition of genetic elements that confer resistance to other previously non-resistant strains (Hawkey and Jones 2009, Sengupta *et al.,* 2013, Bragg *et al.,* 2014). A lack of understanding of the principles behind the mechanism of disinfection and of biosecurity leads to inadequate use. This allows for disinfectant use at sub-MIC levels (Ventola, 2015). Over the time, this results in the selection of bacteria, able to withstand a given concentration of the disinfectant therefore, increasing the prevalence of resistant strains. A higher presence of these resistant strains in the population leads to contamination and mortalities (Spellberg and Gilbert, 2014).

The increased demand for less preservatives, less processed food and more ready-to-eat food; coupled with a lack of good biosecurity will

inevitably lead to outbreaks of food borne disease. The South African outbreak of listeriosis in 2017 (caused by *Listeria monocytogenes*) resulted in a total of 674 confirmed cases and 183 reported deaths, although these numbers are assumed to be much greater in reality (WHO 2018). The source of the outbreak was traced back to a ready-to-eat, processed meat product called '***Polony***'. The food processing company and its associated retailers distribute this product throughout South Africa and to 15 other African countries. The economic implications of extensive recalls, widespread public panic and a loss of consumer confidence can be crippling for any company (Piet *et al.,* 2016). The risks by far outweigh the advantages of implementing good bio-security in any industry.

Biosecurity is the least understood and studied aspect of disease control in both the poultry industry and hospital environments (Dancer, 2011, Lin *et al.,* 2011, Bragg *et al.,* 2014). In hospital environments, bacterial clinical isolates are found to be less susceptible to common disinfectants compared to the culture collection type strains (Dancer 2011). This is usually due to confusion over dis-infecting responsibilities in hospitals which lead to poor disinfection and the emergence of multi-drug resistant strains of nosocomial pathogens (Dancer 2011). In 2011, the fourth leading cause of disease in developed countries was nosocomial infections (Guggenbichler *et al.,* 2011). In the United States about 90000 fatalities occur every year due to hospital acquired infections. This number is assumed to be substantially worse in developing countries (Lin *et al.,* 2011). These resistant bacteria are not isolated in the hospital environment as they have also been identified in outpatients (Buffet-Bataillon *et al.,* 2012). The agricultural industry is under the same stress as it is perceived as normal to lose livestock as a result of multi-drug resistant bacterial infections. This is in spite of extensive use of antibiotics and dis-infectants (Bragg *et al.,* 2014).

Antibiotic and disinfectant resistance is possibly the most threatening problem in human and animal healthcare. It remains unchecked, every surgery, whether a major or minor procedure, would have the potential to be a high risk and life threatening process (Davies *et al.,* 2011). In 2001, this prompted the experts at the WHO to release a document that explained preventative measures to slow down the emergence of highly resistant strains called the WHO global strategy

for containment of antimicrobial resistance (Buffet-Bataillon *et al.,* 2012). It is important to note that any use of disinfectants whether they are used correctly or not, results in selective pressure for resistance (Tansirichaiya *et al.,* 2018).

4. IS THERE ANY LINK BETWEEN ANTIBIOTIC AND DISINFECTANT RESISTANCE?

There has been speculation of a link between resistances to different anti-microbial, known as cross-resistance. Templeton and co-workers (2009) found that antibiotic resistant in *Escherichia coli* strains were more chlorine tolerant than strains that were not resistant to antibiotics. Khan and co-workers (2016) went on to test the MIC values of chlorine tolerant bacteria against a variety of antibiotics. It was concluded that a significant (but weak) correlation between tolerance to the disinfectant and antibiotics did exist.

This cross-resistance may be due to a common underlying mechanism such as the multidrug membrane efflux pump or due to an accumulation of genetic mobile elements that confer resistance or tolerance to antibiotics and antimicrobials including disinfectants (Russel, 2002, Zhou *et al.,* 2017). Efflux pumps are the most prevalent mechanism of resistance to disinfectants and can be specific or used for many antimicrobial compounds in multidrug resistance pumps (Piddock, 2006, Hassan *et al.,* 2010). However, the proposed link is inconsistent and contradicting studies have also shown that this correlation is only present on rare occasions (Li *et al.,* 2012, Zhou *et al.,* 2017). Cross-resistance is highly variable, dependant on the type of antimicrobial and the bacterium. Simply because a bacterium is resistant to an antibiotic does not necessarily mean it will exhibit higher tolerance to disinfectants (Russel, 2002, Zhou *et al.,* 2017).

5. MECHANISMS OF RESISTANCE TO DISINFECTANTS

It is believed to be very rare for bacteria to develop resistance to disinfectants, as many disinfectants are complexes to have multiple target sites (Gnanadhas *et al.,* 2013). It is assumed that some microorganisms are prone to be more resistant than others spore forming bacteria (*Bacillus* spp., *Clostridium* spp.), naked viruses and gram-negative

bacteria are usually more tolerant of disinfectants (Russel and Maillard 1997, Sattar 2007). This is due to differences in cellular structure and composition as well as the physiology of the bacterial cells (Bragg *et al.,* 2014), although variation within the nature of the disinfectants as well as the actual organisms do exist (Russell 1997). When resistance is discovered, understanding the mechanism is important so that we know how to overcome it. Intrinsic resistance refers to the natural ability of a bacterial cell to be less susceptible to an antimicrobial agent whereas acquired resistance is due to genetic changes by mutation or acquisition of genetic material through horizontal gene transfer (plasmids) (Russel and Maillard 1997). In order for any disinfectant or antimicrobial agent to be effective, it needs to reach its target site in high enough concentration. Therefore, many mechanisms work on decreasing the concentration of antimicrobials in the cell. The mechanisms of resistance or tolerance to disinfectants include:

5.1 Phenotypic Changes

In nutrient poor environments, bacteria form capsules, spores and/or biofilms which increase tolerance and resistance to numerous disinfectants (Brown and Gilbert, 1993, Gilbert *et al.,* 2002). The resistance of *Pseudomonas aeruginosa* against benzalkonium chloride increased 100-fold while it was a part of a biofilm (Gilbert *et al.,* 1990, Gilbert and McBain 2003). Numerous cases are recorded where bacteria have grown inside bottles of disinfectants and antiseptics themselves (Marrie and Costerton, 1981). *Serratia marcescens* has been found on multiple occasions in such cases (Marrie and Costerton, 1981). Biofilm formation was responsible for the prolonged survival of *S. marcescens* in 2% chlorhexidine (Marrie and Costerton, 1981). Cells within biofilms have different characteristics to free planktonic cells, this often includes an increased resistance to disinfectants. Various conditions contribute to resistance of biofilms, these include slow growth and/or induction of the *rpoS*-mediated stress response (Gilbert *et al.,* 2002). In addition, changes to biofilm architecture and physical or chemical variations of exo-polysaccharides can confer resistance by excluding disinfectants from the bacterial population. Bacteria in biofilms may exhibit a unique resistance profile dependant on the heterogeneous nature of the biofilm, with several mechanisms at work simultaneously (Mah and O'Toole 2001).

The envelope organisation and architecture differences in Gram-negative and Gram-positive bacteria, contribute to disinfectant susceptibility and tolerance. It has been suggested that Gram-negative bacteria are less susceptible to some disinfectants because of the presence of Outer Membrane Proteins (OMPs). OMPs impair the diffusion and uptake of some disinfectants into the cell (Murtough *et al.,* 2001). This is seen in *P. aeruginosa*, *S. marcescens* and *Proteus* sp. as an increase in tolerance is attributed to hindered uptake of disinfectants (Russel and Maillard 1997). A change in the charge of the bacterial cell surface can also act to hinder disinfectant uptake. Tansirichaiya and co-workers (2018) found that in the presence of *gal* E (involved in lipopolysaccharide production) the host cell surface of *E. coli* was more positively charged. This in turn impaired the binding of positively charged disinfectants. This is the first time a housekeeping gene such as *gal* E has been shown to confer resistance to QACs and other cationic antimicrobials (Tansirichaiya *et al.,* 2018).

5.2 Efflux Pumps

Efflux pumps are the most widespread mechanisms of disinfectant resistance. Efflux proteins actively pump the antimicrobial agents out of the cell, therefore lowering the intracellular concentration (McDonnell and Russell 1999, Piddock 2006, Buffet-Bataillon *et al.,* 2012). Broad-specificity efflux pumps are over expressed and are responsible for cross-resistance in many bacteria by exporting antimicrobials out of the cell (Nikaido 1998a). One example is the chromosomally encoded acrAB efflux pump in *E. coli* (and other gram-negative bacteria). This allows for intrinsic resistance to a variety of antibiotics (ciprofloxacin, tetracycline, fluoroquinolone, β-lactams) and other chemicals (deoxycholate, SDS, phenylethylalcohol, acriflavine, ethidium bromide) (Ma *et al.,* 1993, 1994; Nikaido 1998b).

5.3 Inactivation of Disinfectants

Along with cell wall modification and efflux pumps, another intrinsic method of resistance involves inactivation of disinfectants. Chlorhexidine, used for skin disinfection prior to surgery, is degraded by *Serratia marcescens* and *Pseudomonas aeruginosa*, posing a threat to bio-security in operating theatres (Ogase *et al.,* 1992). Strains of *Pseudomonas fluorescens* were found to have the ability to degrade

didecyldimethyl ammonium chloride to decyldimethylamine and dimethylamine as intermediates. These strains were able to degrade QACs by a process of N-dealkylation, resulting in increased resistance to these disinfectants (Nishihara *et al.,* 2000).

The crystal structure of the functional unit or monomer of a representative protein of each group is shown where available. The MFS and SMR families crystal structures include the substrate represented as a green sphere. The orientation of these proteins within the cytoplasmic membrane (CM) and outer membrane (OM) of Gram-negative bacteria are shown. The ABC super family requires hydrolyses of ATP to provide energy for active transport, which is catalysed by the nucleotide binding domains (NBDs) of the transporter. The crystal structure of *S. aureus* Sav 1866 (pdb: 2HYD) functional unit of the ABC transporter consists of two trans-membrane domains (TMDs) and two NBDs. The five singlet system secondary active transporter families/ super families are MFS, MATE, PACE, SMR and AbgT. Representative transport proteins are shown as crystal structures of *E. coli* MFS Mdfa (pdb:4ZOW), *V. cholerae* MATE NorM (NorM_VC) (pdb: 3MKT), *E. coli* SMR EmrE (pdb:3B5D) and *N. gonorrhoeae* AbgTM trF (pdb: 4R1l). The RND superfamily has multiple components spanning across the cytoplasmic membrane and outer membrane each highlighted in a different colour. Crystal structures of these components are of *E. coli* AcrB (pdb: 2DRD) representing the integral CM protein, AcrA (pdb: 2F1M) the membrane fusion protein (MFP), and TolC (pdb: 2VDE) the OM factor (OMF) of the RND efflux system are illustrated. (Chitzas and Brown 2017).

5.4 Target Alteration

Instead of inactivating the disinfectants themselves, target sites of the disinfectants can be altered to inhibit their activity. Triclosan, a component of surface disinfectants with antibacterial and antifungal properties inhibits fatty acid synthesis in bacteria. Triclosan targets Fab-I, an enoyl-acyl carrier protein reductase essential for bacterial fatty acid synthesis. A missense mutation in the Fab-I gene, alters the target site of the protein, and is responsible for resistance of the bacteria to triclosan (Heath *et al.,* 1999). Additional studies have shown that over expression of Fab-I in *E. coli* can confer resistance as well (Tansirichaiya *et al.,* 2018). In *Mycobacterium tuberculosis*, inhA (an

analogue of fabI) is the target for ethionamide and isoniazid. However, a point mutation within this gene (inhA) confers resistance to this bacterium (Banerjee *et al.,* 1994). It has also been suggested that in *E. coli*, phenol resistance was seen to be conferred by changes in the outer membrane proteins as well as permeability changes in the lipid membrane (Zhang *et al.,* 2011). Altering the targets of typical phenols and therefore, their antimicrobial ability. This ability to modify the outer membrane is one reason why gram-negative bacteria have a greater advantage in increasing resistance and tolerance to antimicrobials and disinfectants (Murtough *et al.,* 2001). In addition, complex cell walls such as those in *Mycobacteria* have unique target sites for disinfectants and thus added targ*et al*teration opportunities. *Mycobacteria* possess water-filled porins that are necessary for the transport and activity of various components of disinfectants and sanitising products (methylenbisoxazolidine, isothiazolinones and octenidinedihydrochloride) (Frenzel *et al.,* 2011). The absence of these porins (MspA and MspaA-like porins) is responsible for reduced susceptibility to these compounds without compromising physiological function in the bacterium (Frenzel *et al.,* 2011).

5.5 Horizontal Gene Transfer

Horizontal gene transfer is a well-known mechanism for the development of disinfectant resistance. Resistance genes can be passed from one bacterium to another, plasmids can be transferred conferring resistance and transposable elements have been known to play a role as well (Cooksey 1987, Russel 1997). *Staphylococcus* species such as *S. aureus* decrease susceptibility to disinfectant compounds by plasmid-mediated mechanisms (Townsend *et al.,* 1983a, b, 1984). In addition to this, reduced susceptibility observed in *S. aureus* can be due to the acquisition of thesh-fab I allele from *Staphylococcus haemolyticus* by horizontal gene transfer (Ciusa *et al.,* 2012). The increased prevalence of these transferable elements in a bacterial population is due the positive selective pressure brought about by the presence of antimicrobial compounds present in disinfectants at sub MIC concentrations (Ciusa *et al.,* 2012). Bacteria in aquaculture environments have been shown to accumulate mobile genetic elements (integrating conjugative elements) which can confer resistance to QACs, heavy metals and rifampicin (Rodriguez-Blanco *et al.,* 2012). Additional genetic material coding for degradative enzymes was also reported.

These enzymes include o-phospho transferases, n-acetyl transferases and o-adenyl transferases capable of degrading antimicrobial compounds (Wright *et al.,* 1998).

5.6 QAC Resistance Genes

Quaternary Ammonium Compounds (QACs) are widely used antimicrobials with an effect against a broad range of microorganisms (Hegstad *et al.,* 2010). The bactericidal effect of QACs is brought about by disrupting the cell wall which subsequently leads to leakage of cytoplasm out of the cells. The systemic use of QACs in several products and across different industries makes the emergence of QAC resistance alarming. Various mechanisms of QAC resistance have been discussed already in this review. However, the genetic side of resistance in particular is becoming more important due to the sequencing technology available today.

Acquired resistance to QACs is well documented in *S. aureus* and *E. coli* (Lyon and Skurray 1987). The QAC genes in *Staphylococcal* species are mostly plasmid borne and encode for efflux pumps, in particular the membrane proteins that export harmful molecules conferring resistance against various compounds including disinfectants (QACs after which the genes are named) and some antibiotics (Langsrud *et al.,* 2003, Hegstad *et al.,* 2010, Wassenaar *et al.,* 2015). The QAC resistance genes were named as they were discovered, Qac A is the protein encoded by the gene *qac* A in the pSK1 family of plasmids (Wassenaar *et al.,* 2015). Staphylococcal multidrug-resistant gene qacA has been associated with resistance to monovalent and divalent cations, ethidium bromide, intercalating dyes, benzalkonium chloride and chlorhexidine (Littlejohn *et al.,* 1992). However, Qac B (encoded by *qac* B) differs as it confers resistance to QACs and intercalating dyes but little or no resistance to diamidines or chlorhexidine (Lyon and Skurray 1987, Grinius *et al.,* 1992, Grinius and Goldberg 1994). Both *qac* A and *qac* B require a transcriptional regulator *qac* R, the transcriptional repressor QacR is needed for expression of both these genes (Peters *et al.,* 2009).

The QacC protein is encoded for by the *qacC* gene (also known as smr), this protein has been identified as a homolog to an efflux protein in *E. coli* named EmrE (Grinius and Goldberg 1994). The protein name QacD is no longer in use as it was identified to be the same as

QacC and now is referred to as QacC (Wassenaar *et al.,* 2015). The next addition to the Qac resistance gene family was QacE, it was first described in *E. coli* of which functionally active derivatives (QacEΔ) have been identified in other bacterial genera (Kazama *et al.,* 1998). Subsequently, QacF was described, it was detected in several gram-positive bacteria but no homologs have been detected in *Staphylococcus* yet (Ploy *et al.,* 1998). QacG and QacH were detected at similar times both conferring high level ethidium bromide resistance with QacG additionally conferring resistance to benzalkonium chloride (Heir *et al.,* 1999a). QacJ was subsequently described and there after QacZ was detected in *Enterococcus faecalis* (Bjorland *et al.,* 2003, Braga *et al.,* 2011). There is a definite division within these QAC resistance genes and the proteins they encode for- QacA and QacB are members of the major facilitator super family (MFS); whereas the rest (QacC, QacE, QacF, QacG, QacH, QacJ and QacZ) are members of the small multidrug resistance (SMR) protein family (Heir *et al.,* 1999b, Wassenaar *et al.,* 2015). These two families evolved separately and therefore are genetically different; the functional similarities are an illustration of parallel evolution. QacE, QacF and QacZ are the only proteins (and corresponding genes) not present in *Staphylococcal* species (Gillings *et al.,* 2009, Zmantar *et al.,* 2011, Wassenaar *et al.,* 2015).

In clinical isolates the *qac* gene is commonly found in class 1 integrons, in environmental isolates the class 1 integrons contain diverse *qac* gene sets (Gillings *et al.,* 2009). The integrons collect resistance cassettes and can readily pick up new resistance determinants on plasmids, proving to be an efficient system to travel between different species (Hegstad *et al.,* 2010, Bragg *et al.,* 2014). The *qac* genes are not the only ones that encode for QAC resistance. In *E. coli*, five additional chromosomally-encoded genes confer QAC resistance namely: sugE, emrE, ydgE/ydgF and mdfA (Bay and Turner 2009). The gene mdfA like qacA/B, belongs to the MFS super family whereas the rest belong to the SMR family (Edgar and Bibi 1997, Bay and Turner 2009). Among all of these QAC resistance genes sugE (p), qacE, qacEΔ1, qacF and qacG are located on mobile genetic elements along with different antibiotic resistance genes allowing for the development of cross-resistance (Zou *et al.,* 2014).

Identifying and characterising genes that confer disinfectant resistance has never been more important. The advances in sequencing techno-

logy has allowed for a deeper understanding of resistance mechanisms and shed light on the possible ways to combat resistance.

6. THE USE OF SEQUENCING TECHNOLOGY IN ANALYSIS AND CHARACTERISATION OF RESISTANCE TO DISINFECTANTS

Next generation sequencing is new technology that can help with an old problem, as full genome sequencing is proving to be a vital tool in the race against bacterial resistance (Koser *et al.,* 2014). Sequencing technology has allowed for identification and analysis of bacteria without the need to culture them (Abdallah *et al.,* 2017). This allows for rapid identification and characterisation of bacteria that have never been cultured and/or that no one has ever seen before (Buermans and Dunnen 2014, Austin 2017). Whole genome sequencing is allowing for advances in new treatments due to the identification and characterisation of resistance mechanisms. In 2005, 454 pyrosequencing allowed for the identification of the target site of the anti-TB agent Bedaquiline (Andries*et al.,* 2005). It identified the F0 subunit of ATP synthase as the target region. Thereafter, hundreds of TB-causing *Mycobacterium* strains were sequenced to confirm this target gene was conserved. This information subsequently allowed for the approval of the first novel class of anti-TB agents in 40 years (Andries *et al.,* 2005, Zumla *et al.,* 2013).

In clinical settings, sequencing is used to distinguish between re-infection from a primary infection, or infection with a new bacterium. Comparative analysis of the genome sequence can confirm the presence of a new bacterium or one from a previous infection. This allows clinical staff to know if their environment or equipment is still harbouring the pathogen or if the staff themselves is carriers of the infection allowing for confirmation of effective biosafety measures or if improvements are needed (Niemann *et al.,* 2009, Eyre *et al.,* 2014, Koser *et al.,* 2014).

Whole genome sequencing could be especially important for surveillance of reoccurring diseases and those with high mutation rates and variants. Sequencing allows for identification of various resistant strains and tracking their movement between industries and countries (Bryant *et al.,* 2013, Nurwidya *et al.,* 2018). New mutations conferring resistance and mutation rates can be monitored as the genomes are

sequenced over time (WHO 2014, Grundmann 2014). Systemic studies at the genomic, epigenomic and transcriptomic levels of more than 25 000 cancer genomes revealed a range of oncogenic mutations (Roukos 2010). This data is currently being used in the development of new cancer therapies.

Bacterial isolates that are of interest, due to their resistant capabilities can be analysed using sequencing techniques. Parallel sequencing with related or type strains followed by a bioinformatics-based approach will allow the sequences to be aligned. Once a reference alignment has been formed, the sequences can be examined for variants, mutations and the presence of antibiotic resistance genes or pathogenicity islands by comparison with the reference genome and those in public databases. Any variation found can be evaluated in a functional context and could point to specific disinfectant resistance mechanisms (Mardis 2008).

Safi and co-workers (2013) used whole genome sequencing to study the evolution of ethambutol resistance in *Mycobacterium tuberculosis* complex (the causative agent in TB). In doing so, it was discovered that resistance in this bacterium developed in a step-wise manner and a novel resistance mechanism was described for the first time (Safi *et al.,* 2013). Once the mechanism of resistance is known, this may help in deciding which treatment options are best to combat this particular strain (Koser *et al.,* 2014). Thus, personalised medicine may be enhanced with sequencing methods which may become a primary diagnostic tool in the future (Harris *et al.,* 2010, Grad *et al.,* 2014).

The advancements in sequencing methods provide us with a greater insight into bacterial genomics and the resultant resistance it confers. The emergence and re-emergence of multi-drug resistant bacterial infections is a growing public health problem, accentuated by a decrease in antimicrobial drug discovery (Pop and Salzberg 2008). The genomic data obtained is a useful tool in developing novel diagnostic assays, as well as new therapeutic options at a personal and population level (Punina *et al.,* 2015). The amount of data that can be generated with sequencing technology is infinite and so new software and algorithms will need to be developed in parallel. Genome sequencing is currently used to supplement real-time diagnostic methods in clinical settings (Pop and Salzberg 2008). In order for its use to become more

systemic, integrated and easy-to-use bioinformatics toolsets for simple data interpretation needs to be established, as well as standards for verification and validation of results (Pop and Salzberg 2008). Nevertheless, this technology has the potential to direct day to day infection control and may even give rise to the field of personalised genomic epidemiology (Koser *et al.,* 2014, Punina *et al.,* 2015).

7. CONCLUSIONS

Disinfectants are currently used on a large scale in many industries. A few decades ago resistance to disinfectants was unheard of. Now resistance to disinfectants is becoming more prevalent and we need to start acting soon if we want to save our disinfectants. There are three things we can do to prevent this, firstly we need to reduce the overuse of disinfectants and therefore lower the selective pressure on bacteria to develop resistance (Murtough *et al.,* 2001). Efforts should be made to ensure that only disinfectants of high standard with proven efficacy should be used in the agricultural and health-care sectors. There are restrictions on the use of antibiotics but none on the use of other antimicrobials. Therefore, disinfectant stewardship should be implemented as it is with antibiotics. Secondly, infection control measures need to be put in place to reduce human to human and animal to human transmission (Hawkey and Jones 2009). Finally, emphasis must be put on research into alternative methods of bio-control and safety (Allen *et al.,* 2014).

Sequencing technology has allowed for the identification and characterisation of numerous genes and the subsequent conferred resistance. These genes can be passed between different strains, species and genera as demonstrated by the QAC resistance genes (Ciusa *et al.,* 2012). The prevalence of resistance to disinfectants is not a rare occurrence as it used to be. If we cannot look after the disinfectants we are currently using, we will be facing the same problems that we have now with antibiotics. However, with proper bio-security measures and the responsible use of disinfectants this can be avoided.

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