Trend Analysis of Antibiotic Resistance in in KwaZulu Natal: A Retrospective Study 2011- 2015

By

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This is the dissertation in which the chapter is written as a research publication, with an overall introduction and final summary.

This is to certify that the content of this dissertation is the original research work of Ms Miriam Patel.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABR	Antibiotic Resistance
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Testing
BSI	Blood Stream Infection
CAESAR	Central Asian and Eastern European Surveillance of Antimicrobial Resistance
CDC	Centers for Disease Control
CLSI	Clinical Laboratory Standards Institute
CPE	Carbapenemase-Producing Enterobacteriaceae
CRE	Carbapenem Resistant Enterobacteriaceae
EARS-Net	European Antimicrobial Resistance Network
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centers for Disease Control
ESBL	Extended Spectrum β-Lactamase
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa*, and *Enterobacter spp.*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GAP	Global Action Plan (on Antimicrobial Resistance)
GARP	Global Antibiotic Resistance Partnership
GASP	Gonococcal Antimicrobial Surveillance Programme
GERMS-SA	Group for Enteric Respiratory and Meningeal Disease Surveillance in South Africa
GLASS	Global Antimicrobial Surveillance System
HAI	Hospital Acquired Infection
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
MCC	Medicines Control Council
MDR	Multi-drug Resistant
MIC	Minimum Inhibitory Concentration

MRSA	Methicillin-resistant *Staphylococcus aureus*
MYSTIC	Meropenem Yearly Susceptibility Test Information Collection
NDoH	National Department of Health
NDP	National Drug Policy
NGO	Non-Governmental Organization
NICD	National Institute for Communicable Diseases
NTS	Non-typhoidal Salmonella
PAHO	Pan American Health Organization
SAB	*Staphylococcus aureus* Bacteremia
STG	Standard Treatment Guidelines
STI	Sexually Transmitted Infection
TB	Tuberculosis
TICU	Trauma Intensive Care Unit
UTI	Urinary Tract Infection
WHO	World Health Organization

ABSTRACT

Objective: Antimicrobial resistance is a global phenomenon which is limiting treatment options for common infections resulting in poor clinical outcomes, increased mortality and increased cost of healthcare. Antibiotic resistance trends in pathogen-drug combinations stipulated in the Global Antimicrobial Surveillance System (GLASS) of the World Health Organization were investigated for the period 2011-2015 in the province of KwaZulu Natal, South Africa.

Methods: Antibiotic susceptibility data from blood, urine, faecal and urethral/cervical samples was retrospectively analyzed from six public hospitals. Pathogens included *Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Salmonella spp., Acinetobacter baumannii, Staphylococcus aureus, Shigella spp. and N. gonorrhoea.* Results were analyzed as MIC50, MIC90, percentage resistance, incidence of monitored infections in the population and proportion of non-susceptible infections per pathogen. Results were also evaluated against South African treatment guidelines. Significant differences in resistance proportions by year were identified using the Pearson χ^2 test. Comparison of MIC50 were analysed using the equality-of-medians test.

Findings: Urine samples were most abundant (61.22%, n= 33 018) and *E. coli* (52%) was the most common pathogen. Most isolates were multi-drug resistant. Resistance to third and fourth generation cephalosporins and fluoroquinolones increased in *K. pneumoniae, E. coli* and *Shigella spp*. over the 5-year period. Notable changes in resistance were: *K. pneumoniae* from blood samples to carbapenems (1 – 26%, p< 0.001) and *A. baumannii* to carbapenems (69% - 50%, p-value not available). Susceptibility to antibiotics recommended in treatment guidelines was >50% for most pathogen-drug combinations.

Conclusion: The results of this study show that antibiotic resistance in hospitals in KwaZulu-Natal generally increased from 2011 to 2015, although some pathogen-drug combinations showed a plateau or decline in resistance necessitating a review of the existing treatment guidelines. To our knowledge, this is the first South African report on ABR using GLASS metrics. There is a need for more extensive research in order to build an accurate, comparable picture of ABR in South Africa.

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Chapter 1 Introduction and Literature Review

1.1.Introduction

The increase in the incidence and spread of infectious diseases has led to an increase in the use of antimicrobial drugs, in turn causing an increase in antimicrobial resistance (AMR) (Mendelson & Matsotso, 2015). The term AMR applies broadly to resistance in all microbial pathogens including bacteria, parasites, viruses and fungi while antibiotic resistance (ABR) refers specifically to resistance of bacterial pathogens to antibiotics (Shaban, *et al*., 2013). While ABR is an anticipated result of antibiotic use, the spread of resistance is accelerated by preventable factors such as poor infection control, irrational antibiotic use and the use of substandard drugs (Hoffman, *et al*., 2015) (World Health Organisation, 2015 (c))

ABR is a global concern affecting both developed and developing countries to varying extents. While efforts are being made to delineate the incidence, prevalence and mechanisms of ABR, there is a need for quality, standardised, comparable data to provide an accurate picture of global trends in ABR (World Health Organisation, 2015 (b)).

The impact of the increasing incidence of ABR is far reaching and if measures are not implemented now, the resulting financial burden and mortality due to ABR is projected to be gravely high. The World Health Organisation (WHO) has released various publications with suggested action plans in order to curb the increase in resistance and in developed regions research around ABR has already been established for some years (Hoffman, *et al*., 2015).

ABR surveillance is vital in providing information to guide the development of strategies to curb the spread of ABR. Established surveillance programmes such as the Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) and the European Antimicrobial Resistance Surveillance Network (EARS-Net) have provided useful data but are limited in a global context due to differences in data collection methods, limited data-sharing and the tendency to focus on specific pathogens or regions (World Health Organisation, 2014 (a)). Limited resources in terms of funding, laboratory capacity and human resources are obstacles in obtaining useable surveillance data in developing countries (Vernet, *et al*., 2014).

In May 2015 the Global Action Plan (GAP) on AMR was adopted by the World Health Assembly committing WHO member states to developing National Action Plans to combat AMR. A key component of the GAP is surveillance, specifically a uniform system of surveillance and reporting of ABR. Subsequently the Global Antimicrobial Surveillance System (GLASS) was developed, aimed specifically at ABR in specified bacterial pathogens and the WHO has called on member states to implement this programme. The key to the success of GLASS is in the cooperation of as many countries as possible to as large an extent as possible in order to gain baseline data from which further strategies can be developed. The GLASS manual for early implementation was published in 2015 and outlines a comprehensive system which aims to provide comparable, validated data which can be shared on a global scale in order to guide future interventions against the spread of ABR World Health Organisation, 2015(b)).

In South Africa, AMR in pathogens other than tuberculosis (TB) and Human Immunodeficiency Virus (HIV) has increasingly gained attention. Treatable illnesses such as diarrhoeal infections and urinary tract infections (UTIs) are among the common conditions burdening our public health system (Mendelson & Matsotso, 2015). Budget constraints, especially in public sector healthcare facilities, limit the choice of antibiotics to those stipulated in the Standard Treatment Guidelines published by the national Department of Health. There is a concern that the susceptibility of pathogens to the empiric antibiotics listed in the STGs is limited, which raises concerns about exacerbating resistance, wastage of money on ineffective treatment and most importantly, treatment failure. The treatment strategies for common infections can be greatly enhanced if informed by current surveillance data. (Perovic, *et al*., 2014) While surveillance studies have been conducted, mainly by academic institutions, the data available is not representative enough to guide national strategies.

1.2. Literature Review

1.2.1. Surveillance of Antimicrobial Resistance:

Public health surveillance can be defined as the collection and analysis of data in order to monitor and manage public health threats and concerns (World Health Organisation, 2017 (a)). The global surveillance report published in 2014 by the WHO, showed the burden of AMR in Member States to be extensive. The report also illustrated the lack of standardised methodology that limited the usefulness of existing surveillance data. Data from hospital based healthcare settings was shown to be more readily available than data from community based facilities and there was also a bias in existing data in that the samples submitted for sensitivity analyses come from patients who were severely ill and were thus more likely to be infected with resistant pathogens. The survey upon which the report was based included the bacterial infections that exhibit high levels of resistance and the corresponding antibiotics commonly used to treat such infections (World Health Organisation, 2014 (c)).

While existing surveillance programmes have contributed towards combatting ABR, it is spreading such that there is still a deficit in the information required in order to develop evidence-based strategies on a global scale.

Surveillance of ABR is needed to guide clinical interventions to provide an optimal level of care, while limiting the spread of resistance. Rapidly spreading resistance is limiting treatment options for common infectious diseases, as is the case with the emergence of strains of untreatable gonorrhoea (World Health Organisation, 2014 (c)). While comprehensive surveillance of all infections caused by pathogens within a population would be ideal, sentinel surveillance is more realistic and sustainable. Sentinel surveillance is conducted on the population of a limited region seen to be representative of the rest of the population and can thus provide more detailed data over a longer period of time (World Health Organisation, 2016).

When conducting surveillance on ABR, samples that are representative of both hospital and community healthcare settings must be included as different infections are prevalent in each setting. While blood stream infections are prevalent in hospitals, gonorrhoea and food-borne diarrhoea are the common infections seen in a community health setting and urinary tract infections are prevalent in both. (World Health Organisation.(b), 2015) (World Health Organisation, 2014 (c)).

In resource limited regions such as Africa, Latin America and the Eastern Mediterranean region there are big gaps in information available as they are still in the early stages of combatting ABR (World Health Organisation, 2016). In such settings, there is limited financial capacity and inter-institutional collaboration to facilitate the implementation of strategies to curb ABR. In this regard, the Global Antimicrobial Surveillance System (GLASS) methodology provides the resources and guidelines to assist in the development of surveillance programmes. (Hoffman, *et al*., 2015)

1.2.2. The Global Antimicrobial Surveillance System (GLASS)

Increasing globalisation has increased the ease with which infections, and thus resistance, can spread. This makes ABR in any one region of the world a global concern and the WHO has made much progress in developing means to address this problem. The primary goal of the GAP is to sustain the ability to treat infectious diseases with safe medicines. The following strategic objectives were outlined in order to achieve this goal: “(1) improve awareness and understanding of antimicrobial resistance through effective communication, education and training, (2) strengthen the knowledge and evidence base through surveillance and research , (3) reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures, (4) optimize the use of antimicrobial medicines in human and animal health, (5) develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other interventions” (World Health Organisation, 2015 (a)). The GLASS programme aims to address the objective of obtaining standardised, comparable, validated data on ABR which can be shard on a global platform in order to guide prevention and control programmes (World Health Organisation, 2015 (b)).

Differences in Antimicrobial Sensitivity Testing (AST) methods, variable quality control and differences in the choice of pathogens for surveillance are just a few of the shortfalls of existing surveillance data. Inadequate laboratory facilities to perform pathogen testing as well as limited human resourced has contributed to the lack of surveillance data in under resourced regions. The

GLASS early implementation phase between 2015 and 2019 aims to set up basic surveillance standards and provide baseline data on ABR on a global scale based on national reports submitted by participating member states. While environmental and animal data is also needed in order to address the problem of ABR holistically, for the initial phase the focus will be on human pathogens (World Health Organisation, 2015 (b)).

The WHO has outlined priority pathogens based on specimen types, to be investigated in this phase of GLASS. Specific pathogen-drug combinations have been indicated in the GLASS manual, illustrated in Table 1. The rationale for selecting blood, urine, faeces and cervical/urethral swabs as sample types is that they represent the incidence of bloodstream infections, urinary tract infections, gastrointestinal infections and gonorrhoea respectively. The pathogens included for each specimen type are commonly encountered in both a community and hospital setting and represent infections that have important public health implications. These samples are easy processed and pathogen identification is straightforward (World Health Organisation, 2015 (b)).

Table 1: Pathogen–antimicrobial combinations on which GLASS will gather data

Pathogen	Antibiotic Class	Antibiotic
Escherichia coli	Sulfonamides and trimethoprim Fluoroquinolones Third-generation cephalosporins Fourth-generation cephalosporins Carbapenems Polymyxins Penicillins	Cotrimoxazole Ciprofloxacin or levofloxacin Ceftriaxone or cefotaxime and ceftazidime Cefepime Imipenem, meropenem, ertapenem or doripenem Colistin Ampicillin
Klebsiella pneumoniae	Sulfonamides and trimethoprim Fluoroquinolones Third-generation cephalosporins Fourth-generation cephalosporins Carbapenems Polymyxins	Cotrimoxazole Ciprofloxacin or levofloxacin Ceftriaxone or cefotaxime and ceftazidime Cefepime Imipenem, meropenem, ertapenem or doripenem Colistin
A. baumannii	Tetracyclines Aminoglycosides Carbapenems Polymyxins	Tigecycline or minocycline Gentamicin and amikacin Imipenem, meropenem, ertapenem or doripenem Colistin
S. aureus	Penicillinase-stable β-lactams	Cefoxitin
S. pneumoniae	Penicillins Sulfonamides and trimethoprim Third-generation cephalosporins	Oxacillin Penicillin G Cotrimoxazole Ceftriaxone or cefotaxime
Salmonella spp.	Fluoroquinolones Third-generation cephalosporins Carbapenem	Ciprofloxacin or levofloxacin Ceftriaxone or cefotaxime and ceftazidime Imipenem, meropenem, ertapenem or doripenem

Shigella spp.	Fluoroquinolones Third-generation cephalosporins Macrolides	Ciprofloxacin or levofloxacin Ceftriaxone or cefotaxime and ceftazidime Azithromycin
N. gonorrhoeae	Third-generation cephalosporins Macrolides Aminocyclitols Fluoroquinolones Aminoglycosides	Cefixime Ceftriaxone Azithromycin Spectinomycin Ciprofloxacin Gentamicin

Patient data must be collected along with AST results and population data from the sample site. The surveillance data will be aggregated at a national level before reporting to the WHO annually (World Health Organisation, 2015 (b)).

Existing resources can be used in the implementation of GLASS but to facilitate proper data collection where surveillance has not yet been established, the WHO has made the WHONET software available as a data capturing and analysis tool. This software includes the capacity for core patient data as well as AST results, the analysis of which can provide trends in the distribution of resistance as well as comparable data to view the change in resistance patterns over time (World Health Organisation, 2015 (b)).

The implementation of GLASS will pave the way for the establishment of reliable surveillance systems. In the future, surveillance may be expanded to include other infections once the protocol and system for data collection and reporting has been established (World Health Organisation, 2015 (b)).

In using the GLASS methodology, the following information can be obtained:

- Population in which most AMR infections are presenting in terms of age and origin of infection (community or hospital)
- The extent of resistance based on epidemiological and laboratory data
- Changes in resistance patterns based on comparison of data year to year

Using the outlined method of data collection and analysis we can obtain an overview of trends in resistance in KwaZulu Natal which can be compared to data available from other regions, nationally and globally and may be used as baseline data for further studies.

These are also pathogens highlighted in the global priority pathogens list developed by the WHO in order to prioritise research and development into new antibiotics to treat antibiotic-resistant bacteria. Pathogens were grouped according to species and type of resistance and were classified as critical, high and medium priority. The following pathogens are classified as critical priority: carbapenem-resistant *Acinetobacter baumannii,* carbapenem-resistant *Pseudomonas aeruginosa and Enterobacteriaceae* resistant to carbapenems and third generation cephalosporins. The following pathogens were classified as high priority: vancomycin-resistant *Enterococcus faecium,* *Staphylococcus aureus* resistant to methicillin and vancomycin, clarithromycin-resistant *Helicobacter pylori*, fluoroquinolone-resistant *Campylobacter,* fluoroquinolone-resistant *Salmonella spp.* and *Neisseria gonorrhoea* resistant to fluoroquinolones and third generation cephalosporins. The following pathogens were classified as medium priority: penicillin-non-susceptible *Streptococcus pneumoniae,* ampicillin-resistant *Haemophilus influenzae and* fluoroquinolone-resistant *Shigella spp.* (World Health Organisation, 2017 (b)).

1.2.3. Existing Surveillance Programs:

Surveillance of ABR is essential in order to determine the extent of the problem and using that information, formulate measures to curb the incidence and spread of resistance. Surveillance programmes are carried out nationally in various countries as well as by independent organisations. In addition, many studies are carried out based on data from samples routinely sent to laboratories for clinical purposes. Existing surveillance programmes implement varying methodologies and cover different pathogens, antibiotics, specimen types and settings. Outlined below is an overview of various surveillance programmes and studies with a special focus on those that have included GLASS pathogens (World Health Organisation, 2014 (b)).

1.2.3.1. WHO Programmes

African Region

The Integrated Disease Surveillance and Response (IDSR) system was adopted in the WHO Africa region in 1998, initially to monitor severe outbreaks of preventable infections. The project has since expanded to a regional surveillance network which aims to provide information on priority infections by strengthening surveillance programmes in member states and improving the use of surveillance data to guide clinical interventions. Measures include personnel training, integrating surveillance systems to promote efficient use of resources and improving the sharing of surveillance data. The contribution of data to the network is variable, depending on the resources and laboratory capacity in each participating country. The Gonococcal Antimicrobial Surveillance Programme (GASP) was initiated by the WHO in order to facilitate the collection of isolates in African countries by providing technical assistance and training (Centres for Disease Control and Prevention , 2015).

The WHO instituted pilot programmes in Durban and Brits, South Africa, as part of a global pilot study in five cities in under-resourced countries. In addition to the two South African sites, data was collected from three sites in India. The objective of the study was to investigate the feasibility and potential for ABR surveillance in resource limited settings by collecting prospective and retrospective drug usage data. The South African sites included *E. coli* isolates from urine samples and *S. pneumoniae* and *H. influenzae* isolates from sputum samples. These isolates were tested against ampicillin, trimethoprim/sulfamethoxazole, cefalexin/cefuroxime, chloramphenicol and erythromycin. In terms of GLASS pathogens, at the Brits surveillance site, *E. coli* isolates were found to be 50 – 65% resistant to ampicillin, trimethoprim/sulfamethoxazole and cefalexin/cefuroxime. At the Durban site, *S. pneumoniae* isolates showed the highest resistance to trimethoprim/ sulfamethoxazole (55%) followed by erythromycin (19%) and chloramphenicol (2%). The results highlighted the extensive use of antibiotics in both India and South Africa, with certain drugs more commonly used in community health centres in the public sector compared to the private sector and vice versa. In terms of the capacity for surveillance it was found that all sites were able to provide data, however methodological and logistic constraints were potential barriers to obtaining comparable data over long periods of time (Holloway, *et al*., 2011). These sites would be ideal for the implementation of GLASS methodology, which would enhance the quality and reliability of the data obtained and address some of the shortcomings of this study.

A review of the extent to which countries in the WHO African region have implemented the WHO Policy Package on AMR showed that none of the countries had robust, representative national surveillance programmes on antimicrobial use and resistance. Pilot projects in various countries include but are not limited to (1) a study implemented in Gambia investigating the prevalence of and risk factors for the faecal carriage of resistant *Enterobacteriaceae* by food handlers in schools; a study involving the characterisation and susceptibility of *E.coli* in street food and raw beef in Tamale, Ghana; sentinel surveillance in Togo of ESBL producing *Enterobacteriaceae* in children under the age of five hospitalised for acute gastroenteritis and the correlation of antibiotic drug use and resistance found in humans, food-producing animals and retail foods in Uganda. In Rwanda, Kenya, Burundi and Tanzania a study investigating the prevalence and characterisation of ESBL producing *E. coli* in animals, humans and the environment has also been implemented. While the results of most of the abovementioned studies are not yet available, they highlight the capacity and potential for surveillance in Africa and provide useful data in terms of ABR as well as in terms of identifying limitations of surveillance methods. (Essack, *et al*., 2016).

The 2014 WHO report on AMR noted the following resistance ranges from 2-13 countries of the 47 member countries in the Africa Region):

- *E. coli* to third generation cephalosporins: 2 – 70% (13 countries)
- *E. coli* to fluoroquinolones: 14 – 71% (14 countries)
- *K. pneumoniae* to third generation cephalosporins: 8 – 77%(13 countries)
- *K. pneumoniae* to carbapenems: 0 – 4% (4 countries)
- Incidence of methicillin-resistant *Staphylococcus aureus* (MRSA): 12 – 80% (9 countries)
- *S. pneumoniae* to penicillin: 3 – 16% (5 countries)
- Non-typhoidal *Salmonella* to fluoroquinolones: 0 – 35% (9 countries)

- *Shigella spp.* to fluoroquinolones: 0 – 3% (4 countries)
- Decreased susceptibility to fluoroquinolones in *N. gonorrhoea:* 0 – 12% (2 countries) (World Health Organisation (a), 2014).

Region of the Americas

Latin American Antimicrobial Resistance Surveillance Network (ReLAVRA) is a surveillance network focussing on Latin America and was implemented by the Pan American Health Organisation (PAHO) in conjunction with the WHO. The system was implemented in 1996 and involves 21 countries, which submit surveillance data via National Reference Laboratories. There are four indicators pathogens, viz., *E. coli*, gonococci, *Klebsiella spp* and *S. aureus*. Based on the country-specific data available on the PAHO website, several countries appear to contribute to the surveillance network but to different degrees. For example, in 2013 there was data from 15 countries for *Klebsiella spp.* but only from 7 countries for gonococci. The general trend observed between the year 2000 and 2013 was an increase in ABR although the percentage of resistance fluctuated over the years as well as between countries. In 2013 resistance to third generation cephalosporins in *Klebsiella spp.* ranged from 19 – 84%, resistance to oxacillin, in hospital acquired *S. aureus* which indicates MRSA ranged from 34 – 78% and resistance to penicillin and ciprofloxacin in gonococci was more than 40% in most countries. Data on *E. coli* resistance was minimal, but data from Panama indicated that resistance to cefalotin, ciprofloxacin and trimethoprim/sulfamethoxazole ranged between 28 – 54% (World Health Organisation.(b), 2015) (Pan American Health Organisation, 2016).

The 2014 WHO report on AMR reported the following resistance ranges from 4-17 countries of the 35 member countries in the region of the Americas:

- *E. coli* to third generation cephalosporins: 0 – 48% (14 countries)
- *E. coli* to fluoroquinolones: 5 – 58% (16 countries)
- *K. pneumoniae* to third generation cephalosporins: 4- 71% (17 countries)
- *K. pneumoniae* to carbapenems: 0 – 11% (17 countries)
- Incidence of MRSA: 21 – 90% (50 countries)
- *S. pneumoniae* to penicillin: 0 – 48% (15 countries)
- Non-typhoidal *Salmonella* to fluoroquinolones: 0 – 96% (13 countries)
- *Shigella spp*. to fluoroquinolones: 0 – 8% (14 countries)
- Decreased susceptibility to fluoroquinolones in *N. gonorrhoea:* 0 – 31% (4 countries) (World Health Organisation, 2014 (a))

Eastern Mediterranean Region

Surveillance in this region so far has been largely disease-focussed and there is limited reliable data on the broader AMR situation. While the Eastern Mediterranean Regional Committee adopted resolutions to address AMR, political and economic unrest in this region makes the co-ordination of broad surveillance studies difficult. (World Health Organisation, 2014)

The 2014 WHO report on AMR reported the following resistance ranges from 2-5 countries of the 21 member countries in the Eastern Mediterranean region:

- *E. coli* to third generation cephalosporins: 22 – 63% (5 countries)
- *E. coli* to fluoroquinolones: 21 – 62% (4 countries)
- *K. pneumoniae* to third generation cephalosporins: 22 – 50% (4 countries)
- *K. pneumoniae* to carbapenems: 0 – 54% (4 countries)
- Incidence of MRSA: 10 – 53% (4 countries)
- *S. pneumoniae* to penicillin: 13 -34% (3 countries)
- Non-typhoidal *Salmonella* to fluoroquinolones: 2 – 49% (4 countries)
- *Shigella spp*. to fluoroquinolones: 3- 10% (2 countries)
- Decreased susceptibility to fluoroquinolones in *N. gonorrhoea:* 0 – 12% (2 countries (World Health Organisation, 2014 (a))

European Region

Many countries within the European Union have established national surveillance programmes and all are participants in the European Antimicrobial Resistance Surveillance Network – EARS-Net. Countries outside of the European Union tend to have less well-established systems. The Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) is a WHO initiative involving countries who are not part of the European AMR Surveillance Network. The first report was published in 2014 from data submitted by five of the CAESAR participating countries while the remaining countries are in various stages of implementation of the surveillance program (World Health Organisation, 2014 (b)).

AST results are obtained from blood and cerebrospinal fluid samples on eight bacterial pathogens of particular interest in terms of public health implications. These included *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter spp.*, *S. aureus*, *S. pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium*. Data includes AST information on selected antimicrobial groups and resistance patterns reported as country-specific data. The data for each country is given a level of reliability based on the extent to which the data was representative of the entire population (World Health Organisation, 2014 (b)).

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The WHO 2014 report on surveillance reported the following resistance ranges from 10-36 countries of the 53 member countries in the European Region:

- *E. coli* to third generation cephalosporins: 3 – 82% (35 countries)

- *E. coli* to fluoroquinolones: 8 – 48% (35 countries)
- *K. pneumoniae* to third generation cephalosporins: 2 – 82% (33 countries)
- *K. pneumoniae* to carbapenems: 0 -68% (31 countries)
- Incidence of MRSA: 0.3 – 60% (36 countries)
- *S. pneumoniae* to penicillin: 0 – 61% (31 countries)
- Non-typhoidal *Salmonella* to fluoroquinolones: 2 – 3% (29 countries)
- *Shigella spp*. to fluoroquinolones: 0 – 47% (10 countries)
- Decreased susceptibility to fluoroquinolones in *N. gonorrhoea:* 0 – 36% (17 countries)

(World Health Organisation, 2014 (a))

South-East Asia Region

AMR data in this part of the world appears to be limited aside from a few countries such as Thailand and India. In the Regional Strategy on Prevention and Containment of Antimicrobial Resistance published by the WHO in 2010 it was reported that no systematic prospective AMR studies had been conducted in the region database (World Health Organisation, 2010 (a)). In August 2012 "A Roadmap to Tackle the Challenge of Antimicrobial Resistance – A Joint meeting of Medical Societies in India" was conducted with the purpose of developing a plan for combatting ABR in Chennai, India. The Chennai Declaration, made by all delegates at the meeting, highlighted the roles of individuals in encouraging rational drug use, ABR surveillance, AST to guide treatment and improving infection control. The WHO was urged to provide technical and financial support in resource limited settings and most importantly co-ordinate initiatives on a global scale. In 2013 all 11 Member States of the region signed the Jaipur Declaration on AMR and agreed to contribute data towards a regional (Ghafur, *et al*., 2013).

The WHO regional strategy for the period 2010-2015 aimed to increase awareness about AMR, encourage the rational use of antibiotics as well as to institute surveillance systems with inter-regional collaboration. A subsequent report on a meeting of 20 member states of the SEA region indicated that the available resistance data was mainly on HIV, tuberculosis, measles and diarrhoeal diseases. Data also commonly included extended spectrum β-lactamase (ESBL) producing bacteria, MRSA and *N. gonorrhoea* as well as specific antibiotics such as penicillin and ciprofloxacin. The general trend was an increase in resistance with specific concern regarding the increasing resistance to cephalosporins and carbapenems (World Health Organisation, 2013).

Various organisations have conducted AMR surveillance in the SEA region including independent laboratories, academic institutions and NGO's. The Sri Lanka College of Microbiologists conducted surveillance on AMR in Gram-negative organisms from several surveillance sites and planned to expand the program to include Gram-positive organisms as well as more surveillance sites. In the Maldives the Indira Gandhi Memorial Hospital (IGMH) Laboratory information system was able to provide limited resistance data showing the increasing patterns of resistance in the country. Other countries which reported some form of AMR data analysis included Nepal, Timor-Leste, Bangladesh, and Indonesia. (World Health Organisation, 2013) The surveillance data available in the SEA region was often as a result of activity by groups such as the International Network on Rational Use of Drugs (INRUD), ReAct- Action on Antibiotic Resistance, International Network for the Demographic Evaluation of Populations and Their Health in Under-Resourced Countries (INDEPTH), Alliance for Prudent Use of Antibiotics (APUA), Health Action International (Asia Pacific) (HAIAP) and the Global Antibiotic Resistance Partnership (GARP). Since the call for action on AMR was made, countries within the region are reported to have adopted national action plans and have made progress in implementing AST, awareness and surveillance projects. (World Health Organisation South-East Asia, 2010)

The WHO 2014 report on surveillance reported the following resistance ranges from 2-5 countries of the 11 member countries in South-East Asia region:

- *E. coli* to third generation cephalosporins: 16 – 68% (5 countries)
- *E. coli* to fluoroquinolones: 32 – 64% (5 countries)
- *K. pneumoniae* to third generation cephalosporins: 34 – 81% (4 countries)
- *K. pneumoniae* to carbapenems: 0 – 8% (4 countries)
- Incidence of MRSA: 10 – 26% (3 countries)
- *S. pneumoniae* to penicillin: 47 – 48% (2 countries)
- Non-typhoidal *Salmonella* to fluoroquinolones: 0.2 – 4% (2 countries)
- Decreased susceptibility to fluoroquinolones in *N. gonorrhoea:* 0 – 5% (5 countries)
  (World Health Organisation, 2014 (a))

Western Pacific Region

In the 1980s, 14 Member States in this region agreed to collect AMR data on key pathogens, however this was disrupted due to other emergencies and co-ordination of AMR efforts between states was diminished although some countries continued surveillance on a national level. More recently, efforts have been made to re-establish AMR surveillance at a regional level (World Health Organisation (a), 2014).

The WHO 2014 report on surveillance reported the following resistance ranges from 4-16 countries of the 27 member countries in the Western Pacific region:

- *E. coli* resistance to third generation cephalosporins: 0 – 77% (13 countries)
- *E. coli* resistance to fluoroquinolones: 3 – 96% (16 countries)
- *K. pneumoniae* to third generation cephalosporins: 1 – 72% (14 countries)
- *K. pneumoniae* to carbapenems: 0 – 8% (9 countries)

- Incidence of MRSA: 4 – 84% (16 countries)
- *S. pneumoniae* to penicillin: 0 – 47% (10 countries)
- Non-typhoidal *Salmonella* to fluoroquinolones: 0 – 14% (9 countries)
- *Shigella spp*. to fluoroquinolones: 3 – 28% (4 countries)
- Decreased susceptibility to fluoroquinolones in *N. gonorrhoea:* 0 – 31% (12 countries) (World Health Organisation, 2014 (a))

1.2.3.2. Global and Regional Surveillance Programmes

The Alexander Project and the Survey of Antibiotic Resistance (SOAR)

Initiated in 1992 by GlaxoSmithKline, the Alexander Project gathered data until 2001 and included three prominent respiratory pathogens: *H. influenza, Moraxella catarrhalis and S. pneumoniae.* Isolates from adult patients with community-acquired respiratory tract infections were obtained from surveillance sites in the USA, Mexico, Brazil, South Africa, Saudi Arabia, Hong Kong and European countries making it the first of its kind to compare standardised, quality data on an international level. MICs were established for a panel of 15 antibiotics including β-lactams, macrolides, fluoroquinolones and trimethoprim/sulfamethoxazole. Trends in resistance for each pathogen within and across the different classes of antibiotics were established, with the MIC values providing key information for many papers published based on the project. (Felmingham, *et al*., 2005)

Between 1992 and 2001 there was an increase in multi-drug resistant infections with differences in resistance patterns between regions. Of specific interest is the data on *S. pneumoniae,* a GLASS pathogen, which showed high levels of penicillin-erythromycin co-resistance but a low prevalence of fluoroquinolone resistance in Europe and the USA. (Felmingham, *et al*., 2005).

After the Alexander Project was concluded in 2001, GlaxoSmithKline initiated the Survey of Antibiotic Resistance Study (SOAR) in 2002 which also tracks ABR in respiratory infections. The latest findings for the SOAR project were published in 2016 and data for the next cycle of publications is being collated. Results from the African countries, including the Democratic Republic of Congo, Ivory Coast, Republic of Senegal and Kenya, showed that resistance to penicillin and trimethoprim/sulfamethoxazole in *S. pneumoniae* ranged from 0 – 35% and 21 – 57% respectively. Although other antibiotics were included in the panel, penicillin and trimethoprim/sulfamethoxazole are reported here as part of the GLASS panel of antibiotics for *S. pneumoniae* (Kacou-Ndouba *et al.*, 2016)

SENTRY

The SENTRY Antimicrobial Programme is an ongoing initiative that was started in 1997 and is funded by various pharmaceutical companies. Data on both nosocomial and community acquired infections are submitted by sentinel hospitals in the Americas, Europe and the Asia-Pacific region.

The project includes the collection of surveillance data relating both to susceptibility data as well as investigation into resistance mechanisms. Reports on the data have been used as an indicator of the status of ABR in various regions of the world including South Africa (Masterton, 2008). Results cited in various studies will be discussed in more detail in the sections below.

Five major objectives of the programme were to monitor bacteraemia, outpatient respiratory tract infections, pneumonia in hospitalised patients, wound infections and urinary tract infections. The inclusion of these infections provided good insight into common infectious diseases which are of significance in terms of public health and the corresponding demographic and epidemiological data allowed for extensive comparison of resistance trends in each geographical region (Masterton, 2008).

A wide range of gram-positive and gram-negative bacteria have been investigated and numerous articles based on SENTRY data highlight trends in terms of specific pathogens, types of infections and antimicrobial agents. Of the GLASS pathogens, *S. aureus, A. baumannii, S. pneumoniae* and *E. coli* have been extensively monitored and the data shows that *S. aureus* was the most common causative pathogen of blood stream infections, pneumonia and soft tissue infections in almost all participating regions. The emergence of multi-drug resistant infections was clear, as was the distribution of resistance and variations in susceptibility patterns of pathogens across geographical regions. Between 1997 and 1999, susceptibility of *S. pneumoniae* isolates to penicillin and cefpodoxime varied from 6.8% and 9.2% in Canada to 17.8% and 22.9% in the Asia-Pacific region (Hoban, *et al*., 2001). A study conducted by Diekema *et a*l., (2001) reported that isolates obtained from sites in Canada and the USA were generally more susceptible to all recorded drugs but that the nosocomial isolates showed higher resistance rates to β-lactam antibiotics than community acquired isolates. This was in contrast to Latin America where higher rates of resistance were reported with no major difference in β-lactam susceptibility between nosocomial and community acquired isolates. (Diekema, *et al*., 2001)

Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program

The MYSTIC programme was started in 1997 and focussed on the susceptibility of nosocomial infections to meropenem and other antibiotic agents by measuring MIC values. The study focussed on the Americas, Europe and the Middle East and the data was used together with antibiotic pharmacokinetic/pharmacodynamics data in the Optimizing Pharmacodynamic Target Attainment using the MYSTIC Antibiogram (OPTAMA) Program to provide insight into the optimal dosage to prevent the development of resistance. In addition, meropenem usage data was also collected in order to correlate usage and resistance patterns. Both Gram-positive and Gram-negative bacterial isolates were tested including *S. aureus, P. aeruginosa, A. baumannii, Enterobacteriaceae* and many others. Over the years the results of studies based on MYSTIC data have shown that meropenem is one of the more active broad-spectrum antibiotics but resistance to carbapenems is on the increase (Turner, 2000). A study published in 2008 looking at 11 years of data from a paediatric ICU found that a number of MDR isolates were susceptible to meropenem but resistant to all other agents. The study also found that the consumption of cephalosporins decreased over the study period, however the use of carbapenems increased significantly, which can be correlated with the increasing incidence of carbapenem resistance that has emerged in recent years (Patzer, *et al*., 2008).

European Antimicrobial Resistance Surveillance Network (EARS-Net)

The European Antimicrobial Resistance Surveillance Network (EARS-Net) includes 30 countries and is a continuation of the European Antimicrobial Resistance Surveillance System (EARSS). Participating countries provide reports on the pathogens and antimicrobial agents under surveillance and have contributed towards a growing network of increasingly comparable AMR data. Representatives from Member States collate the AMR susceptibility data with regard to isolates from cerebrospinal fluid and blood samples from national surveillance sites. (European Centre for Disease Prevention and Control, 2015)

The standard of surveillance in the EU Member States was streamlined by the introduction of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which provides guidelines on susceptibility testing. This has allowed for greater uniformity and thus more comparable data to be collected from EARS-Net Member States. (European Centre for Disease Prevention and Control, 2015) (European Committee on Antimicrobial Susceptibility Testing, 2016)

The 2016 annual report included the AMR data for 2016 as well as a trend analysis of resistance in the region based on data from 2013 to 2016. Varying resistance patterns were noted throughout Europe between 2013 and 2016, but a general increase in resistance in *E. coli* to third generation cephalosporins and aminoglycosides was observed while resistance in *K. pneumoniae* appeared to stabilise with several countries reporting a decrease in resistance in *K. pneumoniae* to most antibiotic groups. For *E. coli* in 2016, over 50% of isolates were resistant to at least one antibiotic with the highest resistance reported to aminopenicillins (57%) while resistance to carbapenems remained low (< 0.1%). Resistance in *E. coli* to third generation cephalosporins remained stable at 12 - 13% while resistance to fluoroquinolones decreased from 22.5% to 21% over the 4 years. In *K. pneumoniae* resistance to fluoroquinolones decreased from 29.3% to 24.6%, resistance to third generation cephalosporins decreased from 30.1% to 25.7% and resistance to carbapenems decreased from 8.2% to 6.1%. Carbapenem resistance was found to be more common in *Acinetobacter spp.* isolates with an average of 35% resistance reported in 2016 with at least 6 countries reporting resistance greater than 70%. In *S. pneumoniae* isolates, resistance varied greatly between countries with non-susceptibility to penicillin ranging from 0.4% to 41.1% in 2016. Resistance to macrolides was 0 – 60% but in most countries, was higher than non-susceptibility to penicillins. The incidence of MRSA varied between countries, ranging between 1.2% and 50.5% in 2016, while the average incidence of MRSA in the region decreased from 18.1% in 2013 to 13.7% in 2016 (European Centre for Disease Prevention and Control, 2017) (European Centre for Disease Prevention and Control, 2015).

In addition to EARS-Net which it administrates, the European Centre for Disease Control (ECDC) also funded the European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) which collected data between 2013 and 2014 and highlighted the increase in carbapenem resistance in *K. pneumoniae* as a cause for concern. Out of all the carbapenemase-producing isolates identified, the ratio between *K. pneumoniae* and *E. coli* was 11:1 (Grundmann *et al.,* 2017*).*

1.2.3.3. National Surveillance Programmes

English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR)

In 2013, the United Kingdom published a strategy on AMR surveillance and ESPAUR was subsequently started as part of the strategy. ESPAUR collaborates with the ECDC and EARS-Net in order to enhance surveillance methods and contribute towards the broader AMR information network. Data from routine susceptibility testing is entered into a national database from hospitals across England and results are reported as pathogen-drug combinations (England Surveillance Programme for Antimicrobial Use and Resistance (ESPAUR), 2015).

Due to the increasing incidence of carbapenem resistance, an Electronic Reporting System (ERS) was implemented in 2015 for the enhanced surveillance of carbapenemase-producing Gram-negative bacteria. Between May 2015 and May 2017 there were 3 166 confirmed carbapenemase-producing organisms reported out of the 6 208 organisms submitted for testing. Sixty four percent of carbapenemase-producing *Enterobacteriaceae* (CPE) were isolated from rectal or faecal specimens (England Surveillance Programme for Antimicrobial Use and Resistance (ESPAUR), 2017).

The ESPAUR 2017 report, which included data between 2012 and 2016, highlighted the trends in resistance with respect to the different types of infection as well as by pathogen with specific focus on *E. coli*, *K. pneumoniae* and *P. aeruginosa.* In terms of results relevant to the GLASS methodology, bacteraemia caused by *E. coli* and *K. pneumoniae,* UTIs caused by *E. coli* and resistance in *N. gonorrhoea* was reported on. The proportion of non-susceptible *E. coli* isolates from blood samples remained stable between 2012 and 2016 although the incidence of bacteraemia caused by *E. coli* increased by 24.3% during this period with 40 272 cases reported in 2016. Isolates showed the highest resistance to amoxicillin/clavulanate (37.3% - 40.8%) followed by ciprofloxacin (18.1% - 18.7%) and third generation cephalosporins (10.8% - 12.4%) while carbapenem resistance remained low (0.07% - 0.14%). A similar trend of stable proportions of resistant isolates between 2012 and 2016 was observed in bloodstream infections caused by *K. pneumoniae* with resistance to ciprofloxacin and third generation cephalosporins fluctuating within the range of 10.0 – 12.3%. Carbapenem resistance was slightly higher than in *E. coli* isolates ranging from 0.8% to 1.15% (ESPAUR, 2017).

It was found that the underlying cause of approximately 50% of cases of bacteraemia was a UTI. Isolates were included from the community setting as well as the acute hospital setting. The majority of isolates (97%) were susceptible to first-line treatment nitrofurantoin but 34-37% of isolates were found to be resistant to trimethoprim, which is recommended where there is a low risk of resistance. Resistance to ciprofloxacin, the recommended treatment for complicated UTIs and pyelonephritis, was found to be 12% to 15% (ESPAUR, 2017).

The data regarding *N. gonorrhoea* showed that resistance to the first line drugs ceftriaxone and azithromycin was low. No isolates were found to be resistant to ceftriaxone and resistance to azithromycin showed a slight decline from 9.8% in 2015 to 4.7% in 2016 (ESPAUR, 2017).

India

Antimicrobial susceptibility testing is conducted by a number of public and private laboratories in India, however systematic surveillance is limited. A surveillance study supported by the WHO was carried out in Mumbai, New Delhi and Vellore between 2002 and 2005 looking at resistance in *E. coli.* There was high resistance to ampicillin (46 – 50%) and trimethoprim/sulfamethoxazole (45 – 65%) (Global Antibiotic Resistance Partnership-India National Working Group, 2011). Initiatives which have been active in India include the Indian Clinical Epidemiology Network (IndiaClen) which implemented the Invasive Bacterial Infection Surveillance (IBIS) project, the Indian Initiative for Management of Antibiotic Resistance (IIMAR) and the Indian Network for Surveillance of Antimicrobial Resistance (INSAR). These initiatives have spanned both the public and private sector and have included various pathogens (World Health Organisation South-East Asia, 2010).

Diarrhoeal and respiratory infections are responsible for 8% and 6% of deaths in India respectively and the associated resistance of the causative pathogens to antibiotics make the surveillance of antibiotic use and resistance a necessity in trying to improve public health outcomes. Studies have been carried out at various health facilities with the focus on hospital acquired infections (HAIs) by ward type as well as pathogen specific studies (Global Antibiotic Resistance Partnership-India National Working Group, 2011).

In the GARP India situational analysis published in 2011, *S. aureus* and *P. aeruginosa* were reported to be the most common causative pathogens in terms of HAIs and resistance data showed multi-drug resistance as high as 96% in a study including burn patients. *P. aeruginosa* isolates showed especially high resistance to tobramycin (83.6%) and amikacin (55.1%). The incidence of MRSA throughout India from past and present surveillance studies ranged from zero to almost 100%. A study carried out in Vellore between 1993 and 1994 found that 24% of *S. aureus* isolates were methicillin resistant and that resistance was >75% to gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole. Of the other GLASS pathogens, studies involving *A. baumannii* showed >80% resistance to third generation cephalosporins with the presence of ESBLs but a relatively smaller percentage of organisms were resistant to carbapenems (8%). High levels of third generation cephalosporin resistance was also found in a study on *K. pneumoniae* from urine samples *(*68%*)*. Results of several studies showed that up to 60% of *E. coli* isolates obtained from various surveillance sites were resistant to at least one antibiotic. A study conducted in New Delhi found that 67% of *S. typhi* isolates from children admitted to hospital with typhoid fever were multidrug resistant. Resistance among *Salmonella* species was found to be high (>70%) against ampicillin, ciprofloxacin and chloramphenicol but relatively lower against gentamicin (>90%). The data available regarding the susceptibility of *N. gonorrhoea* was of concern as a study conducted in a community setting between 2002 and 2003 found 78% of the isolates resistant to ciprofloxacin, 51% to tetracycline and 47% to penicillin (Global Antibiotic Resistance Partnership-India National Working Group, 2011).

Thailand

In Thailand surveillance of AMR has been implemented on a national level with respect to both human and animal health. The National Antimicrobial Resistance Surveillance in Thailand (NARST) was started in 1997 and has since expanded to include more surveillance sites. Surveillance data available from the NARST web page indicated a general trend of increasing resistance. *Enterococcus spp.* showed 84.1% and 69.7% resistance to tetracycline and erythromycin respectively in 2015 while vancomycin and teicoplanin showed the greatest sensitivity with only 3% of isolates showing resistance. Thirty-nine percent of *S. pneumoniae* were found to be resistant to erythromycin in 2016 as opposed to 48% in 2009, with >30% isolates resistant to erythromycin and/or clindamycin. Resistance of *S. aureus* isolates to erythromycin, clindamycin, ciprofloxacin and cefoxitin was found to be high (26 – 36%) in 2016 with resistance to cefoxitin being the highest (36.8%). Resistance to ampicillin was found to be especially high in *E. coli* and *K. pneumoniae* isolates which showed 86% and 99% resistance in 2016 respectively. There was a sharp drop in resistance to ampicillin/sulbactam in non-typhoidal *Salmonella* isolated from blood, dropping from 80% in 2002 to 7% in 2016, however resistance to ampicillin remained high in 2016 at 66%. Of special concern was the trend in resistance of *A. baumannii* isolates to multiple antibiotic agents. In 2016 greater than 50% resistance was found to amikacin, cefepime, ciprofloxacin, piperacillin/tazobactam, ampicillin/sulbactam and imipenem (National Antimicrobial Resistance Surveillance Centre, Thailand, 2015).

An Invasive Bacterial Infection Surveillance (Thai-IBIS) was also established in 2005. The United States Centres for Disease Control (CDC) works closely with the Thailand Ministry of Public Health to improve the prevention strategies for infectious diseases (World Health Organisation South-East Asia, 2010). The CDC is also active in the rest of the Southeast Asia region and has established the Immigrant, Refugee, and Migrant Health Program (IRMHP). There are several awareness programmes aimed at encouraging the rational use of antibiotics through educational courses as well as through an initiative by the Thai Food and Drug Administration (FDA) "Antibiotics Smart Use –

ASU" Project supported by the WHO, however awareness within the general community is still limited (U.S. Centers for Disease Control and Prevention, 2009).

National Surveillance and Reporting of Antimicrobial Resistance and Antibiotic Usage for Human Health in Australia

While Australia has a wealth of laboratories which provide antimicrobial susceptibility testing, a report published in 2013 stated that there was limited aggregation or analysis of the data at a national level and little standardisation and co-ordination occurred between surveillance sites. The Australian Group on Antimicrobial Resistance (AGAR), the National Neisseria Network (NNN) and the National Antimicrobial Utilisation Surveillance Program (NAUSP) are examples of surveillance programmes in Australia which have collected susceptibility data on several pathogens in the hospital and community setting, most commonly covering *Enterobacteriaceae* and *S. aureus.* Data from the *S. aureus* 2011 Antimicrobial Susceptibility Report noted MRSA in 29 – 36% of isolates, while the data from the Gram-negative Bacteria 2011 Hospital-onset Susceptibility Report evidenced high levels of resistance in *E. coli* to ampicillin (50.5%) while resistance in *K. pneumoniae* was highest to cefazolin (18%) and trimethoprim (18%) (Shaban, *et al*., 2013). Resistance in *N. gonorrhoea* isolates to fluoroquinolones in 2000 was reported to be 10% (Tapsall, *et al*., 2008). Based on existing surveillance networks such as EARS-Net, the Australian government outlined a national action plan with clear objectives to collect comparable and validated AMR (AMR) data (Shaban, *et al*., 2013).

National Antimicrobial Resistance Monitoring System- Enteric Bacteria (NARMS)

The NARMS programme was started in the USA by the Centres for Disease Control (CDC), the Food and Drug Administration (FDA) and the US Department of Agriculture. NARMS collects data regarding enteric bacteria including *Salmonella, Campylobacter, Shigella, E. coli* from humans, retail meat and food animal sources. NARMS investigates emerging resistance trends in order to guide health policies, inform consumers about food-borne diseases and encourage rational use of antibiotics. The CDC oversees a number of other surveillance programmes including the Gonococcal Isolate Surveillance Project (GISP) and the Healthcare-Associated Infections-Community Interface. The NARMS 2014 Human Data Report showed that resistance to ciprofloxacin in non-typhoidal *Salmonella* and *Shigella spp*. was 0.4% and 2.4% respectively and resistance in *E. coli* was highest to tetracycline and sulfisoxazole (7%) (Centres for Disease Control, 2016). The CDC also released a report in 2013 tracking the status of resistance in all pathogens of public health concern. The data showed that carbapenem resistance especially in *K. pneumoniae* (2%) and *E. coli* (11%) was of urgent concern as it translated to increasing difficulty in treating carbapenem-resistant *Enterobacteriaceae* (CRE) infections. MDR gonorrhoea was also highlighted as a serious concern with 30% resistance to cephalosporins, tetracycline and azithromycin in *N. gonorrhoea*. Other pathogens which were highlighted were MDR *Acinetobacter,* ESBL producing *Enterobacteriaceae,* drug resistant *Shigella* *spp, Salmonella spp, S. pneumoniae, P. aeruginosa* and MRSA (Centres for Disease Control and Prevention, 2014).

1.2.4. ABR and ABR Surveillance in South Africa:

South Africa has a national AMR strategy framework, which is supported by antibiotic stewardship, increased surveillance and measures to prevent the spread of infection through vaccination and infection prevention and control. The framework also aims to take a comprehensive approach in dealing with AMR by implementing regulatory measures to improve the use of medicines through the Medicines Control Council (MCC) and the National Drug Policy (NDP). The national framework also highlights the need for co-operation from health professionals, members of the agricultural community as well as professionals in the veterinary field (Mendelson & Matsotso, 2015).

The surveillance indicated in the framework is specifically aimed at resistance data, drug consumption trends, drug quality as well as the occurrence of medication errors. Through the education measures suggested, the stage has already been set for the implementation of antibiotic surveillance according to WHO surveillance standards. Through the guidelines set forth in the GLASS early implementation manual, the existing AMR strategy framework can be optimised in order to provide data that is useful on both a national and international scale (Mendelson & Matsotso, 2015).

As the two infectious diseases which cause the highest numbers of deaths in South Africa, HIV and TB have been the focus of numerous studies. The National Institute for Communicable Diseases (NICD) undertakes surveillance on diarrhoeal diseases, which have a direct impact on infant mortality as well as epidemic-prone infections such as cholera, typhoid fever and meningococcal disease. An analysis of AMR in South Africa carried out in 2011 by the Global Antibiotic Resistance Partnership–South Africa (GARP–SA) found that there was an urgent need for action in establishing standardised surveillance methods. There are limitations in population representation in existing data which stems mainly from central academic sites. Existing surveillance data thus does not represent the status of AMR in the entire population. The Centre for Healthcare Associated Infections, Antimicrobial Resistance and Mycoses, a branch of the NICD, conducts laboratory based antimicrobial resistance surveillance (LARS) since 2010 and collects data from sentinel sites. Electronic surveillance was implemented in 2013 and collects data from laboratory information systems in order to generate resistance maps. Enhanced surveillance has been implemented for methicillin-resistant *S. aureus (MRSA)* in order to determine the prevalence and extent of nosocomial and community-acquired MRSA infections. Enhanced surveillance is also underway for carbapenem-resistant Enterobacteriaceae (CREs) (Mendelson & Matsotso, 2015).

Existing surveillance data in South Africa which include pathogens of interest recommend by GLASS will be discussed below per infection type.

Blood Stream Infections

Enhanced surveillance of *S. aureus* bacteraemia (SAB) was conducted in 3 public sector hospitals in Gauteng, South Africa between 2012 and 2013. Core patient data, treatment information as well as antimicrobial susceptibility data were included in the study which provided a comprehensive and detailed picture of the incidence of SAB in the hospitals involved. AST results showed that 36% of the isolates were MRSA and that longer hospital stays, HIV infection, frequent hospitalisation and recent antibiotic use were predictors of MRSA infection (Fortuin-de Smit, *et al*., 2015). A wider study was conducted between 2010 and 2012 including thirteen academic hospitals around South Africa which looked at AMR trends and molecular epidemiology of SAB. *S. aureus* isolates were obtained from blood cultures over two years from academic hospitals in the public sector. There was a greater incidence of MRSA in Gauteng than in the other three provinces represented in the study and a variety of MRSA clones were present in South Africa. Methicillin resistance was found in 46% of isolates, but the incidence of MRSA decreased from 53% in 2010 to 40% in 2012 (Perovic, *et al*., 2015). The GLASS methodology recommends cefoxitin to be included in the panel for *S. aureus* as it is an indicator for methicillin resistance (World Health Organisation (b), 2015).

A national surveillance study was carried out in 2014 based on data obtained from public laboratories conducting AST for various hospitals in different provinces. Results from laboratory data using the Vitek and disk diffusion tests were analysed according to Clinical Laboratory Standards Institute (CLSI) guidelines. The study focussed on bloodstream infections caused by eight pathogens and the susceptibility profiles obtained were analysed. *A. baumannii* isolates showed the greatest resistance to piperacillin/tazobactam with more than 80% resistance, while susceptibility to colistin was retained at almost 100% followed by amikacin and levofloxacin which evidenced approximately 60% susceptibility. The other antibiotics including cephalosporins and carbapenems amongst others, showed resistance ranging between 70% and 80%. *S. aureus* isolates showed almost 0% susceptibility to penicillin, however the susceptibility to all other agents was found to be greater than 50%. The presence of ESBL producing pathogens including *E. coli* and *K. pneumoniae* was also detected, but both pathogens were almost 100% sensitive to carbapenems and amikacin (Perovic, *et al*., 2014).
The 2015 annual report of the Group for Enteric Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) published by the NICD reported national surveillance data from 36 enhanced surveillance hospital sites. Diseases under surveillance included opportunistic, nosocomial, epidemic-prone and vaccine-preventable infections throughout the 9 provinces. The GERMS-SA surveillance project includes susceptibility results from over 200 laboratories and covers a population of approximately 54.9 million. Methods such as the electronic capture on mobile phones of enhanced surveillance case report forms by surveillance officers has improved the ease of data capture and

surveillance site audits ensure that quality control is maintained (GERMS-SA, 2013) (GERMS-SA, 2015).

The GERMS-SA 2015 report included data on invasive pneumococcal disease, which in addition to blood samples, included isolates from the cerebro-spinal fluid (CSF) and other sources. The resistance data showed low levels of penicillin resistance (4%) in *S. pneumoniae.* Isolates have shown high levels of penicillin resistance in some studies, while significantly lower levels in others, leaving the overall level of penicillin resistance in the country at intermediate (GERMS-SA, 2015). Resistance to other classes of antibiotics has also been reported. Data from Johannesburg obtained as part of the Alexander Project in 1999 reported 79% of isolates to be penicillin-resistant and other data from the same year reported MDR in 37% of isolates (Crowther-Gibson, *et al*., 2011). The GERMS-SA 2015 report found MRSA in 33% of isolates, an increase from 29% reported in 2013 and resistance to clindamycin was 29% (GERMS-SA, 2015) (GERMS-SA, 2013).

Diarrhoeal Infections

The GERMS-SA 2015 report found that *Salmonella typhi* isolates showed a slight increase in resistance to ciprofloxacin, the first line treatment for diarrhoeal infections, from 10% in 2013 to 14% in 2015. No resistance was reported to azithromycin which could be used as alternative treatment options in cases of treatment failure with ciprofloxacin. In non-typhoidal Salmonella isolates resistance to ciprofloxacin was 21% while resistance to azithromycin was reported to be 1%. *Shigella* isolates showed low resistance (1%) to ciprofloxacin and azithromycin, although this is greater than the 0.1% resistance to ciprofloxacin reported in 2013 (GERMS-SA, 2015).

The GARP-SA situation analysis on ABR published in 2011 reported a decline in resistance to ampicillin in *Salmonella typhi* from 40% in 2006 to 10% in 2010. Resistance declined in non-typhoidal *Salmonella* isolates as well from 64% to 16% between 2003 and 2010. Resistance to ciprofloxacin was <1% in non-typhoidal *Salmonella* and *S. typhi* isolates in 2010. In *Shigella spp.,* high resistance rates to older antibiotics such as ampicillin, tetracycline and sulfamethoxazole was reported (≥50%), including a high prevalence of MDR isolates, while resistance to the current first-line treatment, naladixic acid, remained low (1%) (Gelband & Duse, 2011).

Urinary Tract Infections

A study conducted in Gauteng by Lewis et.al in 2013 investigated the aetiology of UTI’s in women from public and private healthcare facilities and included antibiotic prescribing data. Gram-negative bacteria, including *E. coli* and *K. pneumoniae,* were the most common causative pathogens. Resistance in Gram-negative bacilli was higher for trimethoprim/sulfamethoxazole (59%) and amoxicillin-clavulanate (19%) than ciprofloxacin (6%). Susceptibility to cephalosporins was greater than 90% (Lewis *et.al.* 2013 (a)).

Another study was carried out between 2010 and 2012 with *K. pneumoniae* as the sentinel organism in 13 public sector healthcare sites in Gauteng, KwaZulu Natal, Limpopo, Free State and Western Cape provinces. Resistance to third generation cephalosporins was found to be greater than 70% while susceptibility to carbapenems and colistin was greater than 85% in 2012 (Perovic, *et al*., 2014).

Coetzee et al. reported in 2016 that increasing colistin resistance in *E. coli* isolates was becoming a serious concern and that the use of colistin as a last resort was becoming more common due to resistance to other antibiotics. Resistance was thought to be spread by the presence of the mcr-1 gene in food animals such as pork and poultry, the presence of which has been confirmed by surveillance of poultry operations in South Africa. The mcr-1 gene has also been detected in colistin-resistant *E. coli* in hospitalised and outpatient-based patients in South Africa (Coetzee *et al.*, 2016).

Such studies show that there is the capacity for the collection of usable, detailed data as required for GLASS as *E. coli* and *K. pneumoniae* are the pathogens of interest for urine samples. These existing findings are useful as a means of comparing the results of this study to the existing knowledge base.

Gonorrhoea

Gonorrhoea is the only STI to be investigated in terms of ABR in South Africa. Isolates were found to be susceptible to ciprofloxacin until 2003, when resistance emerged in Durban. A subsequent study carried out by the STI Reference Centre in several cities including Durban, Cape Town and Johannesburg showed varying levels of quinolone resistance but a general trend of increasing resistance to ciprofloxacin. Studies in Gauteng have also found high levels of tetracycline resistance which excludes its use as well as the use of penicillin, due to the reports of penicillinase-producing gonococci. The current first line therapy was changed from ciprofloxacin to cephalosporins in 2008, which showed good activity against *N. gonorrhoea.* Treatment is recommended as either oral cefixime or intramuscular ceftriaxone but while widespread resistance to these agents has not been reported in Africa, resistance to oral cephalosporins has emerged in the Western Pacific region as well as Europe, a trend which may spread to South Africa in the near future (Crowther-Gibson, *et al*., 2011). In 2012 the first two cases of confirmed extended-spectrum-cephalosporin-resistant *N. gonorrhoeae* were reported in Johannesburg (Lewis *et al.* 2013 (b)).

Other Research in South Africa

Statistics from the Johannesburg Antimicrobial Resistance Laboratory and Culture Collection (AMRL-CC) of the Centre for Opportunistic, Tropical and Hospital Infections at the NICD describe CPE isolates. The majority of isolates were *K. pneumoniae*, *E. coli* and *Serratia marcescens* and the presence of these CPE isolates indicates the need for formal surveillance on a national level in order to inform public health policy (Centre for Opportunistic, Tropical, and Hospital Infections, 2015). A study in five hospitals in the Eastern Cape, investigated carbapenem resistant *Enterobacter cloacae* and found that 72% of isolates harboured carbapenem resistance genes and strains from the same geographic location were genetically similar (Singh-Moodley, *et al*., 2015).

Both these studies indicate a cause for concern that the incidence of carbapenem resistance is increasing and that this resistance may be conferred by mobile genetic elements. These studies also provide confidence in the capacity South Africa has to conduct systematic surveillance and provide usable data for strategy development and that the implementation of WHO recommendations, as will be discussed below, are achievable based on the resources available.

1.2.5. ABR surveillance in KwaZulu Natal

A study was published in 2005 looking at data from 16 public hospitals in KwaZulu Natal including district, regional and tertiary hospitals and the resistance in isolates from the different levels of hospitals was compared. Ninety percent of isolates were found to be multi-drug resistant and the incidence of MRSA was 17 – 28% in district, regional and tertiary hospitals. Resistance to ampicillin was high (>80%) as compared to meropenem (<10%) according to the combined percentage resistance for all species included in the study (Essack *et al.*, 2005).

There are several public healthcare facilities in KZN which serve as sentinel surveillance sites including enhanced surveillance site such as Addington Hospital amongst others as mentioned above. These sites provide susceptibility data on a number of pathogens, including the ESKAPE pathogens and this information has been included in national surveillance reports (GERMS-SA, 2013) (Perovic, *et al*., 2014).

The WHO established pilot projects in two cities in South Africa: Durban and Brits and each site implemented a protocol for collecting community-based AMR data every month for at least 12 months, using one or two indicator bacteria. In Durban *S. pneumoniae* and *H. influenzae* were the indicators and resistance was measured in terms of the MIC values (Holloway, *et al*., 2011).

A study conducted at Inkosi Albert Luthuli Central Hospital (IALCH) in 2009 investigated the susceptibility of nosocomial infections in the Trauma Intensive Care Unit (TICU) and reviewed the accuracy of empiric antibiotics. IALCH employs antibiotic stewardship and the study found that such programmes promoted rational use of antibiotics which in turn limit the progress of AMR. Many of the GLASS pathogens of interest were isolated during the study period. *A. baumannii* isolates were most susceptible to amikacin (43%), susceptibility in *S. aureus* isolates to clindamycin, cloxacillin, erythromycin, gentamicin and vancomycin was 62 -100% while only 11% and 16% susceptibility to penicillin and ampicillin was reported respectively. *S. pneumoniae* isolates showed 7% resistance to penicillin, 33% resistance to ampicillin and 100% resistance to cloxacillin. Susceptibility in *E. coli* isolates was >80% for all antibiotics except amoxicillin-clavulanate (45%) while susceptibility in *K. pneumoniae* isolates was 58 – 92% for all antibiotics (Ramsamy, *et al*., 2013).

Although limited surveillance has been conducted specifically in KwaZulu Natal, data from surveillance sites in the province have contributed towards national surveillance studies. Antimicrobial susceptibility data is available from public sector hospitals via data from VITEK machines which carry out automated antimicrobial susceptibility testing (GERMS-SA, 2013).

1.2.6. Antimicrobial Susceptibility Testing (AST)

In order to identify and quantify drug resistance in pathogens, there are various methods of carrying out susceptibility testing. The most common methods of AST are disc sensitivity testing, broth microdilution and rapid automated instrument methods. Each method has benefits and shortcomings and the accuracy of testing of certain organisms varies between methods (Barth Reller *et al*., 2009).

Broth macrodilution includes preparing two-fold dilutions of antibiotics in a liquid growth medium and identifying the MIC after incubation. The benefit of this method is that a quantitative result is obtained, however this method is labour intensive and there is the possibility for human error. Microdilution works on the same principle, but the process in miniaturised and mechanised and multiple antibiotics can be tested on one tray. This method is that the results are reproducible, trays with prepared antibiotic panels are available and the cost of testing is relatively low (Barth Reller *et al*., 2009).

Another method of testing is the antimicrobial gradient method whereby an antimicrobial concentration gradient in an agar medium is established. An example of this is the Etest which uses test strips impregnated with dried antibiotics of increasing concentration. After overnight incubation, MIC is determined based on the growth inhibition area. This method allows for the flexibility to choose which drugs to test, however the test strips are costly so it is not practical for testing multiple drugs. While the results from this method of testing are generally comparable with broth microdilution, there are some systematic biases to higher or lower MICs in certain antibiotic-pathogen combinations (Barth Reller *et al*., 2009).

Disk diffusion involves applying a bacterial inoculum to a Mueller-Hinton agar plate and antibiotic disks of fixed concentration are placed on the inoculated surface. Results are determined from the diameter of the growth inhibition zone after incubation as per the interpretive criteria in CLSI/ EUCAST guidelines or as per the product insert for the disks. This method of testing produces qualitative results categorised as susceptible, intermediate or resistant instead of MIC values. The testing procedure is simple and requires no specialised equipment and it is the cheapest of all testing methods. The disadvantages are that not all bacteria can be accurately tested by this method and all preparation is manual (Barth Reller *et al*., 2009).

Automated microbiological testing provides pathogen identification and AST processing of specimens and is used to provide the necessary information for clinical interventions. Automated systems are a useful tool in antimicrobial surveillance and allows for easy acquisition of specimen analysis reducing the workload of the researcher. Automated instrument systems allow for rapid susceptibility testing and because computer software is used to interpret results, the reading of endpoints is standardised. The disadvantage is that there is a lessened ability to detect resistance in certain cases such as vancomycin resistance and inducible β-lactamases. An example of an automated testing system in the VITEK 2 machine, which is the method used for susceptibility testing in this study (Ligozzi, *et al*., 2002) (Barth Reller *et al*., 2009).

The VITEK 2 machine uses reagent cards with 64 wells containing a test substrate meaning that a single card can hold up to 64 organism-substrate combinations. The pure culture for analysis first needs to be prepared as a suspension before it can be inserted into the Vitek machine for inoculation and incubation. The incubator can hold up to 60 cards at a time and readings are collected by the machine at 15minute intervals. MIC results are displayed as "+" or "-" based on automatic calculations done on the raw data that are compared to test thresholds (Ligozzi, *et al*., 2002).

The VITEK 2 system has been shown to provide accurate identification and AST results for use in hospitals where microbiological testing is in high demand and results are needed rapidly. This is especially useful in resource-limited settings where there may not be sufficient human resources to conduct manual microbiological testing for clinical use (Ligozzi, *et al*., 2002).

1.3. Conclusion

With the escalation of multi-drug resistant infections, treatment options are becoming more limited, making commonly encountered infections increasingly difficult to treat. The purpose of this study was to illustrate antibiotic resistance trends in pathogen-drug combinations stipulated in GLASS in the province of KwaZulu Natal, South Africa over a 5-year period.

It is clear from the literature that while surveillance systems do exist, there is variability in the quality and reliability of data and differences in methodology, pathogens and drugs of interest hinder the establishment of the true extent of ABR. Specifically, surveillance in Africa is less well established than in other regions such as Europe, North America and Australia and inadequate funding, human resources and infrastructure limit the capacity for surveillance in under-resourced countries. As such, there is a great need for surveillance in order to obtain information on the true extent of antibiotic resistance in the region.

The GLASS manual for early implementation provides data compilation methods, resistance markers for global reporting and a stepwise implementation plan that allows for the gradual induction into the program. By applying the GLASS methodology, simplified data collection and reporting can be established to provide baseline information on the extent of ABR which can later be expanded once the surveillance methodology is established. This study aims to address the gap in knowledge regarding ABR in GLASS pathogen-drug combinations to some extent. The results can be used to guide future studies and build on the existing knowledge base to provide comparable, validated data which can be shared on a global scale in order to guide future interventions against the spread of ABR.

1.4. Aims and objectives

Aims:

To elucidate the extent and trends in ABR in blood stream infections, urinary tract infections (UTIs), diarrhoea and gonorrhoea in KwaZulu Natal using guidelines delineated in the Global Antimicrobial Surveillance System (GLASS) of World Health Organisation (WHO) over the period 2011-2015.

Objectives:

- To create a database of blood stream infections (BSI), urinary tract infections (UTI), diarrhoeal infections and gonorrhoeal infections from extracted from the provincial database of the KwaZulu-Natal National Health Laboratory Services including but not limited to:
    - Causative bacteria and their associated antibiotic susceptibility profiles
    - Clinical data on specimen source, diagnosis (if available), date of admission, date of specimen collection, date of discharge etc.
    - Demographic data on age, gender, co-morbidity (if available).
    - Facility data such as hospital level and ward type
- To identify the proportion of BSIs, UTIs, diarrhoeal infections and gonorrhoea caused by each pathogen of interest.
- To observe any changes/trends in the proportion of causative pathogens for each specimen type per year.
- To analyse the trends in ABR over the study period and identify changes in susceptibility profiles if evident.
- To compare the susceptibility profiles obtained with the antibiotics recommended in the Standard Treatment Guidelines published by the Department of Health with a view to informing amendments per hospital level as appropriate.
- To create a baseline for the WHO GLASS platform at provincial level

1.5. Dissertation Structure

The dissertation is set out as follows:

Chapter 1: Introduction and Literature Review

Chapter 2: Manuscript entitled "A Retrospective Trend Analysis of Antibiotic Resistance in GLASS Pathogens in KwaZulu Natal: 2011- 2015" intended for submission to the Bulletin of the World Health Organisation:

Chapter 3: Conclusion, Limitations and Recommendations

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CHAPTER 2. MANUSCRIPT

The findings are reported in the following manuscript intended for submission to the Bulletin of the World Health Organisation:

A Retrospective Trend Analysis of Antibiotic Resistance in GLASS Pathogens in KwaZulu Natal: 2011- 2015

Patel, M, Mlisana, KP, Ramsamy, Y, Sartorius B and Essack, SY.

- Miriam Patel, as the principal investigator, co-conceptualized the study, undertook data cleaning, alignment and calculations, compilation of tables and figures and drafted the manuscript
- Koleka Mlisana, as co-supervisor, co-conceptualised the study and undertook a critical review of the manuscript.
- Yogandree Ramsamy, undertook data extraction, cleaning and alignment, assisted in review of results and undertook a critical review of the manuscript
- Ben Sartorius, undertook statistical analysis and critical review of the manuscript
- Sabiha Yusuf Essack, as principal supervisor, co-conceptualised the study, reviewed data analysis and undertook a critical review of the manuscript.

A Retrospective Trend Analysis of Antimicrobial Resistance in GLASS pathogens in KwaZulu Natal: 2011- 2015

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Abstract

Objective: Antibiotic resistance trends in pathogen-drug combinations stipulated in the Global Antimicrobial Surveillance System (GLASS) of the World Health Organization were investigated for the period 2011-2015 in KwaZulu-Natal, South Africa.

Methods: Antibiotic susceptibility data from blood, urine, faecal and urethral/cervical samples was retrospectively analyzed from six public hospitals. Pathogens included *Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Salmonella spp., Acinetobacter baumannii, Staphylococcus aureus, Shigella spp. and N. gonorrhoea.* Results included MIC50, MIC90, percentage resistance, incidence of infections in the population and proportion of non-susceptible infections. Results were also evaluated against South African treatment guidelines. Significant differences in resistance proportions by year were identified using the Pearson χ^2 test. Comparison of MIC50 were analysed using the equality-of-medians test.

Findings: Urine samples were most abundant (61.22%, n= 33 018) and *E. coli* (52%) was the most common pathogen. Most isolates were multi-drug resistant and resistance to cephalosporins and fluoroquinolones increased in *K. pneumoniae, E. coli* and *Shigella spp*. Notable changes in resistance were: *K. pneumoniae* from blood samples to carbapenems (1 – 26%, p< 0.001) and *A. baumannii* to carbapenems (69% - 50%, no p-value). Susceptibility to antibiotics recommended in treatment guidelines was >50% for most pathogen-drug combinations.

Conclusion: Although resistance in some pathogen-drug combinations plateaued or declined, antibiotic resistance in hospitals in KwaZulu-Natal increased from 2011 to 2015, necessitating a review of the existing treatment guidelines. To our knowledge, this is the first South African report on ABR using GLASS metrics. There is a need for more extensive research in order to build an accurate picture of ABR in South Africa.

Introduction

Antibiotics are essential for treating bacterial infections however antibiotic resistance (ABR) has become a fast spreading phenomenon which is limiting treatment options for common infections. A key strategic objective of the Global Action Plan (GAP) on Antimicrobial Resistance (AMR), adopted by member states of the World Health Organisation (WHO) in 2015, is to strengthen the knowledge and evidence base through surveillance and research. Surveillance was highlighted as an important tool in mapping out the prevalence, trends and mechanisms of ABR to understand the extent of the problem on a global scale [(1)]. The 2014 surveillance report on antimicrobial resistance published by the WHO highlighted the scarcity of reliable data on ABR in Africa as not many countries in the region carry out surveillance compared to the relatively well-established surveillance systems found in the European region. Inadequate funding, human resources and infrastructure limit the capacity for surveillance in under-resourced countries [(2)]. As such, there is a great need for surveillance in order to obtain information on the true extent of antibiotic resistance in the region.

ABR surveillance is more extensive in South Africa compared to other countries in the WHO Africa region. Existing literature from both public and private sector facilities indicate a decline in efficacy of older antibiotics such as ampicillin and tetracycline and more recently, cephalosporins and fluoroquinolones. This has necessitated the increased use of carbapenems to treat resistant infections.[(3)] In addition, methicillin resistant *Staphylococcus aureus* (MRSA) as well as extended spectrum β-lactamase (ESBL) producing pathogens are prevalent. With the escalation of multi-drug resistant infections, treatment options are becoming more limited, making commonly encountered infections increasingly difficult to treat. [(4)]

While many countries are already conducting surveillance, there is a lack in uniformity in surveillance methods as well as the pathogens and antibiotics investigated. In order for data to be meaningful and comparable on a global scale, the WHO published the Global Antimicrobial Surveillance System (GLASS) manual which aimed to standardise surveillance methods to yield validated data reported in a uniform manner. Specific pathogen-drug combination for four specimen types namely blood, urine, stool and cervical/urethral swabs, were outlined and WHO member states are encouraged to implement GLASS as far as possible in order to provide a baseline database of ABR on a global scale that can be built on as surveillance methods become more firmly established [(2)].

The purpose of this study was to illustrate antibiotic resistance trends in pathogen-drug combinations stipulated in GLASS for the period 2011-2015 in six public hospitals in the province of KwaZulu Natal, South Africa from blood, urine, stool and cervical/urethral samples indicative of blood stream infections (BSIs), urinary tract infections (UTIs), diarrhoeal infections and gonorrhoeal infections respectively. To our knowledge, this is the first South African report on ABR using GLASS metrics.

Methods

Study Design

Antibiotic susceptibility data from 2011 to 2015 was retrospectively extracted from the KwaZulu-Natal National Health Laboratory Services (NHLS) computerized database from six public sector hospitals in KwaZulu Natal, South Africa and included all levels of care from the first point of contact at district level o the centralized, specialized care at tertiary and quaternary levels.

The extracted de-duplicated data included specimen type, bacterial identity and antibiotic susceptibility results in the form of minimum inhibitory concentrations (MICs). The data was analysed to determine the trends in MICs and resistance in selected isolates during the study period. Only blood, urine and faecal samples positive for the pathogens of interest highlighted in the GLASS manual were included in the study. In terms of gonorrhoea, the data collected did not define urethral or cervical swabs as a specimen type and thus all *Neisseria gonorrhoea* specimens classified as "other" or with the specimen type missing were included. Priority pathogens for blood specimens included *Escherichia coli*, *Streptococcus pneumoniae, Klebsiella pneumoniae, Salmonella spp., Acinetobacter baumannii* and *Staphylococcus aureus.* Priority pathogens for urine samples were *E. coli* and *K. pneumoniae*. Priority pathogens for faecal samples were *Salmonella spp.* and *Shigella spp.*

The population data for KwaZulu Natal required for the calculation of GLASS metrics was obtained from the results of the 2011 national census. [5]

Ethical Considerations

The study was approved by the Biomedical Research Ethics Committee (BREC) (REF: BE085/12). All data was anonymized in order to maintain patient confidentiality.

Antimicrobial Susceptibility Testing

Pathogen identification and susceptibility testing was carried out at each participating hospital on the VITEK 2 system (bioMerieux) according to the Clinical Laboratory Standards Institute (CLSI) guidelines and isolates were classified as sensitive or resistant using CLSI-approved breakpoints. Antibiotic panels per pathogen appear in Supplementary Table 1 as recommended by GLASS.

External Quality Assurance

The participating hospital laboratories subscribe to the NHLS Proficiency Testing Scheme (PTS) coordinated by the PTS Managers(Microbiology) at the NHLS Academic Affairs, Research and Quality Assurance(AARQA) Unit. The scheme entails quarterly evaluations for, amongst others, bacteriology. Microscopy, culture and identification methods and manual and automated antimicrobial susceptibility testing (AST) methods are evaluated based on samples prepared with the assistance of the National Institute for Communicable Diseases (NICD) – Centre for Opportunistic, Tropical and Hospital Infections, a division of the NHLS.

Statistical Analysis

All data processing and analyses were performed using Stata 13.0 software SE (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.). Categorical GLASS pathogen data were presented using stratified frequency tables (n and %). Differences in MIC50 levels over the period of 2011-2015 were assessed using a non-parametric equality-of-medians test. Differences or trends or association for resistance types and year for example was assessed using the standard Pearson's chi-square ($\chi 2$) test. If expected cell count in the cross tabulation contained fewer than 5 observations (sparse numbers) then the Fishers exact test was utilized instead. A p-value of <0.05 was considered statistically significant.

Results

Table 1 illustrates the number of isolates per specimen type and pathogen between 2011 and 2015. Isolate numbers for 2014 are not accurate as six months of data from one of the participating hospitals was missing from the NHLS database. The number of samples positive for any of the GLASS pathogens increased more than two-fold from 4 737 collected in 2011 to 10 289 collected in 2015. Blood (n=11 722) and urine (n=20 212) samples formed the vast majority of specimens. The most frequently isolated pathogen was *E. coli* from urine specimens constituting 56.08% (n=33018) of the isolates over the five-year study period.

Table 1: Number of isolates per specimen type and pathogen between 2011 and 2015 (percentage indicated in parenthesis)

Specimen Type	2011	2012	2013	2014	2015	Total	P-value*
Blood	**1792 (37.83)**	**2391 (37.26)**	**2823 (37.42)**	**1353 (33.56)**	**3363 (32.69)**	**11722 (35.50)**	<0.001
E. coli	397 (8.38)	441 (6.87)	417 (5.53)	221 (5.48)	609 (5.92)	2085 (6.31)	<0.001
K. pneumoniae	373 (7.87)	577 (8.99)	698 (9.25)	358 (8.88)	1053 (10.23)	3059 (9.26)	<0.001
A. baumanii	248 (5.24)	341 (5.31)	483 (6.4)	147 (3.65)	353 (3.43)	1572 (4.76)	<0.001
S. aureus	543 (11.46)	773 (12.05)	890 (11.8)	471 (11.68)	1110 (10.79)	3787 (11.47)	<0.001
S. pneumoniae	188 (3.97)	179 (2.79)	238 (3.15)	118 (2.93)	170 (1.65)	893 (2.7)	<0.001
Salmonella spp.	43 (0.91)	80 (1.25)	97 (1.29)	38 (0.94)	68 (0.66)	326 (0.99)	<0.001
Urine	**2808 (59.28)**	**3859 (60.15)**	**4451 (58.99)**	**2494 (61.87)**	**6600 (64.15)**	**20212 (61.22)**	<0.001
E. coli	2202 (46.49)	2936 (45.76)	3257 (43.17)	1907 (47.31)	4913 (47.75)	15215 (46.08)	<0.001
K. pneumoniae	606 (12.79)	923 (14.39)	1194 (15.83)	587 (14.56)	1687 (16.40)	4997 (15.13)	<0.001
Faeces	**102 (2.15)**	**142 (2.22)**	**237 (3.14)**	**163 (4.04)**	**272 (2.64)**	**916 (2.77)**	<0.001
Salmonella spp.	47 (0.99)	46 (0.72)	78 (1.03)	46 (1.14)	82 (0.80)	299 (0.91)	<0.001
Shigella spp.	55 (1.16)	96 (1.50)	159 (2.11)	117 (2.90)	190 (1.85)	617 (1.87)	<0.001
Urethral/cervical	**35 (0.74)**	**24 (0.37)**	**34 (0.45)**	**21 (0.52)**	**54 (0.52)**	**168 (0.51)**	<0.001
N. gonorrhoea	35 (0.74)	24 (0.37)	34 (0.45)	21 (0.52)	54 (0.52)	168 (0.51)	<0.001
Total	**4737 (100)**	**6416 (100)**	**7545 (100)**	**4031 (100)**	**10289 (100)**	**33018 (100)**	

*Pearson's chi^2 p-value

Antibiotic Resistance

The antibiotic resistance data was stratified by year and analysed on 3 levels: (1) a trend analysis of resistance including MIC50, MIC90, MIC range and percentage resistance over 5 years was conducted; (2) metrics recommended in the GLASS manual were calculated and (3) susceptibility data was compared with existing standard treatment guidelines. Table 2 illustrates the AST results over the study period for each specimen type (See Supplementary Table 2 for overall AST results for the study period).

The trends in resistance observed over the five years varied between the different pathogen-drug combinations. While the percentage resistance across most pathogen-drug combinations increased from 2011 to 2015, the MIC50 and MIC90 remained stable, with changes observed in only a few pathogen-drug combinations. *K. pneumoniae* isolates showed the most changes in MIC50 with statistically significant increases in MIC50 in isolates from urine samples for ceftazidime (≤1µg/ml – 8µg/ml, p= < 0.001), cefotaxime (≤ 1µg/ml - ≥ 64µg/ml, p < 0.001) and cefepime (≤1µg/ml – 2µg/ml, p< 0.001). Other pathogen-drug combinations that showed fluctuations in MIC50 were *A. baumannii* for - amikacin (4µg/ml – 8µg/ml, p= 0.001), gentamicin (≥16µg/ml – 8µg/ml, p-value not available) and imipenem (≥16µg/ml - ≤1µg/ml, p-value not available); *E. coli* from urine samples for imipenem (≤1µg/ml - ≤0.25µg/ml, p< 0.001); *Salmonella spp.* from faeces for cefotaxime (4µg/ml - ≤1µg/ml,

$p < 0.001$). These changes in MIC50 corroborated the changes in percentage resistance observed. The majority of pathogens showed an increase in percentage resistance from 2011 to 2015, with the exception of *A. baumannii* isolates from blood samples as well as *Salmonella spp.* isolates from both blood and faecal samples. All *A. baumannii* isolates showed a decrease in resistance to all the antibiotics tested while *Salmonella spp.* isolates showed a decrease or plateau in resistance to all antibiotics tested except for ciprofloxacin in isolates from blood samples which only showed a 0.33% increase in resistance. A statistically significant ($p \leq 0.05$) increase in percentage resistance between 2011 and 2015 was observed in the following pathogen-drug combinations: *E. coli* isolates from blood samples for ceftazidime, cefotaxime, ciprofloxacin, imipenem; *K. pneumoniae* isolates from blood samples for ciprofloxacin, cefepime, ertapenem, imipenem, meropenem; *E. coli* isolates from urine samples for ceftazidime, cefotaxime, ertapenem, cefepime; *K. pneumoniae* isolates from urine samples for ceftazidime, ceftazidime, cefepime, ertapenem, imipenem, meropenem; and, *Salmonella spp.* isolates from faecal samples for ciprofloxacin. The escalating resistance to the broad spectrum cephalosporins, carbapenems and fluoroquinolones was evident. A statistically significant ($p \leq 0.05$) decrease in resistance between 2011 and 2015 was observed in the following pathogen-drug combinations: *Salmonella spp.* isolates from blood samples – ciprofloxacin, cefotaxime, ertapenem; *Salmonella spp.* isolates from faecal samples – cefotaxime, ceftazidime, ertapenem.

Table 2: AST Results by Year 2011 – 2015

		2011					2012					2013					2014					2015						
Blood: E. coli		MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	p-value MIC50	p- value %R
	AM	≥32	≥32	≤2 - ≥32	157	79.62	≥32	≥32	≤2 - ≥32	175	85.14	≥32	≥32	≤2 - ≥32	220	86.82	≥32	≥32	≤2 - ≥32	158	84.18	≥32	≥32	≤2 - ≥32	236	87.29	-	0.98
	CAZ	≤1	16	≤1- ≥64	153	10.46	≤1	16	≤1- ≥64	174	18.39	≤1	16	≤1- ≥64	216	12.96	≤1	16	≤1- ≥64	156	16.03	≤1	16	≤1- ≥64	235	26.81	<0.001	0.004
	CIP	≤0.25	≥4	≤0.25 - ≥8	158	19.62	≤0.25	≥4	≤0.25 - ≥8	177	26.55	≤0.25	≥4	≤0.25 - ≥8	220	26.82	≤0.25	≥4	≤0.25 - ≥8	161	27.95	≤0.25	≥4	≤0.25 - ≥4	237	37.97	<0.001	0.051
	CS	≤0.5	1	≤0.5 - ≥16	*	*	≤0.5	≤0.5	≤0.5 - 8	*	*	≤0.5	≤0.5	≤0.5 - ≤0.5	*	*	≤0.5	≤0.5	≤0.5 - ≥16	*	*	≤0.5	≤0.5	≤0.5 - 8	*	*	<0.001	-
	CTX	≤1	≥64	≤1- ≥64	153	14.38	≤1	≥64	≤1- ≥64	174	27.59	≤1	≥64	≤1- ≥64	216	22.69	≤1	≥64	≤1- ≥64	156	28.85	≤1	≥64	≤1- ≥64	235	39.57	<0.001	0.001
	ETP	≤0.5	≤0.5	≤0.5 - ≤0.5	153	0,00	≤0.5	≤0.5	≤0.5 - 1	171	0.58	≤0.5	≤0.5	≤0.5 - ≤0.5	215	0,00	≤0.5	≤0.5	≤0.5 - 4	155	1.29	≤0.5	≤0.5	≤0.5 - 4	232	0.86	0.396	0.373**
	FEP	≤1	2	≤1- ≥64	153	5.23	≤1	8	≤1- ≥64	174	9.77	≤1	2	≤1- ≥64	216	5.09	≤1	8	≤1- ≥64	157	8.28	≤1	16	≤1- ≥64	235	10.64	<0.001	0.192
	IPM	≤1	≤1	≤1- ≤1	153	0,00	≤1	≤1	≤0.25 - ≥16	174	1.72	≤0.25	≤0.25	≤0.25 - ≤1	216	0,00	≤0.25	≤0.25	≤0.25 - ≤1	156	0,00	≤0.25	≤0.25	≤0.25 - ≥16	234	1.71	<0.001	0.044*
	MEM	≤0.25	≤0.25	≤0.25 - ≤0.25	153	0,00	≤0.25	≤0.25	≤0.25 - ≥16	174	1.15	≤0.25	≤0.25	≤0.25 - ≥16	216	0.46	≤0.25	≤0.25	≤0.25 - 1	155	0,00	≤0.25	≤0.25	≤0.25 - ≥16	216	0.93	0.563	0.591*
	SXT	≥320	≥320	≤10 - ≥320	157	81.53	≥320	≥320	≤20 - ≥320	177	84.75	≥320	≥320	≤10 - ≥320	220	82.27	≥320	≥320	≤10 - ≥320	160	76.88	≥320	≥320	≤10 - ≥320	237	67.93	-	0.587
		2011					2012					2013					2014					2015						
Blood: K. pneumoniae		MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	p-value MIC50	p- value %R
	CAZ	12	≥64	≤1-≥64	140	50.71	16	≥64	≤1-≥64	227	53.30	16	≥64	≤1-≥64	291	59.79	16	≥64	≤1-≥64	234	64.10	16	≥64	≤1-≥64	349	73.64	<0.001	0.080
	CIP	≤0.5	≥4	≤0.25-≥4	141	35.46	1	≥4	≤0.25-≥8	232	41.38	1	≥4	≤0.25-≥8	305	42.95	≤0.5	≥4	≤0.25-≥8	247	36.03	2	≥4	≤0.25-≥8	356	53.93	<0.001	0.043
	CS	≤0.5	2	≤0.5-≥16	*	*	≤0.5	2	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	≤0.5	1	≤0.5-2	*	*	≤0.5	4	≤0.5-≥16	*	*	<0.001	-
	CTX	8	≥64	≤1-≥64	140	58.57	≥64	≥64	≤1-≥64	227	66.96	≥64	≥64	≤1-≥64	291	64.95	≥64	≥64	≤1-≥64	232	72.41	≥64	≥64	≤1-≥64	349	81.09	-	0.205
	ETP	≤0.5	≤0.5	≤0.5-≥8	139	1.44	≤0.5	≤0.5	≤0.5-≥8	219	4.11	≤0.5	≤0.5	≤0.5-≥8	282	5.32	≤0.5	≤0.5	≤0.5-≥8	231	2.60	≤0.5	≥8	≤0.5-≥8	339	26.25	<0.001	<0.001**
	FEP	≤1	24	≤1-≥64	140	10.71	2	≥64	≤1-≥64	227	20.7	2	≥64	≤1-≥64	281	26.33	2	32	≤1-≥64	232	21.98	≤4	≥64	≤1-≥64	349	39.83	<0.001	<0.001
	IPM	≤1	≤1	≤1-4	140	1.43	≤1	≤1	≤0.25-≥16	227	5.29	≤0.25	0.5	≤0.25-≥16	291	4.81	≤0.25	≤0.25	≤0.25-≥16	235	2.13	≤0.25	≥16	≤0.25-≥16	349	26.07	<0.001	<0.001**
	MEM	≤0.25	≤0.25	≤0.25-≥16	140	1.43	≤0.25	≤0.25	≤0.25-≥16	227	4.41	≤0.25	≤0.25	≤0.25-≥16	291	4.81	≤0.25	≤0.25	≤0.25-≥16	230	1.74	≤0.25	≥16	≤0.25-≥16	281	24.91	<0.001	<0.001**
	SXT	≥320	≥320	≤10-≥320	141	71.63	≥320	≥320	≤10-≥320	232	76.72	≥320	≥320	≤10-≥320	305	74.75	≥320	≥320	≤10-≥320	246	74.39	≥320	≥320	≤10-≥320	355	81.41	-	0.906
		2011					2012					2013					2014					2015						
Blood: A. baumanii		MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	p-value MIC50	p- value %R
	AN	4	32	≤2-≥64	101	16.83	8	≥64	≤2-≥64	161	19.25	16	32	≤2-≥64	229	15.28	12	16	≤2-≥64	114	9.65	8	32	≤2-≥64	139	11.51	0.001	0.302
	CS	≤0.5	2	≤0.5-≥16	101	5.94	≤0.5	2	≤0.5-≥64	161	6.83	≤0.5	≤0.5	≤0.5-≥16	229	5.24	≤0.5	2	≤0.5-≥16	115	8.70	≤0.5	≤0.5	≤0.5-≥16	139	5.76	<0.001	0.827
	GM	≥16	≥16	≤1-≥16	102	69.61	≥16	≥16	≤0.5-≥16	156	73.08	≥16	≥16	≤0.5-≥16	230	60.87	≥16	≥16	≤1-≥16	115	60.00	8	≥16	≤0.5-≥16	142	50.70	-	0.363
	IPM	≥16	≥16	≤1-≥16	101	69.31	≥16	≥16	≤0.25-≥16	155	71.61	≥16	≥16	≤0.25-≥16	229	65.94	≥16	≥16	≤0.25-≥16	115	58.26	≤1	≥16	≤0.25-≥16	139	49.64	-	0.342
	MEM	≥16	≥16	≤0.25-≥16	101	69.32	≥16	≥16	≤0.25-≥16	155	72.26	≥16	≥16	≤0.25-≥16	229	65.94	≥16	≥16	≤0.25-≥16	114	60.53	4	≥16	≤0.25-≥16	121	50.41	-	0.453
	TGC	1	4	≤0.12-≥8		*	1	2	≤0.12-≥8		*	1	2	≤0.12-≥8		*	1	2	≤0.12-4		*	≤0.5	4	≤0.12-4		*	<0.001	-
		2011					2012					2013					2014					2015						
Blood: S. pneumoniae		MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n		MIC50	MIC90	Range	n		p-value MIC50	p- value %R
	CTX	≤1	≤1	≤1-≤1	1	0,00	≤1	2	≤1-2	3	33.33	≤1	≤1	≤1-≤1	3	0,00	≤1	≤1	≤0.5-≤1	0	0,00	≤1	≤1	≤1-≤1	5	0	0.351	0.615**
	OX1	0.5	0.5	0.5-0.5	*	*	≤0.25	≤0.25	≤0.25-≤0.25	*	*	.	.	.	*	*	.	.	.	*	*	.	.	.	*	*	0.223	-
	P	≥0.5	≥0.5	≥0.5-≥0.5	1	0,00	2.25	4	≥0.5-4	2	50.00	.	.	.	0	0,00	.	.	.	1	0,00	≥0.5	≥0.5	≥0.5-≥0.5	1	0	0.599	1.000**
	SXT	≥320	≥320	≥320-≥320	2	100,00	170	≥320	≤10-≥320	4	100,00	≤20	≤20	≤20-≤20	3	100,00	≤20	≤20	≤20-≥320	1	100,00	≤20	≥320	≤20-≥320	6	100,00	0.196	1.000**
		2011					2012					2013					2014					2015						
Blood: Salmonella spp.		MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	MIC50	MIC90	Range	n	%R	p-value MIC50	p- value %R
	CAZ	≤1	≤1	≤1-≤1	13	0,00	≤1	≤1	≤1-≤1	41	0,00	≤1	≤1	≤1-≤1	56	0,00	≤1	≤2	≤1-4	20	0,00	≤1	≤1	≤1-≤1	39	0,00	<0.001	-
	CIP	≤0.25	2	≤0.25-2	42	9.52	≤0.25	≤0.25	≤0.25-≤0.5	76	26.32	≤0.25	≤0.25	≤0.25-≤0.25	89	29.21	≤0.25	≤1	≤0.25-2	25	28,00	≤0.25	≤0.25	≤0.25-≤0.5	61	3.28	<0.001	0.001**
	CTX	≤1	4	≤1-4	17	29.41	≤1	≤1	≤1-≤1	41	0,00	≤1	≤1	≤1-≤1	56	0,00	≤1	≤1	≤0.5-≤1	20	0,00	≤1	≤1	≤1-≤1	39	0,00	<0.001	<0.001**
	ETP	≤0.5	≤1	≤0.5-≤1	17	23.53	≤0.5	≤0.5	≤0.5-≤0.5	41	0,00	≤0.5	≤0.5	≤0.5-≤0.5	56	0,00	≤0.5	≤0.5	≤0.5-≤0.5	19	0,00	≤0.5	≤0.5	≤0.5-≤0.5	39	0,00	<0.001	<0.001**
	IPM	≤1	≤1	≤0.25-2	17	5.88	≤1	≤1	≤0.25-≤1	41	0,00	≤0.25	≤0.25	≤0.25-≤0.25	56	0,00	≤0.25	≤0.25	≤0.25-≤1	19	0,00	≤0.25	0.5	≤0.25-≤1	39	0,00	<0.001	0.104**
	MEM	≤0.25	≤0.25	≤0.25-≤0.25	13	0,00	≤0.25	≤0.25	≤0.25-≤0.25	41	0,00	≤0.25	≤0.25	≤0.25-≤0.25	56	0,00	≤0.25	0.5	≤0.25-1	19	0,00	≤0.25	≤0.25	≤0.25-≤0.25	39	0,00	0.003	-

		2011					2012					2013					2014					2015						
Urine: E. coli		**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**p-value MIC50**	**p-value %R**
	AM	≥32	≥32	≤2-≥32	1131	81.70	≥32	≥32	≤2-≥32	1896	83.23	≥32	≥32	≤2-≥32	2301	81.66	≥32	≥32	≤2-≥32	1601	82.26	≥32	≥32	≤2-≥32	2504	81.63	-	0.993
	CAZ	≤1	4	≤1-≥64	1124	8.01	≤1	16	≤1-≥64	1887	11.55	≤1	16	≤1-≥64	###	11.67	≤1	16	≤1-≥64	1595	12.54	≤1	16	≤1-≥64	###	13.13	<0.001	0.002
	CIP	≤0.25	≥4	≤0.25-≥4	1133	27.10	≤0.25	≥4	≤0.25-≥8	1902	31.13	≤0.25	≥4	≤0.25-≥8	2305	31.58	≤0.25	≥4	≤0.25-≥4	1601	32.29	≤0.25	≥4	≤0.25-≥8	2504	33.91	0.011	0.058
	CS	≤0.5	2	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	<0.001	-
	CTX	≤1	8	≤1-≥64	1124	11.92	≤1	≥64	≤1-≥64	1888	16.10	≤1	≥64	≤1-≥64	###	18.77	≤1	≥64	≤1-≥64	1598	19.21	≤1	≥64	≤1-≥64	###	22.86	<0.001	<0.001
	ETP	≤0.5	≤0.5	≤0.5-4	1123	0.36	≤0.5	≤0.5	≤0.5-≥8	1879	0.32	≤0.5	≤0.5	≤0.5-≥8	###	0.39	≤0.5	≤0.5	≤0.5-≥8	1595	0.25	≤0.5	≤0.5	≤0.5-≥8	###	11.65	<0.001	0.001**
	FEP	≤1	≤1	≤1-≥64	1129	3.54	≤1	2	≤1-≥64	1893	5.28	≤1	≤4	≤1-≥64	###	6.46	≤1	≤4	≤1-≥64	1609	6.59	≤1	≤4	≤1-≥64	2504	7.67	<0.001	<0.001
	IPM	≤1	≤1	≤1-≥16	1128	0.71	≤1	≤1	≤0.25-≥16	1892	0.63	≤0.25	≤0.25	≤0.25-≥16	2287	0.66	≤0.25	≤0.25	≤0.25-≥16	1591	0.31	≤0.25	≤0.25	≤0.25-≥16	###	0.64	<0.001	0.622
	MEM	≤0.25	≤0.25	≤0.25-≥16	1125	0.18	≤0.25	≤0.25	≤0.25-≥16	1887	0.42	≤0.25	≤0.25	≤0.25-≥16	2285	0.66	≤0.25	≤0.25	≤0.25-≥16	1590	0.50	≤0.25	≤0.25	≤0.25-≥16	2297	0.57	<0.001	0.416**
	SXT	≥320	≥320	≤10-≥320	1130	76.64	≥320	≥320	≤10-≥320	1897	75.33	≥320	≥320	≤10-≥320	###	70.51	≥320	≥320	≤10-≥320	1597	72.39	≥320	≥320	≤10-≥320	2501	69.53	-	0.267
		2011					2012					2013					2014					2015						
Urine: K. pneumoniae		**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**p-value MIC50**	**p-value %R**
	CAZ	≤1	≥64	≤1-≥64	314	35.03	≤1	≥64	≤1-≥64	522	40.80	≤1	≥64	≤1-≥64	654	41.28	≤1	≥64	≤1-≥64	500	44.20	8	≥64	≤1-≥64	866	50.58	<0.001	0.021
	CIP	≤0.25	≥4	≤0.25-≥4	318	35.22	≤0.25	≥4	≤0.25-≥8	529	37.81	≤0.25	≥4	≤0.25-≥8	664	34.49	≤0.25	≥4	≤0.25-≥8	508	36.61	≤0.5	≥4	≤0.25-≥8	874	40.39	0.568	0.561
	CS	≤0.5	2	≤0.5-8	*	*	≤0.5	2	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	≤0.5	1	≤0.5-≥16	*	*	≤0.5	≤0.5	≤0.5-≥16	*	*	<0.001	-
	CTX	≤1	≥64	≤1-≥64	315	42.54	≤1	≥64	≤1-≥64	522	44.64	≤1	≥64	≤1-≥64	654	47.71	2	≥64	≤1-≥64	503	50.50	≥64	≥64	≤1-≥64	866	56.47	<0.001	0.047
	ETP	≤0.5	≤0.5	≤0.5-4	309	0.65	≤0.5	≤0.5	≤0.5-≥8	519	2.31	≤0.5	≤0.5	≤0.5-≥8	649	2.62	≤0.5	≤0.5	≤0.5-≥8	498	3.21	≤0.5	≥8	≤0.5-≥8	862	11.37	<0.001	<0.001**
	FEP	≤1	32	≤1-≥64	320	10.63	≤1	32	≤1-≥64	557	18.13	2	32	≤1-≥64	658	18.69	≤1	32	≤1-≥64	535	20,00	2	≥64	≤1-≥64	947	27.67	<0.001	<0.001
	IPM	≤1	≤1	≤1-≥16	315	1.27	≤1	≤1	≤0.25-≥16	523	1.53	≤0.25	≤0.25	≤0.25-≥16	654	0.92	≤0.25	0.5	≤0.25-≥16	501	2.59	≤0.25	≤1	≤0.25-≥16	866	9.82	<0.001	<0.001**
	MEM	≤0.25	≤0.25	≤0.25-≥16	315	1.27	≤0.25	≤0.25	≤0.25-≥16	522	1.15	≤0.25	≤0.25	≤0.25-≥16	653	2.45	≤0.25	≤0.25	≤0.25-≥16	501	2.79	≤0.25	8	≤0.25-≥16	798	11.53	<0.001	<0.001**
	SXT	≥320	≥320	≤10-≥320	316	70.25	≥320	≥320	≤10-≥320	528	64.58	≥320	≥320	≤10-≥320	664	61.90	≥320	≥320	≤10-≥320	508	65.94	≥320	≥320	≤20-≥320	874	65.10	-	0.836
		2011					2012					2013					2014					2015						
Faeces: Salmonella spp.		**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**p-value MIC50**	**p-value %R**
	CAZ	≤1	≥64	≤1-≥64	15	20,00	≤1	≤1	≤1-≥64	26	7.69	≤1	≤1	≤1-≤1	31	0,00	≤1	≤1	≤1-≤1	27	0,00	≤1	≤1	≤1-16	46	2.17	0.010	0.024**
	CIP	≤0.25	≥16	≤0.25-≥16	44	11.36	≤0.25	1	≤0.25-2	45	28.89	≤0.25	≤0.25	≤0.25-1	73	46.58	≤0.25	≤0.25	≤0.25-≤0.5	39	17.95	≤0.25	≤0.25	≤0.25-1	77	11.69	<0.001	0.001*
	CTX	4	≥64	≤1-≥64	26	53.85	≤1	≤1	≤1-≥64	26	7.69	≤1	≤1	≤1-≤1	31	0,00	≤1	≤1	≤1-≤1	27	0,00	≤1	≤1	≤1-8	46	4.35	<0.001	<0.001**
	ETP	≤0.5	≤1	≤0.5-≤1	26	42.31	≤0.5	≤0.5	≤0.5-≤0.5	26	0,00	≤0.5	≤0.5	≤0.5-≤0.5	31	0,00	≤0.5	≤0.5	≤0.5-≤0.5	27	0,00	≤0.5	≤0.5	≤0.5-≤0.5	46	0,00	<0.001	<0.001**
	IPM	≤1	≤1	≤0.25-≤1	26	0,00	≤1	≤1	≤0.25-≤1	26	0,00	≤0.25	≤0.25	≤0.25-0.5	30	0,00	≤0.25	≤0.25	≤0.25-0.5	27	0,00	≤0.25	≤0.25	≤0.25-0.5	46	0,00	<0.001	-
	MEM	≤0.25	≤0.25	≤0.25-≤0.25	15	0,00	≤0.25	≤0.25	≤0.25-≤0.25	26	0,00	≤0.25	≤0.25	≤0.25-≤0.25	31	0,00	≤0.25	≤0.25	≤0.25-≤0.25	25	0,00	≤0.25	≤0.25	≤0.25-≤0.25	46	0,00	-	-
		2011					2012					2013					2014					2015						
Faeces: Shigella spp.		**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**p-value MIC50**	**p-value %R**
	CAZ	≤1	≤1	≤1-≤1	22	0,00	≤1	≤1	≤1-≤1	58	0,00	≤1	≤1	≤1-≤1	75	0,00	≤1	≤1	≤1-4	56	0,00	≤1	≤1	≤1-16	108	0.93	0.067	1.000**
	CIP	≤0.25	≤0.25	≤0.25-≤0.25	22	0,00	≤0.25	≤0.25	≤0.25-1	58	0,00	≤0.25	≤0.25	≤0.25-≤0.25	75	0,00	≤0.25	≤0.25	≤0.25-≥4	56	1.79	≤0.25	≤0.25	≤0.25-≥4	108	1.84	0.261	0.617**
	CTX	≤1	≤1	≤1-≤1	22	0,00	≤1	≤1	≤1-≤1	58	0,00	≤1	≤1	≤1-4	75	2.67	≤1	≤1	≤1-≥64	56	7.14	≤1	≤1	≤1-≥64	108	6.48	0.151	0.201**
		2011					2012					2013					2014					2015						
Urethral-cervical: N. gonorrhoea		**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**MIC50 µg/ml**	**MIC90 µg/ml**	**Range**	**n**	**%R**	**p-value MIC50**	**p-value %R**
	CIP	.	.	.	0	0,00	≤0.25	≤0.25	≤0.25-≤0.25	1	0,00	.	.	.	0	0,00	≤0.25	≤0.25	≤0.25-≤0.25	1	0,00	≤0.5	≤0.5	≤0.5-≤0.5	1	100,00	-	1.000**
	GM	.	.	.	*	*	≤1	≤1	≤1-≤1	*	*	.	.	.	*	*	≤1	≤1	≤1-≤1	*	*	≤0.5	≤0.5	≤0.5-≤0.5	*	*	-	

AM- Ampicillin, CAZ- Ceftazidime, CIP- Ciprofloxacin, CS- Colistin, CTX- Cefotaxime, ETP- Ertapenem, FEP- Cefepime, IPM- Imipenem, MEM- Meropenem, SXT- Trimethoprim-sulfamethoxazole, AN- Amikacin, GM- Gentamicin, TGC- Tigecycline, FOX- Cefoxitin, OX1- Oxacillin, P- Penicillin (CLSI Breakpoint for non-meningitis S. pneumoniae)
* CLSI Breakpoint not available
**Pearson chi-square p-values
Note: MIC50 p-values are based on the results of a non-parametric equality-of-medians test.

The following GLASS metrics were generated: proportion of non-susceptible samples out of all samples positive for GLASS pathogens, number of infections caused by GLASS pathogens per specimen type per 100 000 inhabitants, number of infections caused by GLASS pathogens per organism per 100000 inhabitants and number of resistant infections per pathogen and drug per 100 000 inhabitants.

Table 3 illustrates the proportion of non-susceptible samples out of all samples positive for selected GLASS pathogens (See Supplementary Table 3 for all pathogens). While Table 2 reflects the percentage resistance with the total number of positive samples per pathogen-drug combination as a denominator, Table 3 reflects the resistance rates with the total number of samples positive for GLASS pathogens per specimen type as a denominator. Table 3 serves to illustrate the trends in resistance within each specimen type. A statistically significant increase in the proportion of *E. coli* from blood that were non-susceptible to ceftazidime, cefotaxime, ciprofloxacin and trimethoprim/sulfamethoxazole was evident as was the increase in the proportion of non-susceptible K. pneumoniae against all antibiotics tested. A statistically significant increase in the proportion of *Salmonella spp.* from faeces that were non-susceptible to ceftazidime, cefotaxime, ciprofloxacin and ertapenem was also observed. Out of 69 pathogen-drug combinations, only 19 had no resistant isolates in at least one year between 2011 and 2015. Most of the pathogens were multi-drug resistant i.e. resistant to three or more classes of antibiotics. *E. coli, K. pneumoniae, A. baumannii, Salmonella spp.* and *S. pneumoniae* were multi-drug resistant (*S. aureus, Shigella spp.* and *N. gonorrhoea* were tested against less than 3 classes of antibiotics). An escalation of ABR elucidated by GLASS metrics confirmed the general increase in ABR resistance trends described above.

Table 3: Proportion of non-susceptible samples out of all samples positive for GLASS pathogens per specimen type 2011-2015

Blood: ***E. coli***	**2011**	**2012**	**2013**	**2014**	**2015**	**P-value**
AM*	6,98	6,23	6,77	9,83	6,13	0
CAZ	0,89	1,34	0,99	1,85	1,87	0.007
CIP	1,73	1,97	2,09	3,33	2,68	0.013
CTX	1,23	2,01	1,74	3,33	2,77	<0.001
ETP	0,00	0,04	0,00	0,15	0,06	0.197**
FEP	0,45	0,71	0,39	0,96	0,74	0.143
IPM	0,00	0,13	0,00	0,00	0,12	0.131**
MEM	0,00	0,08	0,04	0,00	0,06	0.818**
SXT	7,14	6,27	6,41	9,09	4,79	<0.001
Total GLASS pathogens	**1792**	**2391**	**2823**	**1353**	**3363**	

Urine: ***K. pneumoniae***	**2011**	**2012**	**2013**	**2014**	**2015**	**P-value**
CAZ	3,92	5,52	6,07	8,86	6,64	<0.001
CIP	3,99	5,18	5,14	7,46	5,35	<0.001
CTX	4,77	6,04	7,01	10,18	7,41	<0.001
ETP	0,07	0,31	0,38	0,64	1,48	<0.001**
FEP	1,21	2,62	2,76	4,29	3,97	<0.001
IPM	0,14	0,21	0,13	0,52	1,29	<0.001**
MEM	0,14	0,16	0,36	0,56	1,39	<0.001**
SXT	7,91	8,84	9,23	13,43	8,62	<0.001
Total GLASS pathogens	**2808**	**3859**	**4451**	**2494**	**6600**	

Faeces: ***Salmonella spp.***	**2011**	**2012**	**2013**	**2014**	**2015**	**P-value**
CAZ	2,94	1,41	0	0	0,37	0.010**
CIP	4,90	9,15	14,35	4,29	3,31	<0.001
CTX	13,73	1,41	0	0	0,74	<0.001**
ETP	10,78	0	0	0	0	<0.001**
IPM	0	0	0	0	0	-
MEM	0	0	0	0	0	-
Total GLASS pathogens	**102**	**142**	**237**	**163**	**272**	

N. gonorrhoea	**2011**	**2012**	**2013**	**2014**	**2015**	**P-value**
CIP	0	0	0	0	1.85	-
Total GLASS pathogens	**35**	**24**	**34**	**21**	**54**	

* AM- Ampicillin, CIP- Ciprofloxacin, CTX- Cefotaxime, CAZ- Ceftazidime, FEP- Cefepime, IPM- Imipenem, ETP- Ertapenem, MEM- Meropenem, SXT- Trimethoprim-sulfamethoxazole
**Fisher's exact p-value, all others are Pearson chi-square p-values

Table 4 illustrates the number of resistant infections for one pathogen per specimen type per 100 000 inhabitants. (See Supplementary Table 4 for all pathogens). The number of resistant infections per 100 000 inhabitants generally increased across all pathogen-drug combinations with the exception of *Salmonella spp.* from faecal samples noting that relatively few faecal samples were included in the study (n=916) and *Salmonella spp./*ciprofloxacin was the only pathogen-drug combination for which resistant isolates were identified every year in the study period. Between 2011 and 2015 UTIs and BSIs were the most common infections causing 197 and 119 infections per 100 000 inhabitants (n=10 267 300) respectively while the most common causative pathogen was *E. coli* from urine samples, causing 148 infections per 100 000 inhabitants (n= 10 267 300). In BSIs, *S. aureus* caused the most number of infections (37 per 100 000 inhabitants, n= 10 267 300) while in the most common cause of diarrhoeal infections was *Shigella spp. (*6 per 100 000 inhabitants, n= 10 267 300). *E. coli* isolates from urine samples were responsible for the most number of resistant infections (75 per 100 000 inhabitants resistant to ampicillin, n= 10 267 300) while *K. pneumoniae* infections resistant to trimethoprim-sulfamethoxazole were most common among BSIs (10 per 100 000 inhabitants, n= 10 267 300). Diarrhoeal infections were most commonly caused by *Salmonella spp.* isolates resistant to ciprofloxacin (1 per 100 000 inhabitants, n= 10 267 300).

Table 4: Number of Resistant infections per pathogen and drug per 100 000 inhabitants

Blood: *E. coli*							**Urine: *K. pneumoniae***						
	2011	**2012**	**2013**	**2014**	**2015**	**p-value***		**2011**	**2012**	**2013**	**2014**	**2015**	**p-value***
AM	1,22	1,45	1,86	1,30	2,01	<0.001							
CAZ	0,16	0,31	0,27	0,24	0,61	0,00	**CAZ**	1,07	2,07	2,63	2,15	4,27	<0.001
CIP	0,30	0,46	0,57	0,44	0,88	<0.001	**CIP**	1,09	1,95	2,23	1,81	3,44	<0.001
CTX	0,21	0,47	0,48	0,44	0,91	<0.001	**CTX**	1,31	2,27	3,04	2,47	4,76	<0.001
ETP	0,00	0,01	0,00	0,02	0,02	0,18	**ETP**	0,02	0,12	0,17	0,16	0,95	<0.001
FEP	0,08	0,17	0,11	0,13	0,24	0,09	**FEP**	0,33	0,98	1,20	1,04	2,55	<0.001
IPM	0,00	0,03	0,00	0,00	0,04	0,16	**IPM**	0,04	0,08	0,06	0,13	0,83	<0.001
MEM	0,00	0,02	0,01	0,00	0,02	0,82	**MEM**	0,04	0,06	0,16	0,14	0,90	<0.001
SXT	1,25	1,46	1,76	1,20	1,57	<0.001	**SXT**	2,16	3,32	4,00	3,26	5,54	<0.001
Faeces: *Salmonella spp.*							***N. gonorrhoea***						
	2011	**2012**	**2013**	**2014**	**2015**			**2011**	**2012**	**2013**	**2014**	**2015**	
CAZ	0,03	0,02	0,00	0,00	0,01	0,06							
CIP	0,05	0,13	0,33	0,07	0,09	<0.001	**CIP**	0,00	0,00	0,00	0,00	0,01	NC
CTX	0,14	0,02	0,00	0,00	0,02	<0.001							
ETP	0,11	0,00	0,00	0,00	0,00	<0.001							
IPM	0,00	0,00	0,00	0,00	0,00	NC							
MEM	0,00	0,00	0,00	0,00	0,00	NC	**Total Population: 10 267 300**						

* Pearson chi-square p-value
NC- Not calculated

Resistance data was also compared to the standard treatment guidelines available in South Africa, published by the National Department of Health and the Federation of Infectious Diseases Societies of

Southern Africa (FIDSSA). Table 7 shows the proportion of infections that would be treatable using the antibiotics recommended by these guidelines. The antibiotics in the Table are those used in the Vitek 2 panel of antibiotics. In UTIs, only two thirds of the infections caused by *E. coli* and *K. pneumoniae* could be successfully treated by ciprofloxacin and amoxicillin-clavulanate, which are the first line agents recommended in the treatment guidelines. Nitrofurantoin showed only a 31% susceptibility against UTIs caused by *K. pneumoniae.* Ciprofloxacin, the recommended first line treatment for diarrhoeal infections, would only treat 75% of infections caused by *Salmonella spp*., however, 99% of diarrhoeal infections caused by *Shigella spp.* were shown to be treatable with ciprofloxacin.

Table 5: Percentage of Infections Susceptible to Antibiotics Recommended as per Treatment Guidelines

Blood Stream Infections*	**Cloxacillin**	**Vancomycin**	**Ceftriaxone**	**Gentamicin**	**Clindamycin**
S. aureus	-	96.79% (n=1368)	-	-	96.85% (n= 1367)
E. coli	-	-	-	80.13% (n= 936)	-
K. pneumoniae	-	-	-	40.72% (n= 1196)	-
S. pneumoniae	-	-	-	N/A	-
A. baumannii	-	-	-	37.45% (n= 745)	-
Salmonella spp.	-	-	-	N/A	-
Urinary Tract Infections **	**Ciprofloxacin**	**Amoxicillin-clavulanate**	**Gentamicin**	**Fosfomycin**	**Nitrofurantoin**
E. coli	68.31% (n-953)	67.15% (n=9397)	85.37% (n=9399)	-	87.41% (n=9383)
K. pneumoniae	62.67% (n=2893)	50.98% (n=2864)	59.01% (n=2776)	-	30.87% (n=2860)
Diarrhoeal Infections **	**Ciprofloxacin**				
Salmonella spp.	75.54% (n=145)				
Shigella spp.	99.06% (n=319)				
Gonorrhoea*	**Ceftriaxone**	**Azithromycin**			
N. gonorrhoea	-	-			

* SAASP Guidelines (Wasserman, et al., 2015)
** Government published Standard Treatment Guidelines (Department of Health Republic of South Africa, 2015)
- Pathogen-drug combination not included in study or no samples found for pathogen-drug combination

Discussion:

Antibiotic resistance trends were investigated in pathogen-drug combinations stipulated in GLASS for the period 2011-2015 in six public hospitals in the province of KwaZulu Natal, South Africa in putative BSIs, UTIs, diarrhoeal infections and gonorrhoeal infections. The antibiotic resistance data was stratified by year and analysed on 3 levels: (1) a trend analysis of resistance including MIC50, MIC90, MIC range and percentage resistance over 5 years was conducted; (2) selected metrics recommended in the GLASS manual were calculated and (3) susceptibility data was compared with existing standard treatment guidelines.

We generated a database of MIC and percentage resistance data including the majority of pathogen-drug combinations listed in the GLASS manual. From this data we were able to observe a general increase in percentage resistance during the study period in the majority of pathogen-drug combinations, calculate the proportion of infections caused by each pathogen of interest, calculate the rate of resistance in terms of the population in KwaZulu Natal and correlate the resistance rates with existing treatment guidelines in South Africa.

In South Africa diarrhoeal diseases and sexually transmitted infections (STIs) are major concerns, the former because it is one of the leading causes of infant mortality and the latter because it increases the risk of HIV infection [(6)]. UTIs are amongst the most common infections encountered in both the community and hospital setting and the emergence of resistance in various bacterial species has become a concern worldwide. BSIs are one of the leading nosocomial infections leading to poor treatment outcomes especially in children. In addition to the clinical importance of these infections, blood, urine, faecal and urethral/cervical samples are relatively easy to collect on a routine basis which is why these infections were identified for inclusion in the early implementation of GLASS. Existing ABR surveillance in South Africa has shown a decline in efficacy of older antibiotic drugs such as ampicillin and tetracycline and the emergence of resistance to carbapenems. In BSIs methicillin resistant *Staphylococcus aureus* (MRSA) as well as extended spectrum β-lactamase (ESBL) producing pathogens are prevalent, while in terms of diarrhoeal infections resistance to fluoroquinolones and third generation cephalosporins is increasing [(3)]. In UTIs there is increasing resistance to third generation cephalosporins and carbapenems and in gonorrhoeal infections first line treatment was changed to oral or intramuscular cephalosporins due to resistance to ciprofloxacin [(4)(7)]. We observed similar resistance trends in our study.

In order to address the problem on AMR, South Africa is amongst the few African countries to have a national AMR strategy framework and the only country with laboratory-based surveillance [(6) (8)] with ABR research on the increase. The Centre for Healthcare Associated Infections, Antimicrobial Resistance and Mycoses, a branch of the NICD, conducts laboratory based antimicrobial resistance surveillance (LARS) and. It was established in 2010 and collects data from sentinel sites. Electronic surveillance was implemented in 2013 and collects data from laboratory information systems in order to generate resistance maps. Enhanced surveillance has been implemented for methicillin-resistant *S. aureus* (MRSA) in order to determine the prevalence and extent of nosocomial and community-acquired MRSA infections. Enhanced surveillance is also underway for carbapenem-resistant Enterobacteriaceae (CREs). [9]

The results of this study were stratified, as per GLASS requirements, according to specimen type and pathogen-drug combination. These included BSIs, UTIs, diarrhoeal infections and gonorrhoeal infections. The total number of isolates increased more than two-fold from 4737 in 2011 to 10289 in 2015, which could indicate that there is an increase in the use of AST results to guide treatment and rational prescribing. Isolates from blood and urine samples comprised 96.72% (n=33018) of the samples included in the study, suggesting a focus on the treatment of bacteraemia and UTIs guided by AST results. Treatment guidelines published by the South African National Department of Health recommends symptomatic management of acute diarrhoea and only recommends antibiotic therapy in severe cases or where there is a co-morbidity. Gonorrhoeal infections are similarly managed empirically which could explain the limited number of isolates from faecal and urethral or cervical samples. [10] [11]

Of the isolates obtained from blood samples, *S. aureus* was the most commonly isolated pathogen followed by *K. pneumoniae* and *E. coli*. While over 3000 *S. aureus* isolates were identified, only 29 were tested against cefoxitin, the antibiotic recommended in the GLASS manual, and the percentage resistance was not calculated as there is no listed CLSI MIC breakpoint for this pathogen-drug combination. As such, resistance in *S. aureus* isolates will be discussed with reference to the MIC data.

As a common cause of nosocomial infections with a known prevalence of multi-drug resistance, existing data has shown that *A. baumannii* is becoming increasingly difficult to treat. A study published in 2012 by Ballot et al. reported that *A. baumannii* was the third most common cause of sepsis-related deaths in neonates in a public hospital in Johannesburg, South Africa. [12] Crowther-Gibson et al. reported that only 20 – 40% of *A. baumannii* infections were susceptible to carbapenems in public hospitals in South Africa in 2009 and that in the private sector, the use of carbapenems was becoming necessary due to increasing resistance to fluoroquinolones and third generation cephalosporins. The same paper reported that in the private sector, resistance in *A. baumannii* to imipenem and meropenem was already approximately 33% in 2006. [7] It is encouraging to note that resistance to all antibiotics in *A. baumannii* isolates in this study declined between 2011 and 2015 and that colistin and amikacin showed sensitivity of 94% and 89% respectively in 2015. A study conducted in a trauma intensive care unit in Durban, South Africa, in 2009 reported that *A. baumannii* isolates were most susceptible to amikacin, with 43% of isolates being susceptible, suggesting that susceptibility of *A. baumannii* to amikacin has since improved. *A. baumannii* isolates showed an 18 –

20% decrease in resistance to carbapenems and gentamicin, however, the percentage resistance to these antibiotics in 2015 was still in the region of 50%. These figures are slightly lower than those reported from sentinel public hospitals in South Africa in 2014 carried out by Perovic et al., which reported 66% and 77% resistance to gentamicin and carbapenems respectively and the 2009 study carried out by Ramsamy et al. which reported 87% and 90% resistance in *A. baumannii* isolates to gentamicin and meropenem respectively. (13) (14)

S. aureus is a common pathogen isolated in humans and is a leading cause of nosocomial bacteraemia and more recently there have been reports of MRSA isolates from the community which is a cause for increased concern. Fortuin de-Smit et al. reported that MRSA was associated with higher mortality than methicillin sensitive *S. aureus* and that MRSA isolates were more likely to be resistant to multiple antibiotics from multiple classes. (15) In 2006 the incidence of MRSA was reported to be 36% based on data from 5 private hospitals while Ballot et al. reported that 70% of *S. aureus* isolates were methicillin resistant from blood cultures from neonates at a public hospital South Africa between 2009 and 2010. Perovic et al. also reported methicillin resistance in 46% of *S. aureus isolates* from blood samples between 2010 and 2012 in 13 public hospitals around South Africa. (3) (7) (12) Data from the public sector obtained in 2009 indicated that *S. aureus* isolates from blood cultures showed a 16 – 76%, 11 – 83% and 28 – 85% susceptibility to cloxacillin, erythromycin and clindamycin respectively. Results from the Ramsamy et al. study in trauma intensive care unit patients reported a relatively high susceptibility of *S. aureus* isolates to clindamycin, cloxacillin, erythromycin, gentamicin and vancomycin (62 -100%) while only 11% and 16% susceptibility to penicillin and ampicillin was reported. (7) (14) The results the Perovic et al. study published in 2015 showed that while 99.8% of methicillin sensitive *S. aureus* isolates were sensitive to cefoxitin, only 5% of MRSA isolates were susceptible. Cefoxitin was the only antibiotic included in the study for *S. aureus* isolates as it is a surrogate for testing susceptibility to oxacillin, meaning it is a means of detecting MRSA. Fernandes et al. suggested in a study published in 2005 that cefoxitin resistance could be an easier method of detection methicillin resistance as opposed to the detection of the mecA gene by PCR, which is costly and requires specialised equipment. (2) (16 The Vitek 2 system provides results of a cefoxitin screen instead of MIC50 and MIC90 readings. A positive cefoxitin screen implies that the isolate is MRSA. Regrettably we were unable to extract and include this data in the results and it is an important limitation that should be addressed in future studies. *S. pneumoniae* is the leading cause of community-acquired pneumonia but is also the cause of invasive diseases such as bacterial meningitis and sepsis which are associated with high mortality. Resistance to penicillin and other β-lactam antibiotics in *S. pneumoniae* has been associated with poor clinical outcomes in patients with pneumococcal meningitis, but the implication is less apparent in terms of BSIs. The WHO Antimicrobial Resistance Global Report on Surveillance 2014 report mentions that further resistance data is required in order to establish the effect of reduced susceptibility to penicillins on clinical outcomes in patients with BSIs. The report noted that in the Africa region, 3% of invasive *S. pneumoniae* (including BSIs and meningitis) isolates showed resistance to penicillin while Ramsamy et al. reported that *S. pneumoniae* isolates from the trauma intensive care unit showed 7% resistance to penicillin, 33% resistance to ampicillin and 100% resistance to cloxacillin. (14) (17) In this study AST results were included for *S. pneumoniae* isolates from blood samples against cefotaxime, oxacillin, benzylpenicillin and trimethoprim-sulfamethoxazole. As compared to the other pathogens included in the study, antimicrobial susceptibility data for *S. pneumoniae* was minimal for the GLASS antibiotic panel, with only 11, 5 and 16 isolates tested against cefotaxime, penicillin and trimethoprim-sulfamethoxazole respectively. Oxacillin is used to screen for penicillin non-susceptibility in *S. pneumoniae*, thus there is no CLSI breakpoint for this pathogen-drug combination. Between 2011 and 2015, only one isolate each was found to be resistant to cefotaxime and penicillin while 100% resistance was found to trimethoprim-sulfamethoxazole.

K. pneumoniae is a common cause of UTIs and BSIs and is also a major cause of carbapenem resistant infections. *K. pneumoniae* is also reported to have the highest prevalence of resistance to third and fourth generation cephalosporins out of all of the Enterobacteriaceae. Ballot et al. found that *K. pneumoniae* was the second most common cause of BSIs in neonates at a public hospital in South Africa with 65% of these isolates being ESBL positive. (12) In terms of UTIs, *K. pneumoniae* isolates showed the most notable increase in resistance to all antibiotics except trimethoprim-sulfamethoxazole, with the greatest increase in resistance being a 17% increase in resistance to cefepime. Resistance to carbapenems in isolates from urine samples increased from ≤ 1% in 2011 to 10% in 2015 and increased by more than 20% in isolates from blood samples. This is more than double the figure of 3-5% resistance to carbapenems reported by Perovic et al. in 2014, while the WHO Antimicrobial Resistance Global Report on Surveillance 2014 reported 0 – 4% resistance to carbapenems in the Africa Region. *K. pneumoniae* isolates from blood samples showed an increase in resistance of 18% or more to all the antibiotics with the exception of trimethoprim-sulfamethoxazole. These findings are similar to those reported by Perovic et al. in their study of resistance in *K. pneumoniae* isolates from national sentinel site surveillance in South Africa between 2010 and 2012 which reported that the majority of isolates were ESBL positive and were resistant to third and fourth generation cephalosporins. Resistance to the cephalosporins was found to be in the region of 70% with the exception of cefoxitin, which displayed high sensitivity (>80%) along with the carbapenems, amikacin, colistin, tigecycline and fosfomycin. It is apparent from the results of this study that carbapenem resistance in *K. pneumoniae* isolates is increasing with an 8 – 11% and a 23 – 25% increase in resistance to carbapenems in isolates from urine and blood samples respectively. (6) (13) (17)

E. coli is the most common cause of both community and hospital acquired UTIs and is a common cause of BSIs. In this study *E. coli* isolates accounted for 52% of the isolates, mainly from urine samples. The WHO Antimicrobial Resistance Global Report on Surveillance 2014 showed 2 – 70%

portion of resistance in *E. coli* isolates to third generation cephalosporins and 14 – 71% resistance to fluoroquinolones from national data from the Africa Region. More specifically, the data from AMR surveillance conducted at sentinel public hospitals in South Africa in 2014 reported a 24% resistance to third generation cephalosporins and cefepime, a fourth-generation cephalosporins. (13) It was reported by Ballot et al. that all but one of the *E. coli* isolates from blood cultures from neonates at a public hospital in South Africa were resistant to ampicillin, although all isolates were found to be susceptible to third generation cephalosporins and aminoglycosides. (12) The results of this study show that resistance to ampicillin was >80% in isolates from urine and blood samples by 2015 and that there was an increase in resistance in isolates from blood samples from 18% to 27% for ceftazidime and 26% to 40% for ceftriaxone between 2011 and 2015. Resistance to cefepime was found to be lower than the figure reported from 2014 surveillance data with a reported resistance of 11%. (17) Resistance in isolates from urine samples was found to be lower than in isolates from blood samples with a 13% resistance to ceftazidime, 23% resistance to ceftriaxone and 8% resistance to cefepime in 2015. Susceptibility to carbapenems was found to be >90% in isolates from blood and urine samples which is consistent with the findings of Ramsamy et al. in 2009. (14) Coetzee et al. reported in 2016 that increasing colistin resistance in *E. coli* isolates is becoming a serious concern and that the use of colistin as a last resort is becoming more common due to resistance to other antibiotics. Resistance is thought to be spread by the presence of the mcr-1 gene in food animals such as pork and poultry, the presence of which has been confirmed by surveillance of poultry operations in South Africa. The mcr-1 gene has also been detected in colistin-resistant *E. coli* in hospitalised and outpatient-based patients in South Africa. The percentage resistance data for *E. coli* and colistin was not calculated as there is currently no CLSI breakpoint for this pathogen-drug combination and the MIC50 and MIC90 results remained stable throughout the study period, however, future studies should monitor resistance in this pathogen-drug combination as resistance to colistin would severely limit treatment options in multi-drug resistant *E. coli* infections. (18)

Salmonella spp. pathogens are a common cause of foodborne illnesses such as gastroenteritis and enteric fever in the case of the *Salmonella enterica* serotypes Typhi and Paratyphi. *Shigella spp.* is also major cause of diarrhoeal infections which can be life threatening in children, especially in developing countries. The 2015 GERMS-SA Annual Report identified resistance to ciprofloxacin in *Salmonella typhi* as well as the emerging resistance to fluoroquinolones in *Shigella spp*. as a concern.(8) This was also highlighted in the WHO Antimicrobial Resistance Global Report on Surveillance 2014 which stated a 0 – 35% and a 0 – 3% proportion of resistance to fluoroquinolones in non-typhoidal *Salmonella* and *Shigella spp.* respectively.(17) Ciprofloxacin is the antibiotic recommended in the standard treatment guidelines in South Africa for acute diarrhoeal infections caused by *Salmonella spp.* and *Shigella spp.* Resistance of *Shigella spp.* isolates increased from 0%(n=22) in 2011 to 2%(n=108) in 2015, which is comparable to the 1% resistance reported in the

GERMS-SA 2015 Annual Report. *Salmonella spp.* isolates from both blood and faecal samples showed either a decrease in resistance or no net change in resistance to all antibiotics and showed a considerable decrease in resistance of 50% to cefotaxime in isolates from faecal samples. In 2015, the resistance of *Salmonella spp.* isolates to ciprofloxacin was 12%, which is less than the figure reported in the GERMS-SA 2015 report of 14% in *S. typhi* isolates and 21% in non-typhoidal *Salmonella* isolates. [8]

As an STI, gonorrhoea is a public health concern in South Africa due to the associated increased risk of HIV infection. Resistance to first line therapy for gonorrhoea resulted in the change from ciprofloxacin to cephalosporins in 2008, however, the WHO Antimicrobial Resistance Global Report on Surveillance 2014 reported a decreased in susceptibility to third generation cephalosporins in the Africa Region in 2014.[17] In this study only three *N. gonorrhoea* isolates were included over the five years, of which only one showed resistance to ciprofloxacin. Ceftriaxone and cefixime, the third generation cephalosporins recommended in the GLASS antibiotic panel for testing, were not included in this study.

GLASS metrics were consistent with the percentage resistance data and reinforced the existing literature which suggests that there is a decrease in the efficacy of older antibiotics such as ampicillin and trimethoprim-sulfamethoxazole and that emerging resistance to current treatment options is a grave concern as is the case with ciprofloxacin, third generation cephalosporins and carbapenems.

The implication of the increasing resistance shown in the results, especially the prevalence of multi-drug resistant infections, is that commonly encountered infections are becoming increasingly difficult to treat. Infections are responsive to a smaller range of broader spectrum antibiotics resulting in their increased use and subsequent selection pressure for resistance. If the current trends continue, untreatable infections will become more common, resulting in poorer clinical outcomes and higher mortality. It is clear that there is an urgent need to implement rational prescribing practices and infection control measures in order to curb the incidence and spread of antibiotic resistance.

The standard treatment guidelines and the essential drugs list are devised and published by the South African National Department of Health for implementation in public health facilities. The aim of these guidelines is to streamline empiric drug therapy and encourage rational prescribing practices. Essack and Connolly carried out a study evaluating the sensitivity of antibiotics listed in the STGs against isolates from a district hospital, regional hospital and tertiary hospital, in Durban, South Africa. Susceptibility was found to be varied across different pathogen-drug combinations as well as between the different facilities. As such institution specific, evidence-based guidelines based on regular surveillance was recommended in order to improve the accuracy and success of empiric therapy.[19]The comparison between the trends in resistance and the antibiotics recommended in treatment guidelines showed >65% susceptibility in most pathogens to the recommended agents, with the exception of *K. pneumoniae* in UTIs which showed 51 – 63% susceptibility to ciprofloxacin, gentamicin and amoxicillin-clavulanate but only a 31% susceptibility to nitrofurantoin, the drug of choice for the treatment of UTIs in the 2^{nd} and 3^{rd} trimester of pregnancy and in patients with a severe penicillin allergy. Susceptibility in *A. baumannii* and *K. pneumoniae* from blood samples to gentamicin was between 30% and 40%.

The data obtained during this study has provided a good overview of the trends in ABR in KwaZulu Natal, but the results may not be accurately representative of the true prevalence of resistance. As a retrospective study, the data obtained was limited by what had already been captured in the NHLS database, which only included results positive for microbial growth instead of all samples tested. In addition, due to technical problems data from one of the hospitals was missing for 2014. Many entries were excluded due to insufficient isolate or demographic data, which is an area for improvement in the routine capturing of AST results. Data was not stratified by demographic data such as age and gender; clinical data such as such as diagnosis and number of patient days and facility data such as hospital level and ward type. These parameters would be useful in providing a more detailed analysis of AMR and should be considered for future studies. The data obtained may also be misrepresentative as not all infections necessarily generate a sample for microbiological evaluations because of time and resource constraints and samples sent for AST are likely from patients who are severely ill and have experienced treatment failure. Additionally, not all ill patients seek treatment due to difficulties in accessing healthcare as well as reliance on cultural healing methods. Only 6 public hospitals out of the 71 provincial public hospitals in KwaZulu Natal were included in the study, meaning that community acquired infections and ABR in outlying regions of the province may not have been represented. Future studies should endeavour to include samples from community health settings, as well as try to include more hospitals so as to more accurately represent the status of ABR in KwaZulu Natal.

While some pathogens showed a decline or plateau in resistance to the various antibiotics, the general trend observed is that of an increase in resistance to most of the antibiotics included in this study. This affirms the need for interventions to curb the incidence and spread of resistance such as better prescribing practices, using AST results to guide treatment and antibiotic stewardship. Resources are available to guide rational antibiotic prescribing including standard treatment guidelines published by the South African National Department of Health and other organisations but there is no guarantee that these recommendations are adhered to at ground level.

This study highlights the potential for further research into the trends in resistance and it is apparent that resources are available to obtain the necessary isolates, AST results and demographic data as recommended by the WHO for the monitoring of ABR. This bodes well for the implementation of GLASS in South Africa, as we were able to calculate various metrics based on the GLASS recommendations and the overall analysis gave us a good indication of the general trends in antibiotic resistance in KZN. The ultimate goal of future studies would be to build on the existing knowledge base and provide comparable, validated data which can be shared on a global scale in order to guide future interventions against the spread of ABR.

SUPPLEMENTARY DATA

Table 1: Antibiotic Panel for AST Analysis per Pathogen

Pathogen	Antibiotic Panel
Acinetobacter baumannii	Amikacin Colistin Ertapenem Gentamicin Imipenem Meropenem Tigecycline
Escherichia coli	Ampicillin Cefepime Cefotaxime Ceftazidime Ciprofloxacin Colistin Cotrimoxazole Ertapenem Imipenem Meropenem
Klebsiella pneumoniae	Cefepime Cefotaxime Ceftazidime Ciprofloxacin Colistin Cotrimoxazole Ertapenem Imipenem Meropenem
Staphylococcus aureus	Cefoxitin
Shigella spp.	Cefotaxime Ceftazidime Ciprofloxacin
Streptococcus pneumoniae	Cefotaxime Trimethoprim/sulfamethoxszole Penicillin G
Salmonella spp.	Cefotaxime Ceftazidime Ciprofloxacin Ertapenem Imipenem Meropenem
Neisseria gonorhhoeae	Ciprofloxacin Gentamicin

Table 2: Overall AST Results between 2011 and 2015 per specimen type, pathogen and drug

Bloodstream Infections

	E. coli					*K. pneumoniae*					*A. baumanii*				
	MIC50 (mg/l)	MIC90 (mg/l)	Range	n	%R	MIC50 (mg/l)	MIC90 (mg/l)	Range	N	%R	MIC50 (mg/l)	MIC90 (mg/l)	Range	n	%R
AM**	≥32	≥32	≤2 - ≥32	946	84.99	-	-	-	-	-	-	-	-	-	-
CIP	≤ 0.25	≥ 4	≤0.25 - ≥8	953	28.54	1	≥4	≤0.25 - ≥8	1281	43.56	-	-	-	-	-
CTX	≤1	≥64	≤1 - ≥64	934	27.52	≥64	≥64	≤1- ≥64	1239	70.54	-	-	-	-	-
CAZ	≤1	16	≤1 - ≥64	934	17.56	16	≥64	≤1 - ≥64	1241	62.29	-	-	-	-	-
FEP	≤1	8	≤1 - ≥64	935	7.91	2	≥64	≤1 - ≥64	1229	26.53	-	-	-	-	-
IPM	≤0.25	≤1	≤0.25 - ≥16	933	0.75	≤0.25	≤1	≤0.25 - ≥16	1242	9.98	≥16	≥16	≤0.25 - ≥16	745	63.33
ETP	≤0.5	≤0.5	≤0.5 - 4	926	0.54	≤0.5	≤0.5	≤0.5 - ≥8	1210	10,00	≤0.5	≥8	≤0.5 - ≥8	*	*
MEM	≤0.25	≤0.25	≤0.25 - ≥16	914	0.55	≤0.25	≤0.25	≤0.25 - ≥16	1169	8.55	≥16	≥16	≤0.25 - ≥16	739	64.17
CS	≤0.5	≤0.5	≤0.5 - ≥16	*	*	≤0.25	2	≤0.25 - ≥16	*	*	≤0.5	2	≤0.5-64	720	6.31
SXT	≥320	≥320	≤10 - ≥320	951	78.13	≥320	≥320	≤10 - ≥320	1279	76.54	-	-	-	-	-
TGC	-	-	-	-	-	-	-	-		-	1	2	≤0.12 - ≥8	*	*
GM	-	-	-	-	-	-	-	-		-	≥16	≥16	≤0.5 - ≥16	745	62.55
AN	-	-	-	-	-	-	-	-		-	8	32	≤2 - ≥64	744	14.78

	S. aureus					*S. pneumoniae*					*Salmonella spp.*				
	MIC50 (mg/l)	MIC90 (mg/l)	Range	n	%R	MIC50 (mg/l)	MIC90 (mg/l)	Range	N	%R	MIC50 (mg/l)	MIC90 (mg/l)	Range	n	%R
CIP	-	-	-	-	-	-	-	-	-	-	≤0.25	≤0.5	≤0.25 - 2	230	25.65
CTX	-	-	-	-	-	≤1	≤1	≤1 - 2	12	8.33	≤1	≤1	≤0.5 - 4	173	2.89
CAZ	-	-	-	-	-	-	-	-	-	-	≤1	≤1	≤1 - 4	169	0
FEP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IPM	-	-	-	-	-	-	-	-	-	-	≤0.25	≤1	≤0.25 - 2	172	0.58
ETP	-	-	-	-	-	-	-	-	-	-	≤0.5	≤0.5	≤0.5 - 1	172	2.33
MEM	-	-	-	-	-	-	-	-	-	-	≤0.25	≤0.25	≤0.25 - 1	168	0
CS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SXT	-	-	-	-	-	≤20	≥320	≤10 - ≥320	16	100	-	-	-	-	-
FOX	≥64	≥64	≤4 - ≥64	*	*	-	-	-	-	-	-	-	-	-	-
OX1	-	-	-	-	-	≤0.25	0.5	≤0.25 - 0.5	*	*	-	-	-	-	-
P	-	-	-	-	-	≥0.5	4	≥0.5 - 4	5	20	-	-	-	-	-

Urinary tract infections

	E .coli					*K. pneumoniae*				
	MIC50 (mg/l)	MIC90 (mg/l)	Range	n	%R	MIC50 (mg/l)	MIC90 (mg/l)	Range	N	%R
SXT**	≥320	≥320	≤10 - ≥320	9431	72.27	≥320	≥320	≤10 - ≥320	2890	64.98
CIP	≤0.25	≥4	≤0.25 - ≥8	9445	31.69	≤0.25	≤4	≤0.25 - ≥8	2893	37.33
CAZ	≤1	16	≤1 - ≥64	9384	11.74	≤1	≥64	≤1 - ≥64	2856	43.84
CTX	≤1	≥64	≤1 - ≥64	9385	18.57	≤1	≥64	≤1 - ≥64	2860	49.72
FEP	≤1	≤4	≤1 - ≥64	9427	6.18	≤1	32	≤1 - ≥64	3017	20.23
IPM	≤0.25	≤1	≤0.25 - ≥16	9386	0.6	≤0.25	≤1	≤0.25 - ≥16	2859	4.06
ETP	≤0.5	≤0.5	≤0.5 - ≥8	9370	0.6	≤0.5	≤0.5	≤0.5 - ≥8	2837	5.11
MEM	≤0.25	≤0.25	≤0.25 - ≥16	9184	0.5	≤0.25	≤0.25	≤0.25 - ≥16	2789	4.73
CS	≤0.5	≤0.5	≤0.5 - ≥16	*	*	≤0.5	1	≤0.5 - ≥16	*	*
AM	≥32	≥32	≤2 - ≥32	9433	82.07	-	-	-	-	-

Acute Diarrhoeal Infections

	Salmonella spp.					*Shigella spp.*				
	MIC50 (mg/l)	MIC90 (mg/l)	Range	n	%R	MIC50 (mg/l)	MIC90 (mg/l)	Range	N	%R
CIP**	≤0.25	≤0.5	≤0.25 - ≥16	145	46.90	≤0.25	≤0.25	≤0.25 - ≥4	319	0.94
CTX	≤1	4	≤1 - ≥64	156	11.54	≤1	≤1	≤1 - ≥64	319	4.08
CAZ	≤1	≤1	≤1 - ≥64	145	4.14	≤1	≤1	≤1 - 16	319	0.31
IPM	≤0.25	≤1	≤0.25 - ≤1	155	0,00	-	-	-	-	-
ETP	≤0.5	≤0.5	≤0.5 - ≤1	156	7.05	-	-	-	-	-
MEM	≤0.25	≤0.25	≤0.25 - ≤0.25	143	0,00	-	-	-	-	-

Gonorrhoea

	N. gonorrhoea				
	MIC50	MIC90	Range	n	%R
CIP**	≤0.25	≤0.5	≤0.25 - ≤0.5	3	33.33
GM	≤1	≤1	≤0.5 - ≤1	*	*

* CLSI Breakpoint not available

** AM- Ampicillin, CIP- Ciprofloxacin, CTX- Cefotaxime, CAZ- Ceftazidime, FEP- Cefepime, IPM- Imipenem, ETP- Ertapenem, MEM- Meropenem, CS- Colistin, SXT- Trimethoprim-sulfamethoxazole, GM- Gentamicin

Table 3: Proportion of non-susceptible samples out of all GLASS Pathogens per specimen type

Proportion of non-susceptible samples out of all Blood samples positive for GLASS pathogens													
Blood: *E. coli*	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
AM	125	6,98	149	6,23	191	6,77	133	9,83	206	6,13	0.000	804	6,86
CAZ	16	0,89	32	1,34	28	0,99	25	1,85	63	1,87	0.007	164	1,40
CIP	31	1,73	47	1,97	59	2,09	45	3,33	90	2,68	0.013	272	2,32
CTX	22	1,23	48	2,01	49	1,74	45	3,33	93	2,77	0.000	257	2,19
ETP	0	0,00	1	0,04	0	0,00	2	0,15	2	0,06	0.197*	5	0,04
FEP	8	0,45	17	0,71	11	0,39	13	0,96	25	0,74	0.143	74	0,63
IPM	0	0,00	3	0,13	0	0,00	0	0,00	4	0,12	0.131*	7	0,06
MEM	0	0,00	2	0,08	1	0,04	0	0,00	2	0,06	0.818*	5	0,04
SXT	128	7,14	150	6,27	181	6,41	123	9,09	161	4,79	0.000	743	6,34
Blood: *K pneumoniae*	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
CAZ	71	3,96	121	5,06	174	6,16	150	11,09	257	7,64	0.000	773	6,59
CIP	50	2,79	96	4,02	131	4,64	89	6,58	192	5,71	0.000	558	4,76
CTX	82	4,58	152	6,36	189	6,70	168	12,42	283	8,42	0.000	874	7,46
ETP	2	0,11	9	0,38	15	0,53	6	0,44	89	2,65	0.000*	121	1,03
FEP	15	0,84	47	1,97	74	2,62	51	3,77	139	4,13	0.000	326	2,78
IPM	2	0,11	12	0,50	14	0,50	5	0,37	91	2,71	0.000	124	1,06
MEM	2	0,11	10	0,42	14	0,50	4	0,30	70	2,08	0.000*	100	0,85
SXT	101	5,64	178	7,44	228	8,08	183	13,53	289	8,59	0.000	979	8,35
Blood: *A. baumanii*	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
AN	17	0,95	31	1,30	35	1,24	11	0,81	16	0,48	0.007	110	0,94
CS (ab)	6	0,33	11	0,46	12	0,43	10	0,74	8	0,24	0.161	47	0,40
GM	71	3,96	114	4,77	140	4,96	69	5,10	72	2,14	0.000	466	3,98
IPM (ab)	70	3,91	111	4,64	151	5,35	67	4,95	69	2,05	0.000	468	3,99
MEM (ab)	69	3,85	112	4,68	151	5,35	69	5,10	61	1,81	0.000	462	3,94
	2011		**2012**		**2013**		**2014**		**2015**			**Overall**	
Blood: *S. aureus*	*	*	*	*	*	*	*	*	*	*			
Blood: *S. pneumoniae*	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
CTX	0	0,00	1	0,04	0	0,00	0	0,00	0	0,00	0.472*	1	0,01
P (sp)	0	0,00	1	0,04	0	0,00	0	0,00	0	0,00	0.472*	1	0,01
SXT	2	0,11	4	0,17	3	0,11	1	0,07	6	0,18	0.919*	16	0,14
Blood: *Salmonella spp.*	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
CAZ	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	-	0	0,00
CIP	4	0,22	20	0,84	26	0,92	7	0,52	2	0,06	0.000*	59	0,50
CTX	5	0,28	0	0,00	0	0,00	0	0,00	0	0,00	0.000*	5	0,04
ETP	4	0,22	0	0,00	0	0,00	0	0,00	0	0,00	0.001*	4	0,03
IPM	1	0,06	0	0,00	0	0,00	0	0,00	0	0,00	0.268*	1	0,01
MEM	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	-	0	0,00
Total Blood stream infections caused by GLASS pathogens	**1792**		**2391**		**2823**		**1353**		**3363**			**11722**	
Proportion of non-susceptible samples out of all Urine samples positive for GLASS pathogens													
Urine: *E. coli*	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
AM	924	32,91	1578	40,89	1879	42,22	1317	52,81	2044	30,97	0.000	7742	38,30
CAZ	90	3,21	218	5,65	267	6,00	200	8,02	327	4,95	0.000	1102	5,45
CIP	307	10,93	592	15,34	728	16,36	517	20,73	849	12,86	0.000	2993	14,81
CTX	134	4,77	304	7,88	429	9,64	307	12,31	569	8,62	0.000	1743	8,62
ETP	4	0,14	6	0,16	9	0,20	4	0,16	29	0,44	0.023*	52	0,26
FEP	40	1,42	100	2,59	148	3,33	106	4,25	192	2,91	0.000	586	2,90
IPM	8	0,28	12	0,31	15	0,34	5	0,20	16	0,24	0.816	56	0,28
MEM	2	0,07	8	0,21	15	0,34	8	0,32	13	0,20	0.134*	46	0,23
SXT	866	30,84	1429	37,03	1626	36,53	1156	46,35	1739	26,35	0.000	6816	33,72

Urine: *K. pneumoniae*	2011		2012		2013		2014		2015			Overall	
	n	%R	n	%R	n	%R	n	%R	n	%R	P-value	n	%R
CAZ	110	3,92	213	5,52	270	6,07	221	8,86	438	6,64	0.000	1252	6,19
CIP	112	3,99	200	5,18	229	5,14	186	7,46	353	5,35	0.000	1080	5,34
CTX	134	4,77	233	6,04	312	7,01	254	10,18	489	7,41	0.000	1422	7,04
ETP	2	0,07	12	0,31	17	0,38	16	0,64	98	1,48	0.000*	145	0,72
FEP	34	1,21	101	2,62	123	2,76	107	4,29	262	3,97	0.000	627	3,10
IPM	4	0,14	8	0,21	6	0,13	13	0,52	85	1,29	0.000*	116	0,57
MEM	4	0,14	6	0,16	16	0,36	14	0,56	92	1,39	0.000*	132	0,65
SXT	222	7,91	341	8,84	411	9,23	335	13,43	569	8,62	0.000	1878	9,29
Total urinary tract infections caused by GLASS pathogens	**2808**		**3859**		**4451**		**2494**		**6600**			**20212**	
Proportion of non-susceptible samples out of all Faecal samples positive for GLASS pathogens													
Faeces: *Salmonella spp*.	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
CAZ	3	2,94	2	1,41	0	0,00	0	0,00	1	0,37	0.010*	6	0,66
CIP	5	4,90	13	9,15	34	14,35	7	4,29	9	3,31	0.000	68	7,42
CTX	14	13,73	2	1,41	0	0,00	0	0,00	2	0,74	0.000*	18	1,97
ETP	11	10,78	0	0,00	0	0,00	0	0,00	0	0,00	0.000*	11	1,20
IPM	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	-	0	0,00
MEM	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	-	0	0,00
Faeces: *Shigella spp.*	**2011**		**2012**		**2013**		**2014**		**2015**				
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**		0	0,00
CAZ	0	0,00	0	0,00	0	0,00	0	0,00	1	0,37	1.000*	1	0,11
CIP	0	0,00	0	0,00	0	0,00	1	0,61	2	0,74	0.617*	3	0,33
CTX	0	0,00	0	0,00	2	0,84	4	2,45	7	2,57	0.102*	13	1,42
Total Acute Diarrhoeal infections caused by GLASS pathogens	**102**		**142**		**237**		**163**		**272**	**2,57**		**916**	
Proportion of non-susceptible samples out of all Urethral/cervical samples positive for GLASS pathogens													
N. gonorrhoea	**2011**		**2012**		**2013**		**2014**		**2015**			**Overall**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**P-value**	**n**	**%R**
CIP (ng)	0	0,00	0	0,00	0	0,00	0	0,00	1	1.85	-	1	0,59524
Total Gonorrhoeal infections	**35**		**24**		**34**		**21**		**54**			**168**	

Table 4: GLASS Population Measures

1. GLASS Measure A1/B1: Number of infections caused by GLASS pathogens per specimen type per 100 000 inhabitants													
Specimen Type	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
	n		**n**		**n**		**n**		**N**		**n**		
Blood	1792	17,45	2931	28,55	2823	27,50	1353	13,18	3363	32,75	12262,00	119,43	<0.001
Urine	2808	27,35	3859	37,59	4451	43,35	2494	24,29	6600	64,28	20212,00	196,86	<0.001
Faeces	102	0,99	142	1,38	237	2,31	163	1,59	272	2,65	916,00	8,92	<0.001
Urethral/cervical	35	0,34	24	0,23	34	0,33	21	0,20	54	0,53	168,00	1,64	0,032
Population		**10267300**											
2. GLASS Measure B2: Number of Infections caused by GLASS pathogens per organism per 100000 inhabitants													
Blood	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
E. coli	397	3,87	441	4,30	417	4,06	221	2,15	609	5,93	2085,00	20,31	<0.001
K. pneumoniae	373	3,63	577	5,62	698	6,80	358	3,49	1053	10,26	3059,00	29,79	<0.001
A. baumanii	248	2,42	341	3,32	483	4,70	147	1,43	353	3,44	1572,00	15,31	<0.001
S. aureus	543	5,29	773	7,53	890	8,67	471	4,59	1110	10,81	3787,00	36,88	<0.001
S. pneumoniae	188	1,83	179	1,74	238	2,32	118	1,15	170	1,66	893,00	8,70	<0.001
Salmonella spp	43	0,42	80	0,78	97	0,94	38	0,37	68	0,66	326,00	3,18	0,001
Urine	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
E. coli	2202	21,45	2936	28,60	3257	31,72	1907	18,57	4913	47,85	15215,00	148,19	<0.001
K. pneumoniae	606	5,90	923	8,99	1194	11,63	587	5,72	1687	16,43	4997,00	48,67	<0.001
Faeces	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
Salmonella spp	47	0,46	46	0,45	78	0,76	46	0,45	82	0,80	299,00	2,91	0,111

Shigella spp	55	0,54	96	0,94	159	1,55	117	1,14	190	1,85	617,00	6,01	0,001
Urethral/cervical	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
N. gonorrhoea	35	0,34	24	0,23	34	0,33	21	0,20	54	0,53	168,00	1,64	0,032
Population		**10267300**											
3. GLASS Measure B3: Number of Resistant infections per pathogen and drug per 100 000 inhabitants													
Blood: *E coli*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
AM	125	1,22	149	1,45	191	1,86	133	1,30	206	2,01	804,00	7,83	<0.001
CAZ	16	0,16	32	0,31	28	0,27	25	0,24	63	0,61	164,00	1,60	0,001
CIP	31	0,30	47	0,46	59	0,57	45	0,44	90	0,88	272,00	2,65	<0.001
CTX	22	0,21	48	0,47	49	0,48	45	0,44	93	0,91	257,00	2,50	<0.001
ETP	0	0,00	1	0,01	0	0,00	2	0,02	2	0,02	5,00	0,05	0,175
FEP	8	0,08	17	0,17	11	0,11	13	0,13	25	0,24	74,00	0,72	0,094
IPM	0	0,00	3	0,03	0	0,00	0	0,00	4	0,04	7,00	0,07	0,164
MEM	0	0,00	2	0,02	1	0,01	0	0,00	2	0,02	5,00	0,05	0,820
SXT	128	1,25	150	1,46	181	1,76	123	1,20	161	1,57	743,00	7,24	<0.001
Blood: *K pneumoniae*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CAZ	71	0,69	121	1,18	174	1,69	150	1,46	257	2,50	773,00	7,53	<0.001
CIP	50	0,49	96	0,94	131	1,28	89	0,87	192	1,87	558,00	5,43	0,002
CTX	82	0,80	152	1,48	189	1,84	168	1,64	283	2,76	874,00	8,51	<0.001
ETP	2	0,02	9	0,09	15	0,15	6	0,06	89	0,87	121,00	1,18	<0.001
FEP	15	0,15	47	0,46	74	0,72	51	0,50	139	1,35	326,00	3,18	<0.001
IPM	2	0,02	12	0,12	14	0,14	5	0,05	91	0,89	124,00	1,21	<0.001
MEM	2	0,02	10	0,10	14	0,14	4	0,04	70	0,68	100,00	0,97	<0.001
SXT	101	0,98	178	1,73	228	2,22	183	1,78	289	2,81	979,00	9,54	<0.001
Blood: *A. baumanii*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
AN	17	0,17	31	0,30	35	0,34	11	0,11	16	0,16	110,00	1,07	0,223
CS (ab)	6	0,06	11	0,11	12	0,12	10	0,10	8	0,08	47,00	0,46	0,067
GM	71	0,69	114	1,11	140	1,36	69	0,67	72	0,70	466,00	4,54	<0.001
IPM (ab)	70	0,68	111	1,08	151	1,47	67	0,65	69	0,67	468,00	4,56	<0.001
MEM (ab)	69	0,67	112	1,09	151	1,47	69	0,67	61	0,59	462,00	4,50	<0.001
Blood: *S. aureus*	*	*	*	*	*	*	*	*	*	*			
Blood: *S. pneumoniae*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CTX	0	0,00	1	0,01	0	0,00	0	0,00	0	0,00	1,00	0,01	0,523
P (sp)	0	0,00	1	0,01	0	0,00	0	0,00	0	0,00	1,00	0,01	0,523
SXT	2	0,019	4	0,04	3	0,03	1	0,01	6	0,06	16,00	0,16	0,392
Blood: *Salmonella spp*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CAZ	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0,00	0,00	NC
CIP	4	0,04	20	0,19	26	0,25	7	0,07	2	0,02	59,00	0,57	<0.001
CTX	5	0,05	0	0,00	0	0,00	0	0,00	0	0,00	5,00	0,05	<0.001
ETP	4	0,04	0	0,00	0	0,00	0	0,00	0	0,00	4,00	0,04	<0.001
IPM	1	0,01	0	0,00	0	0,00	0	0,00	0	0,00	1,00	0,01	0,248
MEM	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0,00	0,00	NC
Urine: *E. coli*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
AM	924	9,00	1578	15,37	1879	18,30	1317	12,83	2044	19,91	7742,00	75,40	<0.001
CAZ	90	0,88	218	2,12	267	2,60	200	1,95	327	3,18	1102,00	10,73	<0.001
CIP	307	2,99	592	5,77	728	7,09	517	5,04	849	8,27	2993,00	29,15	<0.001
CTX	134	1,31	304	2,96	429	4,18	307	2,99	569	5,54	1743,00	16,98	<0.001
ETP	4	0,04	6	0,06	9	0,09	4	0,04	29	0,28	52,00	0,51	0,018
FEP	40	0,39	100	0,97	148	1,44	106	1,03	192	1,87	586,00	5,71	<0.001
IPM	8	0,08	12	0,12	15	0,15	5	0,05	16	0,16	56,00	0,55	0,788
MEM	2	0,02	8	0,08	15	0,15	8	0,08	13	0,13	46,00	0,45	0,108
SXT	866	8,43	1429	13,92	1626	15,84	1156	11,26	1739	16,94	6816,00	66,39	<0.001
Urine: *K. pneumoniae*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CAZ	110	1,07	213	2,07	270	2,63	221	2,15	438	4,27	1252,00	12,19	<0.001
CIP	112	1,09	200	1,95	229	2,23	186	1,81	353	3,44	1080,00	10,52	<0.001
CTX	134	1,31	233	2,27	312	3,04	254	2,47	489	4,76	1422,00	13,85	<0.001
ETP	2	0,02	12	0,12	17	0,17	16	0,16	98	0,95	145,00	1,41	<0.001
FEP	34	0,33	101	0,98	123	1,20	107	1,04	262	2,55	627,00	6,11	<0.001
IPM	4	0,04	8	0,08	6	0,06	13	0,13	85	0,83	116,00	1,13	<0.001
MEM	4	0,04	6	0,06	16	0,16	14	0,14	92	0,90	132,00	1,29	<0.001
SXT	222	2,16	341	3,32	411	4,00	335	3,26	569	5,54	1878,00	18,29	<0.001
Faeces: *Salmonella spp.*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CAZ	3	0,03	2	0,02	0	0,00	0	0,00	1	0,01	6,00	0,06	0,056
CIP	5	0,05	13	0,13	34	0,33	7	0,07	9	0,09	68,00	0,66	<0.001
CTX	14	0,14	2	0,02	0	0,00	0	0,00	2	0,02	18,00	0,18	<0.001

ETP	11	0,11	0	0,00	0	0,00	0	0,00	0	0,00	11,00	0,11	<0.001
IPM	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0,00	0,00	NC
MEM	0	0,00	0	0,00	0	0,00	0	0,00	0	0,00	0,00	0,00	NC
Faeces: *Shigella spp.*	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CAZ	0	0,00	0	0,00	0	0,00	0	0,00	1	0,01	1,00	0,01	1,000
CIP	0	0,00	0	0,00	0	0,00	1	0,01	2	0,02	3,00	0,03	0,646
CTX	0	0,00	0	0,00	2	0,02	4	0,04	7	0,07	13,00	0,13	0,152
N. gonorrhoea	**2011**		**2012**		**2013**		**2014**		**2015**		**Overall**		**p-value**
CIP (ng)	0	0,00	0	0,00	0	0,00	0	0,00	1	0,01	1,00	0,01	NC
Population		**10267300**											

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CHAPTER 3: CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

3.1 Conclusion

A retrospective analysis of trends in antibiotic resistance (ABR) was conducted on data obtained from six public hospitals in KwaZulu Natal. The specimen types, pathogens and antibiotics included in the study were based on the guidelines published in the GLASS manual for early implementation. MIC and percentage resistance data was compiled for priority pathogens from blood, urine, faecal and urethral/ cervical samples representing blood stream infections (BSIs), urinary tract infections (UTIs), acute diarrhoeal infections and gonorrhoeal infections. The antibiotic resistance data was stratified by year and analysed on 3 levels: (1) a trend analysis of resistance including MIC50, MIC90, MIC ranges and percentage resistance over 5 years was conducted; (2) selected metrics recommended in the GLASS manual were calculated and (3) susceptibility data was compared with existing standard treatment guidelines.

The following were the main conclusions in relation to the aim and objectives:

1. A database of BSIs, UTIs, diarrhoeal infections and gonorrhoeal infections was generated including specimen type, isolate identification and AST data.
2. The proportion of BSIs, UTIs and diarrhoeal infections caused by the respective pathogens of interest were summarised as follows:
    - Urine samples accounted for 61% (n= 33 018) of all isolates included in the study
    - *E. coli* from blood and urine samples was the most commonly isolated pathogen (52%, n= 33 018)
    - Of the 11 722 positive blood samples, the most common causative pathogens were *S. aureus* (32%), *K. pneumoniae* (26%) *and E. coli* (18%).
    - Of the 20 212 positive urine samples, the most common causative pathogens were *E. coli* (75%) followed by *K. pneumoniae* (25%).
    - Of the 916 stool samples, the most common causative pathogens were *Shigella spp.* (67%) *and Salmonella spp.* (33%)
    - Only 168 gonorrhoeal infections were explored over the 5year period.

3. The trends in resistance between 2011 and 2015 were as follows:
    - The majority of isolates were multi-drug resistant
    - Resistance to third and fourth generation cephalosporins and fluoroquinolones increased in *K. pneumoniae, E. coli* and *Shigella spp.* isolates as did carbapenem resistance in *K. pneumoniae* and *E. coli*.
    - Resistance in *A. baumannii* and *Salmonella spp.* isolates decreased or plateaued against all antibiotics.
4. In terms of standard treatment guidelines:

- BSIs: Treatment guidelines were only available for *S. aureus.* Susceptibility to vancomycin was 97% (n= 1368)
- Only 50 – 68%% of UTIs were treatable with the first line agents ciprofloxacin and amoxicillin-clavulanate.
- Seventy-five percent and 99% of the 145 and 319 diarrhoeal infections caused by *Salmonella spp.* and *Shigella spp.* could be have been successfully treated with ciprofloxacin

3.2. Limitations:

5. Antibiotic susceptibility data was only available for samples positive for bacterial growth.
6. Only 6 out of 71 public hospitals in KwaZulu Natal were included in the study.
7. Available data did not allow stratification into community and hospital acquired infections.
8. As a retrospective study, the data was limited to what was already captured in the National Health Laboratory Services (NHLS) database
9. The results may not be representative of the true extent of ABR in KwaZulu Natal as not all infections necessarily generate a sample for microbiological evaluations because of time and resource constraints. Additionally, not all ill patients seek treatment due to difficulties in accessing healthcare as well as reliance on cultural healing methods.
10. The data was collected before the release of the GLASS manual by the WHO and therefore does not meet all the requirements stipulated therein.

3.3. Recommendations:

- Robust, representative surveillance is necessary to establish a baseline of ABR in KwaZulu Natal and South Africa.
- More facilities should be included in future studies including community health centres and private facilities in order for data to be accurately representative of the true extent of ABR in community and hospital settings.
- Data capturing standards should be improved to minimise the number of entries that had to be excluded and to allow better correlation of demographic and clinical data with resistance trends.
- Standard treatment guidelines should be informed by surveillance data to ensure the efficacy of empiric treatment.
- Increasing resistance to cephalosporins and fluoroquinolones, which are commonly used in the treatment of infections caused by *E. coli, K. pneumoniae, Salmonella spp. and Shigella spp.*, should be monitored and the treatment guidelines modified according to latest surveillance data.

- The increasing incidence of carbapenem resistance, especially in *K. pneumoniae*, should be monitored.

3.4. Significance:

- To our knowledge, this is the first South African report on ABR using GLASS metrics.
- This study implemented the GLASS methodology and is therefore an indication of the capacity to implement surveillance according to these standards in South Africa. Shortcomings of this study can be used to improve the design of future studies so that better quality and representative data can be obtained.
- According to the 2014 WHO report on surveillance, data on ABR in the Africa region is scarce and there is limited evidence available on the true extent of ABR in the region (World Health Organisation, 2014). This study somewhat addresses this surveillance gap in a single province of KwaZulu-Natal in South Africa.

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