SURVEILLANCE OF ANTIMICROBIAL RESISTANT BACTERIA IN AFRICA AND NAMIBIA

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To my wife and son,
Beata Ndeshihala & Atlas Zane Oletu HAINDONGO

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ABSTRACT

Sub-Saharan Africa faces a growing antimicrobial resistance (AMR) threat. Antimicrobial resistant bacterial organisms pose a serious problem to human infection treatment, particularly urinary tract infections and bloodstream infections. Due to inadequate surveillance systems, information gaps on AMR prevalence in Africa (including Namibia) exist.

A systematic review of the WHO African region (2008-2019) analysed 27 bacteremic *E. coli* and *S. aureus* AMR studies. Additionally, a nationwide retrospective AMR analysis of Namibian female urine (2016-2017, Study II) and all patients blood (2011-2019, Study III) isolates was conducted. Antibiotic susceptibility testing (AST) was performed using disk diffusion and Vitek 2 according to CLSI guidelines.

Study I revealed that *E. coli* median resistance was: cefotaxime (42%), ciprofloxacin (44%) and carbapenems (<1%). The *S. aureus* resistance rates for cloxacillin was 34%. Study II estimated ESBL bacterial isolates prevalence at 22% based on cefotaxime resistance. Nitrofurantoin resistance was low in non-ESBL isolates but increased in ESBL isolates (12.9% to 19%), with one region reaching 59% resistance. Study III found that the *E. coli* resistance was: piperacillin-tazobactam (8%), cefotaxime (32%), ciprofloxacin (29%), gentamicin (18%) and co-trimoxazole (79%). *S. aureus* showed 18.8% oxacillin resistance with low resistance (<10%) to clindamycin, gentamicin and rifampin and none to teicoplanin.

This study highlights the limited bacteremic AMR data from only a quarter of WHO African region countries, thereby emphasising the need for strengthened surveillance. In Namibia, nitrofurantoin remains a useful empirical antimicrobial for female urinary tract infections, although regional variations necessitate enhanced surveillance. The high resistance rates in bacteremic *E. coli* (>20%) for most antimicrobials in Namibia underscore the importance of AST-guided therapy.

KEYWORDS: *Escherichia coli,* ESBL, Extended Spectrum beta-lactamase, MRSA, Methicillin Resistant *Staphylococcus aureus*, Namibia, Africa

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TIIVISTELMÄ

Kasvava antibioottiresistenssi (AMR) uhkaa Saharan eteläpuolista Afrikkaa. AMR-bakteerit muodostavat vakavan ongelman ihmisten infektioiden hoidossa, erityisesti virtsatieinfektioissa ja verenmyrkytyksissä. Riittämättömien seurantajärjestelmien vuoksi Afrikassa (ml. Namibiassa) on puutteita AMR-esiintyvyystiedoissa.

WHO:n Afrikan alueen systemaattisessa katsauksessa (2008–2019) analysoitiin 27 *Escherichia coli* ja *Staphylococcus aureus* –bakteremia resistenssitutkimusta. Lisäksi tehtiin maanlaajuinen retrospektiivinen namibialaisten naisten virtsa- (2016-2017, osatyö II) ja kaikkien potilaiden verilöydösten (2011-2019, osatyö III) mikrobilääkeherkkyysanalyysi. Herkkyys määritettiin kiekkoherkkyysmenetelmällä ja Vitek 2:lla CLSI:n ohjeiden mukaisesti.

Osatyössä I *E. colin* mediaaniresistenssi oli kefotaksiimille 42%, siprofloksasiinille 44% ja karbapeneemeille <1%. *S. aureuksen* resistenssi kloksasilliinille oli 34%. Osatyö II arvioi kefotaksiimiresistenssiin pohjaten ESBL-bakteerikantojen esiintyvyyden olevan 22%. Resistenssi nitrofurantoiinille oli alhainen muissa kuin ESBL:ia tuottavissa kannoissa, mutta lisääntyi tutkimuksen aikana ESBL-kannoissa (13%-19%) yhden alueen saavuttaessa 59% resistenssin. Osatyössä III *E. colin* resistenssi oli piperasilliini-tatsobaktaamille 8%, kefotaksiimille 32%, siprofloksasiinille 29%, gentamysiinille 18% ja sulfatrimetopriimille 7%. *S. aureuksen* resistenssi oksasilliinille oli 19%, alle 10% klindamysiinille, gentamysiinille ja rifampiinille, eikä lainkaan teikoplaniinille

Vain neljännes WHO:n Afrikan alueen maista raportoi verenmyrkytysbakteerien AMR-tietoa, mikä korostaa tarvetta tehostaa seurantaa. Namibiassa nitrofurantoiini on edelleen hyödyllinen empiirinen mikrobilääke naisten virtsatieinfektioiden hoidossa, vaikka alueelliset vaihtelut edellyttävätkin tehostettua seurantaa. Veri-*E. colien* korkeat resistenssiluvut (>20%) useimmille mikrobilääkkeille Namibiassa korostavat herkkyysmäärityksillä ohjatun hoidon merkitystä.

AVAINSANAT: *Escherichia coli*, ESBL, Laajakirjoinen beetalaktamaasi, MRSA, metisilliiniresistentti *Staphylococcus aureus*, Namibia, Afrikka

Table of Contents

Abbreviations

AMR	Antimicrobial resistance
AMRSNET	Anti-Microbial Resistance Surveillance Network
ANOVA	Analysis of Variance
ARB	Antimicrobial resistant bacteria
ARG	Antimicrobial resistance genes
AST	Antimicrobial Susceptibility Test
BCI	bacteriologically confirmed infections
BSI	Bloodstream infection
CC	Clonal complex
CLSI	Clinical Laboratory Standards Institute
DALY	Disability Adjusted Life Years
EARSNet	European Antimicrobial Resistance Surveillance Network
ESBL	Extended-spectrum- ß-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility testing
GLASS	Global Antimicrobial Surveillance System
I	Susceptible, increased exposure
IQR	Interquartile range
LMIC	Low-and-medium income countries
MBL	Metallo-ß-lactamases
MDR	Multi-drug resistance
MRSA	Methicillin Resistant Staphylococcus aureus
NIP	Namibia Institute of Pathology
NSTG	Namibia Standard Treatment Guidelines
PCR	Polymerase Chain Reaction
PDR	Pan-drug resistance
R	Resistant
S	Susceptible
SCC	Staphylococcal Cassette chromosome
ST	Sequence Type
UTI	Urinary Tract Infection
WHO	World Health Organization
XDR	Extensive drug resistance

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I   Haindongo EH, Ndakolo D, Hedimbi M, Vainio O, Hakanen A, Vuopio J. Antimicrobial resistance prevalence of Escherichia coli and Staphylococcus aureus amongst bacteremic patients in Africa: a systematic review. *Journal of Global Antimicrobial Resistance*, 2023;32:35-43.

II   Haindongo E.H., Funtua B, Singu B, Hedimbi M, Kalemeera F, Vainio O, Hakanen A, Vuopio J. Antimicrobial resistance among bacteria isolated from urinary tract infections in females in Namibia, 2016–2017. *Antimicrobial Resistance & Infection Control,* 2022;11(1):33.

III   Haindongo EH, Vainio O, Hakanen A, Vuopio J, Hedimbi M. Antimicrobial resistance trends of bacteremic pathogens in Namibia: A retrospective study. Manuscript

1   Introduction

Antimicrobial resistance (AMR) is a significant threat to public health, particularly in low- and-middle-income countries (LMIC) (Gandra et al., 2020; Lee et al., 2019). There were 4.95 million deaths worldwide associated with antimicrobial resistance in 2019 (Murray et al., 2022), with the burden of infectious diseases being considerably high in sub-Saharan Africa (i.e. accounting for 62.35% of the total global cases) (Institute for Health Metrics and Evaluation (IHME), 2022). Amongst communicable diseases, bloodstream infections (BSI) are associated with high fatality rates (C. Liu et al., 2022; Martinez & Wolk, 2016), whilst urinary tract infections (UTIs) are more common and they disproportionally affect women (Flores-Mireles et al., 2015; John E. Bennett et al., 2014). Beyond mortality, infections caused by AMR pathogens present several other challenges to healthcare, as treatment of these infections often requires costly second-line treatment drugs and extends the hospital admission period. These therefore increase the cost to the health care system and patients, and may ultimately result in treatment failure and death (Dadgostar, 2019).

The two study organisms, *Escherichia coli* and *Staphylococcus aureus* are common pathogens causing BSI (Droz et al., 2019; Viscoli, 2016) and they were among the top contributors to the deaths attributable to AMR in 2019 (Murray et al., 2022). *E. coli* exhibits diverse forms, with both commensal and pathogenic strains colonizing or infecting human hosts. Pathotypes (variants that differ by pathogenicity) such as Extraintestinal pathogenic *Escherichia coli* (ExPEC) are involved in pyelonephritis, cystitis, septicaemia and neonatal meningitis (Allocati et al., 2013; Messerer et al., 2017).

Furthermore, extended-spectrum β-lactamases (ESBLs) are a group of broad spectrum β-lactamases produced by enterobacterales, causing resistance to third-generation cephalosporins and aztreonam, but they are inhibited by clavulanic acid. ESBLs hinder the treatment of *Enterobacterales,* such as *Escherichia coli* infections by the commonly used cephalosporins (Rizvi et al., 2011; Shaikh et al., 2016). The plasmids that carry ESBL genes, also carry other resistance mechanisms thus causing multidrug resistance (MDR). The continuing rise of MDR bacteria poses a threat to the management of UTI, bacteremia and septic infections (Dadashi et al., 2021).

Humans are naturally exposed to *Staphylococcus aureus* on the skin and nasopharynx (Foster, 2002). *S. aureus* constitutes part of the normal nasal microbiome in ca. 30% of the human population. The majority of the human population (60%) are intermittent carriers, 20% are noncarriers and 20% are permanent carriers (Laux et al., 2019). Methicillin-resistant *Staphylococcus aureus* (MRSA) is prevalent in hospitals (Gittens-St Hilaire et al., 2020) and in the community(Cheung et al., 2021; W.-T. Liu et al., 2021), and it is resistant to most available β-lactam antimicrobials. It has the potential to cause a variety of infections to the skin, soft tissues, and internal organs. *S. aureus* enters the body through a skin break or can spread from indwelling devices into the blood (Cheung et al., 2021). Among its many other infections are impetigo, folliculitis, bacteremia, meningitis, endocarditis, pneumonia, skin infections, toxic shock syndrome, scalded skin syndrome and other hospital-acquired infections (Oluyele & Oladunmoye, 2017; Singh et al., 2022). Additionally, food poisoning can occur when enterotoxins are ingested (Foster, 2017).

There is limited information on the true burden of antimicrobial resistance prevalence in Africa in general and Namibia in particular, due to weak surveillance systems and limited laboratory capacity (World Health Organization, 2014; World Health Organization, 2017). Hence, in LMIC settings with high bacterial infection burden, AMR surveillance is critical for guiding the antimicrobial therapy of an individual patient and also for guiding empirical antimicrobial treatment as part of the antimicrobial stewardship (Gandra et al., 2020).

This thesis contributes to AMR surveillance within the WHO Africa Region and Namibia in particular, through the analysis of retrospective laboratory data and a systematic review of published data records.

2 Review of the Literature

2.1 AMR as a concept

Antimicrobial resistance (AMR) is a global public health crisis, presenting therapeutic challenges against bacterial infections (McEwen & Collignon, 2018). Antimicrobial resistance is defined as the ability of microorganisms to resist the action of antimicrobials to which they are inherently sensitive to, this is also known as *acquired resistance* (Christaki et al., 2020; Jindal et al., 2015). Additionally, resistance is also noted in bacteria due to their inherent characteristics, for example, glycopeptide resistance in gram-negative bacteria (known as *intrinsic resistance*) (Christaki et al., 2020). Resistance varies across various antimicrobial classes and groups. Bacteria may further be classified as either multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR). MDR refers to bacteria resistant to antimicrobials in at least three different classes, whereas XDR indicates bacteria resistant to antimicrobials in all but one or two classes. Finally, PDR signifies the most concerning scenario - bacteria resistant to antimicrobials in all classes (Falagas et al., 2006; Magiorakos et al., 2012). Furthermore, extensive drug resistance coupled with virulence has the additional ability to cause major outbreaks (Jindal et al., 2015). This is further worsened as the pace of new antimicrobial drug regimens development is slower than the emergence of resistance (Ferri et al., 2017).

Antimicrobials on the other hand also have a certain spectrum of action. The choice of the therapeutic antimicrobial is largely driven by the cell construct of the pathogen. It is important to choose the drug of choice based on the aetiological agent, the antimicrobial profile and host factors such as site of infection, hepatic and renal clearance (John E. Bennett et al., 2014). There are some marked differences in the cell construct of *E. coli* and *S. aureus.* Hence, it is important that these organisms are identified for the therapeutic antimicrobials to be correctly chosen as their usage and susceptibility vary by organisms. For example, the outer membrane (OM) of gram-negative bacteria acts as an important barrier to antimicrobial sensitivity (Delcour, 2009). The empiric regimens for our study pathogens are presented in table 1.

Table 1. Antimicrobials recommended as 1st or 2nd line use against *E. coli* and *S. aureus* causing urinary tract infections or bloodstream infections.

Syndrome	Micro-organisms	Antimicrobial agent	Comment	Reference
Urinary Tract Infections (UTI)	*E. coli*	Amoxicillin-clavulanate	Cystitis	Simoni et al. (2024)
		Ciprofloxacin	Cystitis	
		Co-trimoxazole	Use for cystitis in developed countries. Consider as first line if resistance is below 20%	Simoni et al. (2024); Hadidi et al. (2024)
		Fosfomycin	1st line treatment for uncomplicated UTI	EAU guidelines
		Nitrofurantoin	1st line treatment for uncomplicated UTI	EAU, NSTG
		Nalidixic acid	1st line UTI treatment in children	NSTG
		Gentamicin	2nd line therapy in Namibia for pyelonephritis	
		Cefuroxime		
Bloodstream infections	*E. coli*	Piperacillin-tazobactam	Recommended for healthcare associated pathogens and immunocompromised patients	Rhodes et al. (2017)
		Ticarcillin-clavulanate		
		Third or higher generation cephalosporins	Multi-drug resistant pathogens	
	MRSA	Vancomycin	Potential risk for MRSA	
		Teicoplanin		

Abbreviations: European Association of Urology (EAU), Namibia Standard Treatment Guidelines (NSTG) and MRSA

Bacteria develop resistance through various mechanisms including genetic changes such as mutations and the uptake of genetic material from other resistant bacterial strains (Ferri et al., 2017). These genes encode various mechanisms such as the enzymatic degradation of antibacterial agents, efflux pumps to extrude antimicrobial drugs, modifying the drug target and developing alternative metabolic pathways (C Reygaert, 2018). Additionally, the resistance mechanisms are also presented separately for the two organisms, *E. coli* and *S. aureus* respectively (Tables 2 and 3). Antimicrobial degradation is the primary resistance mechanism *in E. coli* and other gram-negative bacteria producing β-lactamase enzymes. The degradation of antimicrobials varies due to various resistance enzymes, such as New-Delhi metallo-β-lactamases (NDM), Imipenemase (IMP) and Class D Oxacillinase (OXA), Verona Integron-encoded metallo-β-Lactamase (VIM) and Klebsiella Pneumoniae Carbapenemase (KPC). Their classification is based on the amino acid sequence similarity (Ambler class) and the functional characteristic (Bush-Jacoby-Medeiros) of the enzymes as presented in Table 2 below.

Table 2. Classification of enzymes responsible for antimicrobial resistance by the two common classification systems and the spectrum of activity among Enterobacterales.

Group[1]	Ambler Class[2]	Enzymes	Spectrum
1	C (serine-β-lactamases)	AmpC	Hydrolyse cephamycins and oxymino-β-lactams (Hall & Barlow, 2005)
2	A (serine-β-lactamases)	TEM, SHV, CTX-M, KPC	Hydrolyse narrow and extended-spectrum-β-lactams (ESBL) (Hall & Barlow, 2005; Ahmed et al., 2013)
	D (serine-β-lactamases)	OXA	Hydrolyse oxacillin, oxymino-β-lactams and carbapenems (Hall & Barlow, 2005)
3	B (metallo-β-lactamases)	VIM, IMP, NDM	Hydrolyse carbapenems (Cornaglia et al., 2011; Ju et al., 2018; Palzkill, 2013)

[1]Bush-Jacoby-Medeiros classification (based on biochemical function); [2]Ambler Molecular classification (based on amino acid sequence)

Methicillin resistance is clinically the most relevant among *S. aureus* due to the ability of a single genetic element to confer resistance to a commonly prescribed class of antimicrobials such as the β-lactam antimicrobials (e.g. penicillins, cephalosporins, and carbapenems (Grundmann et al., 2006). The resistance to MRSA alternative antibiotics such as vancomycin, daptomycin and linezolid are presented below (Table 3).

Table 3. The resistance genes (including mutations) and the antimicrobial agent affected (i.e. resistance mechanism).

Antimicrobial agent(s)	Resistance mechanism	Encoded/Genes	Reference
Oxacillin (MRSA)	Altered target, PBP2a; mecC - a low affinity for ß-lactams	*mec*C	Lakhundi & Zhang (2018); Taban et al. (2021)
Cefoxitin (MRSA)	Altered target, PBP2a- a low affinity for ß-lactams	*mec*A	
Vancomycin (VISA or VRSA)	Natural precursor (d-Ala-d-Ala) is replaced with d-Ala-d-lac or d-Ala-d-Ser alternatives to which vancomycin has a low affinity	*vanA, vanB, vanD, vanF, vanM*	Ngo et al. (2022); Stogios & Savchenko (2020)
Linezolid	Modification of drug target	point mutations (G2576T) at the drug target site (23S rRNA) *cfr* (chloramphenicol-florfenicol resistance)	Morales et al. (2010; Sánchez García (2010)
Tigecycline	Overexpression of efflux pump protein	transcriptional repressor *mepR*	Dabul et al. (2018); Dabul & Camargo (2014)
Daptomycin	Changes in the fluidity, thickness and charge of the membrane (permeability)	mutation of various genes (*dltABCD* genes, *mprF* and *rpoB*)	Shariati et al., (2020)
MLS (Macrolide lincosamide, and streptogramin B)	Methylates 23S rRNA	erythromycin resistance methylase (*erm*)	Drinkovic (2001); L. Zhang et al. (2015)

Figure 1 shows the multiple drivers and transmission routes of resistance. These are largely driven by inappropriate antimicrobial use patterns which encompass both overutilisation and underutilisation. Sub-optimal treatment regimens exert strong selection pressure for antimicrobial resistant bacteria and thus significantly contribute to the emergence and dissemination of AMR (Andersson & Hughes, 2014; Baquero et al., 2009; Oz et al., 2014). Antimicrobial use surged by 65% between 2000 and 2015, primarily in LMICs. This high antimicrobial use for potentially non-bacterial illnesses is driven, firstly, by limited access to clean water, sanitation, and diagnostics in LMIC (Walsh et al., 2023). Secondly, the rising global demand for meat protein and intensified farming practices, which has lead to high antimicrobial use especially in poultry production, as well as in swine and dairy cattle (Azabo et al., 2022; Cuong et al., 2018). Finally, the environment acts as a vast reservoir for naturally occurring ARGs as well as those deposited through effluent from healthcare facilities, animal or agricultural waste and wastewater treatment plants (Delgado-Baquerizo et al., 2022; Irfan et al., 2022; Theuretzbacher, 2016).
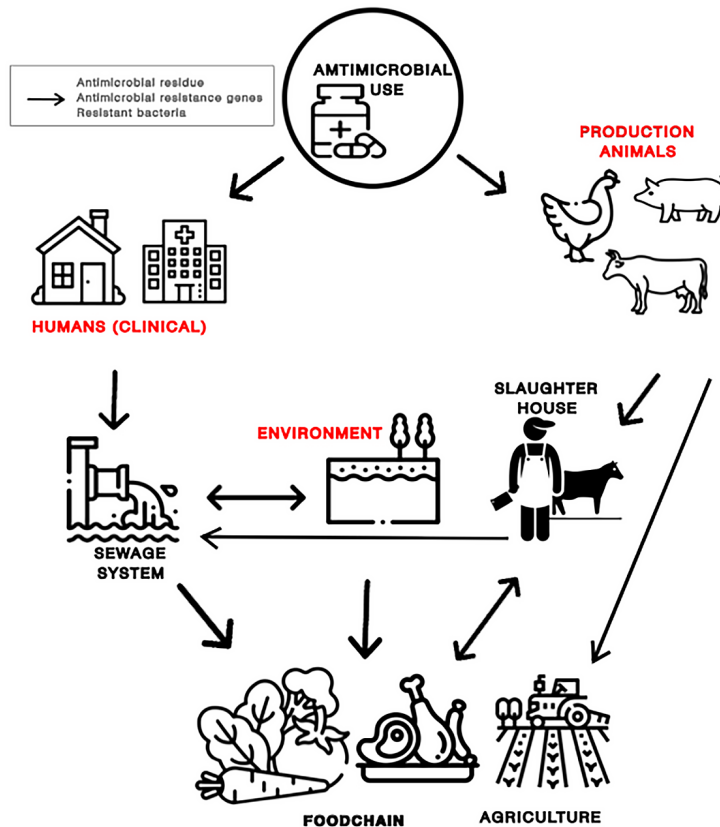
Figure 1. Antimicrobial resistance routes of transmission indicating the interconnectedness between humans, animals and the environment (One Health). Authors own drawing.

Beyond infection, the spread of antimicrobial resistance (AMR) is also driven by carriage. People, livestock, and even wildlife can carry resistant bacteria without necessarily showing infection symptoms (carriage) (Nellums et al., 2018; Subbiah et al., 2020). This carriage can be long-lasting, as seen in travellers who remain colonized with resistant bacteria after returning from high-risk areas (Bokhary et al., 2021). The movement of people and animals, including international travel, migration, and livestock trade, allows resistant bacteria to spread across geographic regions. Studies have shown a high prevalence of AMR bacterial carriage in both healthy humans and livestock, highlighting the complex web of factors contributing to AMR (Muloi et al., 2019; van der Bij & Pitout, 2012).

Europe and Finland in particular, thus offer valuable lessons in reducing antimicrobial use in livestock. Firstly, phasing out the use of antimicrobial growth promoters and implementing stricter legislation like the AGP ban in Europe demonstrably lowered antimicrobial use without compromising animal health. Secondly, consistent monitoring programes like the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) allowed for tracking progress and informed further interventions. Finally, focusing on animal vaccination programmes (Sternberg-Lewerin et al., 2022) and biosecurity measures like hygiene and pest control proved effective in reducing antimicrobial use (Dewulf et al., 2022; Sali et al., 2021).

2.2 AMR Surveillance and Prevalence

2.2.1 Surveillance approaches and considerations

To combat AMR globally, the AMR trends need to be monitored using robust and reliable surveillance approaches in the human, animal and food sectors. Surveillance data is not only used to inform patient care or detect hospital outbreaks but it can be used to inform policy and local antimicrobial stewardship activities, which will ultimately reduce AMR-associated mortality and morbidity (Sugianli et al., 2020).

It has been widely recognised that in LMIC countries, the AMR is worsened by weak national and local policies, a lack of quality diagnostic, and surveillance capacity, and lack of antimicrobial stewardship programs (Sugianli et al., 2020). Deficiencies in data quality thus impede the generation of an accurate picture of the AMR situation. This limitation significantly hinders our capacity to effectively track the spread of resistance, identify early outbreaks, and formulate robust national health policies to address this escalating public health threat (Iskandar et al., 2021).

Two primary approaches to AMR surveillance exist, namely, laboratory-based and population-based.

Laboratory-based surveillance of pathogens is routinely performed on clinical specimens to generate, generating data readily available for local and national analysis. This swift data collection allows for the quicker development of strategies to curb AMR and to assess their effectiveness. However, this method has limitations, for example, specimens reaching the lab may not represent the entire population. As such, this selection bias can skew results, thereby potentially overestimating AMR prevalence, particularly in resource-limited settings (Sugianli et al., 2020).

Population-based surveillance on the other hand offers a more comprehensive picture. By studying individuals with specific symptoms (i.e. clinical case definition) within a defined population, provides more precise data on the true burden of AMR. However, this approach comes with its own drawbacks: it is often considered too laborious and resource-intensive and implementing such a system may prove difficult in areas with limited healthcare infrastructure (Sugianli et al., 2020). Ultimately, a combination of both methods, along with a critical understanding of their strengths and weaknesses, will be most effective in interpreting and guiding public health (Sugianli et al., 2020).

Hence, the demand for effective surveillance is critical. The World Health Organization (WHO) recommends laboratory-based approaches (over population-based) for their practicality in gathering local and national AMR data. This focus aligns perfectly with the second strategic objective of "strengthening the knowledge and evidence base through surveillance and research" - as outlined in the Global Action Plan adopted by the World Health Assembly in 2015. Member states are thus mandated to develop national surveillance systems for antimicrobial resistance, not only in the human clinical sector but also in the animal and environmental sectors (World Health Organization, 2015a).

At a global level, the one key initiative addressing this need is the Global Antimicrobial Resistance and Use Surveillance System (GLASS) which was launched by the World Health Organization (WHO) in 2015. GLASS provides a standardised approach for collecting and analysing data on AMR in humans across participating countries. This system plays a vital role in monitoring AMR trends and informing global efforts to combat this critical public health threat (Abushaheen et al., 2020). GLASS has been expanded to track antimicrobial consumption, and fungal infections, and even incorporates a One Health approach that considers animal and environmental factors. This comprehensive system aims to collect high-quality data on antimicrobial resistance and usage, thereby informing strategies to combat this global health threat (Tornimbene et al., 2022).

Furthermore, there has been an establishment of several regional surveillance networks (World Health Organization, 2020) as shown in the global map below (Figure 2). National and regional action plans recognise surveillance as key to the control and prevention of AMR (Simonsen, 2018). Hence, Europe, Central Asia, and

South America (or Latin America) have established their AMR surveillance networks (Simonsen, 2018; Takaya et al., 2020; PAHO, 2020). Africa has also proposed to establish one through Africa CDC (Africa CDC, 2018). On the contrary, North America (USA and Canada) and the Pacific regions have not established regional but rather have national systems (Aguilar et al., 2023; CDC, 2023; Rudnick et al., 2022). There is variation in collection and sectors involved in the different systems, but this continues to expand in scope in recognition of AMR as a One Health problem.
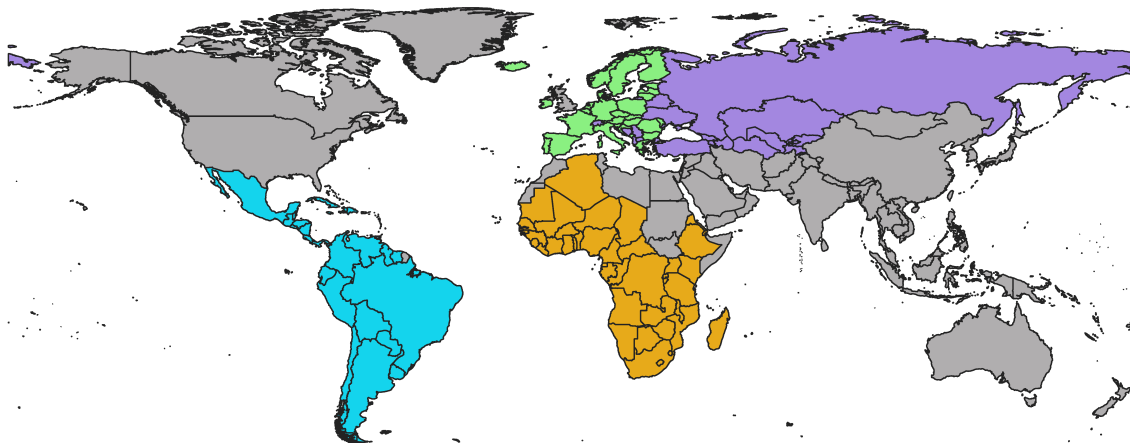
Global Regional AMR Networks

- European Antimicrobial Resistance Surveillance Network (EARS-Net)
- Central Asian and European Surveillance of Antimicrobial Resistance network (CAESAR)
- Latin American and Caribbean Network for Antimicrobial Resistance Surveillance (ReLARVA)
- Anti-Microbial Resistance Surveillance Network (AMRSNET) (proposed)
- Not established

Figure 2. Organization of Regional Antimicrobial Resistance Networks globally. Authors own creation.

2.2.2 Antimicrobial resistance – a global perspective

The development of resistance and increasing AMR has long been recognised and reported on by many actors in the field (Demerec, 1948; Plough, 1945). Unfortunately, a lack of global data on antimicrobial resistance has made it difficult to track trends and design effective interventions (Lobanovska & Pilla, 2017). The United Kingdom's government review on AMR in 2015 highlighted, that this is a global crisis requiring a united effort from the medical community, society, and international collaboration to tackle the rising tide of resistant bacteria and to develop new treatment strategies (Neill, 2016).

Recognizing the urgency of the AMR crisis, the 58th WHO Assembly of 2015 stressed the devastating potential consequences such as developmental setbacks and millions of deaths if AMR goes unaddressed. The assembly proposed an integrated approach, endorsing a "One Health" approach that tackles AMR across human, animal, and environmental sectors. This global effort requires nations to dedicate resources, raise awareness, and implement programmes to combat this growing threat (World Health Organization, 2014).

The global deaths attributable to AMR every year is estimated to be 700,000 and this is expected to increase to 10 million every year, by the year 2050, with the greatest burden being expected to be on continental Africa with 4,150,000 deaths (Neill, 2014, 2016). Latest estimations indicate that annually, there have been 4.95 million deaths worldwide associated with antimicrobial resistance (Murray et al., 2022). Amidst the increasing prevalence of non-communicable diseases, in sub-Saharan Africa infectious diseases continue to dominate, accounting for 62.35% of the total global deaths in this area (Gouda et al., 2019; Institute for Health Metrics and Evaluation (IHME), 2022; Murray et al., 2022). These deaths were mainly caused by *Escherichia coli*, followed by *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. These were responsible for 929,000 deaths attributable to AMR and 3.57 million deaths associated with AMR in 2019 (Murray et al., 2022). Methicillin-resistant *S. aureus* caused more than 100,000 deaths attributable to AMR in 2019. Whereas the deaths attributable to third-generation cephalosporin-resistant *E. coli* and *fluoroquinolone-resistant E. coli* were in the range of 50,000 –100,000 (Murray et al., 2022).

For this study, antimicrobial resistance rates that are reported are confined to *Escherichia coli* and *Staphylococcus aureus* from bloodstream infections. The WHO's 2022 GLASS report, drawing on data submitted by various countries, territories and areas in 2020, reveals insights into global antimicrobial resistance. The report includes bloodstream data from 85 countries, territories and areas for *E. coli*, with a total of 283,030 bacteriologically confirmed infections (BCI). Similarly, data on *S. aureus* blood cultures was submitted by 82 countries, territories and areas, totalling 135,631 BCI.

At the end of July 2019, from a total of 196 countries, territories and areas, there were 82 (41.8%) countries that had enrolled on GLASS and 77 (39.2%) had submitted National Surveillance information (World Health Organization, 2015b). The 2022 bloodstream AMR data from various countries collected in GLASS have been presented earlier in section 2.2.1. This indicates that the GLASS platform is increasingly becoming a surveillance tool for capturing AMR data.

The GLASS data shows that there is wide variation in AMR rates across different settings globally, for different antimicrobial groupings (Table 4). For example,

among *E. coli,* the range of resistance varies among carbapenems (0-58%), fluoroquinolones (9-78%) and third-generation cephalosporins (5-95%). Resistance to methicillin against *S. aureus* had a median of 35% (0-100%). This highlights the need for setting-specific surveillance and response, notwithstanding the importance of concerted efforts.

Table 4. Global level median and range of resistance to various antimicrobials among 283,030 bacteriologically confirmed *E. coli* bloodstream infections. Aggregate data from Countries, Territories, and Areas that reported at least 10 *E. coli* bloodstream infections. Extracted from GLASS Report, 2022.

ANTIMICROBIAL	MEDIAN PERCENTAGE RESISTANCE (%)	RESISTANCE RANGE (%)
AMPICILLIN	72	35-100
CEFOTAXIME	39	5-90
CEFTRIAXONE	48	5-90
CEFTAZIDIME	30	5-95
CEFEPIME	30	5-93
ERTAPENEM	1	0-58
MEROPENEM	1	0-41
IMIPINEM	2	0 - 30
DORIPENEM	5	0 - 10
CIPROFLOXACIN	41	9 - 77
LEVOFLOXACIN	32	10 - 78
CO-TRIMOXAZOLE	57	9 - 100
COLISTIN	1	0-21

2.2.3   Antimicrobial resistance prevalence in Africa

As per the 2020 WHO GLASS report, 40.4% (19/47) of countries within the WHO African Region were enrolled for GLASS but only 31.9% (15/47) had submitted their AMR data. However, GLASS does not disaggregate data by region but presents global median resistance data, as previously shown in Table 4. Without the establishment of the proposed AMRSNET for Africa, the AMR prevalence in Africa is, therefore, established through publications and is presented here below.

AMR in 1990 - 2013: A study by Leopold et al. (2014), analysing data from 1990-2013 on febrile illnesses (bacteremiae, typhoid fever, invasive pneumococcal infection and neonatal infection) revealed a median prevalence of resistance to third-generation cephalosporins in *E. coli* ranging between 0% and 25% across

Africa. *S. aureus* resistance to oxacillin, a marker for methicillin-resistant *S. aureus* (MRSA), showed a median prevalence of 13.4% in West Africa and 8.0% in East Africa. However, concerns arose due to the lack of consistent quality control measures in susceptibility testing reported in less than half (120/256) of the studies.

AMR in 2013 - 2016: Tadesse et al. (2017) reported on *E. coli* from bloodstream infections (BSI) and urinary tract infections (UTI), with a 20-30% third-generation cephalosporin resistance, suggesting a potential rise in extended-spectrum beta-lactamase (ESBL) production when compared to the study by Leopold et al. (2014). This study also reported on *S. aureus* predominantly studied in wound and bloodstream infections. *S. aureus* resistance to cefoxitin was 10.4% (IQR:4.6-33.8). This a potential underestimation due to limited reporting from 8.9% of the studies only. Resistance was generally higher in the West African region.

AMR in 2017 - 2024: Studies by Lester et al. (2020) on bloodstream infections from 20 African countries, confirmed a median resistance to third-generation cephalosporins of 18.4%. The study identified a current gap in understanding how drug-resistant infections impact patients in Africa, and this necessitates future research that links laboratory surveillance data with clinical outcomes/data.

Venne et al. (2023) further revealed a concerning emergence of carbapenem-resistant *E. coli* in some African nations. Carbapenem resistance was generally below 1%, with 2 countries reporting above 5%. Carbapenem resistance was reported in 11 countries in Africa (for countries that have at least 100 isolates tested), and this emphasises the need for enhanced surveillance. These observations are corroborated by the 2020 WHO GLASS report in which only 10 African countries reported on carbapenems, whilst only 4 reported on polymyxins which are considered last resort antimicrobial.

Research by Droz et al. (2019) and Le Doare et al. (2015) underscores that the significant burden of AMR in neonates across Africa. *E. coli* resistance to ceftriaxone was particularly high in Asia compared to Africa for this population. Droz et al., (2019) also highlighted a higher prevalence of *S. aureus* compared to *E. coli* in neonatal bloodstream infections in Africa, with a worrying trend of increased methicillin resistance. Their findings are presented in Table 5 below.

Table 5. Summarised findings of AMR data of systematic reviews from Africa or Low and Low-Middle Income countries focused on *Escherichia coli* and *Staphylococcus aureus*.

	Le Doare et al. (2015)	Williams et al. (2018)	Droz et al. (2019)	Okomo et al., (2019)
Setting	Low- and Low-Middle Income Countries (LMIC) in Africa and Asia	Sub-Saharan Africa	LMIC in Africa and Asia	Africa
Year	2002 – 2013	12 Dec 2012 - 12 Dec 2015	Jan 1990 - Oct 2019	2008-2018
Population	71,326 neonates	Children (# not given)	52,915 neonates	84, 534 neonates
Syndrome/ Specimen	Sepsis, 7056 positive blood cultures	67,451 bacterial cultures	Bloodstream infections, 4,836 bacterial isolates	31,564 blood cultures
Findings: *E. coli*	ceftriaxone median resistance: 0% (Interquartile Range, IQR: 0-50) in Africa; and 80.2% (75.3-100) in Asia.	ESBL producing proportion, CA:9/76 (12%) HA:4/19 (22%); 23/40 (58%) Unknown: 11/22 (50%)	3rd generation cephalosporin resistance: 31.2% (Africa) and 21.2% (Asia)	ESBL-producing proportion: 12% (7/58) in South Africa to 46% (10/22) in Tanzania.
Findings: *S. aureus*	None, Enterobactericiae focus	Oxacillin+ Cefoxitin resistance: 9/32 (28%) CA:15% and HA: 67%	MRSA was 29.5% in Africa and 7.9% in Asia.	Cloxacillin (%R, 95% CI): 40.8% (8-79%) Methicillin: 50% (30-70%)

Abbreviations: CA – Community Acquired, HA- Hospital Acquired and NA-missing.

It has generally been found that there is a paucity of data in the public domain on antimicrobial resistance, particularly in the African region. This is evidenced by a 2017 systematic review by Tadesse that found AMR reports for only 57.4% of the countries in Africa (Tadesse et al., 2017) reporting on AMR for all organisms and syndromes. Therefore, in dealing with the burden of resistance, it is worth noting that, the quantification of the burden of antimicrobial resistance has presented challenges around emergence, distribution and transmission within populations. Both current and historical data on the prevalence of AMR and its impact on health are sparse due to limited surveillance and inadequate laboratory capacity to detect AMR. The true picture is further distorted by selection bias (Hay et al., 2018), where only those with severe infections or those who fail to respond to treatment with empiric regimens are tested and consequently are captured by the laboratory information system. More often than not, the observed prevalence rate is thus an overestimation.

2.2.4   Antimicrobial resistance in Namibia

The Namibian healthcare landscape is comprised of private and public institutions. The public health laboratories provide diagnostic services to most of the population (~85%)(Ministry of Health and Social Services, 2024). These institutions provide individualised diagnostic services to inform patient-level management. The clinical data, therefore, remains unstandardized, unharmonized and fragmented across sites. This highlights the need for a centralised and dedicated national network for resistance surveillance.

Published data on clinical antimicrobial resistance data from Namibia is scarce and yet Namibia faces a growing challenge from AMR, particularly concerning *Staphylococcus aureus* and *Escherichia coli*. The available data is chronologically presented in Table 6. Isolates from these studies remained fully susceptible to critically important antimicrobials like rifampicin, teicoplanin, vancomycin and linezolid (Iileka et al., 2016; Simeon et al., 2021) with exceptions given in the Namibia situational analysis report (Jan-Dec 2015) (Table 7). Additionally, there are also AMR reports on other pathogens such as *Mycobacterium tuberculosis, Chlamydia sp., Neisseria sp.,* Group A *Streptocococi* and *Candida sp.* (Dunaiski et al., 2024; Engelbrecht et al., 2016; Ruswa et al., 2019). Descriptions of this material are, however, out of the scope of this thesis.

Table 6.   Resistance prevalence summarised from various publications on multiple specimens for *E. coli* and *S. aureus* in Namibia.

Year(s)	Setting Population	Specimen	Organism	Antimicrobial	n/N (%R)
2009-2012	Countrywide, meningitis patients[1]	Cerebrospinal fluid (CSF)	*E. coli*	cefuroxime	2/19(10.5)
			S. aureus	cloxacillin	10/29 (34.5)
2012-2014	Countrywide[2]	wound swabs (41.7%), sputum (40.5%)	*S. aureus*	cloxacillin	81/600 (13.5)
2012-2014	Countrywide[3]	Multiple clinical specimens	*S. aureus*	oxacillin	506/3727 (13.6)
2015	Countrywide[4]	All specimens, Windhoek (W): 825 Non-Windhoek (NW): 1441	*S. aureus*	cefoxitin	W: 6% NW: 34%
				vancomycin	W: 1% NW: 8%
2016	272 School children, aged 6-14[5]	Nasal swabs	*S. aureus*	cefoxitin	51/433 (11.8)
2017-2018	Two referral hospital ICU's[6]	Sputum, pleural and bronchial aspirates	*S. aureus*	cefoxitin	51/97 (52.6)
				cloxacillin	26/97 (27.3)
				oxacillin	10/97 (11.1)

References: [1]Mengistu et al. (2013), [2]Iileka et al. (2016), [3]Festus & Moyo(2016), [4]Ministry of Health and Social Services (2017), [5]Walter et al. (2022), [6]Simeon et al. (2021)

Abbreviations: n (number of resistant isolates), N (total number of isolates) %R (percentage resistance)

Furthermore, here below (Table 7) is the tabulated information from the Namibian Situational Analysis on Antimicrobial Resistance Report of the Ministry of Health and Social Services (2017) (unpublished) for *E. coli*. The *E. coli* ESBL proportions in Namibia were as follows: 27% (441/1,589) in Windhoek, 1.6% (50/3,103) elsewhere with a combined total of 10.5%. This indicates the geographic variation which needs to be carefully studied.

Table 7. Namibian resistance prevalence to various antimicrobials among ESBL and non-ESBL *E. coli* isolates from all sources. Extracted from Namibian Situational Analysis on Antimicrobial Resistance, 2017.

	WINDHOEK (CAPITAL CITY)		NON-WINDHOEK	
ANTIMICROBIAL	**non-ESBL percentage resistance (N=1,148)**	**ESBL percentage (%) resistance (N=441)**	**non-ESBL percentage resistance (N=3,053)**	**ESBL percentage (%) resistance (N=50)**
AMPICILLIN	81	100	82	100
CEFOTAXIME	3	100	1	100
CEFTRIAXONE	11	100	25	73
CEFTAZIDIME	3	67	18	76
CEFEPIME	1	28	1	22
ERTAPENEM	1	1	0	0
MEROPENEM	1	1	1	0
IMIPINEM	0	0	1	0
CIPROFLOXACIN	15	79	26	64
AMIKACIN	1	4	5	4
GENTAMICIN	12	57	20	30
CO-TRIMOXAZOLE	76	94	78	84
NITROFURANTOIN	11	41	11	39
COLISTIN	1	1	0	0

Data Gaps: These studies offer valuable insights but a comprehensive, understanding of AMR in Namibia remains limited by the lack of standardized data on resistance patterns across different bacterial species, specimens and various antimicrobials. Also, the number of deaths associated with or attributable to AMR is unreported in Namibia. More research is needed to fill these gaps and to develop a more complete picture of the AMR landscape in Namibia.

Within the One Health framework, there is published data on antimicrobial use (Kaupitwa et al., 2022; Kibuule et al., 2017; Pereko et al., 2015), antimicrobial prescription (Niaz et al., 2020), antimicrobial consumption in humans and animals (Kaupitwa et al., 2022; Shilangale et al., 2012) in Namibia. AMR in food systems

(*E. coli* and *Salmonella enterica* in beef meat) and wastewater (Agrawal et al., 2020) have been studied in Namibia with the findings summarized in Table 8 below.

Table 8. Antimicrobial resistance findings on use, policy, food systems and the environment in Namibia

THEME	FINDING	REFERENCE
Rational Antimicrobial use	• Misalignment of antibiotic use policy between humans and animals • Overuse of tetracycline, penicillin and sulfonamides in animals	Kaupitwa et al., (2022)
	• Wide use of co-trimoxazole, amoxicillin and azithromycin with limited therapeutic indications/policies	Kibuule et al., (2017)
	• Poor awareness of local antimicrobial sensitivity • Poor ownership of Standard treatment guidelines	Pereko et al., (2015)
	• Capital region shows high compliance (73%) to Standard treatment guidelines but high antimicrobial usage (69%))	Niaz et al., (2020)
Food systems	• 71/650 (10.9%) *Salmonella* in animal feed • 19.7% (n=14) were resistant to one or more of the antimicrobials (nalidixic acid, trimethoprim-sulfamethoxazole, sulfisoxazole, streptomycin and/or tetracycline)	Shilangale et al. (2012)
	• 81 *Salmonella* from 9508 beef samples • Observed resistance was sulfamethoxazole (23.46%), trimethoprim-sulfamethoxazole (13.58%), tetracycline (3.7%), amoxicillin-clavulanic acid (1.23%), cephalothin (1.23%) and chloramphenicol (1.23%)	
Environment: Wastewater Treatment Plants	• Comparative study for ARGs between Namibia and Germany • More antibiotic resistance genes found in Namibia (277 vs 93) • Hence higher rate of potential contamination	Agrawal et al., (2020)

2.3 Identification methods and susceptibility determinations

Laboratory equipment and test menus influence which antimicrobial susceptibility testing (AST) methods are used in clinical laboratories. In phenotypic, culture-dependent AST (eg. disc diffusion, broth and agar dilution), an antimicrobial agent is used to examine bacterial responses (K. Yang et al., 2019). The main limitation of culture-dependent methods is the turn-around time of 18-48 hours for isolation, identification and AST (Gajic et al., 2022; Jacobs et al., 2021).

2.3.1 Bacterial identification methods

2.3.1.1 Analytical Profile Index (API)

Automated identification systems for bacterial pathogens have been developed for human clinical use and some veterinary applications. API's are a set of microtubules containing different but standardized, miniaturized dehydrated biochemical reagents on a strip. A sterile saline (NaCl 0.85%)-bacterial suspension mixture is inoculated into the microtubule to reconstitute the dehydrated media. This is incubated for 18-24 hours at 36°C ± 2°C. This incubation results in colour changes in the respective microtubules depending on the bacteria's biochemical profile. The reactions are read in accordance with a reference table and the identification is done using apiweb online. A seven-digit profile is returned which corresponds to the identity of the bacteria with likelihood scores (Topic Popovic et al., 2007).

E. coli is identified using the API20E bacterial identification system for Enterobactericeae and other non-fastidious gram-negative rods. The API20E system is based on 21 biochemical tests (Topic Popovic et al., 2007). The API Rapid E has been developed, thereby shortening the incubation time to 4 hours from 18-24. The identification was comparable between the two systems (98.9% vs 94.0%) (Overman et al., 1985). Equally, the API 10S kit is used to identify *S. aureus,* with 96.9% and 95.9% of isolates correctly identified at genus and species level respectively (Robertson & MacLowry, 1975).

2.3.1.2 Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS offers a rapid and cost-effective method for bacterial identification in clinical microbiology laboratories. Due to its automation, affordability, and speed, several MALDI-TOF instruments have been specifically designed for routine use (Rentschler et al., 2021).

The identification process relies on comparing a bacterium's unique mass spectrum generated during the analysis to a pre-existing database containing spectra of pure bacterial colonies. While conventional biochemical methods typically require a time-consuming culturing step to isolate colonies from clinical samples, MALDI-TOF can bypass this step to a certain extent. The total time-to-result with MALDI-TOF is generally reduced to less than 50 hours compared to the 2-4 days often needed for traditional methods (Rentschler et al., 2021). MALDI-TOF MS can be used for analysing various antimicrobial resistance mechanisms. Studies have also demonstrated its ability to detect the antimicrobial and enzymatic breakdown of products by bacteria, particularly β-lactam antimicrobials hydrolyzed by β-lactamase

enzymes. Carbapenamase has been demonstrated by the detection of meropenem, meropenem sodium salts and corresponding degradation product. This hydrolysis is evident in the mass spectra as a decrease in the peak corresponding to the intact antimicrobial (Florio et al., 2020; Hrabák et al., 2013).

2.3.2 Susceptibility determination methods

2.3.2.1 Disk diffusion

In the disk diffusion susceptibility test, pathogenic bacteria are tested for their susceptibility to various antimicrobials. It works on the principle of growing the bacteria on, for example, Mueller Hinton agar in the presence of antimicrobial filter paper discs. The bacterial inoculum, antimicrobial concentrations, incubation times and culture broth are standardised. The ability of the antimicrobial to inhibit the organism is inferred from the presence (or absence) of growth around the disk (Hudzicki, 2009; Jashmi Chandraker et al., 2022). The zone of clearance is measured and compared to a cut-off value (i.e. breakpoint) for the determination of either resistant or susceptible phenotype (Barnard, 2019). The laboratory result (R, I, or S) guides the physician in the selection of the treatment options for the patient (Hudzicki, 2009; Martins et al., 2020). The method is reliably used to detect various resistance mechanisms, with the aid of pre-defined breakpoints (i.e. zone measurements). Examples are beta-lactamase, MRSA, aminoglycoside, fluoroquinolone resistance and more (Belley et al., 2019; Jashmi Chandraker et al., 2022; Naccache et al., 2019; Nair et al., 2021; X. Yang et al., 2019).

ESBL determinations are also made phenotypically where a standardized suspension is prepared on Mueller Hinton agar as described in the preceding paragraph. The double disk synergy method places 30 μg discs of aztreonam, ceftazidime, ceftriaxone and cefotaxime 15 mm (edge to edge) from amoxycillin-clavulanate, 20/ 10 μg disc. ESBL positive strains, the zone of inhibition between the cephalosporins and clavulanate disc will be enhanced (De Gheldre, 2003; Menon et al., 2006). The Combination Double Disc Test Method is somewhat the same except that there is a corresponding cephalosporin + clavulanate (10 μg, inhibitor) disc for each cefotaxime (30 μg), ceftazidime (30μg) and cefepime (30 μg) used alone (Basu et al., 2014).

2.3.2.2 Broth and Agar dilution susceptibility testing

These approaches determine the minimum inhibitory concentrations (MICs) of antimicrobial agents (i.e. the lowest concentration at which the agent inhibits the growth of microorganisms) (Qaiyumi, 2007). By broth dilution, antimicrobial agents

are diluted two-fold in a liquid broth (e.g. 1, 2, 4, 8, 16 and 32 mg/L) in separate tubes. The minimum volume of the tubes is either 2 mL (macrodilution) or with smaller volumes in a 96-well microtitration plate (microdilution). A known concentration of suspended bacteria (0.5 McFarland) is added to the tubes. The inoculated tubes or 96-well microtitration plates are incubated under suitable conditions (e.g. 37 °C for 6-24 hours) after well-mixing. Finally, bacterial growth is measured by turbidity, thereby allowing visual determination of MIC values (Balouiri et al., 2016; Gajic et al., 2022).

The **agar dilution** method involves adding various concentrations of antimicrobial substances to the nutrient medium before solidification (Wiegand et al., 2008). A standardized bacterial inoculum is then spotted onto the agar and incubated overnight. An assessment is made visually to determine whether there is any growth at the inoculated sites. The lowest concentration of antimicrobials that prevent bacterial growth is then determined (i.e. MIC). This method also allows for the simultaneous testing of different bacterial strains (Jorgensen & Turnidge, 2015; Lo-Ten-Foe et al., 2007; Wiegand et al., 2008).

The **gradient test (E-test) and chromogenic** media are also part of the classical phenotypic approaches used to determine susceptibility. A pre-formed and pre-determined gradient of antimicrobial is immobilised in a dry format onto the surface of a plastic strip. This strip is placed onto an inoculated agar plate and the MIC is determined as the point at which there is complete inhibition of bacterial growth after incubation (Gupta et al., 2022; Matar et al., 2003; Schwalbe et al., 2007). Chromogenic media is coupled with enzymatic substrates that offer direct detection of strains with a defined resistance mechanism (Nahimana et al., 2006; van Belkum et al., 2020).

Using instrumentation one can standardize and produce susceptibility test results in a shorter time frame than manual readings. This relies on sensitive optical detection systems to detect subtle changes in bacterial growth (Reller et al., 2009). To-date four automated instruments have been cleared by the FDA for in vitro diagnostics (IVD), these are: VITEK2 (bioMérieux), MicroScan WalkAway (Siemens Healthcare Diagnostics), BD Phoenix (BD Diagnostics) and Sensititre ARIS 2X (Trek Diagnostic Systems). These systems can generate rapid (3.5–16 h) results, except Sensititre ARIS 2X which takes longer on average to report endpoints (Kaprou et al., 2021; van Belkum et al., 2020).

The VITEK-2 system has routinely been used in Namibia. Vitek2 is an automated antimicrobial susceptibility testing platform that determines MICs by using a combination of optical sensors, databases and algorithms based on the kinetic growth of an organism (Winstanley & Courvalin, 2011).

2.3.2.3   Common testing standards

Standardized testing procedures are essential for accurate and comparable results in clinical microbiology laboratories. Standards are typically provided by the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Gajic et al., 2022; Syal et al., 2017). Standards outline these procedures and high-quality materials such as antimicrobial discs, growth media and reference bacterial strains (i.e. *E. coli* ATCC2592 and *S. aureus* ATCC29213) which are crucial for reliable antibiotic susceptibility testing (AST) (Åhman et al., 2022).

 While these standards provide a framework, their guidelines may differ and their clinical implications can also vary (Gajic et al., 2022). For instance, EUCAST has a stricter resistance threshold or breakpoint for amikacin-resistant *E. coli* (8 mg/L) compared to CLSI clinical breakpoints (16 mg/L) (Rodríguez-Baño et al., 2012). Breakpoints determine whether bacteria are susceptible or resistant and are constantly being revised (Syal et al., 2017). When specific breakpoints are unavailable or unclear, the minimum inhibitory concentration (MIC) is used to classify the bacteria-antibiotic relationship (Gajic et al., 2022).

3   Aims

The overall objective of the study was to assess the antimicrobial susceptibility prevalence among clinical *E. coli* and *S. aureus* isolates causing infections in Africa and Namibia. The specific objectives were to:

1. Describe the antimicrobial resistance prevalence rates of bacteremic *E. coli* and *S. aureus* in Africa by performing a systematic review of published literature.

2. Conduct a 2-year (2016-2017) retrospective study on antimicrobial resistance of urinary *E. coli* isolates among females in Namibia.

3. Determine the 9-year (2011-2019) antimicrobial resistance and trends of *E. coli* and *S. aureus* in Namibia.

4 Materials and Methods

4.1 AMR surveillance in Africa and Namibia

The study was conceptualized in 2018, to understand the historical (10 years ago) AMR landscape in Africa and Namibia. A 12-year understanding of AMR in Africa was determined via systematic searchers of published literature. Thereafter a 2 year and 9-year analysis of local urine and blood culture retrospective data was carried out respectively.

Table 9.   Overview of studies I-III.

STUDY	STUDY TYPE	TIMEFRAME	SETTING	SUBJECTS	SPECIMEN	ISOLATES
I	Systematic Review	2008-2019	WHO African Region, Africa	All sexes, all ages	Blood	*E. coli* *S. aureus*
II	Retrospective Analysis	2016-2017	Nationwide, Namibia	All sexes, all ages	Urine	*E. coli*
III	Retrospective Analysis	2011-2019	Nationwide, Namibia	All sexes, all ages	Blood	*E. coli* *S. aureus*

4.2 Systematic Review Approach (I)

Study I included 23 final papers after screening 562 papers for eligibility. These papers were published in PubMed and Google Scholar and originated from WHO Africa, and the study excluded reviews and case reports. The World Health Organization African Region (WHO AFRO) refers to all African countries with the exception of Algeria, Egypt, Libya, Morocco, Tunisia, Sudan, South Sudan, Somalia, Eritrea and Western Sahara. Antimicrobial resistance of *E. coli* and *S. aureus* in patients with bloodstream infections (bacteremia, bacteremic infections, blood culture, sepsis) across Africa was established through literature searches. The search encompassed epidemiological and surveillance studies from all countries within the WHO African region using keywords such as: 'Antimicrobial or antimicrobial', 'Susceptibility', 'testing', 'non-susceptibility', '*Escherichia coli* (*E. coli*)', '*Staphylococcus aureus* (*S. aureus*)', 'bacteremia*', 'bacteremic infections',

'blood culture', 'bloodstream infection', and 'sepsis'. Antimicrobial count and percentage resistance (n, %R) data were extracted for 12 *E. coli*-drug combinations (N=3,447) and 12 *S. aureus*-drug combinations (N=2,651) of blood cultures performed between 2008 and 2014 but published between 2008 and 2019.

4.3　　Retrospective Review Approach (II & III)

The Namibia Institute of Pathology (the national public health laboratory, NIP) has maintained clinical microbiological data records from multiple specimens over the years. Aerobic blood culture bottles were incubated for 5 days using the automated BACTEC (Becton Dickinson, MD, USA) or manual incubation bottles (Oxoid Ltd., Hampshire, United Kingdom) and cultured for 48 hours on blood or MacConkey agar. Specimens were also routinely plated on Blood, Chocolate, MacConkey and Amikacin Anaerobe agar.

　　Isolate identifications were achieved using Analytical Profile Index (API-bioMériuex, Marcy l'Etoile, France) 10S or 20E GNB and VITEK® 2 GN and VITEK® 2 GP ID (bioMériuex, Marcy l'Etoile, France) cards for Gram-negative and Gram-positive organisms respectively. Antimicrobial Susceptibility Testing has been carried out in accordance with the prevailing Clinical and Laboratory Standards Institute (CLSI) Guidelines and breakpoints. The focus of studies II and III was on the national urine (females only) and blood culture isolates respectively.

　　The following antimicrobial were routinely tested on gram-negatives and positive bacteria at NIP: amikacin (30 μg), amoxicillin/clavulanic acid (20/10 μg), ampicillin (10 μg), cefepime (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefuroxime (30 μg), cephalothin (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), cloxacillin (1 μg), colistin (10 μg), ertapenem (10 μg), erythromycin (15 μg), fusidic acid (10 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), moxifloxacin (5 μg), mupirocin (200 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), ofloxacin (5 μg), penicillin/novobiocin (100/10 μg), piperacillin-tazobactam (30/6 μg), rifampicin (5 μg), sulfamethoxazole-trimethoprim (1.25/23.75 μg), teicoplanin (30 μg), tetracycline (30 μg) and vancomycin (30 μg).

4.4　　Ethics and data analysis

Ethical approval was not sought for Study I as this was based on anonymised publicly available data. The data extraction from eligible papers involved, the extraction of information on the participant/population under study as well as study-specific characteristics such as the laboratory standards, time-frames, etc. The number of resistant isolates (n), total number of isolates (N) and resistant proportion

(%R) were extracted from various papers. The median for each isolate-antimicrobial combination was calculated along with its respective heterogeneity (I^2) assuming random and inverse effect models. The counts of the papers were graphically represented on a map, with the median resistance in each respective year given in bubble plots.

The data for both Studies II and III were obtained from the MEDITECH laboratory information system with permission from the Research Ethics Committee of the Ministry of Health and Social Service, the Government of the Republic of Namibia. No personal identifiers were deposited in public domains. In general, data cleaning and translation of fields were done with Backlink-WHONET. The categorical variables were presented as summarised frequency and percentages of their respective totals. The age demographics were presented as median and ranges. The percentage resistance was also given alongside their 95% confidence intervals for each isolate-antimicrobial combination. Study III had data points for 9 years, to determine the difference between the resistance rates across the years for any given antimicrobial. The One-way Analysis of Variance (ANOVA) test was performed with significance set at 0.05. Data analysis was overall performed with various platforms - WHONET 2020 and 2022, Microsoft® Excel 2021 and R version 3.6.3.

5 Results

5.1 Antimicrobial Resistance in Africa (Study I)

5.1.1 AMR reporting is sparse and unstandardised

Twenty-seven (27) papers were included in the final analysis, after de-duplication and screening against the eligibility criteria. These papers originated from 11 out of 47 member states (23%) of the WHO African region. The highest proportion of the publications were from Ghana (n=5, 18.5%), Nigeria (n=4, 14.8%), Tanzania (14.8%), Uganda (n=3, 11.1%) and South Africa (11.1%) (Figure 3).
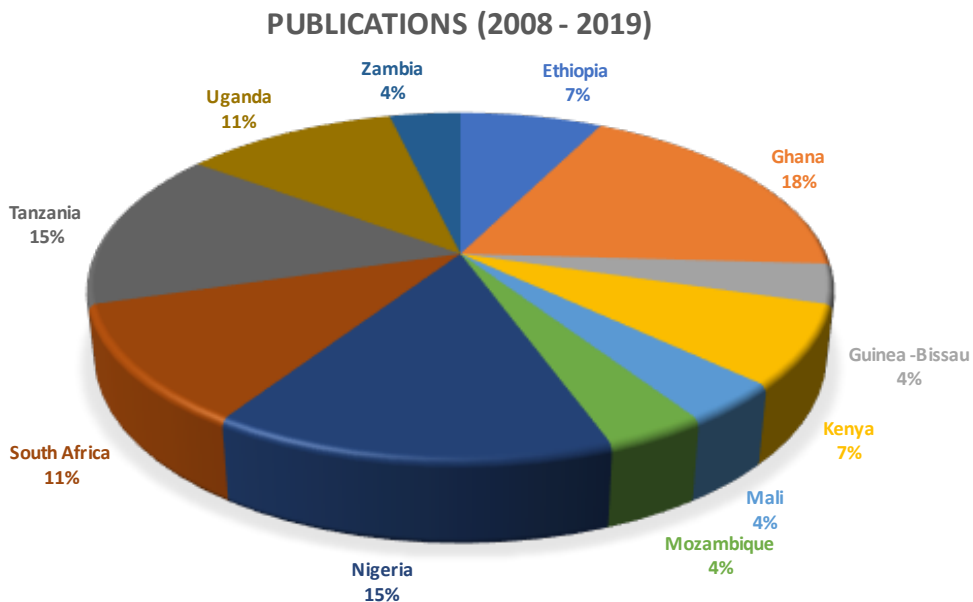
Figure 3. Distribution of 27 publications on antimicrobial resistance of *Escherichia coli* and *Staphylococcus aureus* in patients with bloodstream infections by country of origin in the WHO Africa region (2008-2019).

For the papers published between 2010 and 2019, the majority were published between 2015 (n=8) and 2016 (10), totalling 18 out of the 27(67%) papers. For the rest of the years, 1 (3.7%) or 2 (7.4%) papers were published with no papers in 2012. There was no country with continuous year-to-year publications (Figure 4).
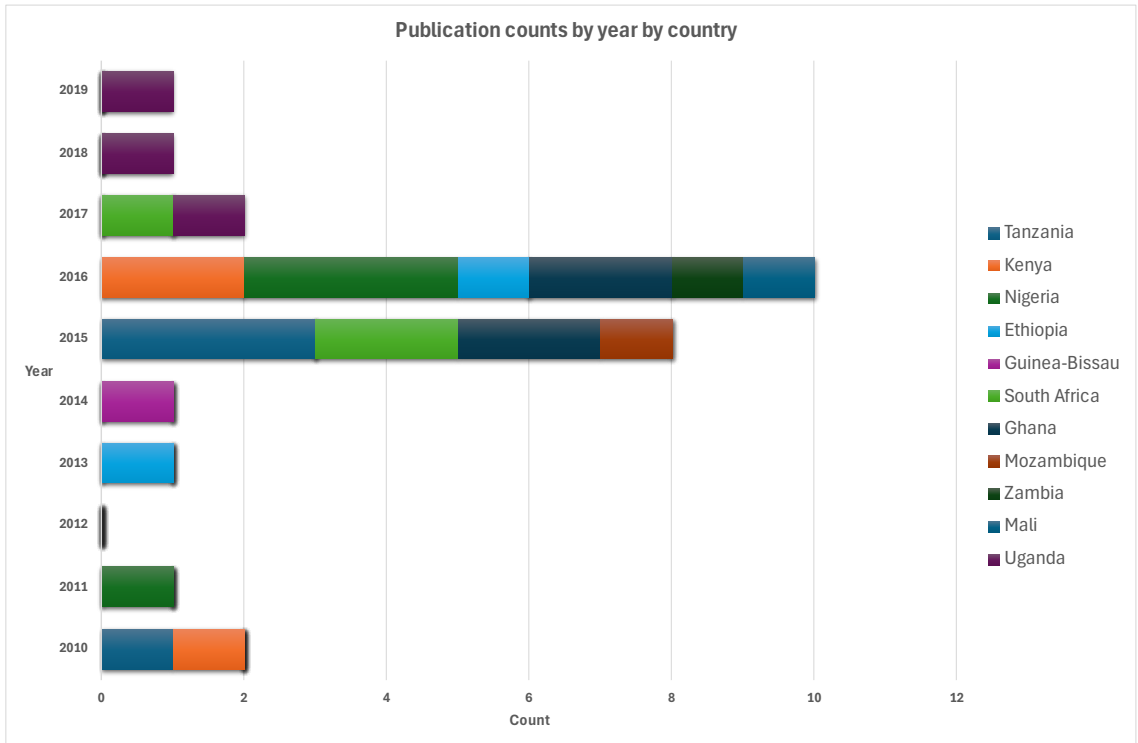
Figure 4. Publication counts concerning antimicrobial resistance of *Escherichia coli* and *Staphylococcus aureus* in patients with bloodstream infections by country across the years (2010-2019)

Table 10 below presents descriptions of the laboratory practices and reporting. The most commonly used guideline in Africa is the Clinical Laboratory Standards Institute guideline (CLSI) representing 21 (77.8%). The duration for almost half (48%) of the studies was less than 12 months (i.e. AST data period coverage). Whereas the duration for seven (25.9%) studies was for more than 1 year but less than 2 years (i.e. 1 < 2), whilst another 7 were for over 2 years. Twenty-two (22) papers reported AST for both *E. coli* and *S. aureus*. Most of the studies (20, 74.1%) did not mention usage of quality control strains.

Table 10. Overview of study and laboratory characteristics.

Characteristics	Count (n)	Percentage (%)
Duration		
< 12 months	13	48.1
1 year < 2 years	7	25.9
>2 years	7	25.9
Quality Control		
Yes	7	25.9
NA[1]	20	74.1
Susceptibility testing guideline		
CLSI[2]	21	77.8
EUCAST[3]	5	18.5
Both	0	0
NA[1]	1	3.7
Microorganisms		
Escherichia coli	2	7.4
Staphylococcus aureus	3	11.1
Both	22	81.5
Abbreviations [1]Missing data [2]The Clinical Laboratory Standard Institute [3]The European Committee on Antimicrobial Susceptibility Testing		

5.1.2   Population under study

The demographic and patient characteristics are reported in Table 11. Newborns (neonates) were present in 37% (10 out of 27) of the studies. Adults and studies with mixed age groups were far less common, each only present in 7.4% (2 out of 25) of the studies. Notably, 22% (6 out of 27) of the studies did not report the age group of participants.

Gender information was rarely included but only mentioned in one study on sepsis after childbirth (puerperal sepsis). Half of the studies involved inpatients only (51.9%), while another third involved both inpatients and outpatients (33.3%).

Most studies (88.9%) involved patients or samples from tertiary care facilities.

Sepsis/septicemia was the most common diagnosis reported in the studies (44.4%). Other studies investigated sepsis alongside other conditions like diabetes, cancer (malignancies), HIV, gastrointestinal infections (gastroenteritis), meningitis, kidney failure, and fever (pyrexia).

Table 11. Patient (n=27) demographic and clinical characteristics. [1]missing data.

Characteristics	Count (n)	Percentage (%)
Age Group		
Neonates/Paediatrics	10	37
Adults	2	7.4
Mixed/all	2	7.4
NA[1]	13	48.1
Gender		
Male	-	-
Female	1	3.7
Both	1	3.7
NA[1]	25	92.6
In-or-Out Patient		
In-patient	14	51.9
Out-patient	2	7.4
Both	9	33.3
NA[1]	2	7.4
Level of Care		
Secondary	1	3.7
Tertiary	24	88.9
NA[1]	2	7.4
Clinical outcome		
Sepsis/Septicaemia	12	44.4
Sepsis + HIV	1	3.7
Neonatal sepsis	3	11.1
Puerperal sepsis	1	3.7
Febrile	2	7.4
Malignancies/Cancer	2	7.4
Multiple	3	11.1
NA[1]	3	11.1

5.1.3 Estimating ESBL prevalence in Africa using cephalosporin data, 2008-2019

From the 2009-2018 publications, the AST was carried out between 2008-2014. The highest resistance percentage stood at 70% (73 out of 104 isolates), and this was recorded for 2nd generation cefuroxime in Ghana. Among the 3rd generation cephalosporins, ceftriaxone resistance was the highest in Uganda being 66% (31/47). Kenya was the only country with continuous year-to-year data (2012-2014). Here, resistance to cefuroxime and cefotaxime fluctuated: cefuroxime (55% to 47% to 58%) and cefotaxime (48% to 45% to 54%).

In 2013, cephalosporin resistance ranged from 40% to 70% in Kenya and Ghana. A notable exception was South Africa, which showed only 12% resistance (cefotaxime). Although generally lower than other cephalosporins, ceftazidime resistance showed a perfect correlation with ceftriaxone resistance in Nigeria and South Africa. In 2008, both ceftriaxone and ceftazidime resistance were 10% in Nigeria. Equally, in 2012, there was 34% resistance for both antimicrobials in South Africa. The latest data from 2014 reports cephalosporin resistance in Uganda and Kenya between 50% and 70% (Figure 5). Concerningly, the data suggests a general upward trend in cephalosporin resistance over the studied period (2008-2014). For example, resistance ranged from 30% to 50% in 2012, but rose to 45-70% in 2013 and further rose to 55-65% resistance in 2014. Previous fixed-effects model analysis (Original publication I, Figure 5) revealed the following median resistance percentages for various cephalosporins between 2008 and 2014, cefuroxime: 58% (95% CI: 47-69%); ceftriaxone: 32% (95% CI: 9-69%); cefotaxime: 42% (28-58%) and ceftazidime: 19% (5-52%). Overall median resistance for ciprofloxacin was 44% (95% CI: 23-67%). Sulfamethoxazole-trimethoprim resistance was 75% (95% CI: 57-87%) and largely absent (below 1%) for imipenem, meropenem and ertapenem.
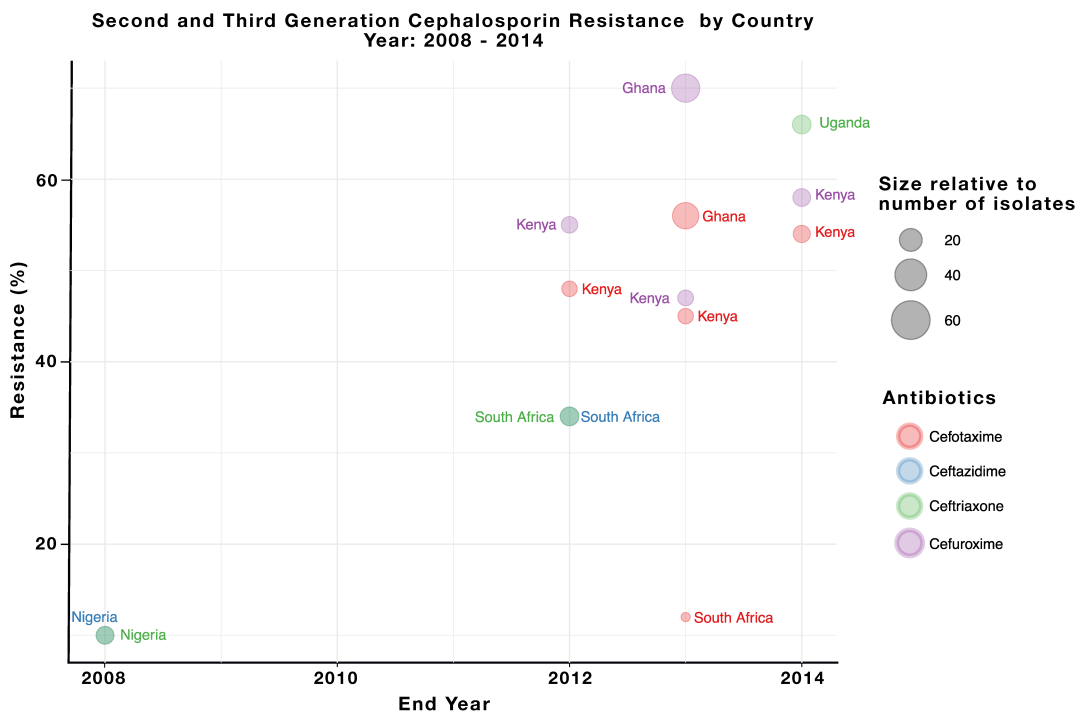
Figure 5. *Escherichia coli* percentage resistance to cephalosporins by country (Isolation End Year: 2008-2014) (not publication year). Bubble size relative to number of isolates.

5.1.4   Methicillin Resistant *Staphylococcus aureus* distribution and levels by country is under-reported

An analysis of resistance to cloxacillin, methicillin, and oxacillin, indicators of MRSA revealed a stable range between 20% and 34% from 2008 to 2014. However, continuous year-to-year resistance data for any particular country was lacking during this period.

While the overall trend remained stable, specific countries and years showed variations. Cloxacillin resistance was as high as 30% (42/140) in Nigeria in 2008, 28% (9/32) in Tanzania in 2009 and 21% (22/103) in South Africa in 2012. The highest resistance to cloxacillin was recorded at 100% (34/34) in the western African country Ghana in 2014. During the same year, in Eastern Africa (Uganda), the resistance rate for methicillin was 32% (41/127) (not shown on figure).

Data on oxacillin resistance was only available for Kenya (2008) and Ghana (2013), with the highest resistance rate (23%) observed in 2013 (Figure 6). Previous analysis (Original Publication I, Figure 4) identified high variability (heterogeneity) within studies for both oxacillin (I^2=92) and cloxacillin (I^2=99).
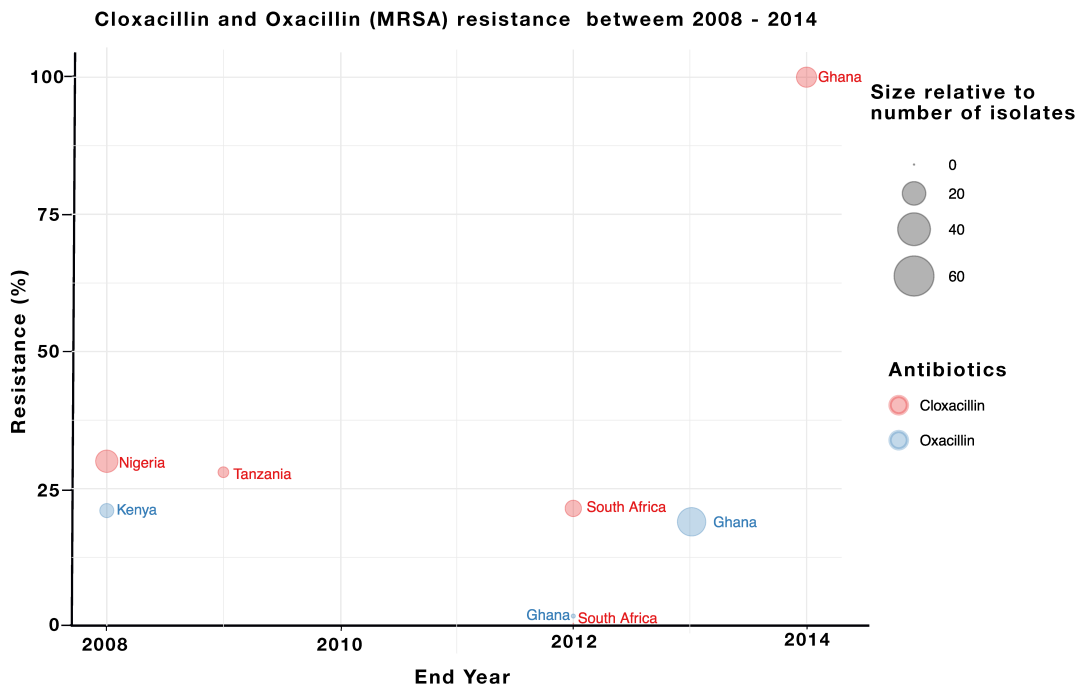
Figure 6. *Staphylococcus aureus* percentage resistance to cephalosporins by country (Isolation End Year: 2008-2014) (not publication year). Bubble size relative to number of isolates.

Table 12 below details the median resistance levels of various other antimicrobials in *E. coli* and *S. aureus*. Although penicillin resistance was found to be 24%, this was reported from Ghana only.

E. coli median resistance to the various antimicrobials, from highest to lowest was as follows: tetracycline (91%), ampicillin (87%), co-trimoxazole (75%) and ciprofloxacin (44%). For *S. aureus* this was: ampicillin (91%), tetracycline (56%), co-trimoxazole (47%), and ciprofloxacin (18%).

Up to 2014, carbapenem and vancomycin resistance was generally unreported across South Africa, Kenya and Ghana. The only exception was a study in Uganda that reported 19% resistance to imipenem (a carbapenem antimicrobial) in 2014.

Table 12. Resistance to various antimicrobials given as median resistance and 95% confidence intervals (CI). [1]Not applicable.

Antimicrobial	*E. coli*	*S. aureus*
Ampicillin	87 (46-98)	91 (37-99)
Penicillin	NA[1]	24 (12-40)
Cefuroxime	58 (47-69)	24 (10-48)
Ciprofloxacin	44 (23-67)	18 (10-29)
Co-trimoxazole	75 (57-87)	47 (13-84)
Tetracycline	91 (82-96)	56 (39-72)
Vancomycin	NA[1]	0 (0-100)
Meropenem	1 (0-3)	NA[1]
Imipenem	4 (0-64)	NA[1]
Ertapenem	1 (0-64)	NA[1]
Colistin	1 (1-8)	NA[1]

5.2 Antimicrobial resistance of *Escherichia coli* from Namibian female urine cultures, 2016-2017 (Study II)

5.2.1 *Escherichia coli* is predominantly found in young-middle aged females

Study II was a 2-year pilot study to understand the useability of the NIP data by using a subset of female urinary data from 2016-2017. In total, 22,259 urine cultures were performed. The total number of *E. coli* isolates was 5,568, with the distribution of isolates being 2,659 (47.8%) and 2909 (52.2%) in the years 2016 and 2017 respectively. A total of 277/2,659 (10.4%) and 326/2,909 (11.2%) of *E. coli* were defined as ESBLs by double disk diffusion or by resistance to any 3rd generation cephalosporin (ceftriaxone, cefotaxime, ceftazidime). In Table 13, the majority of *E. coli* were isolated from females aged 15-59 years (3,709/5,568). ESBL proportion was highest in the 5-14 (12.6% and 13.9%) and over 60 (11.4% and 18.7%) age groups in these two years.

Table 13. Distribution of individuals across different age groups, categorized by *Escherichia coli* for two years (2016 and 2017). [1]missing data.

Year	Category	Age Group					
		0-4	5-14	15-59	60+	NA[1]	Total
2016	Non-ESBL (n, %)	200 (91.7)	76 (87.4)	1,542 (88.7)	281 (88.6)	283 (95.0)	2,382 (89.6)
	ESBL (n, %)	18 (8.3)	11 (12.6)	197 (11.3)	36 (11.4)	15 (5.0)	277 (10.4)
	Total (n, %)	**218 (100)**	**87 (100)**	**1,739 (100)**	**317 (100)**	**298 (100)**	**2,659 (100)**
2017	Non-ESBL (n, %)	183 (93.4)	105 (86.0)	1,751 (88.9)	283 (81.3)	261 (95.6)	2583 (88.7)
	ESBL (n, %)	13 (6.6)	17 (13.9)	219 (11.1)	65 (18.7)	12 (4.4)	326 (11.2)
	Total (n, %)	**196 (100)**	**122 (100)**	**1970 (100)**	**348 (100)**	**273 (100)**	**2909 (100)**

5.2.2 ESBLs exhibited moderate-to-high resistance to first and second-line therapy antimicrobials for cystitis and pyelonephritis

Generally, ESBLs showed higher resistance across all the antimicrobials when compared to non-ESBL *E. coli* (Table 14). For all *E. coli*'s regardless of ESBL status, resistance to ampicillin was very high (>76%). The prevalence of resistance to amoxicillin-clavulanic acid among ESBLs increased from 62% in 2016 to 88% in 2017).

For the first-line therapy drug, nitrofurantoin resistance was below 10% in the non-ESBL groups but was 12.9% and 19% in the ESBL group in 2016 and 2017, respectively. Nalidixic acid which has been recommended for use in children was 36% and 85% in the non-ESBL and ESBL groups respectively.

The second-line therapy drugs are cefuroxime and gentamicin. Cefuroxime resistance was absent in the non-ESBL groups, with resistance of above 97% in the ESBL group. On the other hand, gentamicin exhibited low-moderate resistance of approximately 14% and 50% for non-ESBLs and ESBLs respectively. Noteworthy, low resistance is seen with the other aminoglycoside amikacin at 6% among ESBLs.

Table 14. Percentage resistance of urinary *Escherichia coli* (N=5,568) isolates in years 2016 and 2017, disaggregated by ESBL and Non-ESBLs. Abbreviations, [1]amoxicillin-clavulanate, [2]nalidixic acid.

	2016 (n=2,659)		2017 (n=2,909)	
	ESBL	Non- ESBL	ESBL	Non- ESBL
Antimicrobials	n/N (%R)	n/N (%R)	n/N (%R)	n/N (%R)
AMC[1]	150/242 (62)	180/1814 (9.9)	271/307 (88.3)	319/2187 (14.6)
Amikacin	15/273 (5.5)	32/1735 (1.8)	20/317 (6.3)	40/1935 (2.1)
Ampicillin	215/219 (98.2)	868/1136 (76.4)	278/281 (98.9)	1087/1406 (77.3)
Ceftazidime	170/200 (85)	0/683 (0)	245/255 (96.1)	0/762 (0)
Cephalothin	66/73 (90.4)	415/1394 (29.8)	51/54 (94.4)	513/1291 (39.7)
Ceftriaxone	85/87 (97.7)	0/389 (0)	75/76 (98.7)	0/262 (0)
Cefotaxime	175/181 (96.7)	0/613 (0)	236/240 (98.3)	0/690 (0)
Cefuroxime	229/243 (94.2)	169/1758 (9.6)	295/305 (96.7)	216/1833 (11.8)
Cefepime	116/178 (65.2)	0/601 (0)	227/242 (93.8)	0/694 (0)
Cefoxitin	23/184 (12.5)	1/630 (0.2)	92/244 (37.7)	5/706 (0.7)
Gentamicin	138/271 (50.9)	319/2278 (14)	168/319 (52.7)	338/2442 (13.8)
Imipinem	1/252 (0.4)	5/1026 (0.5)	1/298 (0.3)	5/1357 (0.4)
NAL[2]	217/255 (85.1)	796/2205 (36.1)	265/303 (87.5)	836/2356 (35.5)
Nitrofurantoin	34/263 (12.9)	160/2267 (7.1)	60/316 (19)	201/2420 (8.3)
Ofloxacin	51/84 (60.7)	214/1615 (13.3)	44/66 (66.7)	246/1724 (14.3)

5.3 Retrospective laboratory AMR surveillance of bacteremic isolates in Namibia, 2011-2019 (Study III)

5.3.1 *Escherichia coli* and *Staphylococcus aureus* counts show similar trends over the years

From 2011 to 2019, the national laboratory performed 37,765 blood cultures, identifying 30,526 bacterial isolates. Of these isolates, 22,062 were gram-negative and 8,090 gram-positive. *E coli* and *S. aureus* were the most frequently isolated pathogenic bacteria, accounting for 2,319 (7.6%) and 2,341 (7.7%) of the total isolates, respectively. *E. coli* showed variation over the years, with a minimum of 208 isolates (9%) in 2014 and a maximum of 357 isolates (15.4%) in 2011. Similarly, *S. aureus* isolation varied, with a minimum of 214 isolates (9%) in 2019 and a maximum of 484 isolates (21%) in 2011.

5.3.2 *Escherichia coli* and *Staphylococcus aureus* frequency vary across age groups

During the 9 years, the total proportion of ESBLs was 24.7% and that of MRSA was 23.5%. ESBLs (3rd generation cephalosporin resistance) fluctuated between 16.6% and 29.4% in the various age groups. On the other hand, the MRSA rates fluctuated between 16.7% and 27.4% in the various age groups.

E. coli was commonly isolated from the 15-59 age group with 958/2,319 (41%) isolates. This pattern was maintained in the ESBL and non-ESBL disaggregated data. Proportionally, however, the ESBL percentage was highest in the 5-14 (29.4%) and above 60 (32.4%) age groups (Table 15).

The largest *S. aureus* count of 1,035 out 2,341 was from the 0-4 age group. Equally, MRSAs by count were largest (237/1035, 22.9%) in the 0-4 age group but proportionally smaller than the MRSA proportions in the 15-59 (25.2%) and above 60 (27.4%) age groups (Table 15).

Table 15 Age group distribution of patients categorized by *Escherichia coli* ESBL and MRSA status, 2011-2019. [1]missing data.

Category	Age Group					
	0-4	**5-14**	**15-59**	**60+**	**NA[1]**	**Total**
Non-ESBL (n, %)	579 (83.4)	77 (70.6)	706 (73.7)	242 (67.6)	142 (71.0)	1,746 (75.3)
ESBL (n, %)	115 (16.6)	32 (29.4)	252 (26.3)	116 (32.4)	58 (29.0)	573 (24.7)
Total (n, %)	**694 (100)**	**109 (100)**	**958 (100)**	**358 (100)**	**200 (100)**	**2,319 (100)**
MSSA (n, %)	798 (77.1)	165 (83.3)	597 (74.8)	98 (72.6)	132 (75.4)	1,790 (76.5)
MRSA (n, %)	237 (22.9)	33 (16.7)	201 (25.2)	37(27.4)	43 (24.6)	551 (23.5)
Total (n, %)	**1035 (100)**	**198 (100)**	**798 (100)**	**135 (100)**	**175 (100)**	**2,341 (100)**

5.3.3 Moderate-to-high antimicrobial resistance among *Escherichia coli*

The antimicrobial resistance to various antimicrobials is summarized in Figure 7 below. Resistance rates are particularly high (>80%) for ampicillin and sulfamethoxazole-trimethoprim. Moderate-to-high resistance rates were seen for amoxicillin-clavulanic acid (minimum: 0% – maximum: 37.5%, p-value: 0.05), ceftazidime (4.2%-32.7%, *p:*0.08) and gentamicin (16.3%-35.6%) resistance. Noteworthy, low resistance was observed for amikacin (0%-5.2%), imipenem (maximum: 3.0%), ertapenem (1.1%) and meropenem (0%).
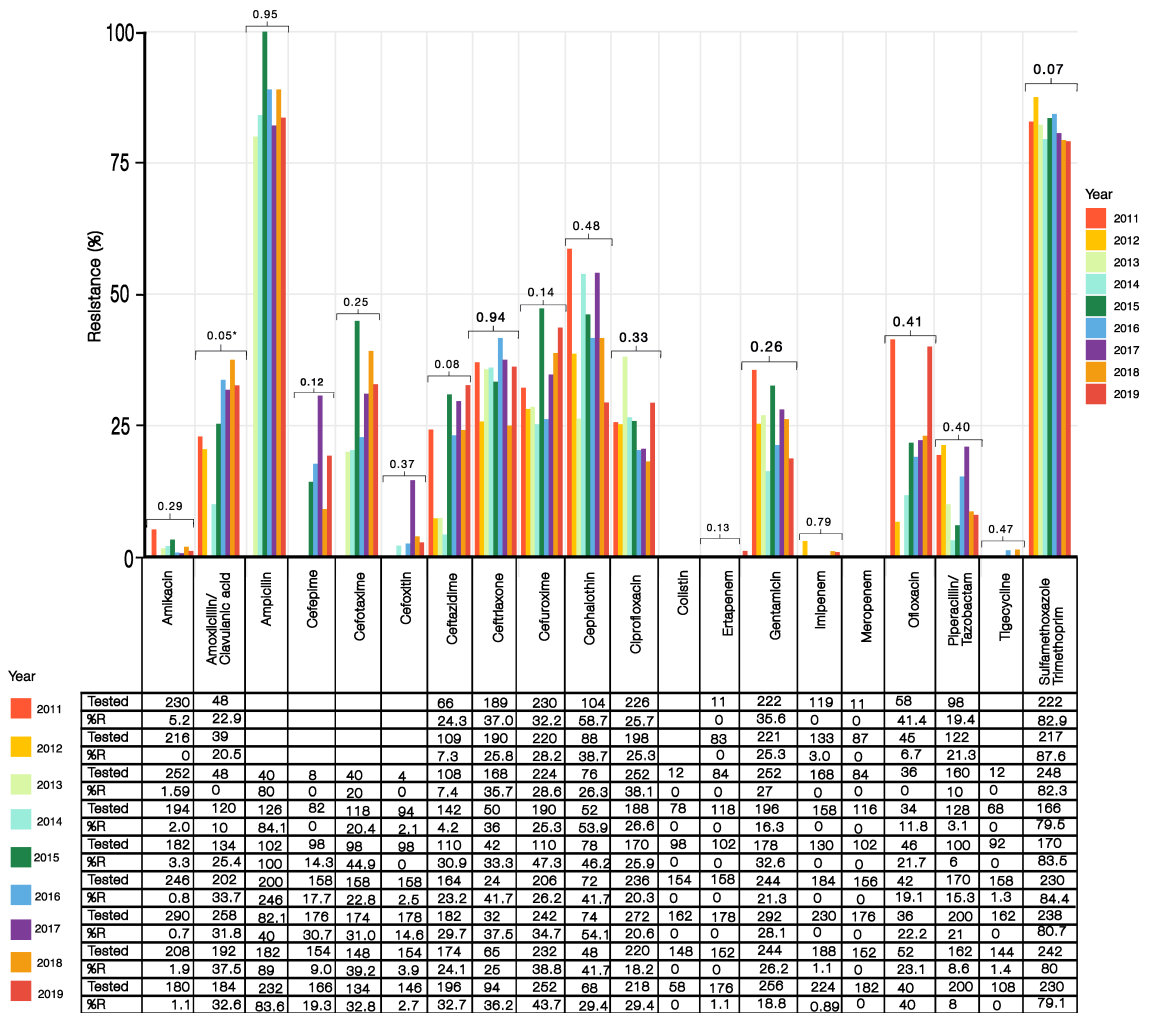
Year		Amikacin	Amoxicillin/Clavulanic acid	Ampicillin	Cefepime	Cefotaxime	Cefoxitin	Ceftazidime	Ceftriaxone	Cefuroxime	Cephalothin	Ciprofloxacin	Colistin	Ertapenem	Gentamicin	Imipenem	Meropenem	Ofloxacin	Piperacillin/Tazobactam	Tigecycline	Sulfamethoxazole-Trimethoprim
2011	Tested	230	48					66	189	230	104	226		11	222	119	11	58	98		222
2011	%R	5.2	22.9					24.3	37.0	32.2	58.7	25.7		0	35.6	0	0	41.4	19.4		82.9
2012	Tested	216	39					109	190	220	88	198		83	221	133	87	45	122		217
2012	%R	0	20.5					7.3	25.8	28.2	38.7	25.3		0	26.3	3.0	0	6.7	21.3		87.6
2013	Tested	252	48	40	8	40	4	108	168	224	76	252	12	84	252	168	84	36	160	12	248
2013	%R	1.59	0	80	0	20	0	7.4	35.7	28.6	26.3	38.1	0	0	27	0	0	0	10	0	82.3
2014	Tested	194	120	126	82	118	94	142	50	190	52	188	78	118	196	158	116	34	128	68	166
2014	%R	2.0	10	84.1	0	20.4	2.1	4.2	36	26.3	53.9	26.6	0	0	16.3	0	0	11.8	3.1	0	79.5
2015	Tested	182	134	102	98	98	98	110	42	110	78	170	98	102	178	130	102	46	100	92	170
2015	%R	3.3	25.4	100	14.3	44.9	0	30.9	33.3	47.3	46.2	25.9	0	0	32.6	0	0	21.7	6	0	83.5
2016	Tested	246	202	200	158	158	158	164	24	206	72	236	154	158	244	184	156	42	170	158	230
2016	%R	0.8	33.7	82.1	17.7	22.8	2.5	23.2	41.7	26.2	20.3	20.3	0	0	21.3	0	0	19.1	15.3	1.3	84.4
2017	Tested	290	258	246	176	174	178	182	32	242	74	272	162	178	292	230	176	36	200	162	238
2017	%R	0.7	31.8	40	30.7	31.0	14.6	29.7	37.5	34.7	54.1	20.6	0	0	28.1	0	0	22.2	21	0	80.7
2018	Tested	208	192	182	154	148	154	174	65	232	48	220	148	162	244	188	152	52	162	144	242
2018	%R	1.9	37.5	89	9.0	39.2	3.9	24.1	25	38.8	41.7	18.2	0	0	26.2	1.1	0	23.1	8.6	1.4	80
2019	Tested	180	184	232	166	134	146	196	94	252	68	218	58	176	256	224	182	40	200	108	230
2019	%R	1.1	32.6	83.6	19.3	32.8	2.7	32.7	36.2	43.7	29.4	29.4	0	1.1	18.8	0.89	0	40	8	0	79.1

Figure 7. Resistance of *Escherichia coli* isolates from blood cultures to various antimicrobials, 2011-2019 (N=2,319). P-values according to one-way ANOVA.

5.3.4 MRSA rates in bacteremic isolates

MRSA rates declined from 48.1% to 16.9% as indicated by cloxacillin (p-value: 0.01) and oxacillin resistance (*p*: 0.01). There was a significant decline in gentamicin resistance from 42.7% to 9.5% (*p*: 0.01). *S. aureus* exhibited high resistance to penicillin/novobiocin (approximately 60%) and sulfamethoxazole-trimethoprim (>40%, *p*: 0.05). Resistance to erythromycin (20%-46%) and rifampicin (1.9% - 50%) was moderate to high. The was no resistance to teicoplanin observed. Clindamycin (6.1%-22.4%) and ciprofloxacin (6.1% - 22.4%) resistance were variable, with the highest resistance being in the year 2011 for both.
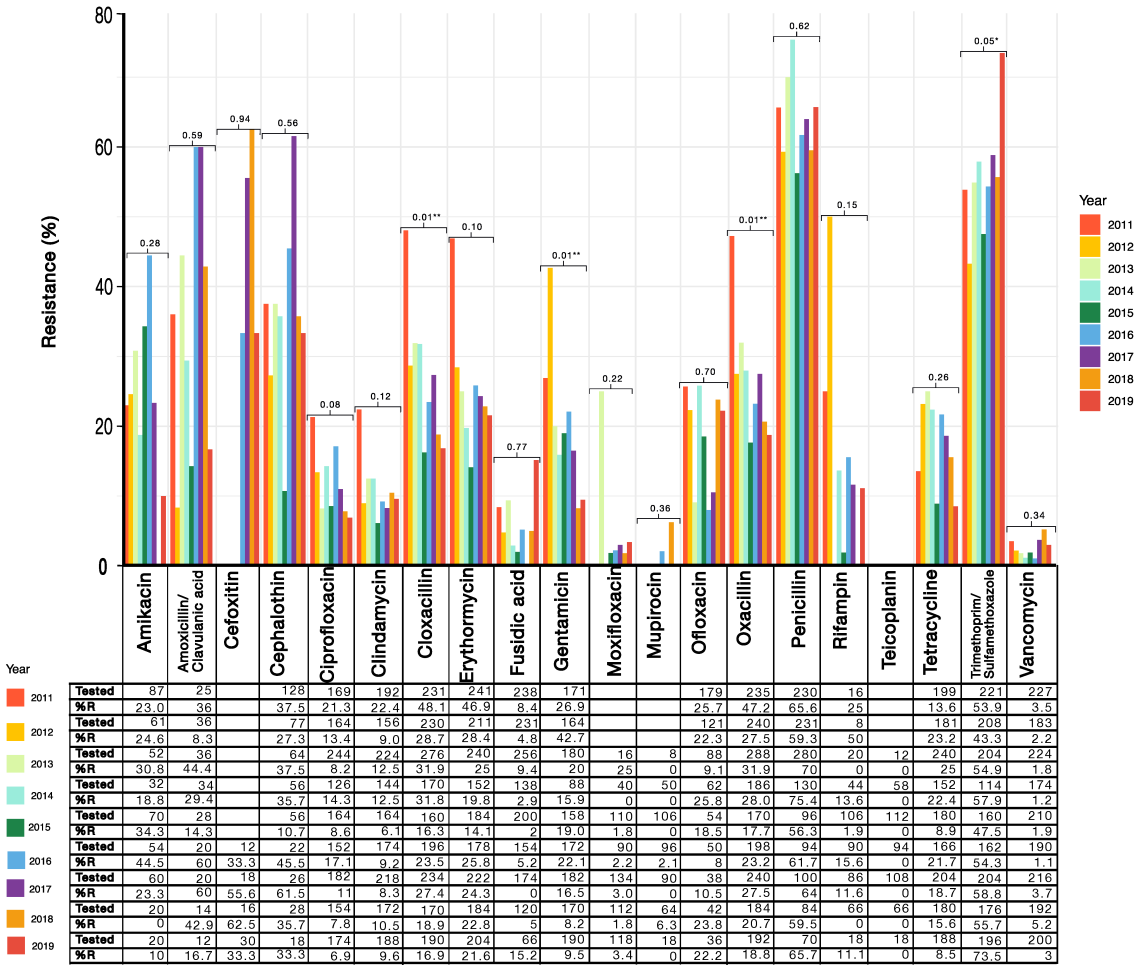
Figure 8. Resistance of *S. aureus* (N=2,341) isolates from blood cultures to various antimicrobials, 2011-2019. P-values according to one-way ANOVA test.

Year		Amikacin	Amoxicillin/ Clavulanic acid	Cefoxitin	Cephalothin	Ciprofloxacin	Clindamycin	Cloxacillin	Erythormycin	Fusidic acid	Gentamicin	Moxifloxacin	Mupirocin	Ofloxacin	Oxacillin	Penicillin	Rifampin	Teicoplanin	Tetracycline	Trimethoprim/ Sulfamethoxazole	Vancomycin
2011	Tested	87	25		128	169	192	231	241	238	171			179	235	230	16		199	221	227
2011	%R	23.0	36		37.5	21.3	22.4	48.1	46.9	8.4	26.9			25.7	47.2	65.6	25		13.6	53.9	3.5
2012	Tested	61	36		77	164	156	230	211	231	164			121	240	231	8		181	208	183
2012	%R	24.6	8.3		27.3	13.4	9.0	28.7	28.4	4.8	42.7			22.3	27.5	59.3	50		23.2	43.3	2.2
2013	Tested	52	36		64	244	224	276	240	256	180	16	8	88	288	280	20	12	240	204	224
2013	%R	30.8	44.4		37.5	8.2	12.5	31.9	25	9.4	20	25	0	9.1	31.9	70	0	0	25	54.9	1.8
2014	Tested	32	34		56	126	144	170	152	138	88	40	50	62	186	130	44	58	152	114	174
2014	%R	18.8	29.4		35.7	14.3	12.5	31.8	19.8	2.9	15.9	0	0	25.8	28.0	75.4	13.6	0	22.4	57.9	1.2
2015	Tested	70	28		56	164	164	160	184	200	158	110	106	54	170	96	106	112	180	160	210
2015	%R	34.3	14.3		10.7	8.6	6.1	16.3	14.1	2	19.0	1.8	0	18.5	17.7	56.3	1.9	0	8.9	47.5	1.9
2016	Tested	54	20	12	22	152	174	196	178	154	172	90	96	50	198	94	90	94	166	162	190
2016	%R	44.5	60	33.3	45.5	17.1	9.2	23.5	25.8	5.2	22.1	2.2	2.1	8	23.2	61.7	15.6	0	21.7	54.3	1.1
2017	Tested	60	20	18	26	182	218	234	222	174	182	134	90	38	240	100	86	108	204	204	216
2017	%R	23.3	60	55.6	61.5	11	8.3	27.4	24.3	0	16.5	3.0	0	10.5	27.5	64	11.6	0	18.7	58.8	3.7
2018	Tested	20	14	16	28	154	172	170	184	120	170	112	64	42	184	84	66	66	180	176	192
2018	%R	0	42.9	62.5	35.7	7.8	10.5	18.9	22.8	5	8.2	1.8	6.3	20.7	59.5		0	0	15.6	55.7	5.2
2019	Tested	20	12	30	18	174	188	190	204	66	190	118	18	36	192	70	18	18	188	196	200
2019	%R	10	16.7	33.3	33.3	6.9	9.6	16.9	21.6	15.2	9.5	3.4	0	22.2	18.8	65.7	11.1	0	8.5	73.5	3

6   Discussion

6.1   Overview of AMR prevalence in the African region (Study I)

Our systematic review aimed at describing the AMR prevalence rates of bacteremic *E. coli* and *S. aureus* in the continent. Reports for the period 2010-2019 were only available for a limited number of African countries (23%), with the majority of them (n=18, 67%) published in two years, 2015 and 2016. This significant gap in data availability has also been reported in other AMR-related systematic reviews from Africa (Kariuki et al., 2022; Mitgang et al., 2018; Okolie et al., 2023). The most common population under study were the neonates/paediatrics group in 10 (27%) studies.

E. coli exhibited moderate to high resistance against third-generation cephalosporins (3GC) (ceftriaxone, cefotaxime, ceftazidime) with a median resistance that was between 19% and 42%. This was variably reported for the years 2008, 2012 to 2014 between countries (*i.e.* there were no year-to-year reports for any one specific country). Gray et al. (2006), Saied et al. (2011), and Tansarli et al. (2014) have reported comparable ranges of 0.7%-75.8% in Africa between 2005 and 2013.

Across European countries, resistance to third-generation cephalosporins was lower in European countries than in African countries. In 2022, Northern countries like Norway, Sweden, and Finland had resistance levels below 7%. Southern countries showed some increase, but still stayed under 10% (including Germany, Belgium, and France). The highest resistance was found in South Eastern Europe, exceeding 15% in Poland and Slovakia. Bulgaria was an exception, with a much higher resistance rate of 40% (ECDC & EARSNet, 2024). In the systematic study, ESBL proportions were inferred from 3GC. It was, however, not possible to ascertain their contribution to the reported resistance rates of tetracycline, ampicillin, co-trimoxazole and ciprofloxacin, as has been done by other scholars (Galindo-Méndez, 2020; Ibrahim et al., 2023; Wu et al., 2021).

Nevertheless, *E. coli* exhibited moderate to high resistance against tetracycline (91%), ampicillin (87%), co-trimoxazole (75%) and ciprofloxacin (44%) generally similar to the reported patterns in Zambia (Bumbangi et al., 2022). The frequent use

of ciprofloxacin and co-trimoxazole has been linked to a rise in bacterial resistance, thus making them less effective as initial treatment options (Bader et al., 2020; Kot, 2019). Studies by Droz et al. (2019) and Ghadiri et al. (2012) show comparable resistance levels for ciprofloxacin (44%). Notably, Africa appears to have a higher ciprofloxacin resistance rate (36.7%) compared to Asia (0%) (Droz et al., 2019). The latest data from EARSNet indicated that fluoroquinolone resistance ranged from 5.8-40.2% among European countries in 2022 (European Centre for Disease Prevention and Control, 2024).

Co-trimoxazole has been routinely used for treating bacterial infections, diarrhoea, HIV complications, and even malaria in sub-Saharan Africa (Al-Hasan et al., 2009; Yamba et al., 2023) and increasing resistance is therefore a growing concern. Our study showed that *E. coli* median resistance for co-trimoxazole was 75% (95% CI: 57-87%). In 2011, before our review, Ashley et al., (2011) found higher co-trimoxazole resistance in Asia (55%) compared to sub-Saharan Africa (25%). By 2017, the Hubei province of China found 72.1% co-trimoxazole resistance which was much closer to our 75% finding (Z. Zhang et al., 2022). In a recent study conducted in Zambia between 2019 and 2021, co-trimoxazole resistance was at 81.5% among *E. coli* isolates and this was even higher among outpatients (59%) compared to inpatients (41%) for combined urine, blood and sputum cultures (Mwansa et al., 2022). The disaggregated findings in Zambia also compared well to those in Ethiopia (50.3%) among diarrheagenic *E. coli* (Embaye et al., 2023). Among uropathogens in central Europe, co-trimoxazole resistance was relatively low at 28.3% in 2019 (Hrbacek et al., 2020). In the UK, co-trimoxazole resistance fluctuated between 30-40% during the years 2011 to 2014, with no significant decreases despite a decline in the use of the antimicrobial (Pouwels et al., 2017).

In our study, MRSA data were from the years 2008-2009 and 2012-2014 (no reports between 2010-2011). We reported concerning MRSA percentages of 20-34%, with a median resistance of 12% and 34%, for oxacillin and cloxacillin respectively. Our 2008-2019 findings compare to an earlier study in Africa (2002-2011), that reported up to 55% MRSA rates (Falagas et al., 2013). The Asia Pacific region's (2000 -2016) overall resistance of 18% for oxacillin and 29% for cloxacillin was comparable to our study findings (Lim et al., 2019). Resistance rates of 1% to 20% were reported between 2000-2010 in European countries (Dulon et al., 2011). In later years, the European region reported MRSA resistance of 19% in 2015 with a decline to 15.5% by 2019 (European Centre for Disease Prevention and Control, 2019).

The MRSA rates in our review were only reported from 8 data points for the 3 antimicrobials - cloxacillin (5), methicillin (1), and oxacillin (3). From these data points, high variability was noted across settings, derived from the heterogeneity (I^2) scores of 92% (cloxacillin), 99% (oxacillin) and impossible to calculate for

methicillin. This high variation has also been reported in the Asia Pacific region (Lim et al., 2019).

The data availability aspects were the major limitations weakening our interpretations. From 27 publications, there was a lot of missing data on the quality control practises (n=20, 74%), age group (n=13, 48%) and sex (n=25, 95%). The sex has only been provided in one study on puerperal sepsis. We speculated that resistance was reported for all sexes if it was not explicitly stated in any given study. About half (n=13, 48%) of the studies reported on a timeframe of less than 12 months whereas the other half reported for at least a year or longer. GLASS recommends that resistance should be reported for 12-month surveillance periods (i.e. one result for each patient per specimen per pathogen) (World Health Organization, 2023). Also, unstandardized microbiological practices, such as collecting cultures predominantly after treatment failure, can inflate resistance rates.

Longer periods do offer valuable information on the resistance patterns but should be clearly disaggregated, in order to be able to compare resistance rates spatio-temporally (in any given year across geographies). Hence, for high quality AMR surveillance, recommendations for minimum variables (i.e. standardization) that should be collected for good information quality and comparability need to be adhered to as reported by Pezzani et al. (2020) and World Health Organization (2023).

6.2 Resistance of UTI pathogens in Namibia (Study II)

From 2016 to 2017, *E. coli* was the most prevalent bacteria found in urine cultures in Namibia, accounting for over 40% (N=5,568) of the isolates. Among these *E. coli* isolates, about 22% (175/794) were resistant to cefoxatime (ESBL indicator). Adult females between 15 and 59 years old accounted for most of the *E. coli* (66%, n=3,709) and the highest ESBL proportions (69%, 416/603) as well when compared to other age groups. Less than 10% of non-ESBL *E. coli* were nitrofurantoin resistant. However, its resistance rate nearly doubled from 12.9% to 19% (2016 to 2017) among ESBLs. In general, ESBL-producing *E. coli* exhibited greater resistance to all antimicrobials tested, except for imipenem (<0.5% resistance).

Whilst the proportion of ESBL was about 22%, the overall resistance varied between ceftriaxone (22%) and ceftazidime (10.5%) in 2017 (Original Publication II, Table 3). India on the other hand has reported a comparable ESBL prevalence of 23.3% (Harwalkar et al., 2013). On the contrary, the proportions of ESBL resistant *E. coli* from Namibia were higher than the 9% ESBL prevalence found in Nepal in 2014 (Pooja Shakya). The ESBL prevalence in Namibia was also higher than the

range of 0-15.7% reported in a 2019 study involving European countries (Ny et al., 2019).

However, the high resistance rates among ESBLs may significantly complicate treatment with commonly used antimicrobials like beta-lactams and quinolones (Shakya et al., 2017) . ESBL *E. coli* has particularly shown high resistance rates to amoxicillin-clavulanic acid (88.3%), ofloxacin (66.75), nalidixic acid (87.5%), gentamicin (52%) and nitrofurantoin (19%) in our study. The ciprofloxacin and ofloxacin rates are somewhat comparable (Nsofor et al., 2021), hence an elaborate discussion has been presented earlier. The pattern of resistance to amoxicillin-clavulanic acid and gentamicin was comparable to that reported in Kenya and South Africa (Heine et al., 2024; Tornberg-Belanger et al., 2022). Resistance rates are higher for second-line therapy regimens (gentamicin and cefuroxime) compared to first-line therapy (nitrofurantoin). These high resistance levels are otherwise worrisome for the management of pyelonephritis. Resistance to cephalosporins may be driving resistance due to co-resistance as has previously been noted for fluoroquinolones, known as 'collateral damage' (Ny et al., 2019).

In our setting *E. coli* was most commonly isolated from female adults (aged 15-59 years) whereas other settings such as Finland, Germany and the USA have reported higher rates in older age groups (>60 age group) (Frisbie et al., 2021; Ilmavirta et al., 2023; Stoltidis-Claus et al., 2023; Toval et al., 2014). Furthermore, the use of terminology such as adults presents comparison problems as the age cut off ranges are sometimes not the same. For example, in our study, the age cut-off between adults and the elderly was set to 59, in contrast to 65 in a Danish study reporting on UTI among females (Waldorff et al., 2022).

Lastly, it would have been interesting to compare the Namibian study to a review of the AMR of *E. coli* in urine/UTI in the WHO Afro region. However, there is no record of such a study as the others are focused on certain parts of the continent only (*i.e.* eastern and western Africa sub-regions, Ethiopia, Tanzania, etc).

6.3   Resistance of bacteremic *E. coli* and *S. aureus* in Namibia

The overall (2011-2019) blood culture positivity rate was as high as 80.9% (30,526/37,765). Among the identified isolates, *S. aureus* (7.7%, n=2,341) and *E. coli* (7.6%, n=2,319) were the most common. Almost half of the *E. coli* were isolated from persons aged, 15-59 (41.3%, n=958) whereas *S. aureus* was isolated most commonly from the age group of 0-4 (44.2%, n=1,035). The overall ESBL *E. coli* and MRSA proportions were 24.7% (573/2,319) and 23.5% (551/2,341), respectively.

ESBLs accounted for about a third of the *E. coli'*s in Namibia (*i.e.* cefotaxime, 32%). This was much higher than the resistance to 3GC reported from the neighbouring country, South Africa (Dramowski et al., 2015). High ESBL proportions have reportedly been associated with healthcare associated sepsis (Machado et al., 2022), which was not defined in our study. Another reason for the lower ESBL rate is that their study population was comprised of adults above the age of 13, thus excluding neonates which have been otherwise associated with high ESBL rates in Asia and Africa (Bah et al., 2023; Mansouri et al., 2024; Mayanja et al., 2023).

Almost half of the *E. coli's* were isolated from the 15-59 years age group, whereas *S. aureus* was isolated from the age group of 0-4, which suggests age group-specific clustering between the two pathogens. Both the bacteremic material in Africa and Namibia show that *S. aureus,* particularly MRSA, is an important pathogen in neonatal bacteraemia and sepsis as it has previously been reported (Dong et al., 2018; Ershad et al., 2019; Kempley et al., 2015). Another contributor to the high neonatal MRSA rates could be the MRSA carriage rates that are reportedly higher in African mothers than in European mothers (Nourollahpour Shiadeh et al., 2022).

In Namibia, the total proportion of MRSA was 23.5% (551/2341) over the 9-year period. These proportions fluctuated between 17% and 40% and finally settled at 27.8% in 2019, comparable to those reported in Italy (34%), Slovakia (27%) and Spain (23%) in 2019 (ECDC & EARSNet, 2024). In the Namibian material, a decline in resistance against cloxacillin, oxacillin and gentamicin (*p*=0.01) has been noted. We cannot ascertain if this represents actual decreases as the total number of isolates has equally reduced from 484 in 2011 to 214 in 2019. However, this declining phenomenon of MRSA (*i.e.* cloxacillin and oxacillin resistant) infections has been reported in parallel in the US and Europe since 2005 (de Kraker et al., 2013; Turner et al., 2019) and is sharply seen in Germany as it reduced from 16% (2011) to 3.9 (2022) (ECDC & EARSNet, 2024). There have been uncertainties around these declines with some scholars citing improvements in hospital-based infection prevention practises, early management of non-invasive infections and changes in the circulating strains (Dantes, 2013; Vihta et al., 2018.

The reasons for MRSA regression could be multi-factorial but there is a need to comprehensively establish the true picture through integrated clinical, laboratory and molecular surveillance. Furthermore, this also demonstrates the weakness of relying on denominators and emphasises the importance of performance indicators and practises that account for the representativeness of the populations (Böhne et al., 2022). Denominators should be harmonised as they may introduce comparison problems, for instance, our study reporting on the resistant isolates over total isolates

(n/N) cannot be compared to an incidence rate of 0.7 MRSA patients per 100 patients reported in a study from Germany (Böhne et al., 2022).

Another issue that weakens the comparisons and reliability of the results is the change of detection and screening with different antimicrobials over the years (*i.e.* oxacillin with shift to cefoxitin, nowadays) and this has different performances depending on the underlying resistance mechanism (*mecA* or *mecC*) (Skov et al., 2014, 2020). Due to these variations, CLSI recommends *mecA* and PBP2a detection as definitive (Humphries et al., 2021).

Lastly, our retrospective analysis indicates, vancomycin resistance of 3.6% and 2.3% in MRSAs and MSSAs overall. MSSAs are generally expected to be susceptible to all antimicrobials but vancomycin resistant MSSAs have previously been reported in Brazil (Panesso et al., 2015). High vancomycin resistance has also been reported across Ethiopia with the main detection method being disk diffusion (Belete et al., 2023). Our retrospective analysis relied on the same approach and vancomycin testing by disk diffusion has been found to be unreliable and overestimates resistance (*i.e.* it is not recommended by both CLSI and EUCAST). As vancomycin resistant *S. aureus* is a priority pathogen, there is a need to utilise reliable and acceptable MIC detection methods in order to quantify the true extent of resistance to vancomycin (Kumar & Sen, 2022).

6.4   Study Strengths and Limitations

Our research using the systematic and retrospective approaches provided us with valuable information on the resistance situation in different African countries, including Namibia. These data mostly relied on routine laboratory data, therefore, highlighting the contribution of laboratories in surveillance as has also previously been stated (Altorf-van der Kuil et al., 2017; Gandra et al., 2016; Musa et al., 2023.) Combining all these gains (existing laboratories and routine susceptibility testing) and issues (unstandardized data collection and reporting) shows that Africa can strengthen its surveillance capacity by drawing lessons from Europe and the international GLASS platforms.

Deficiencies in data quality impede the generation of an accurate picture of the AMR situation. Equally tracking the spread of resistance, identifying early outbreaks, and formulating robust national health policies becomes impeded. The harmonization and standardization of AMR parameters across platforms will thus strengthen surveillance in the African region. There is also a need for regional networks that monitor and understand the spread within a One Health context as seen with EARSNET and European Food Safety Authority (EFSA) in the European settings.

Firstly, very few data points observed in Study I, cannot be used reliably to determine the median resistance for the continent. This data is also not suitable for generalisations/estimations of 3GC resistance or ESBL proportions in other countries and can be strengthened with other explanatory variables such as per capita expenditure on health or other socio-economic conditions.

Countries across Studies I-III, have varying and multiple testing platforms, which adds to the high level of complexity whilst offering higher sensitivity and specificity, especially for variant β-lactamases detection. Comparisons therefore need to take inter-country testing methods into account and this ranges from, screening for reduced sensitivity to cephalosporins or chromogenic selective media, then confirmation by double disk synergy test (DDST), combination disc diffusion, E-test using automated VITEK-2 or classical disk diffusion methods (Réglier-Poupet et al., 2008). Then finally, this has to be placed within the definition of Classical ESBLs (ESBL$_A$) or expanded ESBL definitions of ESBL$_M$ (*i.e.* M-miscellaneous where AmpC is the most common type) and ESBL$_{CARBA}$. The former were restricted to functional enzymes, operationally defined as non-susceptibility to extended spectrum cephalosporins (e.g ceftazidime, ceftriaxone, cefotaxime and oxyimino-monobactam) and clavulanate synergy whereas the expanded definition is described elsewhere (Giske et al., 2008; J. H. Lee et al., 2012).

In all studies, there was a lack of epidemiological and clinical information such as the setting of acquisition, hospital unit, admission characteristics and clinical diagnosis. This has overall made extensive sub-analysis impossible. Hence, the interpretations were restricted by the lack of harmonization between laboratory and hospital management platforms as reported by Pezzani et al., (2020).

The analysis or calculations in studies II and III were mainly based on the total number of isolates (denominator), we could not ascertain whether this was representative of the catchment population or whether the practises in the different health settings and laboratories were in some way contributing to under-or-over sampling combined with under testing or reporting (i.e. test coverage varied).

Lastly, studies performed retrospectively (including systematic reviews) provide resistance data which is usually outdated at the time of publication, with different countries reporting different AMR rates at different time points and timeframes (as shown by the heterogeneity calculations). The proportions of NA's were also worrying and are indicative of the lack of standardisation and data quality checks.

6.5   Future perspectives

African laboratories and surveillance systems need to be strengthened in order to detect resistant pathogens. Surveillance for infectious diseases and AMR by extension has long been recognised as the cornerstone of improved public health care

and practise (Altorf-van der Kuil et al., 2017; Pezzani et al., 2020). Robust surveillance systems are therefore able to detect worrying trends in the ever-changing AMR situation in different sectors and pathogens. This carries the additional benefit of ensuring evidence-based and optimal management of infections, to combat AMR (Bourély et al., 2023; WHO Regional Office for Europe/European Centre for Disease Prevention and Control, 2022)

Along with standardised reporting formats, strong advocacy is necessary to encourage countries in the region to deposit high-quality AMR data in public repositories annually. It is also imperative that hospital, clinical and laboratory data be harmonised to obtain insights into the source of acquisition, patient characteristics, and diagnosis. In order to improve the quality of data and surveillance, there is a need to build coordinated networks such as EARS-Net and CAESAR in Africa. Actions should be taken to harmonise all ASTs to global and open to all EUCAST standards. Although these systems vary in their focus and protocols from GLASS, and they still offer high-quality comparisons across countries (Simonsen, 2018). African surveillance systems can incrementally be built by taking advantage of the economic organisation into different regional blocks – Southern African Development Community (SADC), Economic Community of West African States (ECOWAS), Economic Community of Central African States (ECCAS), and the Eastern African Community (EAC). The collaboration between public health institutions and academia allows for multi-sectoral integration of systems - the One Health approach (Bourély et al., 2023; Ferri et al., 2017).

AMS programmes and infection prevention and control teams will use this information to help manage patients more effectively and devise measures that are responsive and evidence-based in order to curb resistance and better manage outbreaks. The on-going collection of isolates and testing in the laboratories provides an opportunity for in-depth analysis. In order to understand the prevalence of clones and the transmission of resistant pathogens in the African region, molecular epidemiological surveillance is needed. Optimising these data parameters can significantly enhance Namibia's antimicrobial resistance surveillance efforts. Finally, the existing data frames and resistance can be used together with antimicrobial consumption data to enhance the antimicrobial picture, which will undoubtedly bring multi-sectoral teams together for collaboration.

7   Summary/Conclusions

- A review of studies from Africa (2011-2019) showed limited data on bacteremic antimicrobial resistance (23% of countries reported) with high variation across countries.
- From the systematic review, *E. coli* showed moderate resistance to third-generation cephalosporins (19-42%) whereas MRSA rates were 20-34%.
- 22% of *E. coli* from urine cultures were ESBL-producers. Nitrofurantoin is still a good empirical antimicrobial for female UTI (i.e. resistance less than 10% in non-ESBL and 19% in ESBL).
- Most of the *E. coli* cultured from urine in Namibia (2016-2017) were from fertile adult females.
- Almost half of bacteremic *E. coli* isolates were from 15-59 years old, whereas the *S. aureus* isolates were mostly from children aged 0-4 years.
- 32% of *E. coli* blood isolates were ESBL, which were multi-resistant compared to non-ESBLs. MRSA percentage in neonates was 22.9%.

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