R E V I E W

Systematic review in South Africa reveals antibiotic resistance genes shared between clinical and environmental settings

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Abstract: A systematic review was conducted to determine the distribution and prevalence of antibiotic-resistant bacteria (ARB), antimicrobial-resistant genes (ARGs), and antimicrobial-resistant gene determinants (ARGDs) in clinical, environmental, and farm settings and to identify key knowledge gaps in a bid to contain their spread. Fifty-three articles were included. The prevalence of a wide range of antimicrobial-resistant bacteria and their genes was reviewed. Based on the studies reviewed in this systematic review, mutation was found to be the main genetic element investigated. All settings shared 39 ARGs and ARGDs. Despite the fact that ARGs found in clinical settings are present in the environment, in reviewed articles only 12 were found to be shared between environmental and clinical settings; the inclusion of farm settings with these two settings increased this figure to 32. Data extracted from this review revealed farm settings to be one of the main contributors of antibiotic resistance in healthcare settings. ARB, ARGs, and ARGDs were found to be ubiquitous in all settings examined.

Keywords: systematic review, ARB, clinical ARGs, environmental ARGs, ARGDs, South Africa

Introduction

The emergence and spread of antibiotic-resistant bacteria (ARB), antimicrobial-resistant genes (ARGs), and antimicrobial-resistant gene determinants (ARGDs) have been portrayed as one of the leading challenges of the 21st century and a health issue of concern that is rapidly expanding worldwide.[1] Each year in Europe, 400,000 patients experience ill effects due to infection by antibiotic-resistant microorganisms, with an associated mortality of 25,000 patients.[2] More and more enteric bacteria are being reported as being drug resistant in the USA,[3] where antibiotic resistance (AR) is accountable for >2 million hospitalizations and at least 23,000 deaths annually.[4] In addition to the direct cost of hospital services, diseases caused by antimicrobial-resistant microorganisms result in an individual and societal economic burden. In Europe only, reports by the European Centre for Disease Prevention and Control and European Medicines Agency evaluated the general expense of AR to society at €1.5 billion per year.[2] It has been reported that accessible data are lacking to evaluate the financial ramifications regionally or nationally at the point when viable treatment for an infection is totally lost as an aftereffect of such resistance.[1]

The African region has shown negative trends in multiple resistance among key enteric pathogens such as *Escherichia coli*, *Klebsiella*, *Salmonella* spp., *Vibrio cholerae*, and *Shigella* spp. to nearly all commonly available antibiotics.[5–7] Issues related to AR and its magnitude in Africa are hampered by lack of a surveillance system.[8] Many

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African reviews suggest the need for a proper continental surveillance system to gather complete and adequate data on the true extent of the AR problem.[9–11] It is therefore imperative that this rising trend should be controlled, as enteric bacterial infections pose a heavy challenge to human populations, particularly among children and immune-suppressed individuals in developing countries, where malnutrition, HIV/AIDS, and poor sanitation abound.[8] In South Africa (SA), four major outbreaks of AR have been identified at the national level. The National Institute for Communicable Disease[12] reported a methicillin-resistant *Staphylococcus aureus* in 2010;[13] an extended-spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* from 2010 to 2012;[14] vancomycin-resistant Enterococci in 2012, and carbapenemase producing Enterobacteriaceae. In response to the outbreaks, the South African government established the South African Antimicrobial Resistance Strategy framework to combat the spread of AR.[15] Its strategies focus mainly on ARB in clinical and healthcare settings. Currently, there is no strategy in place to contain and track the movement of ARB, ARGs, and ARGDs of environmental isolates in an attempt to prevent them from reaching clinics and healthcare settings. Although ARGs are regarded as emerging environmental contaminants, environments have globally been portrayed as one of the main contributors to the burden of AR.[16]

Several environmental ARB and ARGs resistomes, otherwise referred to as AR hotspots, have been identified. These include the following aquatic environments: surface water bodies (rivers, lakes, and streams),[16,17] groundwater,[18] effluents of hospitals wastewater,[19] and municipal wastewater.[20,21] In terrestrial environments, river sediments[22–24] and antibiotic-treated manure soils[25] have also been identified as contributing to the spread of ARB, ARGs, and ARGDs. In addition to these resistomes, food[26] and drinking water[27] have been found to be direct key reservoirs of ARB, ARGs, and ARGDs associated with human infection. As a result, the presence and dissemination of ARB, ARGs, and ARGDs in environmental settings present a profound threat to public health and highlight the need to be assessed against clinical settings in the South African context, so that the findings can be applied worldwide.

This quinquennial systematic review focused on the genetic epidemiology of ARB, ARGs, and ARGDs in SA from January 1, 2011 to December 31, 2016. The main objective of the current study was to elucidate the distribution and prevalence of ARB, ARGs, and ARGDs in different settings (eg, clinical, environmental, and farm settings) to identify key knowledge gaps in a bid to contain their spread and establish shared genes between the environmental and clinical settings.

Methodology

This quinquennial systematic review on ARGs was compiled using the PRISMA guidelines.[28]

Literature review

The literature search was performed using six online databases: PubMed Database, EBSCOhost Online Research Databases, MEDLINE, ISI Web of Knowledge, African Journals Online, and Scopus in January 2017 by three reviewers (MDE, PB, and VKTP – acknowledged under the "Acknowledgments" section). Predefined terms such as (Antibiotic OR Resistance OR Bacteria OR ARB OR Gene* OR ARG* OR Determinant* OR ARGD*) AND (South Africa OR SA) were used to retrieve relevant articles published from January 1, 2011 to December 31, 2016. No limiters or refiners were applied so to include gray literature in different databases.

Study selection criteria

Figure 1 summarizes the steps taken to conduct the literature search and selection. The first step entailed removing duplicate articles that were found in the six databases. Subsequent to the removal of duplicate articles, the remaining articles were screened based on their title and abstract screening. Full-text articles were read and screened. Studies conducted outside SA were removed, as were those not reporting molecular detection of ARGs' encoding gene. The remaining 53 full-text articles were read and included in the review. Only articles that contained information on the detection of AR encoding genes in clinical, environmental, and farm settings were included in this review. Food ARGs were also excluded to account only for direct environmental ARG input. Articles referring to ARGs detected from viruses and other microorganisms other than bacteria were also excluded.

In this review, ARGs and ARGDs were classified under clinical settings when isolated from hospital inpatient, outpatient, and community settings. Environmental settings constituted all the ARGs and ARGDs that occurred in the natural environments or entered the natural environments (studies reporting food ARGs were not included based on this classification). These environments included aquaculture, freshwater, groundwater, hospital, and municipal wastewater. ARGs and ARGDs isolated from agricultural environments (animal farms, rhizospheres, crop areas, and animal stool) were classified under farm settings.

Data extraction

Relevant data extracted from each of the full-text articles are shown in Table 1. Additionally, three others (MDE, PB, and VKTP – acknowledged under the "Acknowledgments" section) also extracted the same data independently to ensure reliability. When variance occurred, various articles were checked to reach a final decision. Relevant data extracted included first author's name, year of sample collection, location of samples collected, isolated bacteria, AR class targeted, method used for molecular isolation and characterization of the ARGs, samples matrix and settings, and finally the results obtained. The results obtained included bacterial species and strain isolated, number of isolates tested for AR, specific antibiotics tested for resistance, percentage of resistant bacteria, and genetic epidemiology of ARGs detected.

Quality assessment of studies

The quality of the reviewed studies was assessed using the checklist provided by Joanna Briggs Institute.[28] A quality score was obtained from answering each of the 10 questions in the checklist. Consequently, each YES answer gave a point ranging from 0 to 10. A study that scored between 6 and 10 was included in this review.

Results

General overview

This quinquennial (2011–2016) systematic review search retrieved 26,584 combined articles from different online research databases (Figure 1). The six databases were combined, and 21,423 duplicate articles were removed. Of the 5,161 remaining articles, 4,216 articles were removed after title and abstract screening to leave 945 articles. Based on the eligibility criteria, 892 articles were removed based on unspecified locations, lack of molecular or genetic detection of an AR encoding gene, reports on food ARGs, and reports of ARGs and ARGDs in other microorganisms such as viruses, protozoa, and helminths. The remaining 53 articles were included in this qualitative systematic review. Studies reviewed represented seven South African provinces. Figure 2 shows the distribution of resistance patterns by province. No study describing detection and characterization of ARGs and ARGDs in ARB was found for Mpumalanga and the Northern Cape Province. The Eastern Cape Province accounted for 35.8% (n=19), followed by the Western Cape Province with 18.9% (n=10). Gauteng Province and KwaZulu-Natal Province had 17% (n=9) each. North-West represented 3.7% (n=2) and Limpopo Province had 1.9% (n=1). Three articles (5.7%) had data on AR for two or more provinces. Thirty-four articles included in this review were from clinical settings (64.1%) and 10 from environmental settings (18.9%). ARGs isolated from farm settings accounted for 15.1% (n=8). Only one study detected ARGs in food (1.9%) and was excluded from further analysis. Four articles (7.5%) reported AR patterns from two or more sources.[29–32]

Methods used for ARGs detection and characterization

Genetic detection of ARGs in different matrixes was performed using principally three methods: PCR only, PCR coupled with DNA sequencing, and whole genome sequencing (WGS). The PCR technique appeared to be the gold standard involved in 70% (n=37) of detection for ARGs across all sample types, be it clinical, environmental, or from farm settings. DNA sequencing of the detected ARG was performed as a complementary test to the PCR to provide in-depth analysis in 28.1% (n=15) of the reviewed articles.[33–45] The WGS technique was performed in only a single clinical study (1.9%).[46]

ARB distribution

A wide range of ARB was studied for the detection and characterization of ARGs. The most frequently cultured bacterium was *S. aureus,* which accounted for 13.2% (n=7) of all the studies.[31,45,47–51] This was followed by *Mycobacterium tuberculosis* accounting for 11.3% (n=6) of the reviewed articles.[33,34,37,41,42,46] Five reviewed articles (9.4%) studied *E. coli*[40,52,53] and *Enterococcus* spp.[30,54–56] Two different strains of *E. coli* were also studied, namely *E. coli* ST131[35] and O157:H7.[57] *Aeromonas* spp. represented 7.5% (n=4),[58–61] while *K. pneumoniae*,[62–64] *Neisseria gonorrhoeae,*[39,65,66] and *Salmonella* spp.[67–69] each accounted for 5.7% (n=3) in the reviewed articles. Two reviewed articles studied *Vibrio* spp.,[70] of which one article specifically studied *Vibrio cholera* O1 strains[29] and *Pseudomonas* spp.[71] The other sources studied *Pseudomonas aeruginosa.*[36] *Enterobacteriaceae* as a group was also studied in two articles.[72,73]

One article each was studied for the presence of ARGs in *Acinetobacter baumanii*,[74] *Stenotrophomonas maltophilia*,[75] *Ureaplasma parvum*,[76] *Clostridium difficile*,[38] *Bacteroides fragilis*,[77] *Campylobacter* spp.,[78] and *Helicobacter pylori*.[44] Four articles investigated several bacteria in their studies[60,79,80] of which one investigated bacteria as colony forming bacteria assemblages.[43]

Characteristics of ARGs in SA

Studies that only used culture-independent methods reported mostly on the prevalence of ARGs and ARGDs in isolated

Table 1 Characteristics of eligible articles

Isolated bacteria	Year collected	Location	Genes involved	AMR class	Method used	Settings	References
Acinetobacter baumannii	2013	Tshwane region (Gauteng)	bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58}, bla_{VIM}, bla_{KPC}, bla_{IMP}, bla_{GES}, bla_{PER}, bla_{VEB}, bla_{GIM}, bla_{SPM}, bla_{SIM}, bla_{NDM-1}	ESBL resistant	PCR	Clinical	Lowings et al (2015)[74]
Acinetobacter, Aeromonas, Bordetella, Chryseobacterium, Enterobacter, Myroides, Pseudomonas, Salmonella, and *Shewanella*	2012	Stellenbosch (Western Cape)	tetA, tetB, tetC, tetD, tetE, tetH, tetJ, tetY, tetG, Tn1721, TEM β-lactamase	Tetracycline resistance	PCR	Aquaculture (environmental)	Chenia and Vietze (2012)[60]
Aeromonas spp.		Durban (KwaZulu-Natal)	qnrA, qnrB, and qnrS	Quinolone resistant	PCR	Fish (food)	Chenia (2016)[58]
Aeromonas spp.	Not mentioned	Lovedale and Fort Cox farms (Eastern Cape)	psel, bla_{TEM}, blaP1	ESBLs	PCR	Cow stools (farm)	Igbinosa et al (2015)[59]
Aeromonas spp.	Not mentioned	Fort Beaufort and Alice (Eastern Cape)	psel, bla_{TEM}, tetC, class 1 integron, class 2 integron	Multidrug resistant	PCR	Wastewater (environmental)	Igbinosa and Okoh (2012)[61]
Bacteroides fragilis	2011	Cape Town (Western Cape)	nimA-J, tetQ, cfxA, cfiA, ermF	Multidrug resistant	PCR	Clinical	Meggersee and Abratt (2015)[77]
Clostridium difficile	2012–2013	Cape Town (Western Cape)	ermB, gyrA, and gyrB	Erythromycin and moxifloxacin resistant	PCR and DNA sequencing	Clinical	Kullin et al (2016)[38]
Campylobacter spp.	2013–2014	Durban (KwaZulu-Natal)	CjgyrA, tetO, CmeB	Multidrug resistant	PCR	Clinical	Shobo et al (2016)[78]
Escherichia coli	September 2012–August 2013	Amathole District Municipality (Eastern Cape)	strA, aadA, cat I, cmlA1, bla_{TEM}, tetA, tetB, tetD, tetK, tetM, blaZ, cat II, ampC	Multidrug resistant	PCR	Wastewater (environmental)	Adefisoye and Okoh (2016)[52]
E. coli	2012	Kat River (Eastern Cape)	aadA1, Bla, tetA, dfrA1	Multidrug resistant	PCR	River water (environmental)	Nontongana et al (2014)[53]
E. coli	2014–2015	Johannesburg and Pretoria (Gauteng)	mcr-1 plasmid	Colistin resistant	PCR and DNA sequencing	Clinical	Poirel et al (2016)[40]
E. coli O157	2014	Nkonkobe District (Eastern Cape)	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, $bla_{CTX-M \, gp \, 9}$, $bla_{CTX-M \, gp \, 1}$, bla_{ampC}, bla_{VEB}, bla_{CMY}, tet(A), strA	Multidrug resistant	PCR	Animal stool (farms)	Iweriebor et al (2015)[57]
E. coli ST131	2012–2013	Port Elizabeth (Eastern Cape)	bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, qnrA, qnrB, qnrC, qnrD, qnrS, aac (6')-Ib-cr, and qepA	ESBLs resistant	PCR and DNA sequencing	Clinical	Gqunta and Govender (2015)[35]
Enterobacteriaceae	2012–2015	Johannesburg (Gauteng)	bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, bla_{GES}, bla_{KPC}	Carbapenemase resistant	PCR	Clinical	Perovic et al (2016)[72]
Enterobacteriaceae	2012–2015	Various provinces	bla_{NDM}, bla_{KPC}, bla_{OXA-48} and variants, bla_{GES}, bla_{IMP}, and bla_{VIM}	Carbapenem resistant	PCR	Clinical	Singh-Moodley et al (2016)[72]

Organism	Year	Location	Gene(s)	Resistance	Method	Source	Reference
Enterococcus spp.	2011	Motlhabeng, Dibate, and Majemantsho villages (North-West)	vanA and vanB	Vancomycin resistant	PCR	Groundwater (environmental)	Ateba et al (2013)[54]
Enterococcus spp.	2014	Nkonkobe District (Eastern Cape)	vanA, vanB, vanC-1, vanC-2/3, strA, ermB	Multidrug resistant	PCR	Animal stool (farms)	Iweriebor et al (2015)[30]
Enterococcus spp.	2014	Alice (Eastern Cape)	vanA, vanB, vanC1, vanC2/3, and erm(B)	Vancomycin resistant	PCR	Hospital and domestic wastewater effluents (environmental)	Iweriebor et al (2015)[93]
Enterococcus spp.	2013	Cape Town (Western Cape)	vanA, B, and C1, 2 and 3	Vancomycin resistant	PCR	Clinical	Lochan et al (2016)[56]
Enterococcus spp.	2010–2011	Vaal, Harts, Schoonspruit, and Mooi River (North-West)	mefA, tetK, tetL, and msrC	Multidrug resistant	PCR	River water (environmental)	Molale and Bezuidenhout (2016)[55]
Gram-negative bacteria	July 2011–October 2012	Johannesburg (Gauteng)	bla_{NDM-1}	Metallo-β-lactamase	PCR	Clinical	de Jager et al (2015)[79]
Helicobacter pylori	Not mentioned	Eastern Cape	23S-rRNA genes, rdxA and frxA	Metronidazole and clarithromycin resistant	PCR and DNA sequencing	Clinical	Tanih et al (2011)[44]
Klebsiella pneumoniae	2012	Cape Town (Western Cape)	bla_{NDM}, bla_{VIM}, bla_{SPM}, bla_{IMP}, bla_{KPC}, bla_{GES}, $bla_{OXA-48-like}$, bla_{CTX-M}, bla_{TEM}, and bla_{SHV}	Carbapenem and ESBLs resistant	PCR and DNA sequencing	Clinical	Jacobson et al (2015)[62]
K. pneumoniae	Not mentioned	Gauteng	bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{CMY}, bla_{PER}, bla_{VEB}, bla_{OXA}, bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{VIM}, bla_{NDM}, qnrA, qnrS, and qnrB	ESBL and quinolone resistant	PCR	Clinical	Peirano et al (2012)[63]
K. pneumoniae	2010–2012	Gauteng, KwaZulu-Natal, Free State, Limpopo and Western Cape	bla_{CTX-M}, bla_{SHV}, and bla_{TEM}	ESBL and carbapenemase resistant	PCR	Clinical	Perovic et al (2014)[64]
Mycobacterium tuberculosis	January 2012–December 2013	Eastern Cape	katG, rpoB, rrs, and eis	Multidrug and extensively drug resistant	PCR and DNA sequencing	Clinical	Bhembe et al (2014)[33]
M. tuberculosis	2008–2013	KwaZulu-Natal	katG, gidB, rpoB, pncA, rrs, gyrA, embB	Multidrug and extensively drug resistant	Whole genome sequencing	Clinical	Cohen et al (2015)[46]
M. tuberculosis	2005–2009	KwaZulu-Natal	rpoB, katG, inhA, pncA, and embB	Multidrug and extensively drug resistant	PCR and DNA sequencing	Clinical	Dookie et al (2016)[34]
M. tuberculosis	2008–2009	Port Elizabeth (Eastern Cape)	katG, rpoB, inhA, embB, pncA, gyrA, and rrs	Multidrug resistant	PCR and DNA sequencing	Clinical	Klopper et al (2013)[37]

(Continued)

Table I (Continued)

Isolated bacteria	Year collected	Location	Genes involved	AMR class	Method used	Settings	References
M. tuberculosis	2007–2009	Cape Town (Western Cape)	*gyrA*	Quinolone resistant	PCR and DNA sequencing	Clinical	Sirgel et al (2012)[42]
M. tuberculosis	2008–2009	Port Elizabeth (Eastern Cape)	*rrs* A1401 G	Capreomycin resistant	PCR and DNA sequencing	Clinical	Sirgel et al (2012)[41]
Neisseria gonorrhoeae	2008	Johannesburg (Gauteng)	Lactamase encoding plasmids	Penicillin and tetracycline resistant	PCR	Clinical	Fayemiwo et al (2011)[65]
N. gonorrhoeae	2012	Johannesburg (Gauteng)	*penA, mtrR, porB1b* (*penB*), *ponA,* and *pilQ*	Extended-spectrum-cephalosporin resistant	PCR and DNA sequencing	Clinical	Lewis et al (2013)[39]
N. gonorrhoeae	2006–2007	Cape Town (Western Cape)	*gyrA* and *parC*	Ciprofloxacin resistant	PCR	Clinical	Magooa et al (2013)[66]
Pseudomonas aeruginosa	2010–2011	Cape Town (Western Cape)	*bla*$_{IMP}$	VIM-2 metallo-β-lactamase (carbapenem-resistant)	PCR and DNA sequencing	Clinical	Jacobson et al (2012)[36]
P. aeruginosa, Enterobacter cloacae, K. pneumoniae, Burkholderia cepacia, Stenotrophomonas maltophilia	2012–2013	Durban (KwaZulu-Natal)	*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{CMY}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{OXA}$, *bla*$_{KPC}$, *bla*$_{GES}$, *bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{NDM}$	ESBLs resistant	PCR	Clinical	Mhlongo et al (2015)[80]
Pseudomonas spp.	Not mentioned	Kat and Thyme River (Eastern Cape)	*bla*$_{TEM}$, TetC, *bla*$_{OXA}$, *bla*$_{amp}$ C	Multidrug resistant	PCR	Freshwater (environmental)	Igbinosa et al (2012)[71]
Staphylococcus aureus	2007–2008	Cape Town (Western Cape)	*rpoB*	Rifampicin resistant	PCR and DNA sequencing	Clinical	van Rensburg et al (2012)[45]
S. aureus	2013	Cape Town (Western Cape)	*mupA*	Mupirocin resistant	PCR	Clinical	Wasserman et al (2014)[50]
S. aureus (MRSA)	June–August 2015	KwaZulu-Natal	*mecA, bla*$_Z$, *aac* (6′)*-aph* (2″), *ermC, tetK*	Multidrug resistant	PCR	Clinical	Amoako et al (2016)[51]
S. aureus	Not mentioned	Limpopo	*mec* A	Methicillin resistant	PCR	Clinical and drinking water	Samie and Shivambu (2011)[31]
S. aureus	2013–2014	KwaZulu-Natal	*mecA, blaZ, vanA, vanB, tetM, tetK, aacA-aphD, mecC*	Multidrug resistant	PCR	Humans (clinical and animals (farm)	Schmidt et al (2015)[47]
Salmonella spp.	2010	Animal farms (Eastern Cape)	*bla*$_{TEM}$, Tet C, *bla*$_{amp}$ C, *bla*$_{pse1}$, integron conserved segment	ESBLs resistant	PCR	Stools of cows and goats (farm)	Igbinosa (2014)
Salmonella spp.	2014	Nkonkobe District (Eastern Cape)	*ampC, tetA,* and *strA*	Multidrug resistant	PCR	Swine stool (farms)	Iwu et al (2016)[68]
Salmonella spp.	2014	Durban (KwaZulu-Natal)	*Pse–I, ant* (3″)*-Ia, tetA, tetB, Sul1, Sul2*	Multidrug resistant	PCR	Broilers chickens (farm)	Zishiri et al (2013)[69]

Organism	Year	Location	Genes	Resistance	Method	Sample source	Reference
S. aureus	2010–2012	Johannesburg (Gauteng)	*mecA, mecC*	Methicillin resistant	PCR	Clinical	Singh-Moodley et al (2015)[49]
Staphylococcus spp.	Not mentioned	Nkonkobe Municipality (Eastern Cape)	*ermA, ermB, ermC, msrA, mecA, vanA, vanB, and mphC*	Multidrug resistant	PCR	Chicken and pigs (farm)	Adegoke and Okoh (2014)[48]
S. maltophilia		Nkonkobe Municipality (Eastern Cape)	*sul2, sul3*	Sulfonamide resistant	PCR	Rhizosphere (farm)	Adegoke and Okoh (2015)[75]
Total and colony forming bacteria assemblages	2012	eThekwini Metropolitan Municipality (KwaZulu-Natal)	*sul1, sul2, sul3, and tetM*	Sulfonamide and tetracycline resistant	qPCR and DNA sequencing	River, estuaries, and sewage treatment plants (environmental)	Suzuki et al (2015)[43]
Ureaplasma parvum	2009–2010	Pretoria (Gauteng)	*tetM, gyrA, gyrB, parC, and parE*	Multidrug resistant	PCR and DNA sequencing	Clinical	Govender et al (2012)[76]
Vibrio cholerae O1 strains	2008–2009	Not specified	Class 1 integron, class 2 integron (intI2), bla$_{TEM}$, bla$_{SHV}$, bla$_{CTX-M}$, tetA, SXTint, floR, sul2, dfrA1, dfrI8, strA, strB, qnrVC3, gyrA/gyrB, and parC/parE	Multidrug resistant	PCR	4 Environmental, 598 stools, and 118 rectal swab specimens	Ismail et al (2013)[29]
Vibrio spp.	Not mentioned	Fort Beaufort and Alice (Eastern Cape)	SXT integrase, sul2, floR, dfrI8, tet(A), strB, dfrA1, blaPI	Multidrug resistant	PCR	Wastewater (environmental)	Igbinosa et al (2011)[70]

Abbreviations: ESBL, extended-spectrum β-lactamase; AMR, antimicrobial resistance; MRSA, methicillin-resistant *S. aureus*.

ARB. The gene *aac (6′)-aph (2″)* was assessed through PCR for gentamicin resistance in *S. aureus*[51] and it was found that 92.6% of strains harbored the gentamicin resistance gene. The *aac (6′)-lb-cr* gene was studied in *E. coli* ST131 for ESBLs resistance.[35] The *aac (6′)-lb-cr* gene was not detected in negative ESBLs resistance and was found to be carried by most isolates positive for ESBLs resistance. The *aacA-aphD* gene responsible for the aminoglycoside resistance determinant was studied in *Staphylococcus* spp.[47] The authors found a total of nine human isolates that carried the *aacA-aphD* gene, comprising four *Staphylococcus epidermidis* and five *Staphylococcus haemolyticus*.[47] The *aadA* gene conferring resistance to streptomycin (aminoglycosides) was studied in *E. coli* and *K. pneumoniae*.[52,53,81] It was found that six *E. coli* isolates belonging to different pathotypes carried the *aadA* gene,[52] with one reference[53] showing that all the screened *E. coli* colonies were positive for the *aadA* gene. Peirano et al[81] found that all the EBSLs-resistant *K. pneumoniae* positives also carried a plasmid-encoded *aadA* gene cassette. As it confers resistance to gentamycin, the *ant (3″)-la* gene was studied in *Salmonella* spp., shown in 32% of bacterial isolates and detected in 80% of clinical specimens.[69]

In some studies, ARGs were not detected, while phenotypic resistance was observed. In one study, the *tat* gene was not detected although phenotypic results showed high tetracycline resistance.[51] In another study,[52] cat II, *amp*C, and *bla*$_Z$ genes were not detected even when the phenotypic pattern exhibited resistance.

Mutations were mainly assessed by DNA sequencing. The *23S-rRNA* gene was assessed for resistance to clarithromycin in *H. pylori*.[44] Two-point mutations were detected in the *23S-rRNA* gene and assigned as conferring clarithromycin resistance, since isolates showed phenotypic resistance to this antibiotic. A study by Bhembe et al[33] found isoniazid-resistant *M. tuberculosis* strains in 71.4% (100/140) isolates with mutations at codon 315. For the *katG* gene region, seven different mutations were observed: two-point mutation was found in 35.7% (50/140) and 14.3% (20/140) and five-point mutation in 7.1% (10/140). Mutations among the rifamycin-resistant isolates were located at codon 42 (21.4%) followed by 14.3% of mutations on codon 52, codon 87, and codon 531, which is known to be a hotspot for *rpo*B gene mutations. In addition, four types of mutation patterns were observed in the *rrs* gene region; S2170A, R2201G, K2202E, and a deletion in position 2207.[33]

rrs A1401G was further investigated to reveal the extent of capreomycin resistance in *M. tuberculosis*.[41] It was found that 58% of isolates with an *rrs* A1401G mutation showed a
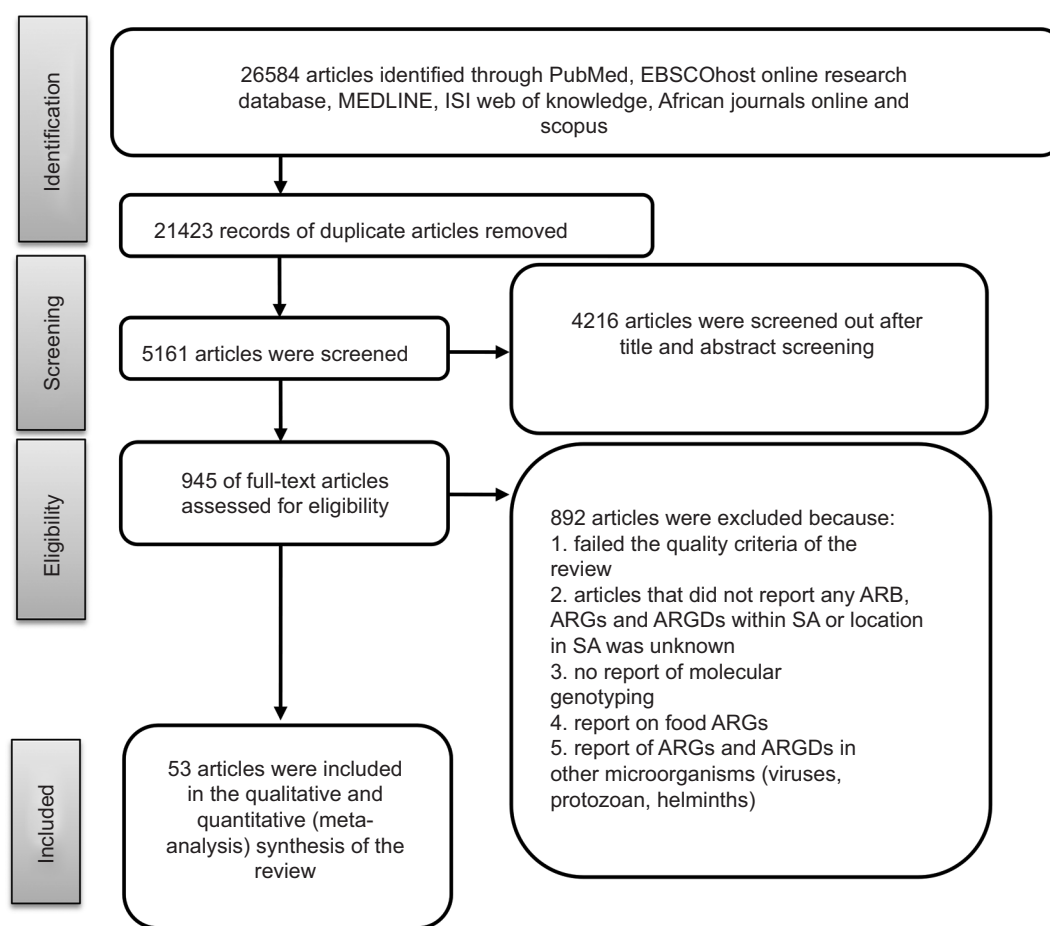
Figure 1 Flow diagram summarizing the process of literature search and selection.
Abbreviations: ARB, antibiotic-resistant bacteria; ARGs, antimicrobial-resistant genes; ARGDs, antimicrobial-resistant gene determinants; SA, South Africa.

high level of resistance to amikacin and decreased phenotypic susceptibility to capreomycin.[41] Only one study made use of WGS technology[46] to study a collection of *M. tuberculosis* isolates from KwaZulu-Natal. These authors could identify the order of acquisition of *katG*, *rpoB*, *gidB*, *pncA*, *rrs*, and *gyrA* genes to the emergence of extensively resistant *M. tuberculosis*. Table 1 lists details of the characteristics of ARGs and ARGDs in isolated ARB.

ARGs clinical and environmental nexus

Shared ARGs in environmental, clinical, and farm settings are presented in Figure 3. Numbers inside shared intersecting circles represent the number of shared ARGs in environmental, clinical, and farm settings. All three settings shared 39 ARGs and ARGDs. Twelve ARGs and ARGDs were shared between environmental and clinical settings. Farm settings shared 32 ARGs and ARGDs in a clinical setting and the same number of ARGs and ARGDs was shared between environments and farms (n=32). Forty-eight out of 140 ARGs and ARGDs were only found in clinical settings. This was

followed by environmental ARGs and ARGDs found only in environmental settings, where there were 22 out 60 ARGs and ARGDs. In farm settings, there were six out of 48 ARGs in total. Table 2 shows the shared ARGs and ARGDs per setting.

Discussion

In this systematic review, the findings of 53 published articles that reported detection and characterization of ARB, ARGs, and ARGDs in different matrixes were summarized. As data from abstracts and full text were screened and extracted by three reviewers, the authors believe this process reduced the possibility of bias. This review was not designed to review phenotypic characteristics or gene sequences of isolates, but rather collected data on the genetic epidemiology of ARB, ARGs, and ARGDs. The Northern Cape and Mpumalanga Provinces were not represented in the data sets and this may be due, in these provinces, to a paucity of research facilities and/or logistical problems regarding transport of specimens to such research facilities. The Eastern Cape was found to be the leading province in the number of articles published
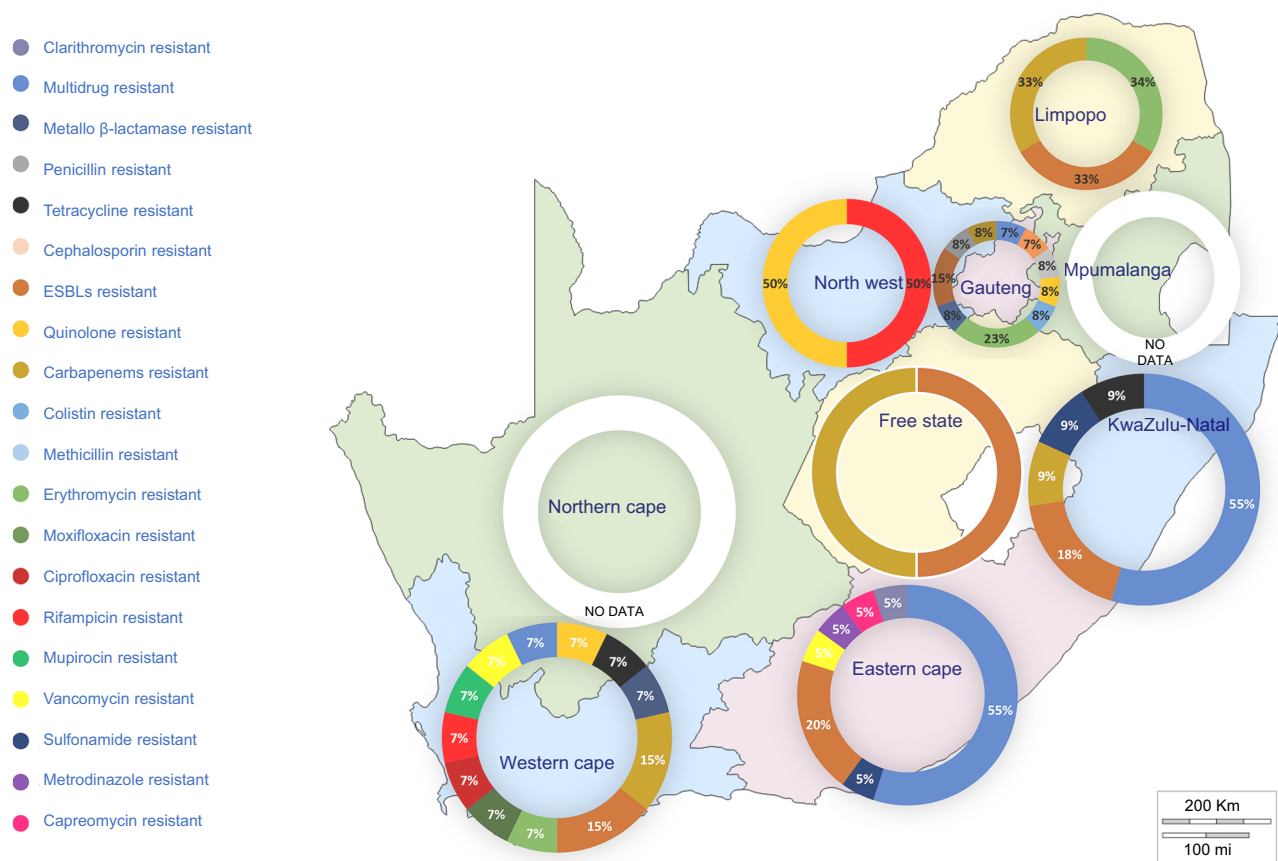
Figure 2 Distribution of genotypic AMR studies in SA: the initial South African map was created using an open source GIS software (QGIS 2.18 – http://www.qgis.org). Subsequent chart was added using PowerPoint® 2016 (Microsoft Corporation, Redmond, WA USA).
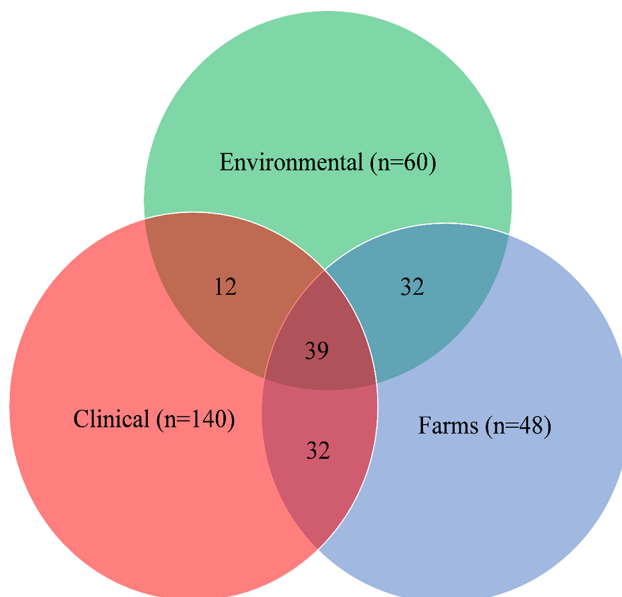**Abbreviations:** ESBL, extended-spectrum β-lactamase; AMR, antimicrobial resistance; SA, South Africa.

Figure 3 Venn diagrams showing shared ARGs and ARGDs between environmental, clinical, and farms settings.
Abbreviations: ARGs, antimicrobial-resistant genes; ARGDs, antimicrobial-resistant gene determinants.

reporting clinical investigations. Most studies were conducted in clinical settings compared to environmental settings. There is a dearth of information on the link between laboratory and clinical data systems and it is thus difficult to identify patterns of environmental and community-acquired ARGs compared to hospital-acquired bacterial resistance.[15]

Three main molecular biology methods were used to detect and characterize ARGs in the different settings, namely PCR, DNA sequencing, and WGS. The PCR technique was found to be the most commonly published technique in articles, probably because of relatively easy access to PCR cyclers and decreasing costs associated with PCR. A study by Ali et al[82] indicated that PCR was the gold standard for identification of ARGs in methicillin-resistant *S. aureus.* Microarray technologies have been used to detect multiple ARGs and ARGDs simultaneously in ARB,[83–85] but this method was not used in any of the reviewed studies where most characterization of specific ARG or ARGD made use of DNA sequencing of ARG and ARGD amplicons.

Table 2 Shared ARGs and ARGDs per setting

Farms, n=48	Environmental, n=60	Clinical, n=140	Farms and environmental	Farms and clinical	Environmental and clinical	All
ant (3″)-la, ermA, mphC, msrA	aadA, aadA1, Bla, cat I, cat II, cmlA1, dfr18, dfrA1, dfrA1, floR, mefA, srC, strB, sul3, SXT integrase, tetD, tetE, tetG, tetH, tetJ, tetL, tetY, Tn1721	23S-rRNA genes, aac (6′)-aph (2″), aac (6′)-lb-cr, bla_GES, bla_GIM, bla_IMP, bla_KPC, bla_NDM, bla_NDM-1, bla_PER, bla_SIM, bla_SPM, bla_VIM, cfiA, cfxA, CjgyrA, CmeB, eis, embB, frxA, gidB, gyrA, gyrB, inhA, katG, mcr-1 plasmid, mtrR, mupA, nimA-J, parC, parE, penA, pilQ, pncA, ponA porB1b (penB), qepA, qnrA, qnrB, qnrC, qnrD, qnrS, rdxA, rpoB, rrs, rrs A1401G, TetO, tetQ	ampC, bla_ampC, bla_VEB, bla_P1, bla_pse1, pse1, class 1 integron, class 2 integron, strA, sul1, sul2	bla_CMY, bla_CTX-M gp 1, bla_CTX-M gp 9, bla_CTX-M, ermC, mecA, mecC	bla_OXA, bla_OXA-23, bla_OXA-24, bla_OXA-48 and variants, bla_OXA-48, bla_OXA-48-like, bla_OXA-51, bla_OXA-58, tetM	bla_SHV, bla_TEM, TEM β-lactamase, bla_Z, tetA, tetB, tetC, tetK, vanA, vanB, vanC1, vanC2/3, ermB, erm

Abbreviations: ARGs, antimicrobial-resistant genes; ARGDs, antimicrobial-resistant gene determinants.

Despite several advantages offered by WGS, it was used only once in all the reviewed articles. In recent years, WGS has been implemented in the USA by the Food and Drug Administration and the Centers for Disease Control and Prevention as a tool for outbreak source tracking and surveillance in clinical and environmental settings and ex post facto sentinel studies.[86] The application of WGS allows the detection of all ARGs with no exception, including ARGDs such as integrons and plasmids.[87] Analysis with WGS has shown the ability to resolve phenotypic and genotypic discrepancies[88–90] and should be coupled with phenotypic tests to circumvent gene expression problems in obtaining a full AR profile of ARB. It is clear from the findings of this review that WGS is now needed in ARB studies in SA and worldwide.

This review shows that more attention is paid to clinical isolates compared to environmental isolates. The same trend is observed internationally, as studies show that ARB are mostly investigated in clinical compared to environmental settings. This is despite the fact that ARGs are thought to originate from environmental bacteria, as clinically relevant resistance genes have been detected in the genomes of environmental ARB.[91] The use of high throughput functional metagenomics techniques[92] showed evidence of recent exchange of ARGs between ARB of environmental and clinical origin. One study in this review demonstrated the line of transmission from clinical to environmental settings.[31] The same study also revealed that water could be a transmission vector of staphylococcal urinary tract infection among HIV and AIDS patients in the Limpopo Province. Iweriebor et al[93]

detected the same ARB, ARGs, and ARGDs in hospital wastewater, sewage treatment plant, and receiving water bodies. High evidence of transmission from the environment to humans can only be established through genetic relatedness of ARGs using molecular typing techniques.[94] In this review, a wide range of ARB were studied, and these bacteria also cover the most important clinical isolates worldwide.

A considerable number of ARGs and ARGDs were found to be shared across all three settings (n=39). Farm settings shared most ARGs with clinical and environmental settings (n=32) compared with clinical vs environmental settings (n=12). An analysis of 71 environmental specimens found soil to have the most diverse ARGs,[95] concluding that soil is a major contributor of ARB, ARGs, and ARGDs. This agrees with the findings of this review, since the soil isolates were classified under farm settings to share ARGs and ARGDs with clinical settings as well. In the same article, it was found that soil and human feces shared more resistance classes with each other than other matrixes. A considerable number of ARGs and ARGDs were only found in clinical isolates (n=48). This indicates that a considerable number of ARGs and ARGDs are still isolated from clinical settings with unknown origins. There is a dire need for development of innovative approaches to track relevant clinically isolated ARB, ARGs, and ARGDs. Important ARB, ARGs, and ARGDs were also found only in environmental and farm settings. A study by Agga et al[96] found that swine and cattle shared ARGs and ARGDs with wastewater settings. This agrees with the findings of this review that farm settings

shared significant ARGs and ARGDs with environmental settings and indicates the need to contain these ARGs and ARGDs to limit their spread into clinical settings.

An approach to ARGs and ARGDs tracking and containment

Based on the current findings, there is a need for high through-put technologies such as WGS and shotgun metagenomics analysis as means for tracking and characterizing ARB, ARGs, and ARGDs better. The WGS sequencing technique is of paramount importance in solving the discrepancy between phenotypic and genotypic types of AR, as well as building an improved WGS database. This molecular approach has been widely used in other countries such as Denmark, where a study compared the use of WGS as a routine technique against phenotypic tests and reported 99.74% accuracy to suggest that WGS be used as an alternative method to phenotypic testing.[97] Another study by Leekitcharoenphon et al[98] used WGS to track the source of *Salmonella* Eko from Nigerian isolates and successfully identified the source of bacteria. A report by Berendonk et al[99] has suggested a standardized system of core parameters to tackle AR and as the world is moving into WGS, SA should also advance in the same direction to track endemic AR patterns better. To obtain an in-depth view of the occurrence and distribution of ARGs and ARGDs, WGS should be complemented by shot-gun metagenomics to observe routes of dissemination better and elucidate their distribution across various environments.

According to a study by Rowe et al,[100] farms and waste-water treatment plants serve as an important reservoir of ARGs, mobile genetic elements, and pathogenic bacteria for river environments. The two metagenomic approaches, WGS and shotgun metagenomics, are now critical in track-ing and characterizing AR in different environments and are also needed in SA to streamline individual investigations of ARB, ARGs, and ARGDs.

Furthermore, an AR genomic epidemiology application is needed in a bid to link environmental ARB, ARGs, and ARGDs with clinical ones. This tool should describe the genomics, laboratory, clinical, and epidemiological contextual information required to support data sharing and integration for AR surveillance and outbreak investigations. The appli-cation should geospatially map ARB, ARGs, and ARGDs between environmental and clinical settings and report on recent antibiogram data from different environments. The application should initially utilize recently published online studies to create an initial database of environmental, farm, and clinical ARB, ARGs, and ARGDs. In addition, geospatial

mapping of ARGs and ARB would permit the identification of national AR hotspots and actions for containment. The application should also provide baseline information nec-essary to link the environmental spread of AR to routes of transmission. The development of this application would aid national clinicians, physicians, scientists, and policy makers to formulate appropriate strategies to combat AR.

Conclusion

Although data could not be found for the Northern Cape and Mpumalanga Provinces, genes conferring AR were found to be ubiquitous across SA. The available data present a broader scope on isolation and prevalence information on ARGs in ARB and emphasize the dire need for surveillance and docu-mentation of ARGs, ARB, and ARGDs in all provinces. Owing to the lack of sequence analysis in the current review, future reviews will investigate shared ARB, ARGs, and ARGDs based on related sequence data. One of the main findings in this review was the discrepancy between phenotypic and genotypic patterns. This calls for a move toward complementing the gold standard PCR with the use of WGS in examining ARB as well as shotgun metagenomics technologies when dealing with a consortium of bacteria. In extending this tracking and genetic characterization of ARB, ARGs, and ARGDs, this study sug-gests the adoption of an AR genomic epidemiology application.

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Disclosure

The authors report no conflicts of interest in this work.

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