Molecular epidemiology and mechanisms of antibiotic resistance in *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. in Africa: a systematic review from a One Health perspective

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Abstract

A systematic review of antibiotic-resistant Gram-positive bacteria in Africa from a One Health perspective is lacking. Here, we report result from a search for English-language articles on the resistance mechanisms and clonality of Gram-positive bacteria in Africa between 2007 and 2019 reported in PubMed, Web of Science, ScienceDirect, and African Journals OnLine; 172 studies from 22 different African countries were identified. Resistance genes, such as *mec*A, *erm(*B), *erm*(C), *tet*(M), *tet(*K), *tet*(L), *vanB, vanA, vanC,* and *tet*(O), were found to be common. *Staphylococcus* spp., *Enterococcus* spp., and *Streptococcus* spp. were the main species reported by the studies, with clones such as *Staphylococcus aureus* ST5 (*n* = 218 isolates), ST8 (*n* = 127 isolates), ST80 (*n* = 133 isolates), and ST88 (*n* = 117 isolates), and mobile genetic elements such as IS*16* (*n* = 28 isolates), IS*256* (*n* = 96), Tn*916* (*n* = 107 isolates), and SCC*mec* (*n* = 4437 isolates) identified. SCC*mec* IV (*n* = 747 isolates) was predominant, followed by SCC*mec* III (*n* = 305 isolates), SCC*mec* II (*n* = 163 isolates), SCC*mec* V (*n* = 135 isolates), and SCC*mec* I (*n* = 79 isolates). Resistance to penicillin (*n* = 5926 isolates), tetracycline (*n* = 5300 isolates), erythromycin (*n* = 5151 isolates), rifampicin (*n* = 3823 isolates), gentamycin (*n* = 3494 isolates), sulfamethoxazole/trimethoprim (*n* = 3089 isolates), and ciprofloxacin (*n* = 2746 isolates) was common in most reports from 22 countries. Clonal dissemination of resistance across countries and between humans, animals, and the environment was observed. Resistance rates ranged from 1.4% to 100% for 15 of the studies; 10 were One Health–related studies. Strict infection control measures, antimicrobial stewardship, and periodic One Health epidemiological surveillance studies are needed to monitor and contain the threat of increasing antibiotic resistance in Africa.

Keywords: *Staphylococcus* spp.; *Enterococcus* spp.; *Streptococcus* spp.; MRSA; VRE; antimicrobial resistance; Africa; One Health; Gram-positive; mobile genetic element

Introduction

Antibiotic resistance—a threat to public health

Antibiotic resistance is evidently a grave threat to humans and animals as their absence or inefficacy will make clinical management and prevention of infections challenging, if not impossible.[1–3] Comparatively, Gram-negative bacterial pathogens, such as carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae, are of higher (critical) priority than Gram-positive pathogens (high priority), such as vancomycin (VAN)-resistant *Enterococcus faecium* (VRE), methicillin-resistant and/or VAN-intermediate *Staphylococcus aureus* (MRSA/VISA), and medium priority penicillin-nonsusceptible *Streptococcus pneumoniae*.[4] This is because Gram-negative bacteria have higher evolution, burdens, and levels of resistance mechanisms than Gram-positive

bacteria.[5] This difference is reflected in the relatively limited antibiotic resistance research and surveillance studies on Gram-positive bacteria, not only in Africa, but also worldwide.[2–4]

This is not to suggest, however, that antibiotic resistance in Gram-positive bacteria is not an imminent and serious threat, particularly in Africa. In a recent review, Gram-positive bacteria were responsible for infections, including sepsis, pneumonia, osteomyelitis, and meningitis among children, with a high rate of resistance to World Health Organization (WHO)-recommended drugs in Africa.[6] In some African regions, 80% of *S. aureus* infections are MRSA, which show resistance to most standard licensed drugs, including fluoroquinolones and peptides, aminoglycosides, macrolides, and tetracycline (TET). Although *Enterococcus* spp. are mostly not as virulent as *S. aureus*, their multidrug resistance (MDR) propensities restrict drug options for clinicians.[7] According to a recent WHO report, the potential for antibiotic resistance to lead to higher mortalities and morbidities in low- and middle-income countries may even be greater as a result of the higher burden of bacterial infections, limited diagnostic capacity, and lower access to second-line antibiotics.[2,8]

Increasingly, many clinical epidemiologists are adopting the One Health concept in molecular surveillance studies due to the increasing realization that antibiotic resistance in the environment can be transferred to animals and humans or vice versa.[9–11] In addition, there has been a debate on the possible impact of agricultural and veterinary use of antibiotics on human medicine;[5,11] as well, the clinical importance of antimicrobial resistance genes found in the environment has been discussed.[5,11]

However, the presence of same clones and plasmid types in bacteria from animal, human, and environmental sources is helping to settle these debates and strengthen the importance of a One Health concept.[12] Resistance genes from *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. have been detected in ground and surface water fed by effluents from hospitals and sewage processing plants, as well as runoff from animal farms that use antibiotics.[13–15] Furthermore, genes that mediate resistance to last-resort Gram-positive bacteria–specific antibiotics, such as VAN, have been recovered from raw milk and other animal products, pigs, wild animals (buffalo, zebra, and cattle), waste water, effluents, and patients, which implicates both veterinary and agricultural (over)use of antibiotics as potential sources of antimicrobial resistance genes in humans.[16–18]

These reports suggest that a larger share of the antibiotics that end up polluting environments and communities emanate from livestock production,[19,20] evincing the interconnectivity between animals, humans, and the environment. To better appreciate the dissemination routes of antibiotic resistance genes to inform appropriate interventions, the One Health concept is offered as the model for future epidemiological research. Even so, studies in Africa from a One Heath–perspective are limited.

The results of the systematic review presented herein (see highlights in Box 1), in which same bacterial clones and mobile genetic elements (MGEs) have been found in humans, animals, and the environment in countries within Africa, should be a wake-up call to researchers to begin pursuing more directly One Health–related studies as a method for finding deeper answers and solutions to the menace of antibiotic resistance—emphasized here for the African continent.[1]

Prior evidence

Several reviews have documented the high prevalence of antibiotic resistance in Gram-positive bacteria in Africa. For instance, varying antibiotic resistance has been recorded among bloodstream infections caused by *S. aureus* in humans from Ghana, Gambia, Burkina Faso, Niger, and Togo.[21] MRSA prevalence ranging from 9.4% to 13.5% has been reported in sepsis and meningitis infections,[22] while *S. aureus* is responsible for causing sepsis and pneumonia at resistance rates of 90%, 29%, and 20% to ampicillin (AMP), gentamycin (GEN), and cloxacillin, respectively, among children in Africa.[6] Furthermore, Founou *et al.* recently reported 100% and 93.8% MDR rates in *S. aureus* and *Enterococcus* spp., respectively.[23] Thus, the relatively high resistance and infection rate of *S. aureus* compared with other Gram-positive bacteria in Africa cannot be overemphasized.[24] The clonal diversity of MRSA in humans in Africa has been described in a single systematic review. MRSA clones ST239III/ST241III, ST80IV, ST8IV, ST88IV, and ST5IV were detected in 15 countries in Africa, including Algeria, Angola, Cameroon, Egypt, Gabon, Ghana, Kenya,

Box 1. Highlights

- Out of 553 articles fully assessed for eligibility, 172 (31.1%) were included in this review.
- Multidrug-resistant (MDR) clones, such as *S. aureus* (ST5, ST80, ST88, and ST247) and *E. faecium* (ST80 and ST901), were isolated from human, animal, and environmental sources.
- *mecA*, *blaZ*, *tet*(K/M), *dfrG*, and *van(A/B)* in *S. aureus*; *ermB*, *van(A/B)*, and *tet*(L/M) in *E. faecium* and *E. faecalis*; and *erm*(B), *tet*(M/O/T), and *mefA/E* in *S. pyogenes* and *S. agalactiae* were common.
- MRSA were mostly isolated from animals (21.5%, 437/2036 isolates), the environment (20.0%, 25/125 isolates), and humans (12.8%, 3485/27,291 isolates) in a descending order.
- Local outbreaks of clonal and polyclonal *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. strains in many African countries occurred within the study period, showing zoonotic and anthroponotic tendencies.
- A One Health approach to studying antibiotic resistance mechanisms and molecular epidemiology of GPB is lacking but warranted.

Madagascar, Morocco, Niger, Nigeria, Senegal, South Africa, São Tome and Príncipe, and Tunisia.[25] However, MRSA clones ST22IV, ST36II, and ST612IV have been reported only in South Africa, with *mecA* and resistant *S. aureus* clones being described.

Purpose of review

Reviews discussing the molecular epidemiology and mechanisms of antibiotic resistance in clinically important Gram-positive bacteria, including *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp., in humans, animals, and environmental isolates, and in the context of common antibiotic resistance genes, clones, and MGEs from a One Health perspective, to the best of our knowledge have not been performed. Our review here sought to fill this gap by analyzing the burden, types, and molecular epidemiology of resistant Gram-positive bacteria within a One Health context.

Methods

Systematic review protocol

The systematic review was compiled using the standard procedures established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Search strategy and data collection

English research articles published within the last 13 years (January 1, 2007–April 4, 2019) and indexed in PubMed, Web of Science, ScienceDirect, and African Journals OnLine were searched with the following keywords: "Enterococcus," "Streptococcus," "Staphylococcus" in permutations and combinations with "resistance" AND the names of each of 54 African countries. Duplicate entries were identified and removed before the final selection of articles.

Inclusion criteria

Studies that did not identify the underlying antibiotic resistance mechanisms/genes, as well as the clonality of the antibiotic-resistant Gram-positive bacteria, were excluded as this review focuses on molecular resistance mechanisms. Thus, studies that only reported on antibiotic sensitivity testing results or undertook antibiotic resistance surveillance studies without further molecular tests to characterize the antibiotic resistance mechanisms and/or clonality of the isolates were excluded (Fig. 1). In all, 381 studies were excluded because they only had minimum inhibitory concentration (MIC) data (see File S1, online only). Data extraction was undertaken independently by both authors in triplicates to ensure the replication of the results.

Data extraction

Data extracted from the articles included year of study, country, Gram-positive species, clones, sample sources, sample size/number of isolates, number of resistant isolates, resistance genes and MGEs, and antibiotics to which the strains were resistant (Table 1 and Tables S1–S3, online only). The rate of antibiotic resistance in each species was determined to identify countries with the highest or lowest levels of antibiotics resistance in Africa (Tables S4–S6 and Fig. S1A–O, online only).

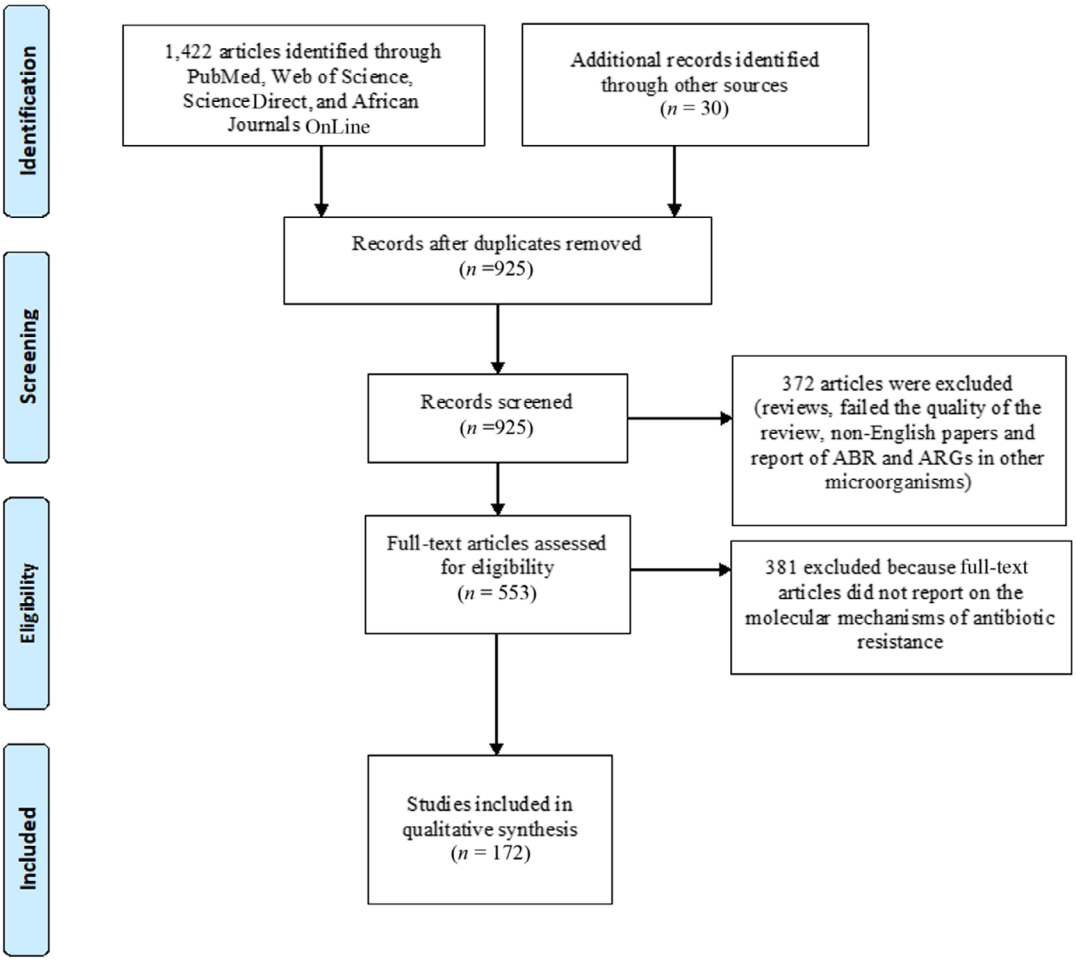

Figure 1. PRISMA-adapted flow chart showing included and excluded articles. All searches were conducted on PubMed, Web of Science, ScienceDirect, and African Journals OnLine, and a final number of 172 articles were included in this review.

Data and bioinformatics analyses

Microsoft Excel 365 was used in curating and calculating the results as well as designing the Tables and charts. Frequencies and resistance rates were calculated using Microsoft Excel 365 (File S2, online only). Genomic sequences of *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. of African origin at PATRIC (https://www.patricbrc.org/) (File S3, online only) were used to draw phylogeny trees with RAxML to demonstrate the molecular epidemiology of Gram-positive bacteria in Africa from a One Health context. Figtree (http://tree.bio.ed.ac.uk/software/figtree/) was used for tree annotations.

Quality assessment of studies

The quality of the reviewed articles was assessed by two independent researchers using a data extraction form to extract predetermined qualitative and quantitative data. Inconsistencies were resolved by consensus. Studies with well-described and appropriate research designs for isolating Gram-positive bacteria from humans, animals, and environmental sources, and further describing the mechanisms of antibiotic resistance were included in this review.

Results

Characteristics of included articles

Out of 553 articles fully assessed for eligibility, 172 were finally included representing 22 out

Table 1. Frequency distribution of Gram-positive bacterial species, resistance genes, and MGEs isolated from animals, humans, and environmental specimens from 2007 to 2019

		Human	Animal	Environment	Total
Species	*E. faecalis*	466	307	138	911
	E. faecium	425	796	606	1827
	E. casseliflavus	2	4	35	41
	E. mundtii	0	12	36	48
	E. gallinarum	17	49	5	71
	E. hirae	0	174	1	175
	E. raffinosus	1	0	0	1
	E. sulfurens	0	0	1	1
	E. durans	1	148	17	166
	S. agalactiae	658	92	0	750
	S. aureus	27,291	2036	125	29,440
	S. haemolyticus	96	43	38	177
	S. pyogenes	585	0	0	585
	S. epidermidis	544	16	≥1	≥561
	S. arlettae	0	0	≥1	≥1
	S. xylosus	0	8	≥1	≥9
	S. schleifer	3	0	0	3
	S. warneri	9	0	≥1	≥10
	S. lugdunensis	4	0	0	4
	S. saprophyticus	10	0	30	40
	S. cohnii	3	0	≥1	≥4
	S. pasteuri	0	0	≥1	≥1
	S. simulans	0	0	≥1	≥1
	S. sciuri	9	0	≥1	≥10
	S. mitis	1	0	0	1
	S. pseudinterendius	0	31	0	31
	S. hominis	2	1	0	3
Antibiotic resistance gene	*mecA* (% MRSA)	3485 (12.8)	437 (21.5)	25 (20.0)	3947 (13.4)
	erm(B)	605	556	245	1406
	erm(C)	239	33	11	283
	tet(M)	639	211	168	1018
	tet(K)	265	249	44	558
	tet(L)	32	73	81	186
	vanB	17	417	82	516
	vanA	56	26	32	114
	vanC-1/2/3	41	900	110	1051
	dfrA/G	454	0	2	456
	aph(3′)-llla	62	7	162	231
	aac(6′)-aph(2′)	350	29	100	479
	ant(6)-la	13	24	38	75
	blaZ	578	227	70	875
MGEs	IS*16*	13	0	15	28
	Tn*916*	63	44	0	107
	IS*256*	92	4	0	96
	SCC*mec*	3821	561	55	4437

of 54 African countries (40.7%). Studies from Tunisia ($n = 39$), South Africa ($n = 27$), Egypt ($n = 26$), Nigeria ($n = 20$), Algeria ($n = 10$), Angola ($n = 6$), Uganda ($n = 6$), Democratic Republic of the Congo ($n = 3$), Zambia ($n = 4$), São Tomé and Príncipe ($n = 5$), Ghana ($n = 5$), Kenya ($n = 3$),

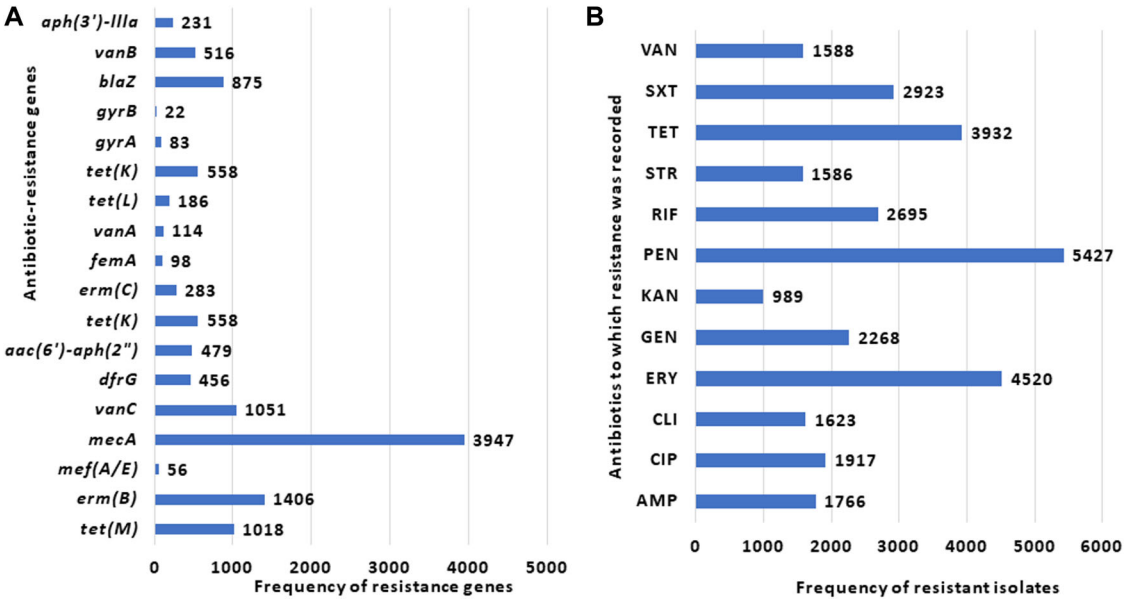

Figure 2. Frequency and distribution of resistance genes, antibiotics, and mobile genetic elements (MGEs) with recorded resistance in Gram-positive bacteria in Africa. (A) Frequency of the various resistance genes found in the drug-resistant Gram-positive bacterial strains. *mecA* and *erm*(B) were the most dominant resistance genes detected. (B) Antibiotics to which the isolates were most resistant: erythromycin (ERY) was the least effective drug, followed by rifampicin (RIF), tetracycline (TET), penicillin (PEN), sulphamethoxazole/trimethoprim (SXT), ciprofloxacin (CIP), GEN (GEN), vancomycin (VAN), ampicillin (AMP), clindamycin (CLI), streptomycin (STR), chloramphenicol (CHL), and kanamycin (KAN).

Gabon ($n = 2$), Morocco ($n = 2$), Sudan ($n = 2$), Tanzania ($n = 4$), Libya ($n = 4$), and single studies each from Cape Verde, Mozambique, Namibia, Gambia, and Senegal were included (Fig. 1). Among the included studies, 116 were from clinical (human) sources only, 45 were from animal sources only, 20 were from environmental sources only, four were from both clinical and animal sources, three were from clinical and environmental sources, two were from animal and environmental sources, and one was from clinical, animal, and environmental sources. A total of 381 papers were excluded because they only had MIC data (File S1, online only).

Distribution of resistance genes, clones, and MGEs

A total of ≥34,487 Gram-positive bacterial samples were isolated from humans, animals, and the environment (Tables S1–S3, online only), with antibiotic resistance rates varying from 1.4% to 100% across the 22 included countries (Tables S4–S6 and Fig. S1A–O, online only). The following drug-resistant Gram-positive bacteria were identified across Africa: *S. aureus* ($n = 29{,}440$); *E. faecium* ($n = 1{,}827$); *Streptococcus agalactiae* ($n = 750$); *Enterococcus faecalis* ($n = 911$); *Streptococcus pyogenes* ($n = 585$); and *Streptococcus haemolyticus* ($n = 177$) (Table 1). Predominant resistant clones among these species were *S. aureus* ST5 ($n = 209$), ST80 ($n = 125$), ST8 ($n = 116$), and ST88 ($n = 109$); *E. faecium* ST317 ($n = 33$), ST51 ($n = 20$), and ST910 ($n = 13$); *E. faecalis* ST78 ($n = 28$); and *S. agalactiae* ST616 ($n = 22$). These species and clones, which were isolated from humans, animals, and the environment, harbored *mec*A ($n = 3947$), *erm*(B) ($n = 1406$), *vanC1/2/3* ($n = 1051$), *tet*(M) ($n = 1018$), *blaZ* ($n = 875$), *tet*(K) ($n = 558$), *vanB* ($n = 516$), *aac(6′)-aph(2′)* ($n = 479$), *dfrA/G* ($n = 456$), *erm*(C) ($n = 283$), *aph(3′)-IIa* ($n = 231$), *tet*(L) ($n = 186$), and *ant(6)-Ia* ($n = 67$) resistance genes (Table 1 and Fig. 2A).

Except for *vanB*, *vanC*, *tet*(L), and *ant(6)-Ia*, which were higher in animals and the environment, all other resistance genes were higher in humans. There were more *E. faecium*, *Enterococcus casselivaflavus*, *Enterococcus mundtii*, *Enterococcus hirae*, *Enterococcus durans*, and *Enterococcus*

xylosus in animals and environmental specimens than in human samples; *Enterococcus gallinarum* was higher in animals than in humans, but higher in humans than in the environment. *Streptococcus schleifer*, *Streptococcus warneri*, *Streptococcus lugdunensis*, *Streptococcus cohnii*, *Streptococcus sciuri*, and *Streptococcus hominis* were higher in humans than in animals and the environment. The country by country distribution of all other species is summarized in Tables S1–S3 (online only).

SCC*mec* ($n = 4437$), Tn*916* ($n = 107$), IS*256* ($n = 96$), and IS*16* ($n = 28$) were the most common MGEs identified in the included studies (Table 1 and Fig. 3). These MGEs were higher in humans except IS*16* that was predominant in environmental isolates than in humans and animals. These MGEs were identified in *Enterococcus* spp., *Staphylococcus*, and *Streptococcus* spp. in six African countries. *IS256* was reported in *S. aureus*, *Streptococcus epidermidis*, and *Enterococcus* spp. in South Africa and Nigeria. Tn*916* was detected in *Enterococcus* spp., *S. agalactiae*, and *S. pyogenes* in Egypt, Kenya, and Tunisia, while IS*16* was only reported in *E. faecium* and *E. faecalis* in Tunisia.

The antibiotics to which the isolates were most resistant to were penicillin (17.0%; $n =$ 5427/32,020), erythromycin (ERY) (13.7%; $n =$ 4520/12,557), TET (12.1%; $n = 3932/32,557$), sulfamethoxazole (SXT)/trimethoprim (9.1%; $n =$ 2923/32,088), rifampicin (RIF) (8.7%; $n = 2695/$ 08.968), GEN (7.0%; $n = 2268/32,454$), ciprofloxacin (CIP) (4.6%; $n = 1917/34,057$), clindamycin (CLI) (5.1%; $n = 1623/31,678$), streptomycin (STR) (5.1%; $n = 1586/31,199$), AMP (5.6%; $n = 1766/31,458$), and VAN (4.7%; $n = 1588/34,057$) (Fig. 2B and Tables S1–S3, online only). VAN-resistant *Enterococcus* spp. (VRE) ($n = 1310$) and VAN-resistant *Staphylococcus* spp. ($n = 278$) were reported in humans, animals, and the environment; and VAN-resistant *S. aureus* was reported in humans ($n = 19$), animals ($n = 215$), and the environment ($n = 15$). A similar situation occurred with VAN-resistant *E. faecium*, which was isolated from the environment ($n = 207$), animals ($n = 120$), and humans ($n = 15$); VAN-resistant *E. faecalis* was also isolated from the environment ($n = 23$), animals ($n = 46$), and human ($n = 10$) (Tables S1–S6 and Fig. S1A–O, online only).

Antibiotic resistance patterns in species and sources

Different types and levels of antibiotic resistance were reported among Gram-positive bacteria from clinical, animal, and environmental sources. Antibiotic resistance for *Enterococcus* spp. was highest from isolates recovered from environmental sources followed by animal sources and humans, while antibiotic resistance for *Staphylococcus* spp. was highest for animal isolates followed by humans and the environment. *Streptococcus* spp. reported lower resistance in human isolates except for TET and ERY, which recorded a resistance of 77.4% (488/693) and 26.2% (354/1335), respectively. Among the *Enterococcus* spp. isolated from environmental sources, 85.3% (297/348) were resistant to penicillin, 54.4% (397/730) were resistant to ERY, 66.6% (247/371) were resistant to AMP, 47.96% (377/786) were resistant to VAN, and 45.9% (200/436) were resistant to CIP. Among *Enterococcus* spp. recovered from animal sources, 86.4% (766/887) were resistant to CLI, 73.3% (650/877) were resistant to penicillin, 67.9% (1099/1618) were resistant to ERY, 45.8% (824/1800) were resistant to VAN, and 36.8% (303/1069) were resistant to TET. Among the *Staphylococcus* spp. isolated from animals, 61.0% (1115/1873) were resistant to penicillin, 53.9% (549/1018) were resistant to STR, 44.6% (835/1873) were resistant to TET, 35.4% (235/664) were resistant to CLI, 34.0% (441/1298) were resistant to ERY, and 12.0% (243/2027) were resistant to VAN. In clinical samples, *Staphylococcus* spp. recorded that 83.2% (3171/3817) were resistant to penicillin, 67.9% (549/808) were resistant to AMP, 40.6% (2000/4925) were resistant to trimethoprim/SXT, 38.2% (483/1264) were resistant to kanamycin, 31.3% (1477/4700) were resistant to TET, and 5.0% (19/373) were resistant to VAN.

Varying antimicrobial resistance to antibiotics was reported in different countries in Africa, with higher resistance reported from different sources in Algeria, Egypt, Kenya, Nigeria, South Africa, Sudan, Tanzania, Tunisia, and Senegal due to higher prevalence of resistant bacterial clones, resistance genes, and SCC*mec*, Tn*916*, IS*16*, and IS*256*. For instance, in environmental samples, $\geq$30% resistance to penicillin, ERY, sulphamethoxazole–trimethoprim, VAN, and TET was found in *Staphylococcus* spp., particularly *S. aureus*, *E. faecium*, and *E. faecalis* in Tunisia, South Africa, and Algeria. Substantial

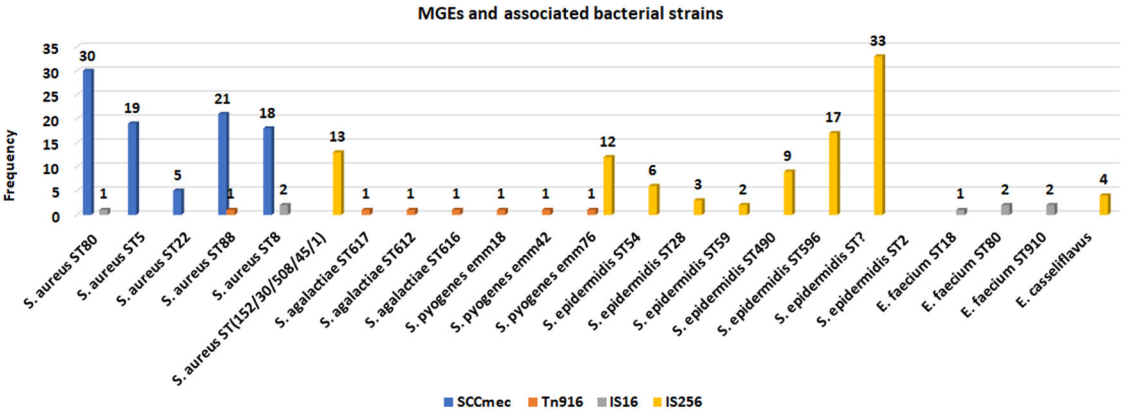

Figure 3. Frequency distribution of mobile genetic elements (MGEs) in Africa. The most common MGE was SCC*mec*, found in *S. aureus* ST5, ST8, ST22, and other clones, followed by IS*256* in *S. epidermidis*, *S. aureus*, and *E. casseliflavus*. IS*16* in *E. faecium* ST18 and ST80; *S. aureus* ST8 and ST80; *S. pyogenes emm*18; as well as Tn*916* in single isolates of *S. agalactiae* ST617, ST616, and ST612; *S. pyogenes emm*118 and *emm*42; and *E. faecium* ST18 were also common. Each color represents a particular resistant clone.

penicillin resistance from animal sources was found in *S. aureus* (Egypt, South Africa, Senegal, Tanzania, and Tunisia) and *Enterococcus* spp., including *E. faecium* (South Africa). Higher VAN resistance was found in *E. faecium* and *E. faecalis* in Tanzania and Tunisia from animal sources, while cefoxitin (FOX) resistance was moderately high in *Staphylococcus* spp. (Nigeria and South Africa) from animals (Fig. 4A and Table 1; see also Tables S1–S6 and Fig. S1A–O, online only).

In human samples, penicillin resistance was most pronounced in *S. aureus* (Angola, DRC, Gabon, Ghana, Morocco, Nigeria, Sao Tome and Principe, Sudan, and Tunisia), *S. epidermidis* (Uganda), and *E. faecalis* (Egypt), while VAN resistance was high in *E. faecalis* (Tanzania) and *E. faecium* (Tanzania and Tunisia). However, FOX resistance was substantially found in only *S. aureus* (Algeria, Angola, Kenya, and Tunisia) from humans.

Molecular epidemiology of antibiotic-resistant Gram-positive bacteria in specific regions

Clonal and polyclonal outbreaks of both resistant and nonresistant strains of *Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. occurred in countries, such as South Africa, Ghana, Tanzania, Algeria, Tunisia, and Kenya. For instance, antibiotic-resistant *S. aureus* ST612 was found in both pigs and humans in South Africa (Figs. 5 and 6); ST15, ST152, ST250, and ST3250 in humans in Ghana; and *S. aureus* ST8 in Tanzania (Fig. 5A). A drug-resistant *S. pneumoniae* ST7052 and ST3214 outbreak occurred in South Africa, while *E. mundtii* circulated in animals and an abattoir (slaughterhouse) in Kenya (Fig. 5B and C). Dissemination of *E. faecium/faecalis* strains within and between countries, animals, humans, and the environment was pronounced in South Africa and Tanzania (Fig. 5C).

***Staphylococcus* spp. (*S. aureus*, *S. haemolyticus*, and *S. saprophyticus*) in North Africa.**

Algeria. *S. aureus* was recovered from six different clinical studies and one animal study in Algeria. In assessing the nasal carriage of *S. aureus* in patients with medical conditions, including pneumonia, urinary tract infections, osteoarthritis, heart diseases, diabetes, and chronic kidney disease, Djoudi *et al.* isolated MRSA;[26] they found the nasal carriage of *S. aureus* to be significantly associated with cancer and previous hospitalization for kidney failure due to immunological suppression and hemodialysis. The MRSA isolates ST239 ($n = 60$), ST80 ($n = 27$), ST5 ($n = 2$), ST22 ($n = 2$), and ST535 ($n = 1$) harbored *mecA*.[27,28] In another study in Algeria, typing of 64 MRSA isolated from human pus ($n = 47$), venous catheters ($n = 7$), tracheal aspirates ($n = 4$), puncture fluids ($n = 3$), blood ($n = 2$), and urine ($n = 1$) in 64 patients found that 50 were hospital-acquired MRSA (HA-MRSA) and 14 were community-acquired MRSA (CA-MRSA);[29] *mecA*, mobilized by SCC*mec*, was the only detected mechanism of resistance.

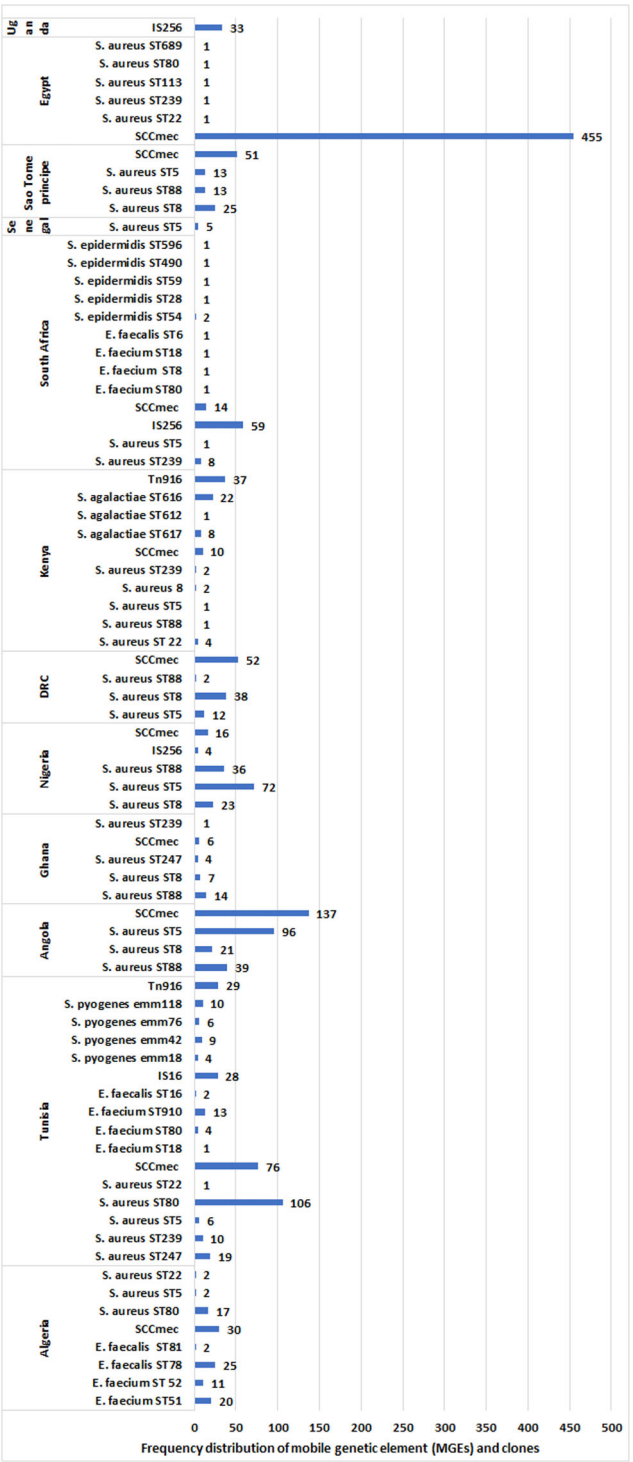

Figure 4. Frequency distribution of resistant Gram-positive bacterial species, clones, and mobile genetic elements (MGEs) per country in Africa. *S. aureus* ST5 is predominant in Tunisia, the DRC and Senegal, while ST22 is highly prevalent in Algeria. SCC*mec* was the commonest MGE in most of the countries except Tunisia, where IS*16* and Tn*916* were higher in prevalence. *S. aureus* ST8 and ST80 were the most common clones reported, followed by *E. faecium* ST317. *(Continued)*

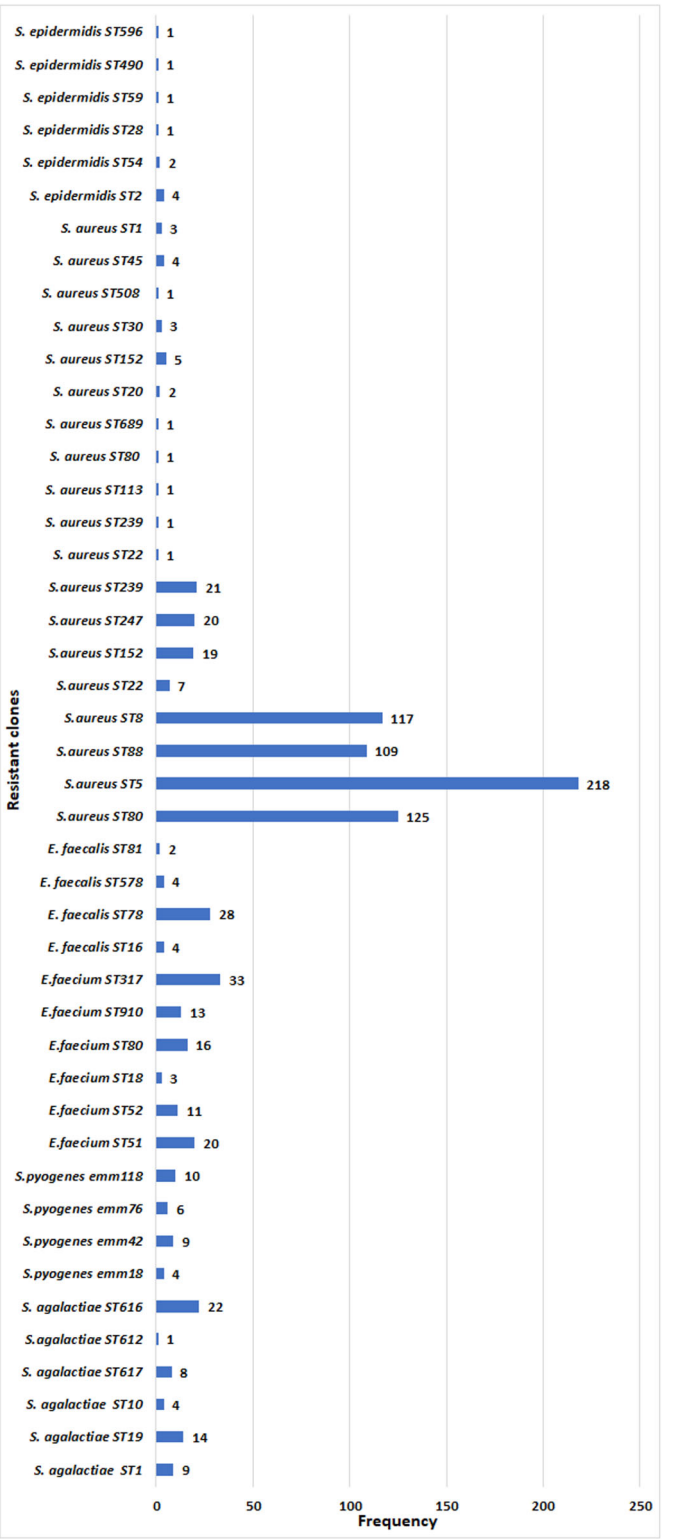

Figure 4. *Continued*

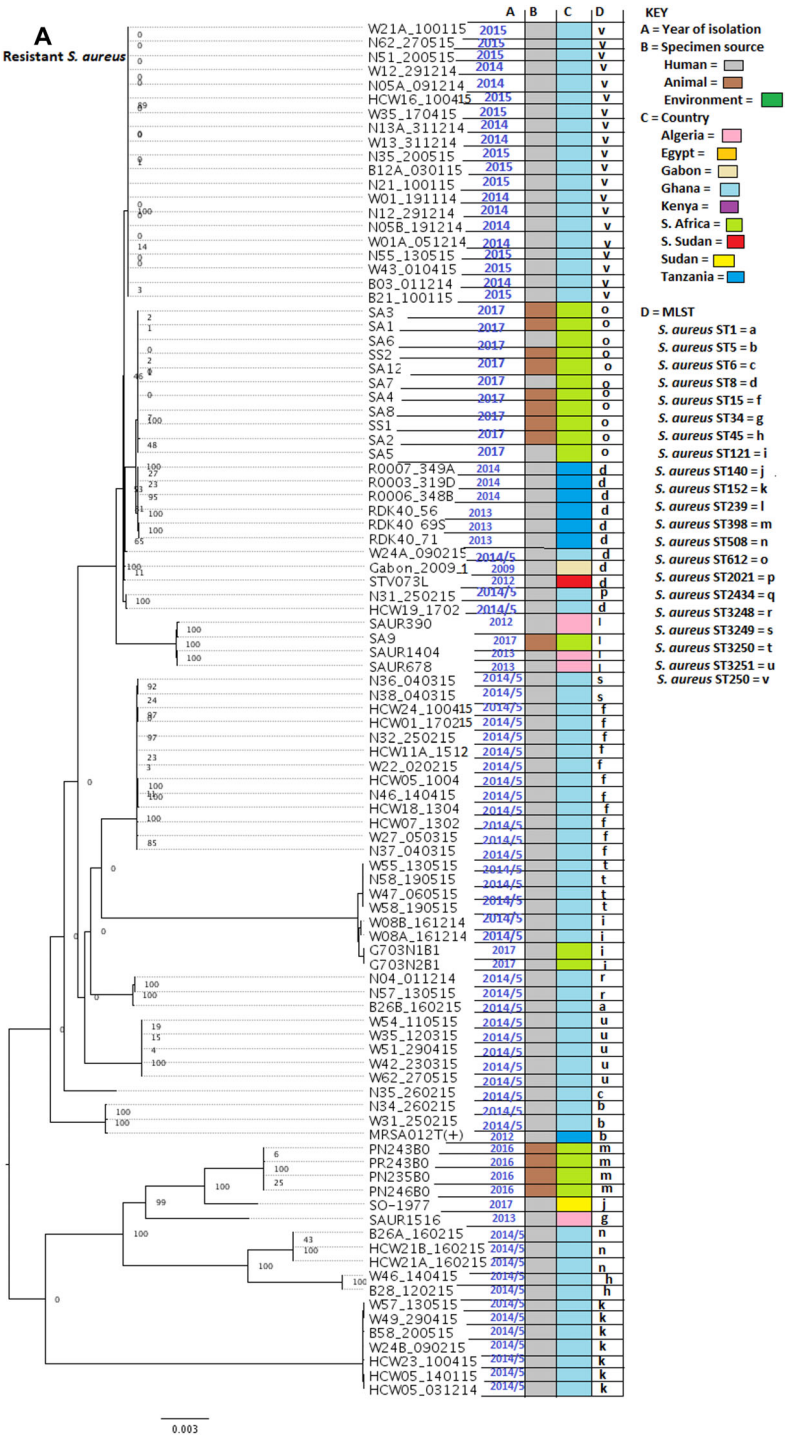

Figure 5. Phylogenomic analyses of *Enterococcus* spp. and drug-resistant *S. aureus* and *S. pneumoniae* isolates from Africa. (A–C) Genomic sequences of drug-resistant *S. aureus*, A, and *S. pneumoniae*, B, strains and of *Enterococcus* spp., C, were downloaded from PATRIC (https://www.patricbrc.org/) and used for phylogenomic analyses using RAxML. The tree shows local clonal outbreaks of all four species within specific countries. Dissemination of same clones or clades between the environment, animals, and humans was also observed in *S. aureus* ST612 and ST8, and *E. faecium/faecalis* in Africa, showing the clonal expansion of ABR in animals, humans, and the environment in Africa. *(Continued)*

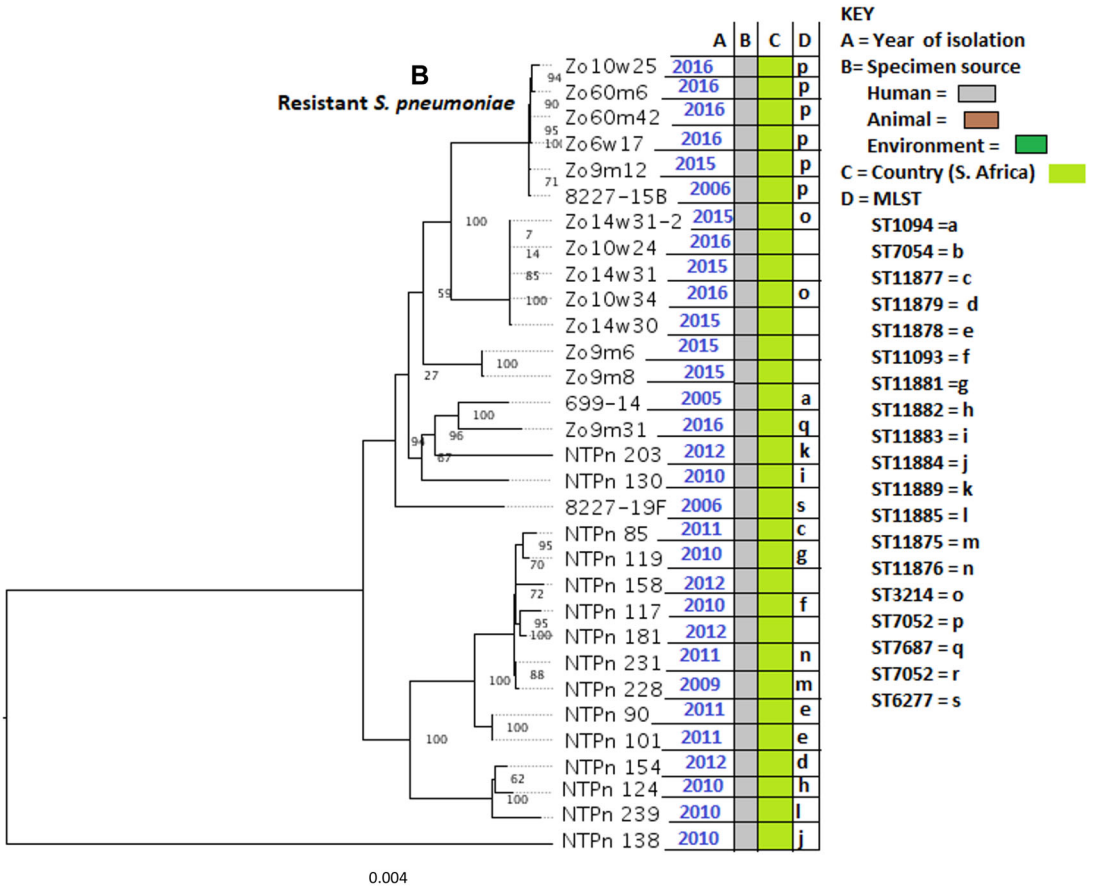

Figure 5. *Continued*

Egypt. MRSA has been isolated in 11 animal-based, nine human-based, and one environmental-based studies in Egypt between 2007 and 2019. Hashem *et al.* isolated 94 *S. aureus* strains from blood and wounds among which 45 were MRSA, while 25 were fluoroquinolone resistant.[30] Mutations in gyrase enzymes, including C2402T, T2409C, T2460G, T1497C, and A1578G, which lead to fluoroquinolone target-site alterations, were implicated in resistance to fluoroquinolones (CIP, levofloxacin, and ofloxacin). The high rate of fluoroquinolone resistance (55.56%) among MRSA infections is rather concerning, as patients unable to tolerate that VAN must be treated with other antibiotics, such as fluoroquinolones. Resistant clones, including ST22 ($n = 1$), ST239 ($n = 1$), ST689 ($n = 1$), ST113 ($n = 113$), and ST80 ($n = 1$), were isolated.[31,32] MDR to drugs, such as GEN, AMP, amoxicillin, cefepime, TET, and chloramphenicol (CHL) in MRSA, is mediated by diverse resistance mechanisms, including impermeability effects and activity of efflux pumps. Unrestricted access to antibiotics and inappropriate prescriptions were responsible for the high rates of drug resistance in study described above.[30] In a similar study, MRSA was isolated from patients suffering from surgical wound infections, diabetic foot, abscess, and burns. Although *mecA* was the only mechanism of resistance, the isolates were additionally multiple-resistant to several antibiotics, including β-lactams, aminoglycosides, fluoroquinolones, macrolides, lincosamides, TETs, and glycopeptides, indicating other mechanisms of resistance.[33]

Al-Ashmawy *et al.* detected a high prevalence of MRSA (53%) in milk and dairy products believed to originate from human contamination rather than from animals. Besides being resistant to β-lactams

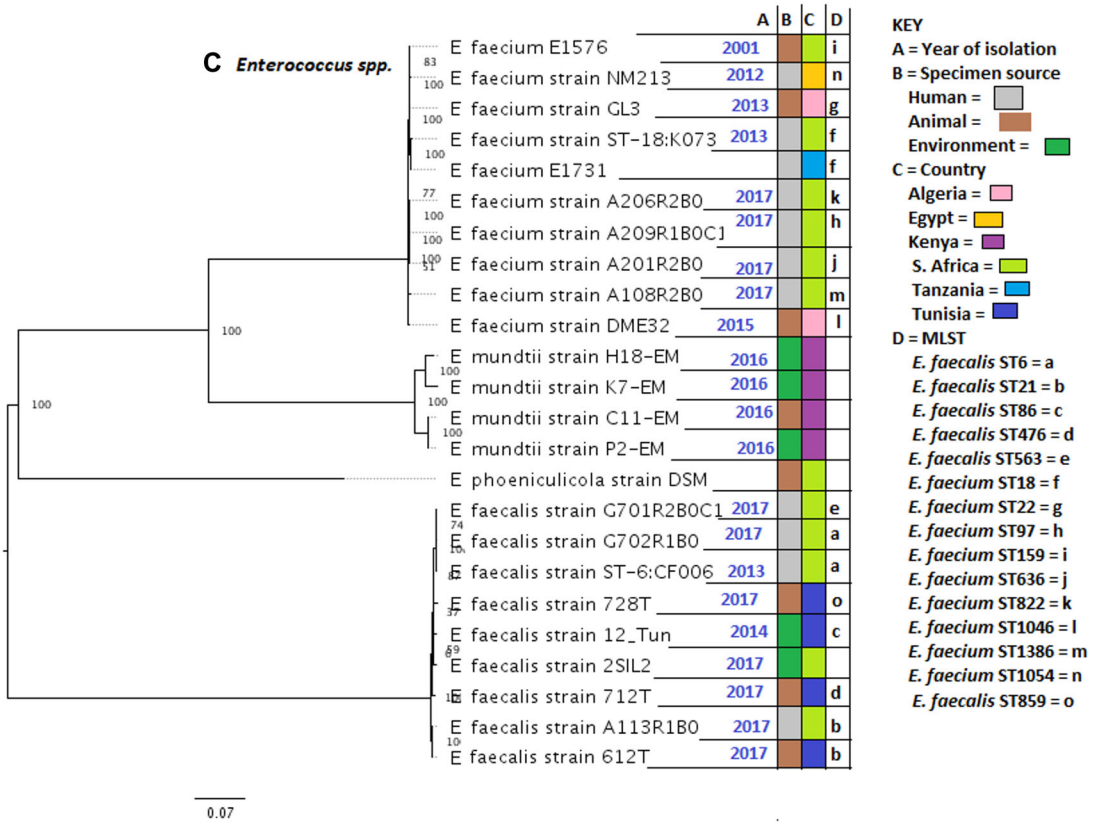

Figure 5. *Continued*

and other antibiotics, 36 of the isolates were resistant to VAN, making milk and dairy products a likely source of MDR/toxigenic *S. aureus* infections. In 2017, Osman and colleagues detected *Staphylococcus* spp. in imported beef; 16 of the isolates were MDR owing to resistance mechanisms, such as *mecA* and mutations in *gyrA* and *gyrB*. Of 133 *S. aureus* recovered from animal origin, more than 70% were MDR and 30 were MRSA and exhibited high resistance to CLI, cotrimoxazole, TET, oxacillin (OXA), FOX, ceftriaxone, and ERY; four of the isolates were resistant to VAN.[34] MRSA ST689 ($n = 1$), *mecA* ($n = 3$), *van*A ($n = 1$), and *van*B ($n = 1$) were found in *S. aureus* isolated from food samples.[35]

Morocco. In a study assessing *S. aureus* carriage among end-stage renal disease patients undergoing haemodialysis, 42.9% participants were found to be carriers, of which one was MRSA. Among the others, methicillin-susceptible *S. aureus* (MSSA) was resistant to many of the local antibiotics; for example, 81.8% of the MSSA were penicillin resistant, which limited the treatment. Being male and age 30 or younger were identified as risk factors of *S. aureus* nasal carriage ($P < 0.001$).[36]

Tunisia. Antibiotic-resistant *S. aureus* was isolated from the environment, animals, and humans between 2011 and 2019. Said *et al.* recovered 12 MSSA from wastewater samples that were resistant to penicillin ($n = 12$), ERY ($n = 7$), TET ($n = 1$), and CLI ($n = 1$) because of the presence of *blaZ* ($n = 7$), *msr*(A) ($n = 7$), and *tet*(K) ($n = 1$). The resistant strains were of ST3245 ($n = 7$), ST15 ($n = 1$),[37] and ST247 ($n = 2$),[38] which have been reported in animals and humans.

In an investigation to evaluate the prevalence of coagulase-negative *Staphylococcus* (CoNS) in the hospital environment, MDR *S. haemolyticus* and *S. saprophyticus* were the most dominant. Methicillin resistance was detected in *S. haemolyticus*, *S. epidermidis*, and *S. saprophyticus*. These isolates were resistant to ERY,

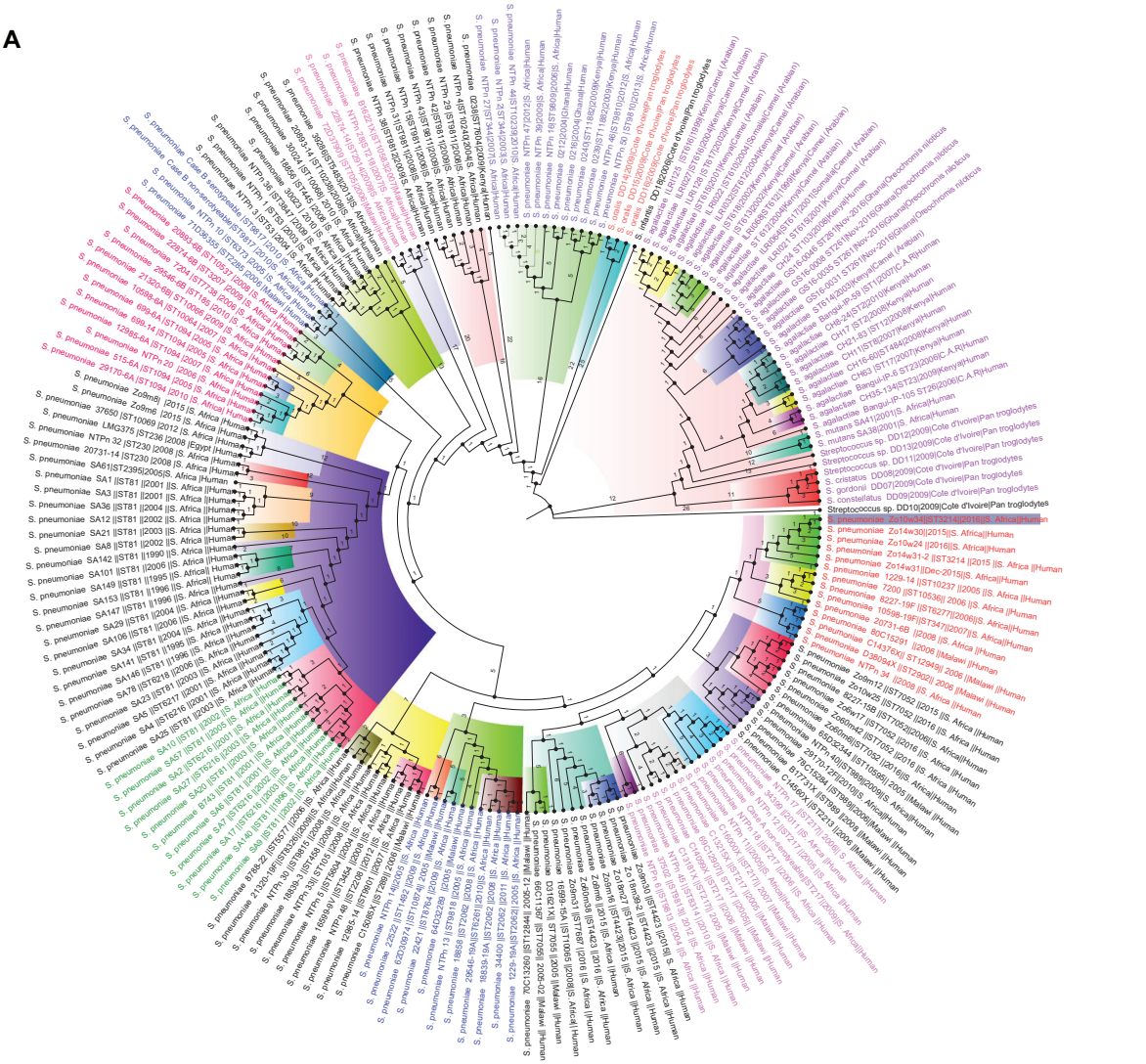

Figure 6. Phylogenomic analyses of *Staphylococcus* spp. and *Streptococcus* spp. in Africa. Genomic sequences of (A) *Staphylococcus* spp. and (B) *Streptococcus* spp. strains were downloaded from PATRIC (https://www.patricbrc.org/) and used for phylogenomic analyses using RAxML. The tree shows local clonal outbreaks of all four species within specific countries. Dissemination of same clones or clades between the environment, animals, and humans was also found. Strains within the same clade/clone are highlighted with the same color. Within these clades, there was the presence of same or very closely related strains in different countries, animals, and humans, showing the clonal expansion and dissemination between humans and animals, as well as across countries. *(Continued)*

TET, GEN, kanamycin, tobramycin, and STR, owing to the presence of *msrA* ($n = 32$), *erm*(C) ($n = 8$), *tet*(K) and *tet*(M), *aac(6′)-Ie-aph(2″)-Ia* ($n = 16$), *aph(3′)-IIIa* ($n = 19$), *ant(4′)-Ia* ($n = 14$), and *ant(6′)-Ia* ($n = 3$).[39] The high prevalence of MDR *Staphyloccoci* spp. isolates may result from the transmission between the staff, patients, and the environment. Infections caused by CoNS are common cause of death, particularly in low birth weight children, and are opportunistic infections in immunocompromised patients.[40] Several resistant *S. aureus* clones, including ST133 ($n = 15$), ST153 ($n = 5$), ST178 ($n = 4$), ST6 ($n = 4$), ST2057 ($n = 4$), and ST15 ($n = 1$), were detected in sheep and carried *blaZ*, *ant(6)-Ia*, *aph(30)-IIIa*, *erm*(C), *tet*(K), and *fusB*, which encode resistance

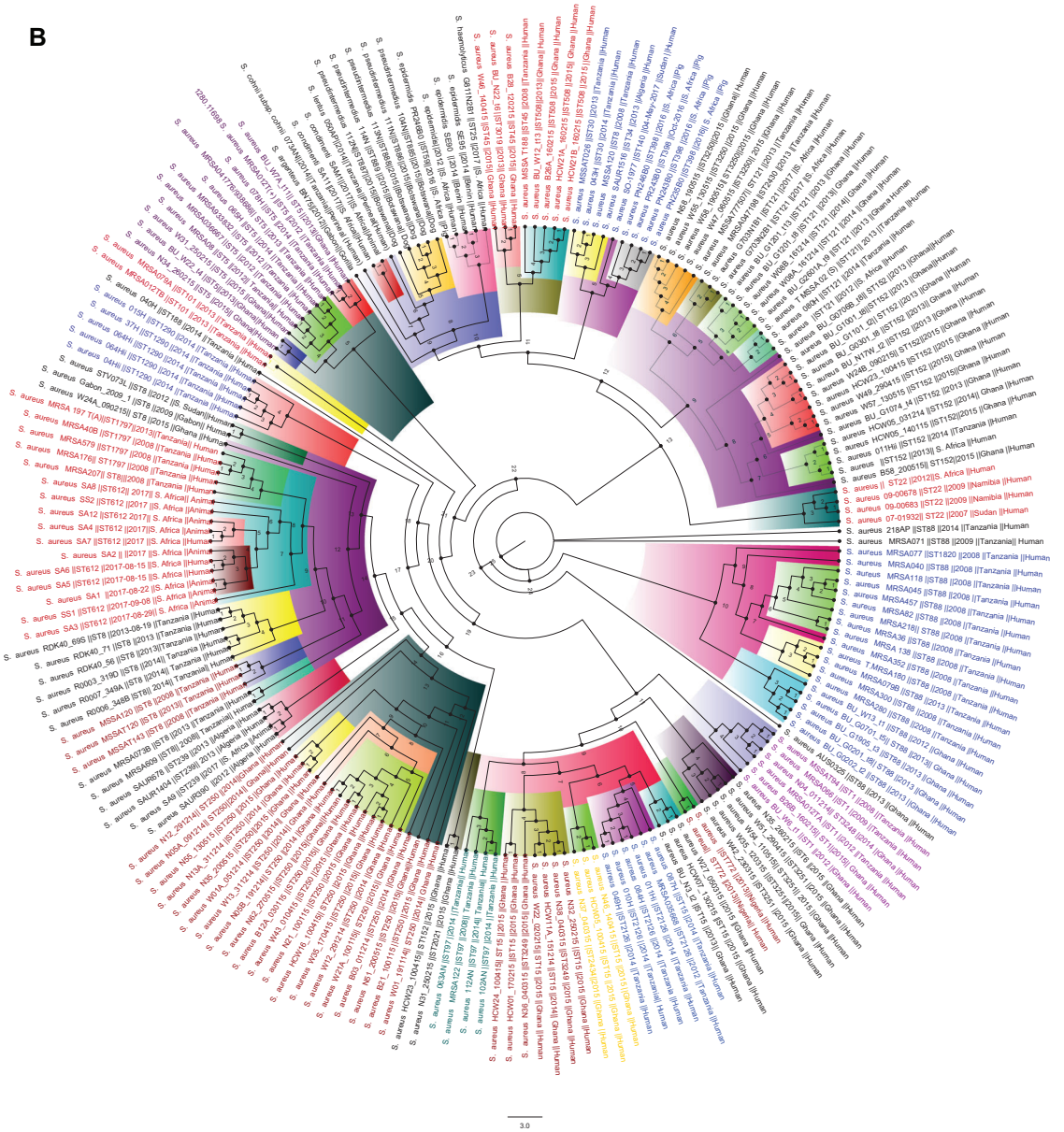

Figure 6. *Continued*

to penicillin, STR, kanamycin, ERY, TET, and fusidic acid, respectively. Thus, the nares of healthy sheep could be reservoirs of MRSA.[41,42]

Between 2011 and 2012, 99 MRSA strains were detected from nasal swabs, blood, catheter, wounds, pleural puncture, and abscess, among which 39 were TET resistant. The isolates were resistant to aminoglycosides, fluoroquinolones, macrolides, and lincosamides, with mechanisms of resistance, including *mecA* ($n = 24$), *tet*(K) ($n = 6$), *tet*(L) ($n = 1$), and/or *tet*(M) ($n = 18$), *erm*(A) ($n = 14$), and *aph(2′)-acc(6′)* ($n = 13$). Identified drug-resistant strains included ST247 ($n = 12$), ST239 ($n = 6$), ST728 ($n = 2$), ST241 ($n = 1$), ST398 ($n = 1$), ST5 ($n = 1$), and ST641 ($n = 1$).[43] For the first time, clonal lineage ST398, which has previously been reported in pigs from several studies in the United States, South America, Asia,

and Canada,[44–47] was found in an MRSA isolate in Africa from a nasal swab of a 74-year-old patient.

Additionally, 69 MRSA strains were isolated from hospital-acquired and CA infections. Although *mecA* ($n = 59$) was the only mechanism of resistance identified, the isolates were resistant to aminoglycosides, TET, fluoroquinolones, macrolides, and RIF. The *S. aureus*–resistant clones were ST80 ($n = 96$), ST247 ($n = 15$), ST5 ($n = 5$), ST22 ($n = 1$), ST97 ($n = 2$), ST153 ($n = 2$), ST239 ($n = 9$), ST241 ($n = 3$), ST256 ($n = 1$), ST1819 ($n = 3$), and ST1440 ($n = 1$).[48,49] Maalej and colleagues isolated five pristinamycin-resistant MDR *S. aureus* strains from patients with skin infections (Table S2, online only), being the first detection of streptogramins resistance due to *vat*(B) and *vga*(B) resistance genes,[50] which emerged due to selective pressure from the use of pristinamycin. Thirty-six methicillin-resistant *S. haemolyticus* (MRSHae) were isolated from neutropenic patients (suffering from febrile neutropenia) with hematological cancer between 2002 and 2004. These MDR isolates carried SCC*mec*-borne *mecA* (Table S2, online only).[51]

Libya. Owing to the fact that MRSA colonization develops into infections in children, nasal samples were collected from children inpatients, their mothers, healthcare workers, and outpatient workers, yielding an MRSA nasal carriage of 8.3%, 11%, 12.3%, and 2.2%, respectively.[52] *S. aureus* isolated from wound, skin, and soft tissue infections, and abscess recorded resistance to antibiotics, including VAN ($n = 13$).[42,53,54]

Staphylococcus spp. (*S. aureus*, *S. haemolyticus*, and *S. saprophyticus*) in West Africa.

Ghana. Among 308 *Staphylococcus* isolates collected across Northern, Central, and Southern Ghana in 2013, low prevalence of antibiotic resistance was reported except for penicillin (97%), TET (42%), and ERY (6%).[55] *MecA* was detected in only nine isolates, implying the presence of other β-lactam resistance mechanisms. MRSA clones included ST8 ($n = 1$), ST72 ($n = 1$), ST88 ($n = 2$), ST239 ($n = 1$), ST250 ($n = 2$), ST789 ($n = 1$), and ST2021 ($n = 1$). In a similar study that characterized 30 MRSA isolates resistant to TET, fluoroquinolones, and macrolides, *tet*(M) ($n = 13$), *tet*(K) ($n = 10$), *aphA3* ($n = 7$), *aacA–aphD* ($n = 5$), and *erm*(C) ($n = 4$) were detected. Both similar and different resistant clones, namely, ST88 ($n = 8$), ST8 ($n = 5$), and ST247 ($n = 4$), were detected,[56] indicating high MRSA clonal diversity in Ghana. These studies show a relatively high prevalence of resistance to non-β-lactams that likely further complicates MRSA treatment. Furthermore, the isolation of USA300 and other epidemic MDR MRSA clones suggests the need for increased surveillance and adequate control measures. Similar clinical studies detected drug-resistant *S. aureus* ST15, ST152, ST5, ST45, ST707, ST121, ST72, ST6, and ST508. In an emergency ward environment, *S. aureus* ST15 and ST508, harboring *blaZ* ($n = 5$) and *dfrG* ($n = 2$), were detected.[57,58]

Nigeria. Different studies reported drug-resistant *S. aureus* from several human anatomical sites and contexts, including throat, soft skin and tissue, urinary tract and respiratory infections, wounds, vagina, otitis, conjunctivitis, and septicaemia. Of $\geq$602 isolates, $\geq$433 were resistant to several antibiotic classes (Table S1, online only); of note, 429 were all resistant to cotrimoxazole or trimethoprim/SXT. The mechanisms of resistance included *mecA* ($\geq$107), *blaZ* ($n = 284$), *dfrA* ($\geq$5), and *dfrG* ($\geq$152). Colonized persons—including immune-compromised individuals—facilitated the spread of *S. aureus* and MRSA ST8, identified as ubiquitous in various geographic areas of Nigeria.[59–61] High utilization of cotrimoxazole or SXT, because of low cost and easy obtainability through lenient medication regulations, was implicated for the high resistance.[62] Besides *S. aureus*, *S. haemolyticus* was a major species isolated; it is considered the second most clinically important *Staphylococci* spp., particularly in immunocompromised patients.[51] All *S. haemolyticus* isolates detected were resistant to at least three antibiotics classes (Tables S4–S6, online only).[63] However, drug-resistant *S. aureus* ST5 ($n = 72$), ST7 ($n = 44$), ST121 ($n = 38$), ST250 ($n = 28$), ST88 ($n = 33$), ST30 ($n = 26$), ST8 ($n = 18$), ST1 ($n = 20$), ST15 ($n = 8$), ST80 ($n = 8$), ST241 ($n = 7$), ST25 ($n = 5$), and ST72 ($n = 3$) were the dominant clones detected.

Ayepola *et al.* reported a prevalence of 20.8% *S. aureus* from UTIs, higher than the reported ranges in Africa (6.3–13.9%)[64] and far exceeding the rate reported from Europe and Brazil (1.1%).[65] In a study to examine the genetic mechanism(s) of

resistance in CoNS in fecal samples, all 53 isolated CoNS were penicillin V–resistant, and between 3 and 19 exhibited MDR (Table S2, online only); *mecA* ($n = 15$), *erm*(C), *tet*(M) ($n = 4$), and *tet*(K) ($n = 6$) were identified.[63] CoNS isolates from feces carrying TET-, macrolide-, and aminoglycoside-resistance genes can transfer them via inter- and intraspecies routes, disseminating MDR in *Staphylococcus* spp.

Senegal. A low prevalence of MRSA (10.5%) has been reported in Senegalese pigs compared with that reported in developed countries. This might be due to a lower veterinary antibiotic use as growth promoters and/or for therapy. However, all the isolates were resistant to penicillin; 27 were resistant to cotrimoxazole; and 16 were resistant to TET.[66] The dominant MRSA clones were ST5 ($n = 5$) and ST88 ($n = 1$).

Cape Verde. In Cape Verde, a low prevalence of 5.6% (6/107) MRSA nasal carriage was documented in 2015, dominated by ST5 ($n = 3$), ST8 ($n = 1$), and ST88 ($n = 2$). These isolates showed significant levels of resistance to ERY, SXT, and penicillin G (PEN).[67]

Staphylococcus spp. (*S. aureus*, *S. haemolyticus*, and *S. saprophyticus*) in Central Africa.

Gabon. In Gabon, *S. aureus* isolated from colonized persons, blood, as well as soft and skin tissue infections, had 49% (104/212) resistance to trimethoprim; *dfrA* ($n = 1$), *dfrG* ($n = 100$), *dfrK+G* ($n = 1$), *dfrB* ($n = 2$), and *mecA* ($n = 1$) were detected in the isolates.[68] Thus, *dfrG* is the most abundant and common trimethoprim resistance mechanism in Africa, overtaking the *dfrB* mutation as the main mechanism of resistance to trimethoprim.[69–71]

D.R. Congo (D.R.C.). A total of 215 (79.3%) drug-resistant *S. aureus* isolates were collected between 2015 and 2017 from nasal swab and bloodstream infections; 70 isolates were MRSA. Other major genes mediated resistance to SXT, aminoglycosides, macrolides, TET, PEN, and CHL included *dfrG* (≥120), *tet*(K) (≥98), and *femA* (≥98). MRSA showed high-level resistance to β-lactams, aminoglycosides, macrolides, and TET, and caused severe infections, such as pneumonia, meningitis, complicated urinary tract infections, gynecological infections, and peritonitis. *S. aureus* ST8 (≥47) was the most common, followed by ST152 (≥17), ST5 (≥2), and ST88 (≥2). MRSA ST8 outnumbered MRSA clone ST88, which is dominant in Africa. The *femA* Y195F mutation mediates high-level OXA resistance in the D.R.C., while *dfrG* is responsible for high-level trimethoprim resistance; SXT is administered prophylactically to immunosuppressed patients to prevent opportunistic infections, such as *Pneumocystis carinii* pneumonia, toxoplasmosis, and bacterial pneumonia.[72] Additionally, there was high-level MDR among MRSA, which is a great concern, as microbiological laboratories and second-line antibiotics are rare in the D.R.C.

Staphylococcus spp. (*S. aureus*, *S. haemolyticus*, and *S. saprophyticus*) in East Africa.

Kenya. In contrast to earlier studies done in Kenya, Omuse and colleagues detected a wide genetic diversity of MRSA and well-established epidemic MRSA clones among clinical isolates. MRSA clonal complexes 5, 22, and 30, implicated in several outbreaks, were described. These clones included ST5 ($n = 1$), ST8 ($n = 2$), ST22 ($n = 4$), ST88 ($n = 1$), ST241 ($n = 12$), ST239 ($n = 2$), and ST789 ($n = 1$). Approximately, 41% of the MRSA in the study were MDR (Table S2, online only) and showed resistance to CLI, ERY, and SXT.[73]

Tanzania. In a study to investigate the molecular epidemiology of trimethoprim resistance in MSSA causing skin and soft tissues infections, *dfrG* was detected in all 32-trimethoprim-resistant isolates. Other reported trimethoprim-resistance mechanisms, such as *dfrA*, *dfrB*, and *dfrK*, were missing, confirming *dfrG* as the main trimethoprim resistance mechanism in sub-Saharan Africa.[68] *S. aureus* isolated from bovine milk had high rates of resistance to SXT, TET, and PEN, with two of the isolates being resistant to VAN.[74]

Uganda. An MRSA carriage of 56.1% (23/41) was detected in milk from pastoral communities in Uganda, 70% of which were TET-resistant; clones ST97 and ST1 were identified. Furthermore, over 90% of the isolates carried genes encoding enterotoxin, which causes food-borne diseases. The weak veterinary delivery system and the high dependency on animals and animal products for food in Uganda were implicated in the high prevalence of MRSA.[75]

S. aureus isolates, including 24 MRSA and 40 MSSA, were isolated from patients with surgical site infections (SSIs); the MRSA isolates were MDR (including resistance to OXA, GEN, CIP, and CHL).

Inducible CLI resistance was found in 17.2% of the isolates, mostly in MRSA. In a multivariate analysis, inducible CLI resistance and cancer were identified as independent predictors of MRSA-SSI.[76] In a similar study, out of 300 clinical *S. aureus* isolates, 22 were resistant to VAN, 187 were SXT-resistant, and 143 were resistant to ERY.[77]

***Staphylococcus* spp. (*S. aureus*, *S. haemolyticus*, and *S. saprophyticus*) in Southern Africa.**

Angola. Conceiçao *et al.* reported a nasal *S. aureus* carriage of 23.7% ($n = 128$), out of which 58.1% ($n = 77$) were MRSA. Fifty-seven of the MRSA clones were of ST5, followed by ST88 ($n = 9$), ST8 ($n = 5$), and ST72 ($n = 3$). This study represents the first description of the spread of MRSA ST5 in Africa. All the 77 MRSA strains were resistant to SXT, FOX, and PEN.[78] In a study to identify OXA-susceptible *mecA*-positive *S. aureus* (OS-MRSA) for the first time in Africa, a prevalence of 17.7% was detected among healthy healthcare workers in Angola and São Tomé and Príncipe, making them potential OS-MRSA reservoirs.[79] The OS-MRSA isolates displayed MDR (Table S2, online only) and were characterized as being ST88 ($n = 15$) and ST8 ($n = 9$). In sub-Saharan Africa, identifying clinically important *S. aureus* is heavily based on phenotypic agar-screening and OXA disk–diffusion methods. Overall, ST5 ($n = 106$), ST ($n = 79$), ST88 ($n = 46$), and ST72 ($n = 10$) were the dominant resistant *S. aureus* clones.[80,81]

Mozambique. The prevalence of HA-MRSA and CA-MRSA in Mozambique was found to be 15.1% and 1%, respectively. MRSA showed high-level resistance to PEN, FOX, GEN, CIP, ERY, SXT, CHL, and TET, compared to MSSA. Additionally, inducible macrolide–lincosamide–streptogramin B (MLS_B) resistance was 41.7% and 10.7% in hospital-acquired *S. aureus* and community-acquired *S. aureus* isolates, respectively,[82] further limiting therapeutic options for *S. aureus* infections. This study, which was the first to detect the emergence of HA-MRSA within postoperative abdominal and burn wounds in Mozambique, reported that patients with infected burn wounds had a significantly longer hospitalization than patients with postoperated abdominal wounds.

Namibia. The dominant resistance gene mediating trimethoprim resistance in MRSA and MSSA in Namibia was *dfrG*, similar to reports in other Africa countries.[68] Moreover, *dfrG* was frequently detected in *S. aureus* from SSTIs in travelers returning from other African countries, suggesting that *dfrG* can be transmitted in populations with low antifolate resistance, such as found in North America and Europe.[83,84]

South Africa. Thirty MDR *S. aureus* isolates were recovered between April 2015 and April 2016 from 10 beaches in the Eastern Cape Province, South Africa (Table S2, online only). Notably, the isolates harbored *mecA*, *femA*, *rpoB*, *blaZ*, *erm*(B), and *tet*(M),[15] making marine environments and public beaches potential depositaries of MDR *S. aureus* that can be transmitted to animals and humans. Further, the 50% resistance to VAN recorded is concerning to global health as it is a last-resort antibiotic for treating MRSA infections.

S. aureus was detected in raw and pasteurized milk at prevalence of 75% and 29%, respectively, likely because of inefficient thermal processing and postprocess contamination. A high proportion (60–100%) of these isolates showed resistance to aminoglycosides, β-lactams, VAN, TET, and ERY, but only 19 isolates were found to carry *mecA*.[85] Both raw and pasteurized milk can harbor MDR *S. aureus*, exposing consumers to colonization and/or infections. Again, *Staphylococcus* spp., including *S. aureus*, *S. haemolyticus*, *Streptococcus xylosus*, and *Streptococcus capitis*, were isolated from healthy pigs and cattle, of which 75–100% were resistant to PEN, TET, SXT, and nalidixic acids, owing to their use as growth promoters in animals; *mecA* and *mphC* were identified. Additionally, 12% of the isolates were resistant to VAN and ERY, evincing the important role of animals in the dissemination of resistance determinants and of commensals to public health.[86]

Van Rensburg *et al.*[87] detected 43.4% (1432/3298) and 3.1% (328/10,448) RIF resistance among MRSA and MSSA, respectively. Similar studies in South Africa have also reported on high RIF resistance in MRSA,[88,89] due to the frequent use of RIF among the relatively high number of tuberculosis patients in South Africa. MRSA ST5 and ST612 were detected, while *rpoB* H481Y/N and I527M mutations were associated with high RIF resistance, similar to reports in Italy;[90] additionally, novel H481N, I527M, and K579R mutations were also detected.

Three studies reported the prevalence of 29.1%,[91] 45.44%,[92] and 100%[93] MRSA recovered from humans; the MRSA expressed resistance to macrolides, TET, aminoglycosides, cotrimoxazole, and RIF. MRSA ST612, ST239, ST36, and ST5 were the dominant strains, similar to other findings in Australia and Europe.[94] The study showed that *S. aureus* bacteremia is common and accounts for high mortality in South Africa. The study by Perovic *et al.*[91] came to a similar conclusion showing that 202 patients died from *S. aureus* bacteremia infections, with HIV patients being more likely to acquire HA-MRSA. The isolates were, however, susceptible to glycopeptides, fluoroquinolones, linezolid, tigecycline, fosfomycin, and fusidic acid.

In another recent study, a high prevalence and genetic diversity of multidrug efflux resistance genes were found in clinical *S. aureus* isolates, including 81 MRSA and 16 MSSA.[95] *norA*, *norB*, *mepA*, *tet*(38), *sepA*, *mdeA*, *imrs*, and *sdrM* were present in at least 86% of the isolates, predicting resistance to broad-spectrum of biocides and fluoroquinolones. Efforts to develop efflux pump inhibitors may mitigate such resistance mechanisms. Resistant *S. aureus* ST612 ($n = 52$), ST239 ($n = 8$), ST5 ($n = 60$), ST152 ($n = 5$), ST45 ($n = 4$), ST35 ($n = 4$), and ST30 ($n = 3$) have been commonly isolated in clinical samples.[96,97]

São Tomé and Príncipe. MRSA prevalence of 26.9%[98] and 25.5%[67] was reported in nasal swabs in 2014 and 2015, respectively, in São Tomé and Príncipe. Additionally, a high prevalence of OS-MRSA was reported in both São Tomé and Príncipe and Angola.[79] Dominant MRSA clones circulating in São Tomé and Príncipe include ST8 ($n = 34$), ST5 ($n = 13$), ST88 ($n = 28$), ST5 ($n = 3$), ST1 ($n = 2$), and ST105 ($n = 2$).[80,81] High genetic variability was found in MSSA strains. Both MRSA and MSSA showed different levels of resistance to SXT, ERY, CIP, and TET; however, all the MRSA isolates were resistant to FOX.

***Streptococcus* spp. (*S. pyogenes*, *S. pneumoniae*, and *S. agalactiae*).** Drug-resistant *Streptococcus* spp., including *S. agalactiae* and *S. pyogenes*, have been identified in Northern, Eastern, and Southern Africa. *S. pyogenes* were reported in humans only, while *S. agalactiae* was reported in both animals (camels) and humans, with a high prevalence of resistance to TET and ERY.

Algeria. A single study has so far detected 44 TET (100%, 44/44)- and ERY-resistant (43.18%, 19/44) *S. agalactiae* from vaginal swabs; *tet*(M) and *erm*(B), respectively, mediated resistance. A high diversity of resistant clones, namely, ST1, ST19, ST10, ST158, ST166, ST233, ST460, ST521, and ST677, were detected,[99] which have been reported worldwide for causing life-threatening invasive diseases, such as meningitis and sepsis.[100,101]

Egypt. Similarly, Shabayek *et al.* detected 98% and between 14% and 17% *S. agalactiae* resistance to TET and macrolides, respectively. *tet*(M) was detected in all 98 TET-resistant isolates, while *erm*(B) and *erm*(A) mediated ERY resistance. Efflux pump genes, such as *tet*(K) ($n = 12$), *tet*(L) ($n = 1$), and *mefA/E* ($n = 1$), were also found.[102,103] This study also showed that VAN and fluoroquinolones are effective replacement for ERY and CLI, as well as for patients allergic to PEN. Although PEN is the antibiotic of choice for treating *S. agalactiae* infections, reports of PEN resistance in the United States and China call for increased surveillance.[103]

Tunisia. From January 2007 to December 2009, 226 *S. agalactiae* samples were isolated from females (genital) and gastric fluid of infected newborns. Of these, 97.35% (220/226), 40% (90/226), and 19.1% (43/226) were resistant to TET, ERY, and RIF, respectively. Additionally, seven isolates were resistant to aminoglycoside (GEN and STR) and CHL. *tet*(M) ($n = 205$) was the main TET-resistance mechanism and was significantly associated with Tn*916* ($P = 0.0002$). Other resistance genes, including *erm*(B) ($n = 79$) and *tet*(O) ($n = 50$), were detected. All isolates were, however, susceptible to β-lactams and quinupristin–dalfopristin.[104] Between 2005 and 2007, 160 ERY-resistant *S. agalactiae* were isolated from humans, with a high resistance rate of 84.3% (135/160) to MLS_B.[105]

North Africa, Tunisia. Hraoui *et al.* reported a low macrolide resistance rate (5%, 5/103) and a high TET resistance rate (70%, 72/103) among human isolates, with *tet*(M), associated with Tn*916*, being responsible for TET resistance.[106] Increased TET use in food animals was implicated in this instance, leading to the selection and dissemination of resistance genes from animals to humans. Macrolide resistance was detected in seven isolates, which was corroborated by the findings of Ksia *et al.*, who detected low-level macrolides

resistance among children.[107] *erm*(B), *tet*(M), and *mefA* were the other resistance genes found in the clones *emm*18 (4), *emm*42 (9), *emm*76 (6), and *emm*118(10).[108]

East Africa, Kenya. In the horn of Africa, camels play significant roles in human survival by providing milk, meat, and transportation. In 2013, Fischer *et al.* detected 36% (37/92) TET resistance in *S. agalactiae* isolates from camel wound infections and mastitis that was mainly mediated by a Tn*916*-borne *tet*(M). ST616 ($n = 22$) was the major resistant clone, followed by ST612 and ST617.[109]

South Africa. In South Africa, *S. agalactiae* colonization of 30.9% was detected from vaginal and rectal swabs of pregnant women. Similar to other reports elsewhere in Africa, high rates of TET (94.5%, 120/128) and macrolide (21.1%, 27/128) resistance were documented. All isolates were, however, sensitive to PEN, AMP, VAN, and GEN. Macrolide and CLI resistance were associated with the presence of *erm*(B) and *mefA*.[110]

Enterococcus spp. (*E. faecium*, *E. faecalis*, *E. hirae*, *E. durans*, and *E. gallinarum*) in North Africa.

Algeria. Enterococcus spp. from urinary tract and wound infections in Algeria revealed a prevalence of resistance ranging from 51.4% to 92.5% to ERY, TET, CIP, and GEN. Only 2.9% (6/210) were VRE, confirming glycopeptides as ideal antibiotics for treating *Enterococcus* infections (an attributable mortality rate of 10% was reported); *erm*(B) ($\geq$92) and *vanC-1*($\geq$7) were the main mechanisms of resistance. A high genetic diversity among strains was seen in *E. faecium* and *E. faecalis*, with *E. faecium* ST78 ($n = 31$) and ST17 ($n = 16$) being the dominant resistant strains. A novel ST317 ($n = 33$) clone was predominant among the *E. faecalis* isolates.[111,112]

Egypt. In a similar study to characterize *E. faecium* and *E. faecalis* from patients, 82% of isolates were MDR, showing high-level resistance to aminoglycosides, β-lactams, and TET. *VanA* ($n = 13$), *vanB* ($n = 3$), and *VanC-2/3* ($n = 3$) were detected in five *Enterococcus* isolates, all were resistant to the antibiotics tested. Bioinformatic (sequence) analyses revealed that *vanA* was transmitted horizontally to *S. aureus*, showing the importance of horizontal gene transfer considerations during the management of enterococci infections, such as bacteremia, endocarditis, and urinary tract infections.[113–115] Similarly, high-level resistance to AMP, CLI, ERY, TET, GEN, CIP, and VAN was reported in *Enterococcus* spp. isolated from chicken, ducks, pigs, fish, and raw milk cheese. *VanA* ($n = 23$), *vanB* ($n = 27$), *vanC* ($n = 38$), and *tet*(M) ($n = 6$) were the dominant resistance genes;[116–118] Tn*916* ($n = 7$) was the MGE detected.

Tunisia. Antibacterial-resistant *Enterococcus* was found in feces of pets and camels, wild birds, irrigation water from farm environments, food vegetables, hospital environments, animal meat, and patients in Tunisia.[119–126] Resistance to VAN, macrolides, aminoglycosides, β-lactams, and TET was detected in the environment, animals and humans, with the majority of the isolates being *E. faecium*, followed by *E. faecalis*. *Tet*(M), *tet*(L), *erm*(B), *ant (6)-la*, *vanA*, and *aph(3′)-llla* were the major resistance mechanisms, with IS*16* being the main MGE disseminating the resistance gene. *E. faecium* ST80, ST910, and ST16, and *E. faecalis* ST848 and ST9 were the dominant resistant clones in Tunisia, showing that meat, animals, pets, hospital environment, and wastewater used for farm irrigation may play a crucial role in the spread of antibiotic-resistant *Enterococcus*.

Enterococcus spp. (*E. faecium*, *E. faecalis*, *E. hirae*, *E. durans*, and *E. gallinarum*) in Nigeria.

Enterococcus spp. isolated from poultry and cattle, as well as their manure, demonstrated high-level resistance to TET, ERY, GEN, AMP, and STR. Sixty isolates were MDR, showing resistance to three or more antimicrobials.[127] Additionally, 48.3% (29/40) of the Enterococcus isolated from chicken feces were resistant to VAN. IS*256* was the main MGE identified.[128] The rate of MDR is a reflection of the substantial use of broad-spectrum antibiotics in Nigeria, raising major public health concerns as practices, such as the use of untreated poultry and cattle manure for fertilizing agricultural soils, particularly vegetables, are a common practice in Africa, which could transfer MDR Enterococci to humans.

Ngbede *et al.* recently characterized 63 AMP- and 37 GEN-resistant *E. faecium* from vegetables, soils, farms, animals, and manures.[129] Approximately 95% (35/37) and 8% (5/63) of the aminoglycoside- and AMP-resistant clones were recognized as

aminoglycoside- and AMP-resistant *E. faecium*, respectively. Genes including *aac(6′)-Ie-aph(2″)-Ia)*, *aph(2′)-1c*, *aph(3′)-llla*, and *ant(4′)-la* accounted for aminoglycoside resistance. Thirteen *Enterococcus* spp. isolated from clinical samples were resistant to VAN and harbored *vanA* ($n = 1$), *vanB* ($n = 2$), *vanC-1* ($n = 9$), and/or *vanC-2* ($n = 1$).

***Enterococcus* spp. (*E. faecium*, *E. faecalis*, *E. hirae*, *E. durans*, and *E. gallinarum*) in Tanzania.** Antibiotic resistance in wild animals, such as buffalo, zebra, and wildebeest, was found to be higher than in cattle, although wildlife is periodically treated with antibiotics. Ten VRE- and AMP-resistant *Enterococcus* were found in the wild animals, but not in cattle. Additionally, Enterococcus isolates from wildlife were highly resistant to TET, RIF, macrolides, aminoglycosides, and cotrimoxazole.[130] *Enterococcus* spp. isolated from humans, cattle, and cattle waste also expressed high-level resistance to GEN, ERY, RIF, SXT, and TET. Nine isolates from cattle and 59 from humans were VAN resistant; *vanA* ($n = 3$), *vanB* ($n = 3$), *tet*(W), and *sull* were identified.[131] The practice of cograzing possibly results in the transmission of antibiotic resistance genes from livestock to wildlife. The relatively high presence of antibiotic resistance bacteria in wildlife was likely due to contact with environmental surfaces that have been contaminated with humans, birds, or animal excreta. Results from this study demonstrate the presence of antibiotic resistance enterococci in wild animals without antibiotic pressure.

***Enterococcus* spp. (*E. faecium*, *E. faecalis*, *E. hirae*, *E. durans*, and *E. gallinarum*) in South Africa.** Multiple antibiotic resistance enterococci were isolated from borehole water, wastewater, pigs, and humans in South Africa. Notably, relatively high-levels of VAN, aminoglycoside, β-lactam, macrolide, and fluoroquinolone resistance were detected among the enterococci isolates, compared with isolates from other countries. *Erm*(B) (≥348), *vanC*-2/3 (162), *vanB* (≥140), *vanC* (≥120), and *strA* (≥120) were the major resistance genes. The VAN-resistant isolates were from patients with hematological malignancies, bacteremia, pigs, wastewater, and underground water.[13,14,17,132] *E. faecium* ST25 and ST23 and *E. faecalis* ST23, ST25, and ST780 were resistant clones isolated from sewage water, treated effluent, and hospital waste.[133,134] Inefficient chlorination to kill bacteria accounted for the high resistance rates in the final effluents discharged into the environment. Subtherapeutic antibiotic usage in animal feed also accounted for the emergence of antibiotic resistance in pigs, while the construction of boreholes near pit toilets resulted in high enterococcal isolation and resistance rates in South Africa.

Discussion

Among Gram-positive bacteria, MRSA, VISA, VRSA (VAN-resistant *S. aureus*), VRE, PEN-resistant, or PEN-intermediate resistant *S. pneumoniae* have been identified by the WHO as high- and medium-priority pathogens needing urgent attention with regard to antibiotic resistance research and antimicrobial discovery.[4] Quinolone-resistant *S. pneumoniae* infections also pose a great challenge to clinicians.[135] For such pathogens, important antibiotics, such as linezolid, daptomycin, and streptogramins (e.g., Synercid, virginiamycin, and pristiniamycin), are critical reserve agents that provide useful alternatives to compounds that are ineffective.[135] Except for quinolone– and PEN–nonsusceptible *S. pneumoniae*, MRSA, VISA, VRSA, and VRE were found in few countries in Africa (Tables S1–S4, online only), mainly in Angola, Egypt, Nigeria, South Africa, Tunisia, and Tanzania, and in humans, animals, and the environment. Concerning as this is, it is perhaps comforting to note that the overall MRSA prevalence was 13.4% (Table 1) and is within the range reported recently by the WHO for Africa (12–80%) and another review,[22] but lower than that of other WHO regions, such as parts of the Americas, Asia, Europe, and the Western Pacific.[4] The prevalence of MRSA Africa is also lower than the minimum rate of 20% in most countries;[4] however, in some African countries, the prevalence was higher (Tables S1–S6 and Fig. S1A–O, online only).

Overall, the resistance of Gram-positive bacteria isolated from humans (ranging from 0.2% to 96.6%), animals (4.2–100%), and the environment (6.7–91.9%) was both country and antibiotic specific. Thus, but for higher resistance rates to, for example, PEN, TET, ERY, and GEN (Fig. 2B), the prevalence of Gram-positive bacteria resistance to reserved antibiotics, such as linezolid, VAN, daptomycin, and streptogramins in Africa, is not that dire.

Nevertheless, the report on pristiniamycin resistance in *S. aureus* in Tunisia is a cause for concern; continuous surveillance and education on antibiotic stewardship is necessary to preserve the efficacy of particularly reserved antibiotics. On the other hand, the limited molecular diagnostics and molecular skill available in Africa may mean that many important resistance determinants could go undetected. More advanced studies and thorough molecular surveillance studies in African countries could increase these resistance rates reported in our review, specifically to reserve antibiotics. The absence of PEN-nonsusceptible *S. pneumoniae* in Africa is a good sign, given the very high mortalities attributed to pneumonia and pneumococcal meningitis in infants and geriatrics in Africa.[4] Contrary to this finding, however, PEN-nonsusceptible *S. pneumoniae* strains were found in all WHO regions.[4]

Although relatively few *vanA* genes (Table 1) in VREs were found in five countries, their prevalence was relatively high; in environmental samples, 10.6% (Tunisia) and 29.4–74.0% (South Africa) demonstrated the presence of *vanA* genes, while in animals, 5.7–8.3% (Tanzania), 46.2% (Egypt), 65% (Nigeria), and 100% (South Africa) were detected. In humans, 2.5% (South Africa), 3.0–3.5% (Egypt), 7.0% (Algeria), 20.8% (Tunisia), and 31.5–44.3% (Tanzania) were reported. These rates show a high prevalence of VREs in animals and humans in the respective countries, posing serious health threats. These rates are also no better than that reported in Europe,[136] and go to suggest that VREs are on the rise globally and should be given much priority in future molecular surveillance studies. Coupled with VREs are VISAs and VRSAs, which are difficult-to-treat pathogens. MDR VREs increase VRE-associated mortality rates above figures caused by sensitive enterococci strains.[17,137] As well, the evolution of macrolide resistance in drug-resistant streptococci is limiting treatment options and resulting in high mortalities.[106,138,139] These data support the need to prioritize antibiotic stewardship and increase One Health molecular studies across Africa to quickly identify and preempt full-scale outbreaks and dissemination of pathogens.

The antibiotic resistance gene frequencies reported largely mirror the antibiotic resistance rates (Fig. 2) for PEN, ERY, and TET. Discrepancies between antibiotic resistance gene frequencies and antibiotic resistance rates could be due to the unbalanced detection of antibiotic resistance genes in all isolates in the included studies, which could be influenced by financial and molecular skill challenges. Interestingly, although a lesser number of Gram-positive bacteria were isolated from environmental sources, they expressed higher antibiotic resistance rates than those from animals and humans. This is despite the higher prevalence of antibiotic resistance genes among human isolates than found in animal or environmental strains (Table 1), which can be explained by the fact that, although the environmental samples were few, most were resistant, while fewer of the larger number of human isolates were resistant. This also underscores the fact that there are increasing antibiotic resistance genes in the environment, which could be due to antibiotic pollution from human activity.[11]

The number of studies sampling humans, animals, and environmental specimens for antibiotic resistance research was woefully inadequate. As well, conjugation studies and bioinformatics analysis to establish the mobility of MGEs and antibiotic resistance genes within and between species, animals, humans, and the environment were lacking, making it difficult to establish the dissemination of antibiotic resistance genes through MGEs from the environment to animals and humans or vice versa. Some studies did, however, establish the association of IS*16*, Tn*916*, and SCC*mec* with *erm*(B), *tet*(M), and *mecA*, respectively, in *E. faecium* (ST18, ST80, and ST910), *S. agalactiae* (ST612, ST616, and ST617), *E. faecalis*, and *S. pyogenes* (*emm*18, *emm*42, *emm*76, and *emm*118) isolated from humans, animals, and the environment. These limitations affect efficient analyses of the role of MGEs in antibiotic-resistant bacteria from a One Health context and further evince the need for genomics-based epidemiological surveillance studies that comprehensively describe the genetic context of antibiotic resistance genes and associated MGEs.

Important international and local clones as well as novel clones of the various species were identified from all three sources. The presence of same clones in almost all the countries from human, animal, and environmental sources suggests that undertaking multicenter One Health molecular studies will yield interesting results. In particular, the widespread distribution of *S. aureus* ST5, *E. faecium*

ST18, ST80, and ST910, *E. faecalis*, and *S. agalactiae* harboring *mec*A, *tet*, and *erm* shows that the spread of antibiotic resistance in Africa is partly, if not totally, clonally mediated. For instance, Djoudi *et al.*,[140] van Rensburg *et al.*,[87] and De Boeck *et al.*[141] in Algeria, South Africa, and Democratic Republic of Congo, respectively, reported on resistant *S. aureus* ST5 in humans, while Fall *et al.*[66] reported on the same clone in pigs from Senegal. Further, Mariem *et al.*[48] isolated the same clone (*S. aureus* ST5) from the environment in Tunisia, suggesting that this clone is widely distributed in Africa in humans, animals, and environment (Figs. 5 and 6). Specifically, *S. aureus* ST5 is among the frequently reported clones in Asia,[142] and recent evidence suggests that it has spread from hospitals into communities, resulting in CA-MRSA.[143] Similarly, Lochan *et al.*[132] isolated resistant *E. faecium* ST80 from humans in South Africa, and this clone was reported for the first time by Dziri *et al.* from environmental samples in a Tunisian hospital[144] and by Elhani *et al.*[124] also in Tunisia. Transmission of this resistant clone to animals is not yet reported, which implies that these resistant species and clones are circulating between humans and the environment, underpinning the broad host range and transmissibility of these strains between humans and the environment.

Owing to the finer resolution of whole-genome sequencing (WGS) over multilocus sequencing, isolates identified as different STs/clones were in some cases shown to be very closely related rather than being of the same clone. Figure 6 shows the possible evolution of certain strains/clones from their ancestors in other countries and even from animals or the environment to humans and vice versa (e.g., see *S. agalactiae* in Kenya, Somalia, and Central African Republic). This pylogenomic relationship between strain and their evolution over time, as they jump between animals, humans, and the environment in same and different countries, portrays the power of WGS-based phylogenomics in epidemiological analyses and the usefulness of undertaking One Health studies to trace the dissemination and sources of resistance and infection outbreaks.

Comparing our analysis to other recent systematic reviews in Africa[6,21,22] and the recent WHO Global Antimicrobial Resistance Surveillance System (GLASS) report,[3] there is close alignment and specific differences. For instance, in five West African countries, Bernabé *et al.*[21] reported 82.7% (95%, CI = 66.9–94.5) resistance to PEN, 44.7% (95%, CI = 29.5–60.3) resistance to SXT, 30.6% (95%, CI = 11.3–54.0) resistance to cloxacillin, 23.0% (95%, CI 4.7–49.1) resistance to CIP, and 19.6% (95%, CI = 10.1–31.2) resistance to ERY. These rates are higher than that obtained in some countries, such as Cape Verde, and lower for ERY, cloxacillin, and SXT than that obtained for *S. aureus* or *E. faecium/faecalis* in countries, such as Algeria and Tunisia (Tables S4–S6 and Fig. S1A–O, online only). Thus, the resistance rates vary by country for species and antibiotics, as recently shown in the WHO GLASS report, making it difficult to provide an overarching conclusion between continents and countries, except for specific antibiotics and species. For instance, OXA and FOX resistance rates among *S. aureus* in the Philippines were 60–70%, while it was lower in 20 African countries included in our study, though not in Kenya (84.1%) and Algeria (100%).[3] There were minor and major conflicts between the WHO GLASS report's findings and some studies reported above, particularly with *S. aureus* in South Africa: as examples, the WHO GLASS report found 25% resistance to FOX, while our analysis found 22.5%; WHO GLASS had 25% and 50% penicillin G and cotrimoxazole resistance rates, respectively, while our analysis found 39.4–96.7% and 11.7–43.3%, respectively. Some of these differences stem from the different study periods and studies included in the respective analyses. The differences further buttress the picture of changing resistance rates over time due to different factors in the healthcare, veterinary, and environmental sectors of each country.

Conclusions: future perspectives and study limitations

Several resistance mechanisms in *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. from environmental, animal, and human sources are driving antibiotic resistance in several African countries; in particular, *S. aureus* ST5, ST8, and ST80; *E. faecium* ST317, ST51, and ST910; *E. faecalis ST78*; and *S. agalactiae* ST616 clones; as well as SCC*mec*, Tn*916*, IS*256*, and IS*16* MGEs were prevalent in most of the included studies. Gram-positive bacterial resistance in Africa is antibiotic and species specific and varies from country to country, which is reflective of what has been observed in

other countries. Clonal and polyclonal outbreaks of drug-resistant strains in different countries were observed to disseminate resistance among animals, humans, and the environment. Many factors in the included countries affect selection for resistance among different species and to different antibiotics used, which are now ineffective and require urgent attention to remedy the situation of increasing antimicrobial resistance. These threats to clinical medicine, economy, and socioeconomic development call for a One Health approach, as well as national and international rules and regulations to contain the problem. While resistance to important antibiotics, such as daptomycin and linezolid, was not found, continued surveillance for it should be done to prevent escalation and dissemination.

Multicenter One Health studies that investigate the molecular epidemiology and the evolution and resistance mechanisms of antibiotic resistance among Gram-positive bacteria in all African countries are necessary to fill the numerous antibiotic resistance gaps on the continent. Such studies should involve the use of WGS to characterize genomic epidemiology and phylogenomics, plasmid mobility and evolution, and other MGEs associated with resistance determinants in isolates from animals, humans, and the environment. Such studies would inform important sectors of the possible dissemination of antibiotic resistance along the farm-to-fork continuum. They would also identify emerging and reemerging resistance mechanisms, zoonoses, pathogens, and opportunistic pathogens in clinical and nonclinical environments to inform public health interventions.

Effective surveillance and monitoring of antimicrobial drug usage and licensing, banning or restricting the prescription of reserved, expired, and substandard drugs, periodic monitoring of pharmacies and veterinary shops, and antibiotic stewardship are recommended measures to contain antibiotic resistance. Periodic monitoring of patients on hemodialysis is crucial as they are at increased risk of *S. aureus* infection due to periodic hospitalization, immunosuppression, and high invasive vascular interventions. Implementation of these policies will decrease the rate of antibiotic resistance in Africa, reduce longer hospital stays, and preempt the resort to expensive but toxic antibiotic alternatives, with a concomitant reduction in morbidity and mortality rates.

Our study here was limited by the relatively few articles describing the molecular mechanisms of Gram-positive bacterial resistance and studies that undertook conjugative assays to establish the mobility of the MGEs described. Furthermore, most of the included publications define sentinel sites that, by their nature, are not representative of whole countries or larger regions. Among Gram-positive bacteria, only *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. were included in our search, and studies reported in non-English languages were excluded. Moreover, the presence of *vanABC* genes in high numbers reported in *S. aureus* in some of the studies is questionable, as such prevalence has never been reported; we therefore recommend caution in drawing conclusions and that additional studies be performed to confirm the data. One Health studies on the molecular determinants of resistance in Gram-positive bacteria in Africa demonstrate the critical need for increased molecular surveillance and more extensive epidemiological studies.

Author contributions

J.O.S. conceived the study; developed the protocol; searched the literature; screened title abstracts and full text; extracted the data; performed quality assessment; designed the tables and figures; performed data, bioinformatics, and phylogenomic analyses; interpreted the results; and wrote, edited, and formatted the paper for publication. E.M. searched the literature; screened title abstracts and full text; extracted the data; performed quality assessment; analyzed the data; interpreted the results; designed the tables; and drafted the paper. Both authors read and approved the final version for submission.

Supporting information

Additional supporting information may be found in the online version of this article.

File S1. Raw data and analysis of extracted information from included articles.

File S2. List of excluded articles on the basis of only phenotypic (antibiotic sensitivity) tests.

File S3. Metadata of strains used for phylogenomic analyses of Figures 5 and 6.

Figure S1A–O. Resistance rates of antibiotics per country, source, and species in Africa (2007–2019). Isolates from humans (H), animals (A), and the environment (E) per country and species are shown in the image. The different resistance rates per antibiotic per species, source, and country are depicted by the bars.

Table S1. Geographical distribution, species, clones, and resistance mechanisms of antibiotic-resistant Gram-positive bacteria isolated from humans in Africa from 2007 to 2019.

Table S2. Geographical distribution, species, clones, and resistance mechanisms of antibiotic-resistant Gram-positive bacteria isolated from animals in Africa from 2007 to 2019.

Table S3. Geographical distribution, species, clones, and resistance mechanisms of antibiotic-resistant Gram-positive bacteria isolated from the environment in Africa from 2007 to 2019.

Table S4. Antibiotic resistance rates of various Gram-positive bacteria species isolated from human sources in Africa from 2007 to 2019.

Table S5. Antibiotic resistance rates of various Gram-positive bacteria species isolated from animal sources in Africa from 2007 to 2019.

Table S6. Antibiotic resistance rates of various Gram-positive bacteria species isolated from environmental sources in Africa from 2007 to 2019.

Competing interests

The authors declare no competing interests.

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