Carbapenem-resistant Enterobacterales in patients with bacteraemia at tertiary academic hospitals in South Africa, 2019 - 2020: An update

M Lowe,[1] PhD; **L Shuping,**[1] MPH; **O Perovic,**[1,2] MMed (Microbiol), FC Path (SA) [iD]

[1] *Centre for Healthcare-associated Infections, Antimicrobial Resistance and Mycoses, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa*

[2] *Department of Clinical Microbiology and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*

Corresponding author: O Perovic (olgap@nicd.ac.za)

Background. The emergence of carbapenem-resistant Enterobacterales (CRE) has become a serious and significant public health threat worldwide, owing to the limited antimicrobial therapy options, and the elevated mortality rates associated with these infections.

Objectives. To present an update on the epidemiology of CRE bloodstream infections among hospitalised patients reported under the Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa (GERMS-SA) between January 2019 and December 2020.

Methods. Patients of all ages with CRE bacteraemia were included and isolates, when available, were sent to the reference laboratory for confirmatory testing and molecular characterisation. Multivariable logistic regression analysis was performed to assess factors associated with in-hospital mortality.

Results. We included 2 144 patients with CRE bacteraemia with a median age of 33 (interquartile range 1 - 51) years, of whom 1 145 (54.2%) were male. *Klebsiella pneumoniae* accounted for 79.8% of infections (n=863/1 082), of which 89.5% (n=611/683) were healthcare associated (HA). The most common carbapenemase genes were carbapenem-hydrolysing oxacillinase-48 ($bla_{OXA-48-like}$) (76.8%; n=761/991), New Delhi metallo-β-lactamase (bla_{NDM}) (21.1%; n=209/991) and Verona integron-encoded metallo-β-lactamase (bla_{VIM}) (1.3%; n=13/991). None of the screened isolates with a colistin minimum inhibitory concentration >2 μg/mL harboured the mobilised colistin resistance (mcr)-1 to mcr-5 genes. The crude in-hospital mortality rate was 36.6% (n=377/1 029). Patients aged ≥60 years (v. 1.6 - 9 years) (adjusted odds ratio (aOR) 4.53; 95% confidence interval (CI) 2.21 - 9.28), those with comorbidities (diabetes, malignancy, renal and/or cardiovascular failure) (aOR 1.72; 95% CI 1.17 - 2.52), those with altered mental state (aOR 5.36; 95% CI 3.21 - 8.92) and those with previous antimicrobial use (aOR 1.88; 95% CI 1.27 - 2.77) had increased odds of in-hospital mortality.

Conclusion. The epidemiology of CRE bloodstream infections remained similar compared with the previous surveillance report. Most infections were HA and caused by OXA-48-like carbapenemase-producing *K. pneumoniae* with no plasmid-mediated colistin resistance. Standard infection control measures should be strengthened.

Enterobacterales are Gram-negative bacteria that cause healthcare-associated (HA) and community-associated (CA) infections.[1,2] With the increased use of carbapenems (i.e. doripenem, ertapenem, imipenem and meropenem), the emergence of carbapenem-resistant Enterobacterales (CRE) has become a serious and significant public health threat worldwide.[1,3] The European Centre for Disease Control and Prevention reported that mortality is >50% in patients with CRE bloodstream infections.[4]

Some Gram-negative bacteria can produce carbapenemases (the most common being Guiana extended-spectrum β-lactamase (GES), imipenem metallo-β-lactamase (IMP), *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), carbapenem-hydrolysing oxacillinase-48 (OXA-48-like) and Verona integron-encoded metallo-β-lactamase (VIM), and they are known as carbapenemase-producing Enterobacterales (CPE).[5] These carbapenemases are commonly harboured on plasmids and can easily be transferred between the members of the Enterobacterales family.[4,6] The first CPE were detected in South Africa (SA) in 2011; the KPC and NDM carbapenemases were detected in 2011, followed by the OXA-48-like, GES and VIM carbapenemases in 2012.[7-10] Since then, there has been an increase in Enterobacterales producing one or more carbapenemases.[11] NDM was the most frequently detected carbapenemase in SA from 2011 to 2015.[11-13] However, towards the end of 2015, there was a substantial increase of *Klebsiella pneumoniae* with OXA-48-like variants across north-eastern SA,[14] and this is currently the most frequently detected carbapenemase in the country, as well as in other regions of the world.[5,8,13,15-19] Routine surveillance for monitoring CRE trends in SA hospitals has been conducted since 2015, and findings from this surveillance have been published previously.[13]

We present an updated overview of CRE infections in selected public hospitals in SA from January 2019 to December 2020.

Methods

Study setting and population

This was a cross-sectional study of patients with CRE bacteraemia reported to the Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa (GERMS-SA) from January 2019 to December 2020. Surveillance methods for the programme are detailed elsewhere.[13] Briefly, the study sites were 16 public sector tertiary academic hospitals across four provinces of SA

(Gauteng (*n*=4), KwaZulu-Natal (*n*=7), Western Cape (*n*=3), and Free State (*n*=2)). All patients with CRE bloodstream infections were included in the surveillance programme, and isolates, when available, were sent to the reference laboratory at the National Institute for Communicable Diseases (NICD) for confirmatory testing. Systematic and independent examinations (i.e. audits) of the diagnostic laboratories' records were done to ensure that all cases of CRE bloodstream infection were reported. Surveillance officers collected demographic and clinical information on patients through medical record reviews and patient/caretaker interviews using standard case report forms (CRFs). As patients were not followed up, the in-hospital outcome was documented at the time of CRF completion.

A case of CRE bacteraemia was defined as any patient with Enterobacterales cultured from blood that was resistant to one or more carbapenem (doripenem, ertapenem, imipenem and/or meropenem) or had a positive result for the modified Hodge test according to the Clinical Laboratory Standards Institute (CLSI) guidelines.[20,21] When a patient had an additional Enterobacterales isolated >21 days after the first confirmed laboratory diagnosis, it was regarded as a new case. HA CRE bacteraemia was defined as collection of a positive CRE blood culture specimen ≥3 days after hospital admission or when a patient had any healthcare contact within 1 year before the current admission. CA CRE bacteraemia was defined as the collection of a positive CRE blood specimen within 2 days of hospital admission when there had been no prior healthcare contact. The source of infection was determined by the surveillance officers based on clinical assessment and/or medical records. Underlying conditions were considered as an acute or chronic comorbidity or condition not related to sepsis and included diabetes mellitus, malignancy, renal failure, cardiovascular diseases, hypertension and tuberculosis. HIV status was reported separately.

Phenotypic and molecular characterisation
Among isolates received at the reference laboratory, viable isolates were identified using an automated system, Microflex matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) (Bruker Daltonik GmbH, Germany). Antimicrobial susceptibility testing was performed using the MicroScan Walkaway system (Siemens Healthcare Diagnostics Inc., USA) with the NM44 card (Beckman Coulter Inc., USA). All colistin-resistant isolates (identified with the MicroScan Walkaway system (Siemens Healthcare Diagnostics Inc., USA)) were re-tested and confirmed with the Sensititre instrument (Trek Diagnostic Systems Ltd, UK) using the FRCOL panel (Separation Scientific SA (Pty) Ltd, SA). The minimum inhibitory concentration (MIC) results were interpreted using the 2020 CLSI guidelines.[21]

DNA was extracted from all CRE isolates using a crude boiling method.[16] The extracted DNA was used to screen for carbapenemase genes (bla_{GES} (GES-1 - 9 and 11), bla_{IMP} (IMP-9, 16, 18, 22 and 25), bla_{KPC}, bla_{NDM-1}, $bla_{OXA-48-like}$ (i.e. OXA-162, 163, 181, 204, 232, 244, 245 and 247) and bla_{VIM} (VIM-1 - 36)) by a multiplex real-time polymerase chain reaction (PCR) assay (LightCycler 480 II; Roche Diagnostics Corp., USA).[16] Colistin-resistant CRE isolates (with an MIC >2 μg/mL) were screened for the presence of mobilised colistin resistance (*mcr*)-1 to *mcr*-5 genes by a conventional multiplex PCR.[22]

Data analysis
We conducted a descriptive analysis of demographic and clinical characteristics of all cases of CRE bacteraemia with available data. Data were summarised using percentages and medians with interquartile ranges (IQRs). We conducted univariate and multivariable logistic regression analysis to explore factors associated with in-hospital mortality. We selected variables *a priori* based on their likelihood to contribute to death (e.g. HIV infection, comorbidities, and prior systemic antibiotics (as a proxy for prior infection/s)) and indicator variables for the severity of illness (e.g. mental status and intensive care unit admission). HIV status was included as one of the covariates possibly contributing to mortality, but this information was not available for patients aged ≤18 months; this analysis was therefore limited to patients aged >18 months. We included variables in the multivariable model if the *p*-value for the association was ≤0.2 in the univariate analysis; sex and age groups were included in the final model regardless of the *p*-value. All statistical analyses were done using the Stata statistical software package, version 14 (StataCorp, USA).

Results

Overview of patients with CRE bacteraemia
A total of 2 144 patients with CRE bacteraemia were reported during the 2-year surveillance period (Fig. 1). The majority of the patients were reported from surveillance sites in Gauteng province (64.6%; *n*=1 385/2 144), followed by KwaZulu-Natal (19.6%; *n*=420/2 144), Western Cape (13.9%; *n*=298/2 144), and Free State (1.9%; *n*=41/2 144) provinces.

Clinical characteristics of patients with CRE bacteraemia
The median (IQR) age was 33 (1 - 51) years. Male patients accounted for 54.2% (*n*=1 145/2 113) of the patients with known sex (Table 1). Of the 2 144 reported patients, 49.6% (*n*=1 063) had completed CRFs and 89.5% (611/683) had HA infections. The proportion representing CA infections in the current report was 10.5% (*n*=72) v. 4.8% (*n*=75/1 554) in the previous report[13] (*p*<0.001). Of those with available information on underlying conditions, 52.3% (*n*=489/935) had at least one. Ninety-eight percent (*n*=961/985) had an invasive device inserted at any point from admission to positive specimen collection. Of the 597 patients with HIV status available, 31.0% (*n*=185) were HIV positive. The majority of the patients (61.0%; *n*=569/933) had received systemic antibiotics in the past 6 months. Outcome data were available for 1 029 patients, and the in-hospital mortality rate was 36.6% (*n*=377). This mortality rate was similar to the previous period[13] (37.8%; *n*=489/1 239) (*p*=0.559). Seven hundred and fifty-two patients (73.1%) with outcome data also had outcome date available: the median (IQR) duration from specimen collection to outcome was 8 (4 - 17) days, that for patients who were still admitted was 8 (6 - 14) days, that for discharged patients was 17 (8 - 32) days, and that for patients who died was 4 (1 - 10) days.

Mortality among patients with CRE bacteraemia aged >18 months
Of the 1 552 patients aged >18 months, 763 (49.2%) had outcome data, and 306 (40.1%) of these patients died. A higher proportion of females compared with males died (41.5% v. 38.9%, respectively), and of adults aged ≥60 years compared with children aged 1.6 - 19 years (56.8% v. 22.2%, respectively) (Table 2). In the multivariable logistic regression model, patients aged ≥60 years (adjusted odds ratio (aOR) 4.53; 95% confidence interval (CI) 2.21 - 9.28; *p*<0.001), those with other comorbidities (such as diabetes, malignancy, renal and/or cardiovascular failure) (aOR 1.72; 95% CI 1.17 - 2.52; *p*=0.006), those with altered mental state (aOR 5.36; 95% CI 3.21 - 8.92; *p*<0.001), and those with previous antimicrobial use (aOR 1.88; 95% CI 1.27 - 2.77; *p*=0.001) had increased odds of in-hospital mortality.

Table 1. Clinical characteristics of all patients with carbapenem-resistant Enterobacterales bacteraemia at sentinel surveillance sites reported to the GERMS-SA surveillance programme, 2019 - 2020

Characteristics	Patients reported, n (%)
Sex (n=2 113)	
Male	1 145 (54.2)
Female	968 (45.8)
Age group (n=2 060)	
Neonates (≤28 days)	294 (14.3)
Infants (29 days - 11 months)	213 (10.3)
Children (1 - 19 years)	227 (11.0)
Adults (20 - 59 years)	1 033 (50.1)
Older adults (≥60 years)	293 (14.2)
ICU admission (n=1 063)	
No	660 (62.1)
Yes	403 (37.9)
Clinical presentation or source of infection (n=829)	
Bacteraemia without focus	58 (7.0)
Diarrhoea	22 (2.7)
Lower respiratory tract infection/pneumonia	128 (15.4)
Sepsis/septic shock	147 (17.7)
Skin/soft-tissue infection	189 (22.8)
Surgery	227 (27.4)
Other	58 (7.0)
Comorbidities (n=935)	
No	446 (47.7)
Yes*	489 (52.3)
Mechanically ventilated (n=985)	
No	625 (63.5)
Yes	360 (36.5)
Medical device inserted (n=981)	
No	20 (2.0)
Yes†	961 (98.0)
Type of medical device inserted (n=2 061)‡	
Central venous catheter	400 (19.4)
Drainage port	127 (6.2)
Intravenous line	785 (38.1)
Nasogastric tube	203 (9.4)
Urinary catheter	427 (20.7)
Other	119 (5.8)
HIV status (n=597)§	
Negative	412 (69.0)
Positive	185 (31.0)
Received antibiotics in the past 6 months (n=933)	
No	364 (39.0)
Yes	569 (61.0)
Infection origin (n=683)	
Healthcare associated	611 (89.5)
Community associated	72 (10.5)
Outcome (n=1 029)	
Still admitted	430 (41.8)
Discharged	222 (21.6)
Died	377 (36.6)

GERMS-SA = Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa; ICU = intensive care unit.
*Patients who had any of the following conditions: diabetes mellitus, malignancy, renal failure, cardiovascular diseases, hypertension and tuberculosis.
†637 patients had 1 - 2 devices inserted and 324 patients had ≥3 devices inserted.
‡The total (n=2 061) includes the combined number of medical devices for all patients with devices (n=961).
§Patients aged ≤18 months (n=619) were excluded because data to confirm appropriate diagnostic method (i.e. polymerase chain reaction test results) were not available.

CRE isolates

Of all patients, 50.5% (n=1 082/2 144) had at least one viable isolate and a confirmatory species identity (Fig. 1). Overall, *K. pneumoniae* (79.8%; n=863/1 082) accounted for the majority of infections, followed by *Enterobacter cloacae* complex (5.7%; n=62/1 082), *Serratia marcescens* (5.0%; n=54/1 082), and *Escherichia coli* (4.1%; n=44/1 082). The proportion of Enterobacterales isolated was similar in 2019 and 2020 (Fig. 2).

Susceptibility to a majority of the antibiotics tested was <30% (Fig. 3). Susceptibility to amikacin (69.4%; n=751/1 082), fosfomycin (95.4%; n=1 032/1 082), minocycline (64.4%; n=697/1 082) and tigecycline (80.2%; n=868/1 082) was >60%. Colistin resistance was reported in 18.6% (n=201/1 079) of the isolates, and 81.4%
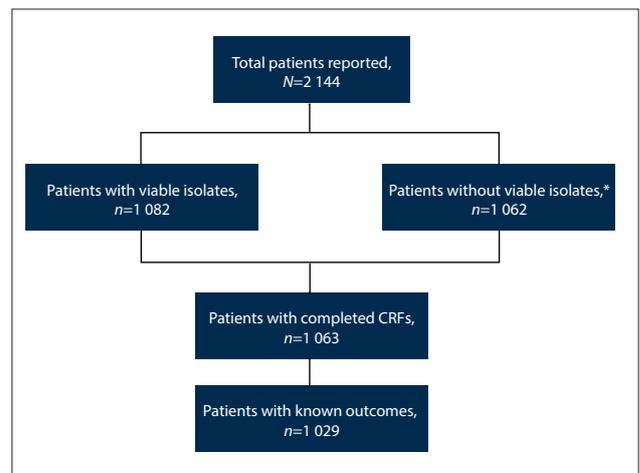
*Fig. 1. Flow diagram for patients with carbapenem-resistant Enterobacterales bacteraemia reported to the GERMS-SA surveillance programme at sentinel surveillance sites in South Africa, 2019 - 2020. (CRFs = case report forms; *Includes cases detected by audit (n=912), non-viable isolates (n=12), missing isolates (n=131), and broken/lost Dorset egg mediums (n=7).)*
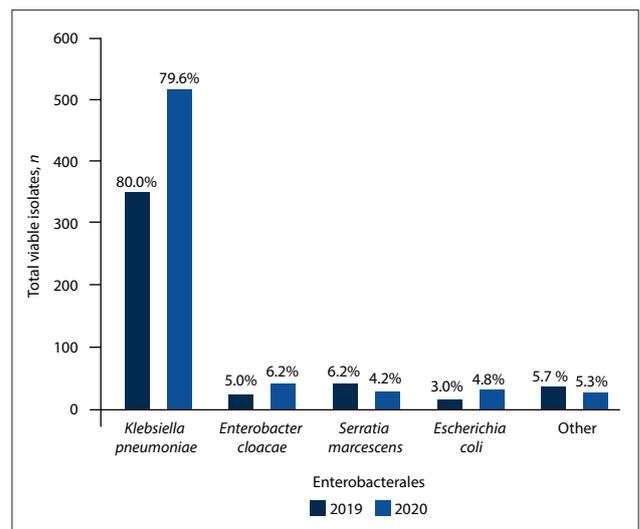
Fig. 2. Carbapenem-resistant Enterobacterales causing bloodstream infections among patients at GERMS-SA sentinel surveillance sites, 2019 - 2020. The percentages of total patients for each year are indicated above the bars. (Other = Citrobacter freundii, Enterobacter aerogenes, Enterobacter asburiae, Enterobacter kobei, Enterobacter species, Escherichia adecarboxylata, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella ozaenae, Klebsiella variicola, Morganella morganii, Proteus mirabilis, Providencia rettgeri and Providencia stuartii.)
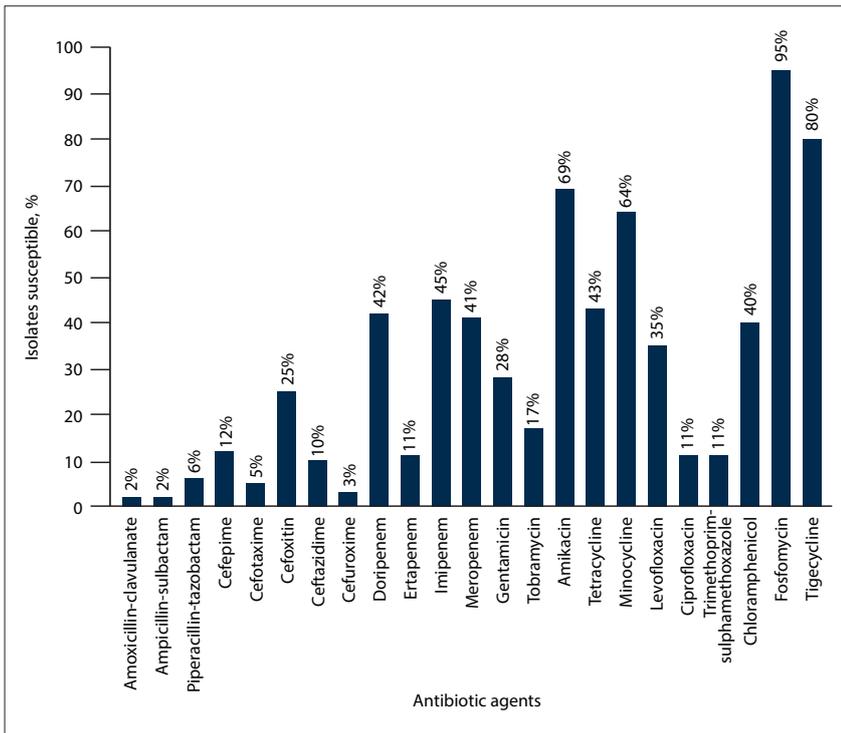
Fig. 3. Antibiotic susceptibility patterns of carbapenem-resistant Enterobacterales isolated from patients with bloodstream infections at the GERMS-SA sentinel surveillance sites, 2019 - 2020. The total percentages of tested isolates that were susceptible are indicated above the bars.
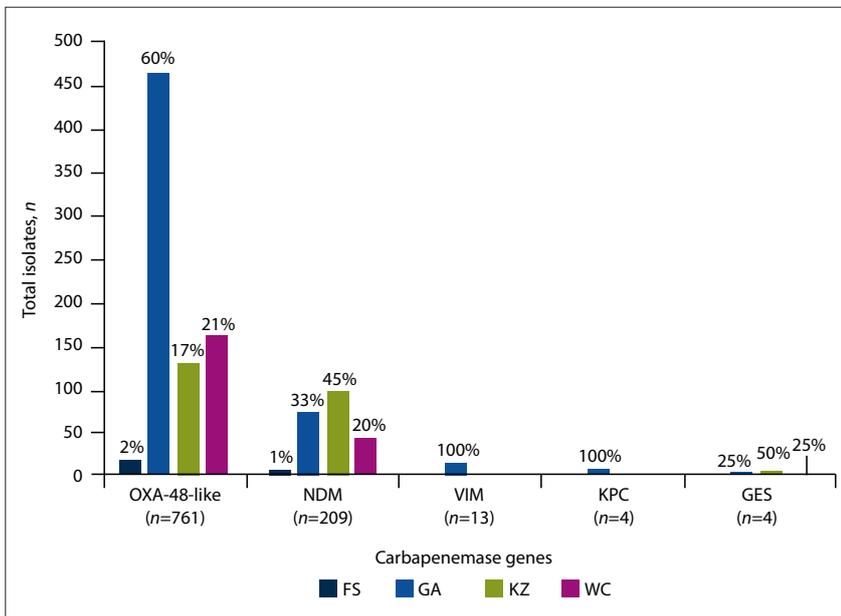
Fig. 4. Carbapenemase genes detected in carbapenem-resistant Enterobacterales from patients with bloodstream infections at the GERMS-SA sentinel surveillance sites, 2019 - 2020. Total percentages of genes detected are indicated above the bars. (FS = Free State; GA = Gauteng; KZ = KwaZulu-Natal; WC = Western Cape; OXA-48-like = carbapenem-hydrolysing oxacillinase-48; NDM = New Delhi metallo-β-lactamase; VIM = Verona integron-encoded metallo-β-lactamase; KPC = Klebsiella pneumoniae carbapenemase; GES = Guiana extended-spectrum β-lactamase.)

(n=878/1 079) of the isolates had intermediate resistance. Of the 201 colistin-resistant isolates, 52 (i.e. *S. marcescens* (n=49), *Morganella morganii* (n=2) and *Proteus mirabilis* (n=1)) were intrinsically resistant (25.9%). The proportion of isolates susceptible to doripenem, imipenem and meropenem ranged from 41.2% to 44.9%, while susceptibility to ertapenem was 11.5% (n=124/1 082).

The MIC_{50} and MIC_{90} (MIC required to inhibit the growth of 50.0% and 90% of organisms, respectively) were the same for doripenem (both ≥4 µg/mL), ertapenem (both >1 µg/mL) and meropenem (both 8 µg/mL), while for imipenem they were 2 µg/mL and 8 µg/mL, respectively. The MIC distribution for all carbapenems is shown in Table 3.

Of the 1 082 patients with viable isolates, 915 (84.6%) had isolates with one carbapenemase gene detected, 38 (3.5%) had isolates with two genes detected and 129 (11.9%) had isolates with no genes detected. All the *Escherichia adecarboxylata*, *M. morganii* and *P. mirabilis* isolates tested negative for all the screened carbapenemase genes. Overall, the most common carbapenemases were $bla_{OXA-48-like}$ (76.8%; n=761/991), followed by bla_{NDM} (21.1%; n=209/991), bla_{VIM} (1.3%; n=13/991), bla_{GES} (0.4%; 4/991) and bla_{KPC} (0.4%; n=4/991) (Fig. 4). The $bla_{OXA-48-like}$ gene was predominant in all four provinces, although the proportion of bla_{NDM} in KwaZulu-Natal was notably high (42%; n=95/224). The bla_{IMP} gene was not detected during this surveillance period. Among the isolates that harboured two carbapenemase genes simultaneously, the combinations were $bla_{OXA-48-like}$ with bla_{NDM} (71.1%; n=27/38), $bla_{OXA-48-like}$ with bla_{KPC} (7.9%; n=3/38), $bla_{OXA-48-like}$ with bla_{VIM} (7.9%; 3/38), $bla_{OXA-48-like}$ with bla_{GES} (5.3%; n=2/38), bla_{NDM} with bla_{GES} (5.3%; n=3/38), and bla_{NDM} with bla_{KPC} (2.6%; n=1/38). The screened CRE isolates (n=159) with colistin MIC >2 µg/mL were negative for the *mcr*-1 to *mcr*-5 genes.

Carbapenem susceptibility was compared with the identified carbapenemase genes (Fig. 5). The majority of the isolates harbouring the $bla_{OXA-48-like}$ gene (n=761) were resistant to ertapenem (n=576) and meropenem (n=377), while the remaining isolates were susceptible to doripenem (n=353) and imipenem (n=388). The majority of the isolates harbouring the bla_{NDM} gene (n=209) were resistant to doripenem (n=200), ertapenem (n=205), imipenem (n=200) and meropenem (n=198). The majority of the isolates harbouring the bla_{VIM} gene (n=13) were intermediately resistant to doripenem (n=6) and ertapenem (n=6), resistant to imipenem (n=10), and susceptible to meropenem (n=9). The majority of the isolates harbouring the bla_{GES} gene (n=4) were resistant to doripenem (n=3), imipenem (n=3) and meropenem (n=3), while all isolates were resistant to ertapenem (n=4). The majority of the isolates harbouring the bla_{KPC} gene (n=4) were resistant to imipenem (n=3), while all isolates were resistant to doripenem (n=4), ertapenem (n=4) and meropenem (n=4).

Table 2. Factors associated with in-hospital mortality among patients with carbapenem-resistant Enterobacterales bacteraemia reported to the GERMS-SA surveillance programme, 2019 - 2020

Characteristic	Alive* (n=457), n (%)	Died (n=306), n (%)	In-hospital mortality Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Sex						
Female	213 (58.2)	151 (41.5)	Ref.	-	-	-
Male	244 (61.1)	155 (38.9)	0.89 (0.67 - 1.19)	0.458	1.02 (0.71 - 1.47)	0.906
Age group						
Children (1.6 - 19 years)	77 (77.8)	22 (22.2)	Ref.	-	-	-
Adults (20 - 59 years)	316 (61.2)	200 (38.8)	2.21 (1.33 - 3.67)	0.002	1.86 (1.00 - 3.43)	0.046
Older adults (≥60 years)	64 (43.2)	84 (56.8)	4.59 (2.58 - 8.16)	<0.001	4.53 (2.21 - 9.28)	<0.001
Comorbidities†						
No	195 (66.8)	97 (33.2)	Ref.	-	-	-
Yes	230 (56.9)	174 (43.1)	1.51 (1.11 - 2.07)	0.010	1.72 (1.17 - 2.52)	0.006
Mental status‡						
Fully conscious and responsive§	283 (79.0)	75 (21.0)	Ref.	-	-	-
Altered mental state¶	146 (43.8)	187 (56.2)	4.83 (3.46 - 6.75)	<0.001	5.36 (3.21 - 8.92)	<0.001
HIV status						
Negative	263 (71.5)	105 (28.5)	Ref.	-	-	-
Positive	143 (66.5)	72 (33.5)	1.26 (0.87 - 1.81)	0.210	-	-
Healthcare-related factors						
ICU admission‡	127 (52.5)	115 (47.5)	1.56 (1.14 - 2.13)	0.005	1.16 (0.73 - 1.84)	0.512
Mechanical ventilation‡	112 (47.5)	124 (52.5)	2.30 (1.67 - 3.15)	<0.001	0.77 (0.43 - 1.38)	0.389
Any medical device inserted‖	435 (61.3)	275 (38.7)	0.72 (0.25 - 2.01)	0.534	-	-
Prior antibiotics**	229 (56.0)	180 (44.0)	1.72 (1.24 - 2.38)	0.001	1.88 (1.27 - 2.77)	0.001

GERMS-SA = Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa; OR = odds ratio; CI = confidence interval; Ref. = reference; ICU = intensive care unit.
*Includes patients who were still admitted and patients who were discharged.
†Diabetes mellitus, malignancy, renal failure, cardiovascular diseases, hypertension and tuberculosis.
‡At the time of positive specimen collection.
§Fully conscious and orientated to time, place and person.
¶Unresponsive/unconscious/sedated/postictal or partial or nearly complete unconsciousness due to drug administration or a seizure, or a deep prolonged unconsciousness where the patient cannot be aroused.
‖From admission up to the day of specimen collection.
**Within 6 months of the current admission.

Table 3. Carbapenem MIC distribution in viable carbapenem-resistant Enterobacterales from patients with bloodstream infections at the GERMS-SA sentinel surveillance sites, 2019 - 2020

Carbapenem	MIC value (µg/mL), n isolates ≤0.5	≤1	>1	2	4	>4	8	>8	MIC$_{50}$	MIC$_{90}$	MIC range reported by Microscan
Doripenem (n=1 082)	-	451	-	75	171	385	-	-	≥4	≥4	≤1 - 8
Ertapenem (n=1 082)	124	139	819	-	-	-	-	-	>1	>1	≤0.5 - 2
Imipenem (n=1 081)*	-	487	-	164	80	-	104	246	2	8	≤1 - >8
Meropenem (n=1 082)	-	446	-	46	42	-	160	388	8	8	≤1 - >8

MIC = minimum inhibitory concentration, determined according to the Clinical Laboratory Standards Institute guidelines;[21] MIC$_{50}$ = MIC required to inhibit the growth of 50.0% of organisms; MIC$_{90}$ = MIC required to inhibit the growth of 90.0% of organisms; - = none reported/zero.
*Imipenem was not readable on Microscan for one isolate.

Discussion

This study showed the current epidemiology of CRE bloodstream infections among hospitalised patients with CRE bacteraemia in SA. Similar to the previous period, most CRE infections occurred in Gauteng, KwaZulu-Natal and Western Cape.[13] However, the actual proportion of patients from Gauteng decreased, while it increased in Western Cape and KwaZulu-Natal. The increase was most notable in Western Cape, where the proportion of patients doubled. This shift may be due to changes in at-risk patients being admitted and/or specimen-taking practices, an increase in the number of surveillance sites, and increasing or decreasing numbers of patients admitted. These data were not collected as part of the surveillance, so the reasons for the changes are unclear. However, the observed increase in patients in some provinces is of concern and should be closely monitored.

The in-hospital mortality rate was high, but similar to the previous surveillance period.[13] Zou et al.[23] reported a higher mortality rate (50%; n=40/80) among hospitalised patients with CRE infections in China. However, a study conducted across 15 states in the USA[3] showed a lower in-hospital mortality rate in patients with a CRE infection (24%; n=107/449) compared with our findings. Our study showed that patients aged ≥60 years, those with other comorbidities, those with altered mental state, and those with previous antimicrobial exposure had increased odds of in-hospital mortality, which is in agreement with previous findings.[24] Our findings show consistently high mortality in patients with CRE infections and highlight patients at increased risk of death who may benefit from intensified prevention measures, such as proper and timely clinical management and treatment, active screening on admission to hospital or a specific high-risk ward, and pre-emptive contact precautions to reduce infections and consequently CRE-related mortality.[25]
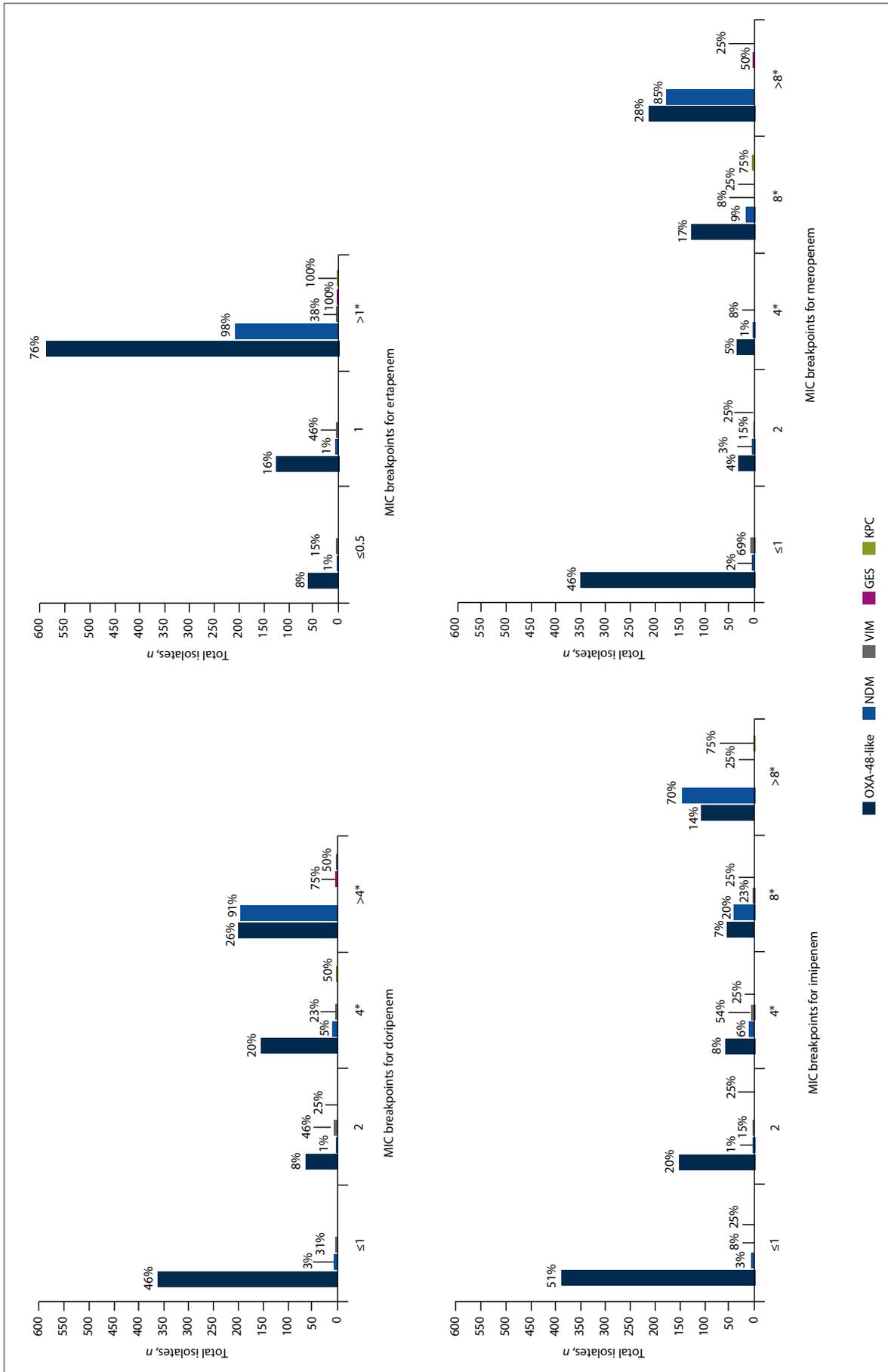
Fig. 5. Correlation of carbapenemase genes and carbapenem susceptibility in viable carbapenem-resistant Enterobacterales from patients with bloodstream infections at the GERMS-SA sentinel surveillance sites, 2019 - 2020. Total percentages of genes detected are indicated above the bars. (OXA-48-like = carbapenem-hydrolysing oxacillinase-48; NDM = New Delhi metallo-β-lactamase; VIM = Verona integron-encoded metallo-β-lactamase; KPC = Klebsiella pneumoniae carbapenemase; GES = Guiana extended-spectrum β-lactamase; MIC = minimum inhibitory concentration (μg/mL), determined according to the Clinical Laboratory Standards Institute guidelines;[21] *Resistant MIC breakpoints.)

K. pneumoniae accounted for nearly 80% of CRE infections, similar to the previous reporting period.[13] Globally, *K. pneumoniae* remains the dominant pathogen among patients with CRE infections; it was the most dominant pathogen reported in 33 European countries, as well as in 15 states in the USA and 25 provinces and municipalities in China.[3,17,26,27]

Overall resistance to antibiotics remained high, but the proportion of isolates resistant to tigecycline and fosfomycin decreased slightly compared with the previous SA surveillance report.[13] More than 80% of the isolates had intermediate resistance to colistin. The change from susceptible to intermediate resistance was due to the changes in the 2020 CLSI guidelines (i.e. the susceptible category was removed from the guidelines; only the intermediate (≤ 2 μg/mL) and resistant (≥ 4 μg/mL) categories remain). The colistin MIC breakpoints differ in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline (i.e. only the susceptible (≤2 μg/mL) and resistant categories (>2 μg/mL) are reported). Compared with the previous surveillance report, colistin resistance has increased by 5.6% (the MIC breakpoint for the resistant category remained the same).[13,21] The high level of carbapenem resistance, especially towards ertapenem, is well described in other SA studies.[11,13,28] This increase in resistance could be due to suboptimal infection prevention and control (IPC) measures and antibiotic stewardship practices. Compared with the previous surveillance report, the MIC_{50} has stayed in the same reported range for ertapenem (>1 μg/mL) but has increased for doripenem (≤1 μg/mL to ≥4 μg/mL), imipenem (≤1 μg/mL to 2 μg/mL) and meropenem (≤1 μg/mL to 8 μg/mL), and the MIC_{90} also stayed in the same reported range for doripenem (≥4 μg/mL), ertapenem (>1 μg/mL), imipenem (≥4 μg/mL), and meropenem (≥4 μg/mL).[13]

The $bla_{OXA-48-like}$, bla_{NDM} and bla_{VIM} remained the most common carbapenemase genes identified in public sector hospitals in SA overall, but their proportions have changed. Compared with the previous surveillance period, the number of CREs harbouring the $bla_{OXA-48-like}$ and bla_{KPC} genes has increased from 52% to 76.8% and 0.1% to 0.4%, respectively. In contrast, the bla_{NDM} gene decreased from 34% to 21.1% and the bla_{VIM} gene from 4% to 1.3%, while the bla_{IMP} gene was not detected compared with being present in 0.2% of the isolates previously. The number of CREs harbouring the bla_{GES} gene remained the same. The increase in the number of CREs harbouring the $bla_{OXA-48-like}$ gene could be due to the fact that plasmids containing this gene can disseminate between various bacterial species via horizontal transmission more efficiently.[17] The current spread of the $bla_{OXA-48-like}$ gene is predominantly driven by the composite transposon Tn*1999* and its variants (i.e. Tn*1999.2* and Tn*1999.3*), which are harboured on the pOXA-48a-like IncL conjugative plasmid.[17,29] The pOXA-48a-like IncL plasmid is highly transmissible and has a transfer frequency 50 times higher than the pNDM-OM IncL plasmid that harbours the bla_{NDM-1} gene.[17] IPC measures such as early identification of CPE, contact precautions, hand hygiene, environmental cleaning, adhering to aseptic techniques and antimicrobial stewardship should be implemented, and CRE- and/or CPE-positive patients should ideally be isolated and treated according to strict standard guidelines to curb transmission.[25,30] Screening and isolation may not always be feasible owing to limited resources and staff. Awareness of the specific carbapenemase detected is critical in decisions around optimal antibiotic treatment.[25] CPE is associated with increased mortality and poor clinical outcomes, and is considered to be more virulent than CRE isolates that do not harbour carbapenemase genes.[25] The majority of isolates harbouring the bla_{GES}, bla_{KPC} and bla_{NDM} genes were resistant to all carbapenems. The majority of isolates harbouring the bla_{VIM} gene were resistant to imipenem, intermediately resistant

to doripenem and ertapenem, and susceptible to meropenem. The majority of isolates harbouring the $bla_{OXA-48-like}$ gene were resistant to ertapenem and meropenem, susceptible to imipenem, and showed an equal susceptible and resistant distribution to doripenem, which corresponds with previously reported SA results.[11,28]

Just over a tenth of the CRE isolates did not harbour any carbapenemase genes, which was similar to the previous period.[13] CRE isolates that do not harbour carbapenemase genes are generally still resistant to multiple antibiotics and can be transmitted between patients. Their detection therefore still warrants targeted infection control interventions, such as contact precautions.[25]

This study has some limitations: (*i*) comprehensive patient demographic details were unavailable, and it was not possible to determine accurate trends in patient age, ward and gender; (*ii*) treatment was not included as a factor associated with mortality in the analysis; (*iii*) data originated from public sector hospitals in SA; (*iv*) owing to missing isolates, it was not possible to determine accurate trends in antibiotic resistance and circulating carbapenemase genes; (*v*) non-carbapenemase-producing Enterobacterales were not screened for other carbapenemases, *Amp*C and/or extended-spectrum beta-lactamases by PCR; and (*vi*) the conventional PCR assay used in this study to screen for the presence of *mcr* genes only covered the *mcr*-1 to *mcr*-5 genes; we did not screen for the *mcr*-6 to *mcr*-9 genes.

Conclusion

The study findings show overall consistent epidemiology of CRE bloodstream infections with slight changes that may become prominent over time. *K. pneumoniae* harbouring the $bla_{OXA-48-like}$ gene remains the most prevalent organism among patients with CRE bacteraemia in SA's public academic hospitals. The findings in this study call for better and improved surveillance programmes nationwide.

1. Sheu CC, Chang YT, Lin SY, Chen YH, Hsueh PR. Infections caused by carbapenem-resistant Enterobacteriaceae: An update on therapeutic options. Front Microbiol 2019;10:80. https://doi.org/10.3389/fmicb.2019.00080
2. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends Mol Med 2012;18(5):263-272. https://doi.org/10.1016/j.molmed.2012.03.003
3. Van Duin D, Arias CA, Komarow L, et al. Molecular and clinical epidemiology of carbapenem-resistant Enterobacterales in the USA (CRACKLE-2): A prospective cohort study. Lancet Infect Dis 2020(6);20:731-741. https://doi.org/10.1016/S1473-3099(19)30755-8

4. World Health Organization. Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. Geneva: WHO, 2017. http://apps.who.int/iris/bitstream/handle/10665/259462/9789241550178-eng.pdf;jsessionid=623130C9C3DE356707C020ED2F5D2CC0?sequence=1 (accessed 8 June 2021).

5. Castanheira M, Doyle TB, Collingsworth TD, Sader HS, Mendes RE. Increasing frequency of OXA-48-producing Enterobacterales worldwide and activity of ceftazidime/avibactam, meropenem/vaborbactam and comparators against these isolates. J Antimicrob Chemother 2021;76(12):3125-3134. https://doi.org/10.1093/jac/dkab306

6. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 2015;59(10):5873-5884. https://doi.org/10.1128/AAC.01019-15

7. Brink A, Coetzee J, Clay C, et al. The spread of carbapenem-resistant Enterobacteriaceae in South Africa: Risk factors for acquisition and prevention. S Afr Med J 2012;102(7):599-601. https://doi.org/10.7196/SAMJ.5329

8. Brink AJ, Coetzee J, Clay CG, et al. Emergence of New Delhi metallo-beta-lactamase (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC-2) in South Africa. J Clin Microbiol 2012;50(2):525-527. https://doi.org/10.1128/JCM.05956-11

9. Brink AJ, Coetzee J, Corcoran C, et al. Emergence of OXA-48 and OXA-181 carbapenemases among Enterobacteriaceae in South Africa and evidence of *in vivo* selection of colistin resistance as a consequence of selective decontamination of the gastrointestinal tract. J Clin Microbiol 2013;51(1):369-372. https://doi.org/10.1128/JCM.02234-12

10. Lowman W, Sriruttan C, Nana T. NDM-1 has arrived: First report of carbapenem resistance mechanism in South Africa. S Afr Med J 2011;101(12):873-875. https://doi.org/10.7196/SAMJ.5329

11. Perovic O, Britz E, Chetty V, Singh-Moodley A. Molecular detection of carbapenemase-producing genes in referral Enterobacteriaceae in South Africa: A short report. S Afr Med J 2016;106(10):975-977. https://doi.org/10.7196/SAMJ.2016.v106i10.11300

12. Singh-Moodley A, Perovic O. Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in Enterobacteriaceae in South Africa. BMC Infect Dis 2016;16(1):536-546. https://doi.org/10.1186/s12879-016-1858-7

13. Perovic O, Ismail H, Quan V, et al. Carbapenem-resistant Enterobacteriaceae in patients with bacteraemia at tertiary hospitals in South Africa, 2015 to 2018. Eur J Clin Microbiol Infect Dis 2020;39(7):1287-1294. https://doi.org/10.1007/s10096-020-03845-4

14. Lowe M, Kock MM, Coetzee J, et al. *Klebsiella pneumoniae* ST307 with $bla_{OXA-181}$, South Africa, 2014 - 2016. Emerg Infect Dis 2019;25(4):739-747. https://doi.org/10.3201/eid2504.181482

15. Strydom KA, Chen L, Kock MM, et al. *Klebsiella pneumoniae* ST307 with OXA-181: Threat of a high-risk clone and promiscuous plasmid in a resource-constrained healthcare setting. J Antimicrob Chemother 2020;75(4):896-902. https://doi.org/10.1093/jac/dkz550

16. Singh-Moodley A, Perovic O. Phenotypic and genotypic correlation of carbapenemase-producing Enterobacteriaceae and problems experienced in routine screening. S Afr Med J 2018;108(6):495-501. https://doi.org/10.7196/SAMJ.2018.v108i6.12878

17. Pitout JDD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. Clin Microbiol Rev 2019;33(1):e00102-19. https://doi.org/10.1128/CMR.00102-19

18. Tangden T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: Clinical perspectives on detection, treatment and infection control. J Intern Med 2015;277(5):501-512. https://doi.org/10.1111/joim.12342

19. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: Epidemiology, genetic context, treatment options and detection methods. Front Microbiol 2016;7:895. https://doi.org/10.3389/fmicb.2016.00895

20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Wayne, Pa.: CLSI, 2019.

21. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, Pa.: CLSI, 2020.

22. Rebelo AR, Bortolaia V, Kjeldgaard JS, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 for surveillance purposes. Euro Surveill 2018;23(6):17-00672. https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672

23. Zou H, Xiong SJ, Lin QX, Wu ML, Niu SQ, Huang SF. CP-CRE/non-CP-CRE stratification and CRE resistance mechanism determination help in better managing CRE bacteremia using ceftazidime-avibactam and aztreonam-avibactam. Infect Drug Resist 2019;12:3017-3027. https://doi.org/10.2147/IDR.S219635

24. Tamma PD, Goodman KE, Harris AD, et al. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae bacteremia. Clin Infect Dis 2017;64(3):257-264. https://doi.org/10.1093/cid/ciw741

25. Ambretti S, Bassetti M, Clerici P, et al. Screening for carriage of carbapenem-resistant Enterobacteriaceae in settings of high endemicity: A position paper from an Italian working group on CRE infections. Antimicrob Resist Infect Control 2019;8:136. https://doi.org/10.1186/s13756-019-0591-6

26. Glasner C, Albiger B, Buist G, et al. Carbapenemase-producing Enterobacteriaceae in Europe: A survey among national experts from 39 countries, February 2013. Euro Surveill 2013;18(28):20525. https://doi.org/10.2807/1560-7917.es2013.18.28.20525

27. Zhang R, Liu L, Zhou H, et al. Nationwide surveillance of clinical carbapenem-resistant Enterobacteriaceae (CRE) strains in China. EBioMedicine 2017;19:98-106. https://doi.org/10.1016/j.ebiom.2017.04.032

28. Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa (GERMS-SA). Annual surveillance report. 2020. https://www.nicd.ac.za/wp-content/uploads/2022/02/2020-GERMS-SA-Annual-Review.pdf (accessed 1 December 2021).

29. Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother 2012;56(1):559-562. https://doi.org/10.1128/AAC.05289-11

30. National Department of Health, South Africa. Practical manual for implementation of the national infection prevention and control strategic framework. October 2021. https://www.knowledgehub.org.za/system/files/elibdownloads/2021-10/National%20Infection%20Prevention%20and%20Control%20Practical%20Manual%20%28Web%20version%29.pdf (accessed 1 December 2021).