

Surveillance of Multidrug-Resistant and Antimicrobial Sensitivity Patterns in Gram-Negative Isolates from Burn Units in the Free State and Northern Cape, South Africa (2018–2024)

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Abstract:

This study investigated Gram-negative bacterial isolates and corresponding antimicrobial resistance (AMR) patterns in burn units across the Free State and Northern Cape provinces during the pre-COVID-19, COVID-19, and post-COVID-19 periods. A total of 1 097 isolates were recorded, with the Free State contributing the majority (n=1057) compared to the Northern Cape (n=40). *Pseudomonas aeruginosa* was the predominant isolate in both provinces, followed by *Proteus mirabilis* and *Acinetobacter baumannii*. The Free State demonstrated fluctuating isolate numbers, with notable decreases during COVID-19 and rebounds post-pandemic, whereas the Northern Cape showed a sharp decline in isolates across all periods, likely reflecting reduced specimen collection rather than true epidemiological trends.

AMR analysis revealed province- and species-specific variations. In the Free State, *P. aeruginosa* resistance increased substantially during COVID-19 (37.39% to 57.49%) before declining post-pandemic, although not to pre-COVID-19 levels. *A. baumannii* exhibited persistently high resistance across all periods, rising to 88.89% post-COVID-19. *Proteus mirabilis* showed a gradual decline in resistance, while several Enterobacterales demonstrated reduced resistance post-COVID-19. In the Northern Cape, meaningful trend interpretation was limited due to sample scarcity; however, pre-COVID-19 resistance levels were generally lower than those observed in the Free State.

Overall, the findings indicate that the COVID-19 pandemic substantially influenced bacterial isolation rates and AMR profiles, particularly in the Free State. The sustained high resistance in *A. baumannii* and elevated resistance in *P. aeruginosa* underscore the urgent need for strengthened antimicrobial stewardship and targeted infection-control strategies in regional burn units.

Keywords: Antimicrobial resistance, Burn units, Gram-negative bacteria, COVID-19 pandemic impact, *Pseudomonas aeruginosa*

INTRODUCTION

Burn injuries are one of the most difficult injuries to treat due to the extreme physical damage to the skin but also due to the high risk of secondary infections for the patient and also for the burn wounds. The extended stay of patients in the hospitals and the open wounds make these patients vulnerable to bacterial infections, particularly Gram-negative organisms that are frequently seen as multidrug-resistant (MDR) (Kabanangi, et al., 2021). Antimicrobial resistance (AMR) causes a significant increase in mortality and morbidity due to the lack of effectiveness of these standard therapies that are used (Tchakal-Mesbahi, Abdouni, & Metref, 2021).

The presence of COVID-19 further increased the resistance of certain organisms and complicated the treatment options for infections. While COVID-19 caused a decrease in staffing, and also put a strain on hospital systems, it also had a broader impact due to the disruptions in infection prevention and control (IPC) and antimicrobial stewardship programs. The effect of these disruptions could be seen by the likely alteration that could be seen in the epidemiology of AMR in different settings (Alotaibi, 2022). As stated in different articles from across the world, there was an increase in antibiotic consumption seen during this COVID-19 period. This could likely explain

the increased resistance of Gram-negative bacteria that was also seen during this period (Alotaibi , 2022) ; (Jeon, et al., 2022).

Based on previous studies done the most common pathogens that were seen in hospital environments, particularly intensive care units, were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. These organisms were also classified as “problematic organisms” (Greatorex & Oosthuizen , 2015). There are however studies from other countries that show what the impact of the COVID-19 pandemic was on the resistance pattern of these organisms, there is not any that has been done in dept in South Africa, particularly in the Free State and Northern Cape provinces.

Burn wound infections that are caused by Gram-negative organisms complicates patient outcomes, but it also causes a considerable burden on healthcare systems. These infections cause risings costs and pressure on limited resources due to prolonged hospitalization, advanced infection control measures and increased use of broad-spectrum antibiotics (Greatorex & Oosthuizen , 2015). In settings such as the Northern Cape and the Free State where healthcare infrastructure is likely strained, the presence of multidrug-resistant organisms can overwhelm these hospital settings. By understanding the impact of COVID-19 on resistance patterns it can further assist in strengthening infection prevention strategies and optimize resource allocations.

This study was therefore done to determine what the bacterial resistance profile was of the Gram-negative organisms that were isolated from the swabs that were sent from the burn units of the public hospitals in the Northern Cape and Free State by comparing the resistance patterns before, during and post-COVID-19. These findings will then be able to provide specific evidence that will be used to guide stewardship programs and infection control.

Problem statement

Patients that are admitted to hospitals for burn wounds are at risk for acquired infections due to prolonged hospitalization, invasive procedures and devices and also due to the compromised skin barriers. Organisms such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the most commonly isolated organisms from burn wounds and they commonly display multidrug resistance (Kabanangi , et al., 2021). Without current local data on the resistance trends that can be used to evaluate the effectiveness of IPC programs the treatments that are given to these patients may be

suboptimal and could contribute to higher mortality rates (Vickers , et al., 2018).

Additional challenges were introduced by COVID-19 to the hospitals due to the disruption of antimicrobials stewardship programs and IPC measures. Studies from other countries show an increase in MDR Gram-negative infections during the COVID-19 timeframe (Jeon, et al., 2022). (Musuroi, et al., 2024). Little to nothing is known about how the pandemic impacted South Africa’s burn units. It is critical that this gap should be addressed, because if there is not an accurate understanding of these resistance profiles the antimicrobial stewardship programs could not effectively be implemented and this could cause a decrease in the number of reliable options when it comes to treating infections (Greatorex & Oosthuizen , 2015). The results of this study will therefore show the resistance pattern of Gram-negative organisms in the Free State and Northern Cape over the three different timeframes (before, during and after COVID-19) to act as evidence for clinicians and policymakers when it comes to IPC.

Aim

This research aimed to determine what the bacterial resistance profile of the Gram-negative organisms from the swabs are, that were taken from the patients in the burn units of the public hospitals in the Free State and Northern Cape was between 1 March 2018 and 31 July 2024.

Objectives

1. To determine the bacterial resistance profile of the Gram-negative organisms from the swabs that were sent to the laboratories from the burn units of the public hospitals in the Free State and Northern Cape before, during and after the COVID-19 pandemic.
2. To determine how the years before, during and after the pandemic compared to one another in terms of the Gram-negative bacterial resistance profile from the swabs that were sent from the burn units to the laboratories.

Literature review

Significance of burn wound infections

Burn wounds are a significant health concern since it contributes to prolonged hospitalization and the morbidity and mortality of patients with severe burn injuries. The skin acts as a protective barrier against infections and when this barrier is compromised, such with burn wounds, these patients become susceptible to opportunistic infections. These burn wounds create optimal

conditions for microbial colonization (Kabanangi, et al., 2021). Infections in burn units commonly lead to a wide range of complications that influence patient outcomes. These complications often result in organ dysfunction, septicemia and even death. A wide range of studies have shown that the deaths caused by infections related to burn wounds causes more deaths than the burn wounds self (Greatorex & Oosthuizen, 2015).

The infections that are seen in burn wound patients come from pathogens from the hospital environment, healthcare workers, invasive devices and even endogenous flora. Infections related to Gram-positive organisms are commonly seen in the early stages post-injury for these patients; however, Gram-negative bacteria frequently cause infections in the later stages of hospitalization for these patients (Tchakal-Mesbahi, Abdouni, & Metref, 2021). The transformation that is seen in the stages of hospitalization and infections is important to understand since it highlights the increase in concern that is caused by these Gram-negative organisms in burn units.

Different studies have confirmed that infections in burn wound patients are amongst the leading causes of morbidity and mortality globally. In Tanzania a high level of infections has been documented in paediatric burn wound patients. Gram-negative organisms were the leading cause of these infections and amongst these isolates it was documented that multidrug resistance was very common (Kabanangi, et al., 2021). Another study that was done in Algeria noted that 70% of isolates that were taken from burn wounds swabs were Gram-negative organisms and the MDR was also high amongst these isolates (Tchakal-Mesbahi, Abdouni, & Metref, 2021).

Burn units are ideal environments for the colonization of MDR organisms. When these burn wound patients stay in the hospital environment for longer periods of time it is more common to see bacterial infections. A study that was done in sub-Saharan Africa noted that patients that were infected with MDR *Enterobacteriaceae* in the hospital had a larger burn surface area, their stay in the hospital was prolonged and an increase in mortality was seen (Greatorex & Oosthuizen, 2015).

Gram-negative organisms in Burn units

Gram-negative organisms are commonly seen to cause hospital-acquired infections especially in immune compromised patients such as burn wound patients. The most common organisms that are seen to cause infections include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter species*, *Escherichia coli* and

Acinetobacter baumannii. These organisms are well adapted to the hospital environment. These organisms thrive especially in burn wounds due to the frequent use of invasive devices and the nutrient-rich tissue environment that is exposed during wound cleaning (Jeon, et al., 2022).

Pseudomonas aeruginosa is the most frequently reported pathogen in burn units worldwide, this is due to the acquired and intrinsic resistance mechanisms of the pathogen. The bacterium has extrinsic resistance to a wide range of antibiotic classes and it can easily acquire resistance through porin modifications, efflux pumps and beta-lactamases. (Musuroi, et al., 2024). *Acinetobacter baumannii* has the ability to form resistance at an alarming rate through plasmids and transposons. This bacterium is classified as a critical threat due to its ability to form resistance and also because it is able to survive on hospital surfaces (Vickers, et al., 2018).

Escherichia coli and *Klebsiella pneumoniae* have been seen to produce extended-spectrum β -lactamase (ESBL) and these bacterium's therefore cause challenges when it comes to treatment of infections in burn settings. Other common mechanisms that have been reported in Gram-negative organisms include AmpC and Carbapenem-resistance (Kabanangi, et al., 2021). *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter species* were the most commonly seen Gram-negative organisms in the hospital environments across the world. However, the prevalence of these organisms varied by region. In South Africa it was documented that the two most common bacteria were *Pseudomonas aeruginosa* and *Acinetobacter species* with high resistance rates to key antimicrobial agents (Greatorex & Oosthuizen, 2015).

In Africa the resistance is just as concerning. In a study done that was done in Kenya and Ghana it showed that *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are both responsible for majority of the hospital acquired infections that were seen in that study. Some of the isolates in this study showed resistance to colistin which is the last-line antibiotic that is used for Gram negative infections (Odoom & Donkor, 2024). Environmental studies that have been done showed that pathogens are able to persist on wound dressings, equipment and burn ward surfaces for long periods of time. This therefore suggests that the bacterium's use biofilm formation as an important survival mechanism (Zafer, et al., 2024).

These biofilm formation by the bacteria in burn wounds are able to withstand antibiotic

concentrations as high as 1000 times higher than their planktonic counterparts and this complicates treatment options (Zafer, et al., 2024). This biofilm formation is especially common in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* since they are able to form polymicrobial biofilms that are able to protect a wide range of species at the same time. These biofilms prevent healing and also facilitate gene transfer that accelerates the spread of resistant genes (website 3).

Clinical challenges and multidrug resistance

Multidrug resistance especially in Gram-negative organisms has adapted to such an extent that it has become difficult to treat these infections in burn units and these modifications limits therapeutic options. Some of these modifications include porin modifications, production of β -lactamases, efflux pumps and carbapenemases (Ruesegger, Xiao, Naziripour, Brown , & Kanumuambidi, 2022). These MDR organisms that are seen in burn units are commonly seen to be resistant to three or more classes of antimicrobials. This only leaves last-resort agents such as carbapenems and colistin (Jeon, et al., 2022).

The presence of MDR organisms that cause infection in burn wounds, complicates the patient outcomes and care, but it also complicates the infection control efforts in the hospital environment. Since patients are in close proximity in these burn units and receive frequent procedures, infections are commonly seen and resistant strains spread rapidly (Vickers , et al., 2018). As a consequence of these MDR infections extended hospital stays, treatment failures and increased treatment costs are commonly seen (Greatorex & Oosthuizen , 2015). The mortality rates increase drastically when the infections are caused by Gram-negative MDR organisms. Due to the lack of effective treatment options for infections with *Klebsiella pneumonia* and carbapenem-resistant *Acinetobacter baumannii*, a poor outcome is commonly seen with patients with these infections (Musuroi, et al., 2024). It is estimated that there are annually one million deaths globally due to MDR infections. This contribute to the global antimicrobial resistance crises that we are currently facing. This confirm that there is a need for context-specific surveillance programs and the current data needs to be updated for these settings such as burn units (Ruesegger, Xiao, Naziripour, Brown , & Kanumuambidi, 2022).

In an effort to manage MDR infections focus has been mainly put on antimicrobial stewardship and infection control programs, but there have been inconsistencies seen when it comes to implementation of these programs in resource limited settings. Studies that were done in South

Africa show that there are a wide range of factors that undermine surveillance and containment programs. These factors include inadequate isolation facilities, shortages of trained IPC personnel and inconsistent antimicrobial susceptibility testing (Khorshidi, Firoozeh, Akbari, Mahmoudi, & Corehtash, 2015).

Impact of the COVID-19 Pandemic on Antimicrobial Resistance

A wide range of effects could be seen in healthcare systems due to the COVID-19 pandemic, and that also influenced the antimicrobial resistance patterns across different regions and sectors. The pandemic cause tremendous pressure in the hospital environment due to increased patient loads, shortage of staff and the stopping of IPC practices. During COVID-19 70% of patients received broad-spectrum antibiotics as part of their empirical therapy when they were hospitalized with COVID-19. This treatment was given without the confirmation of a bacterial co-infection. This caused an increase in the selection pressure for resistant organisms (Alotaibi , 2022).

From other articles it was noted that specifically in burn units the pandemic altered bacterial epidemiology. A study in Europe showed that non-fermenting Gram-negative bacteria showed an increase in prevalence during COVID-19. The same study showed that during COVID-19 the resistance of *Klebsiella* species and *Pseudomonas* increased (Musuroi, et al., 2024). Hospital-based studies noted that an 25% increase in *Acinetobacter baumannii* resistance was seen during the pandemic and a 15% increase in resistance is seen with carbapenem-resistant Enterobacteriaceae during the pandemic (Alotaibi , 2022). A study that was done from 2023 to 2024 in Africa found that MDR Gram-negative infections are higher now post-COVID-19 than baseline levels pre-COVID-19. The cause of this could likely be due to the disruption of surveillance and stewardship programs (Diop, et al., 2025).

Other studies have even gone so far to suggest that COVID-19 may indirectly have increased the resistance through immune dysregulation and prolonged hospitalization among patients with bacterial co-infections. Patient that was critically ill with COVID-19 were ventilated and required catheters and extended critical care admissions. All of these are known risk factors for Gram-negative colonization (Diop, et al., 2025).

Methodology

Study location

The Free State and Northern Cape were chosen as the study site for this research. The burn units of the following hospitals were chosen: Pelonomi,

National, Universitas, Bongani Regional Hospital, Boitumelo and Mofumahadi Manapo Mopeli Hospital. The burn unit of Robert Mangaliso Sobukwe hospital was also chosen in the Northern Cape. The burn units specialize in patients who have burn wounds.

Study design

This was a retrospective study and therefore, retrospective results were collected for samples that had already been tested in laboratories. The data were only collected and no changes were made to these data except that the results were de-identified. The data that were requested were separated into three different timeframes, pre-pandemic (1 March 2018 until 30 February 2020), during the pandemic (1 March 2020 until 30 June 2022), and post-pandemic (1 July 2022 until 31 July 2024).

Study population

Number of participants

The study did not consist of a set number of samples. The samples from the burn units were collected from the public hospitals of the Free State and Northern Cape between 1 March 2018 until 30 February 2020 before the COVID-19 pandemic, between 1 March 2020 until 30 June 2022 during the pandemic, and lastly between 1 July 2022 until 31 July 2024, which was after the pandemic. All samples from these different timeframes were chosen for this study.

Participant identification

An application was submitted on the Academic Affairs and Research Management System (AARMS) to request access to the data from the NHLS central data warehouse (CDW). Only patients from the burn units of Pelonomi, National, Universitas, Bongani Regional Hospital, Boitumelo, Mofumahadi Manapo Mopeli and Robert Mangaliso Sobukwe Hospitals were included in the study. The timeframe from 1 March 2018 until 30 February 2020 before the COVID-19 pandemic, between 1 March 2020 until 30 June 2022 during the pandemic, and lastly between 1 July 2022 until 31 July 2024, which was after the pandemic.

In- and exclusion criteria

Inclusion criteria

1. All participants were admitted patients from the burn units of the following hospitals: Pelonomi, National, Universitas, Bongani Regional Hospital, Boitumelo, Mofumahadi Manapo Mopeli and Robert Mangaliso Sobukwe.
2. The patient were 18 years or older.
3. The organism was Gram-negative
4. The sample were swabs that was sent to the laboratory for antimicrobial susceptibility testing.

5. The sample were collected from 1 March 2018 until 29 February 2020, or from 1 March 2020 until 30 June 2022, or lastly from 1 July 2022 until 31 July 2024.

Exclusion criteria

1. The patient was not admitted to the burn units of the following hospitals: Pelonomi, National, Universitas, Bongani Regional Hospital, Boitumelo, Mofumahadi Manapo Mopeli and Robert Mangaliso Sobukwe.
2. The patient was younger than 18 years.
3. The organism identified was not Gram-negative
4. The sample was not a swab, nor it was it sent for antimicrobial susceptibility.
5. The sample was not collected from 1 March 2018 until 29 February 2020, or from 1 March 2020 until 30 June 2022, or lastly from 1 July 2022 until 31 July 2024.

METHODS AND MATERIAL

Preparation

A data sheet was compiled by the data analyst for the bacterial resistance profile before, during and after the pandemic. The results were split into two tables, one per province and the results were entered into these two tables per timeframe. De-identified data were received from the NHLS CDW so there was no need to remove patient personal information.

Data collection

This was a retrospective study and the data had already been available for this study. After the necessary approval from the NHLS academic affairs and research management system (AARMS) for the data use and the HSREC, data were obtained from the CDW. The inclusion and exclusion criteria were also given to the NHLS to ensure that the data meet the necessary requirements. The organism and the antimicrobial resistance pattern for each patient was then entered into the respective data sheets and other non-essential information was removed from the results.

Data management and statistical analysis

Primary data were extracted from the NHLS CDW from the seven hospitals for the patients that were tested for antimicrobial susceptibility in the burns units that met the criteria. This data was entered into the spreadsheet based on the date of collection. Statistical analysis was used to determine the different Gram-negative organisms and the resistance patterns of each. The change over the periods selected was used to calculate the *p*-value, any sample error margin (SEM) was identified and analysed to determine the trueness of the results obtained.

ETHICAL ASPECTS AND GOOD CLINICAL PRACTICE

Ethical clearance

Ethical approval was requested from the Health Sciences Research Ethics Committee (HSREC) and permission from the manager of Pelonomi National Health Laboratory Services (NHLS) was granted. The research only commenced once the necessary permission was granted.

Confidentiality

All data that was received from NHLS was de-identified data. Therefore, no personal information from patients was obtained and patient confidentiality was maintained throughout this study.

Results

This table shows the number of Gram-negative bacterial isolates from the burn unit swabs across the Free State and Northern Cape during the three timeframes. The Free State had a much higher sample number for each timeframe when compared to the North Cape. The Free State had a total of 1057 samples overall while the Northern Cape only had 40 samples. In the Free State the sample size decreased during COVID-19 (382 to 253) and then increased again to above pre-COVID-19 levels (422). In the Northern Cape the sample size decreased across all three timeframes from 37 to 1 sample.

Table 1 Gram Negative Bacterial Isolates per Province

Gram Negative Bacterial Isolates Per Province									
	Free State				Northern Cape				
	Pre-COVID-19	During COVID-19	Post-COVID-19	T	Pre-COVID-19	During COVID-19	Post-COVID-19	T	Grand total
Pseudomonas aeruginosa	210	139	186	5	12	0	0	1	547
Proteus mirabilis	30	49	100	1	9	0	1	1	189
Acinetobacter baumannii	32	27	64	1	8	1	0	9	132
Klebsiella pneumoniae subs pneumoniae	40	13	27	8	2	0	0	2	82
Escherichia coli	6	11	20	3	1	0	0	1	38
Providencia stuartii	22	1	3	2	0	0	0	0	26
Enterobacter cloacae	6	12	1	1	0	0	0	0	19

ac subs p cloac ae									
Serratia marc esce ns	12	1	3	1	2	0	0	2	18
Pseudom onas putid a	0	0	11	1	1	1	0	2	13
Citrobact er freun dii	12	0	0	1	0	0	0	0	12
Enteroba cter cloac ae com plex	6	0	4	1	1	0	0	1	11
Enteroba cter aero gene s	6	0	3	9	1	0	0	1	10
TOTAL	382	253	422	1	37	2	1	4	109 7

Pseudomonas aeruginosa was the bacterium with the greatest number of samples for both provinces. In the Free State during COVID-19 the sample size decreased (210 to 139) and then increased slightly post-COVID-19 (186). However, in the Northern Cape there were only 12 samples pre-COVID-19. In the Free State the sample size for *Proteus mirabilis* increased gradually over the three timeframes (30,49,100). The sample size decreased during COVID-19 (9 to 0) and then increased post-COVID-19 (1). In the Free State the sample size decreased slightly during COVID-19 (32 to 27) for *Acinetobacter baumannii* and ten increased again (64) post COVID-19. In the Northern Cape the sample size also decreased during COVID-19 (8 to 1).

The sample size decreased during COVID-19 for *Klebsiella pneumoniae* (40 to 13) and then increased again (27) post-COVID-19. In the North Cape there were only 2 samples pre-COVID-19. The sample size for *Escherichia coli* increased gradually over the three timeframes in the Free State (6,11,20). In the Northern Cape there was only one sample pre-COVID-19. The sample size decreased sharply for *Providencia stuartii* during COVID-19 (22 to 1) and then increased again

slightly during COVID-19 (3). There were no samples for this organism in the Northern Cape. In the Free State the sample size increased during COVID-19 for *Enterobacter cloacae* (6 to 12) and then decreased sharply to one post-COVID-19. There were also no samples in the Northern Cape for the bacterium.

In the Free State the sample size for *Serratia marcescens* decreased during COVID-19 (12 to 1) and then increased again post-COVID-19 (3). There were only two samples pre-COVID-19 for this bacterium in the Northern Cape. Post-COVID-19 there were 11 samples for *Pseudomonas putida* in the Free State. The sample size remained the same (1) pre- and during COVID-19 for the bacterium in the Northern Cape. There was only 12 samples pre-COVID-19 for *Citrobacter freundii* in the Free State and no samples in the Northern Cape.

The sample size for *Enterobacter cloacae complex* decreased during COVID-19 (6 to 0) in the Free State and then increased post-COVID-19 (4). There was only one sample pre-COVID-19 in the Northern Cape. The sample size for *Enterobacter aerogenes* decreased during COVID-19 (6 to 0) in

the Free State and then increased post-COVID-19
(3). There was only one sample pre-COVID-19 in the Northern Cape.

Table 2 Free State Gram Negative Bacterial Sensitivity Results

FREE STATE GRAM NEGATIVE BACTERIAL SENSITIVITY RESULTS									
	PRE-COVID-19			DURING COVID-19			POST-COVID-19		
	SENS I T I V E	RESIS T A N T	TO T A L	SENS I T I V E	RESIS T A N T	TO T A L	SENS I T I V E	RESIS T A N T	TO T A L
PSEUDOMONAS AERUGINOSA	1204 (62.61)	719 (37.39)	1923	556 (42.51)	752 (57.49)	1308	1230 (50.51)	1205 (49.49)	2435
PROTEUS MIRABILIS	398 (74.53)	136 (25.47)	534	678 (76.27)	211 (23.73)	889	1184 (79.95)	297 (20.05)	1481
ACINETOBACTER BAUMANNII	77 (21.21)	286 (78.79)	363	53 (15.59)	287 (84.41)	340	84 (11.11)	672 (88.89)	756
KLEBSIELLA PNEUMONIAE	408 (63.35)	236 (36.65)	644	112 (46.67)	128 (53.33)	240	260 (54.97)	213 (45.03)	473
ESCHERICHIA COLI	84 (78.50)	23 (21.50)	107	196 (96.08)	8 (3.92)	204	282 (90.38)	30 (9.62)	312
PROVIDENCIA STUARTII	223 (57.47)	165 (42.53)	388	9 (50.00)	9 (50.00)	18	30 (58.82)	21 (41.18)	51
ENTEROBACTER CLOACAE SUBSP CLOACAE	66 (61.68)	41 (38.32)	107	136 (62.10)	83 (37.90)	219	12 (80.00)	3 (20.00)	15
SERRATIA MARCESCENS	126 (63.32)	73 (36.68)	199	11 (64.71)	6 (35.29)	17	30 (66.67)	15 (33.33)	45
CITROBACTER FREUNDII	180 (78.95)	48 (21.05)	228	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
ENTEROBACTER	81 (72.97)	30 (27.03)	111	0 (0.00)	0 (0.00)	0	50 (76.92)	15 (23.08)	65

CLOACAE COMPLEX									
ENTEROBACTER AEROGES	66 (57.89%)	48 (42.11%)	11 (9.62%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	33 (68.75%)	15 (31.25%)	48 (100%)
KLEBSIELLA OXYTOCA	0 (0.00%)	0 (0.00%)	0 (0.00%)	119 (94.44%)	7 (5.56%)	12 (9.09%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
ACINETOBACTER BAUMANNII COMPLEX	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	22 (18.03%)	100 (81.97%)	12 (100%)
TOTAL	2913 (61.74%)	1805 (38.26%)	47 (1.00%)	1870 (55.64%)	1491 (44.36%)	33 (0.97%)	3217 (55.44%)	2586 (44.56%)	58 (100%)

The resistance for *Klebsiella pneumoniae* increased drastically during COVID-19 (36.65% to 53.33%) and post-COVID-19 a slight decrease in resistance could be seen (45.03%). A sharp decrease in resistance could be seen during OVID-19 for *Escherichia coli* (21.50% to 3.92%) and post-COVID-19 an increase in resistance was seen again (9.62%). The resistance pattern for *Providencia stuartii* stayed relatively constant pre- and post-COVID-19. An increase in resistance could be seen during COVID-19 (42.53% to 50%) and the resistance then decreased again post-COVID-19 (41.18%) to almost the same percentage as pre-COVID-19.

During COVID-19 a slight decrease in resistance could be seen for *Enterobacter cloacae* (38.32% to 37.90%). A more drastic decrease in the resistance could be seen post-COVID-19 (20%). A constant decrease in the resistance could be seen for *Serratia marcescens* over the three timeframes (36.68%, 35.29%, 33.33%). The resistance pre-COVID-19 was 21.05% for *Citrobacter freundii*, however there was no samples during and post-COVID-19 to compare this resistance with.

Enterobacter cloacae complex showed a 27.03% resistance pre-COVID-19 and there were no samples during COVID-19 to compare this result with. Post-COVID-19 a slight decrease could be seen in resistance (23.08%) when comparing it with pre-COVID-19 results. The same results could be seen with *Enterobacter aerogenes*. The resistance was 42.11% pre-COVID-19 and there

were no results during COVID-19 to compare this with, however the resistance post-COVID-19 was 31.25%. There was only a 5.56% resistance seen for *Klebsiella oxytoca* during COVID-19. There was also an extremely high resistance seen for *Acinetobacter baumannii* post-COVID-19 (81.97%).

In the Free State the resistance showed a sharp increase during COVID-19 for *Pseudomonas aeruginosa* (37.39% to 57.49%) and this then decreased slightly post-COVID-19 (49.49%). The resistance however did not decrease back to pre-COVID-19 levels. The resistance for *Proteus mirabilis* stayed relatively the same over the three timeframes. A slight decrease in resistance could however be seen (25.47%, 23.73%, 20.05%). An alarmingly high percentage of resistance could be seen for *Acinetobacter baumannii* across all three timeframes. The resistance increased during COVID-19 (78.79% TO 84.41%) and then increased even further post-COVID-19 (88.89%).

There were only resistance results pre-COVID-19 for *Pseudomonas aeruginosa* (34.72%) in the Northern Cape and no other results for the other timeframes to compare this with. *Proteus mirabilis* showed a 31.65% resistance pre-COVID-19. There were no sample during COVID-19, but post-COVID-19 a 21.05% resistance could be seen which is lower than pre-COVID-19 results. *Acinetobacter baumannii* showed an alarmingly high level of resistance. The resistance decreased slightly during COVID-19 (82.35% to 81.82%)

but there were no samples post-COVID-19 to compare this with.

Table 3 Northern-Cape Gram Negative Bacterial Sensitivity Results

NORTHERN-CAPE GRAM NEGATIVE BACTERIAL SENSITIVITY RESULTS									
	PRE-COVID-19			DURING COVID-19			POST-COVID-19		
	SENS I T I V E	RESIS T A N T	TO T A L	SENS I T I V E	RESIS T A N T	TO T A L	SENS I T I V E	RESIS T A N T	TO T A L
PSEUDOMONAS AERUGINOSA	94 (65.28)	50 (34.72)	144	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
PROTEUS MIRABILIS	108 (68.35)	50 (31.65)	158	0 (0.00)	0 (0.00)	0	15 (78.95)	4 (21.05)	19
ACINETOBACTER BAUMANNII	18 (17.65)	84 (82.35)	102	2 (18.18)	9 (81.82)	11	0 (0.00)	0 (0.00)	0
KLEBSIELLA PNEUMONIAE	32 (88.89)	4 (11.11)	36	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
ESCHERICHIA COLI	16 (88.89)	2 (11.11)	18	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
SERRATIA MARCESCENS	16 (72.73)	6 (27.27)	22	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
ENTEROBACTER CLOACAE COMPLEX	13 (76.47)	4 (23.53)	17	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
ENTEROBACTER AEROGENSES	14 (82.35)	3 (17.65)	17	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
TOTAL	311 (60.51)	203 (39.49)	514	2 (18.18)	9 (81.82)	11	15 (78.95)	4 (21.05)	19

All of the other organisms only had results pre-COVID-19 so there were no results during and post-COVID-19 to compare this with. *Klebsiella pneumoniae* and *Escherichia coli* both showed an 11.11% resistance pre-COVID-19. *Serratia marcescens* had a 27.27% resistance pre-COVID-19 and *Enterobacter cloacae complex* showed a 23.53% resistance. *Enterobacter aerogenes* showed a 17.65% resistance pre-COVID-19.

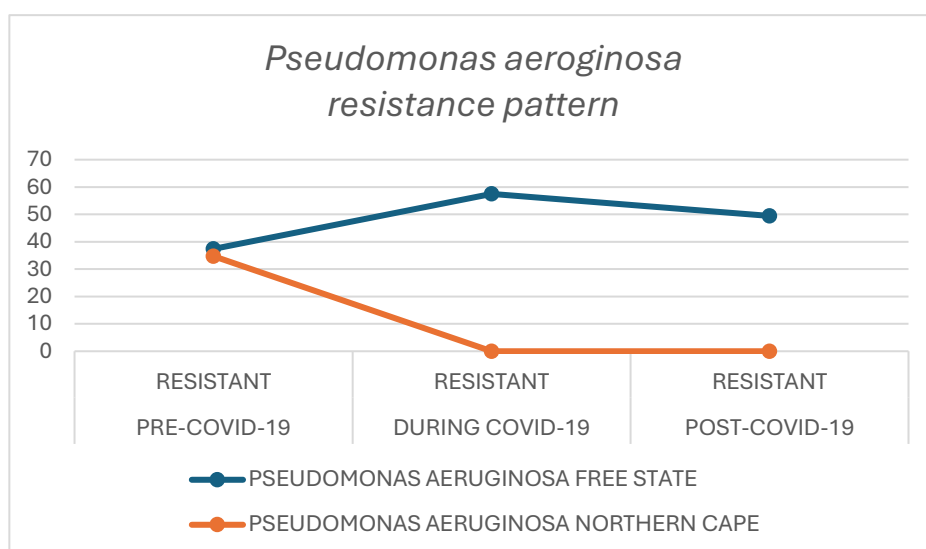


Figure 1 *Pseudomonas aeruginosa* Resistance Pattern

Overall, a higher resistance percentage could be seen for *Pseudomonas aeruginosa* in the Free State when compared to the Northern Cape. In the Free State a sharp increase in resistance was seen during COVID-19 and then a slight decrease in resistance was seen post-COVID-19. In the Northern Cape there was a decrease in resistance seen during COVID-19 and this remained the same post-COVID-19. The decrease in resistance that was seen is due to no samples for these two timeframes.

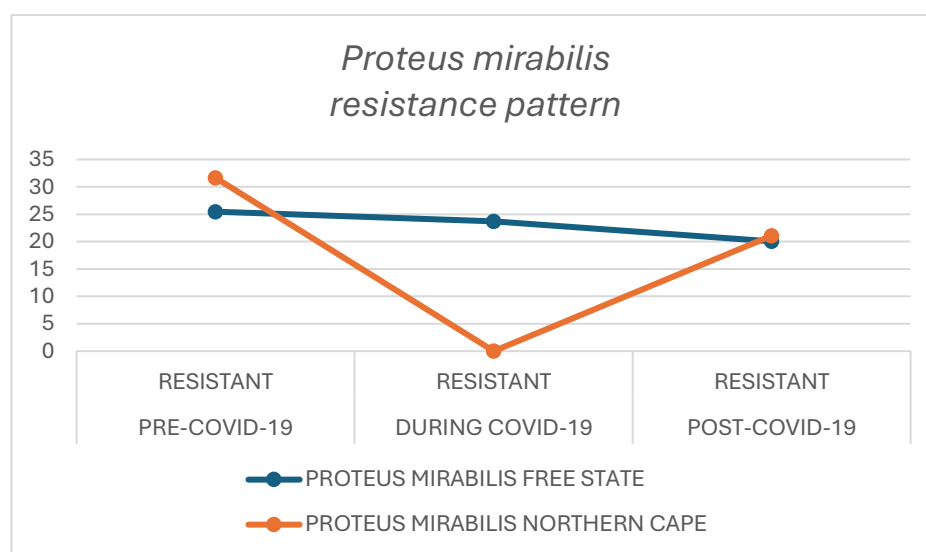


Figure 2 *Proteus mirabilis* Resistance Pattern

In the Free State a constant decrease in resistance could be seen for *Proteus mirabilis* over the three timeframes. A sharp decrease in resistance could be seen in the Northern Cape but this decrease is

due to no samples during the pandemic. The resistance then increases again post-COVID-19. The resistance percentage pre- and post-COVID-19 are similar for both provinces.

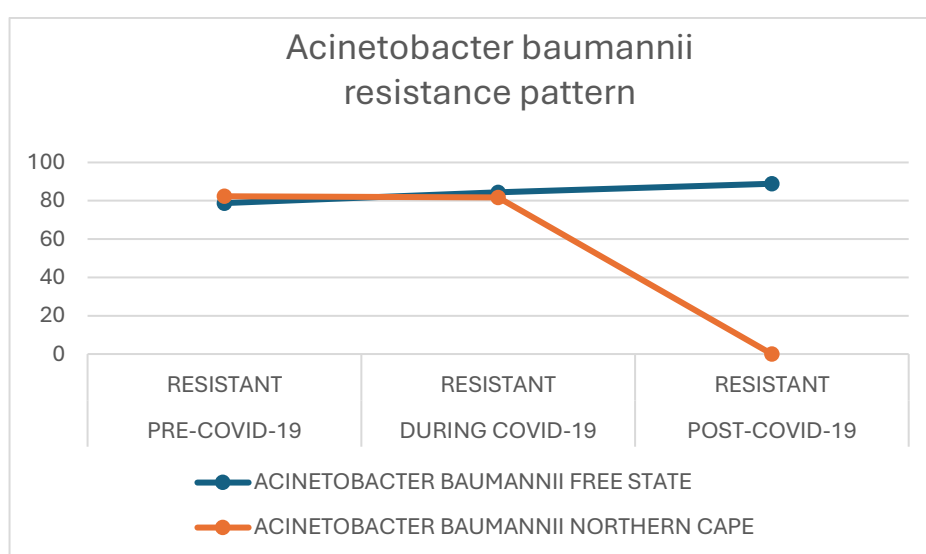


Figure 3 *Acinetobacter baumannii* Resistance Patter

The resistance percentage for *Acinetobacter baumannii* are similar pre-COVID-19 for both provinces. A slight decrease could be seen during COVID-19 in the Northern Cape while a slight increase is seen

in the Free State. A further increase in resistance is seen post-COVID-19 in the Free State while there are no samples in the Northern Cape to compare this with.

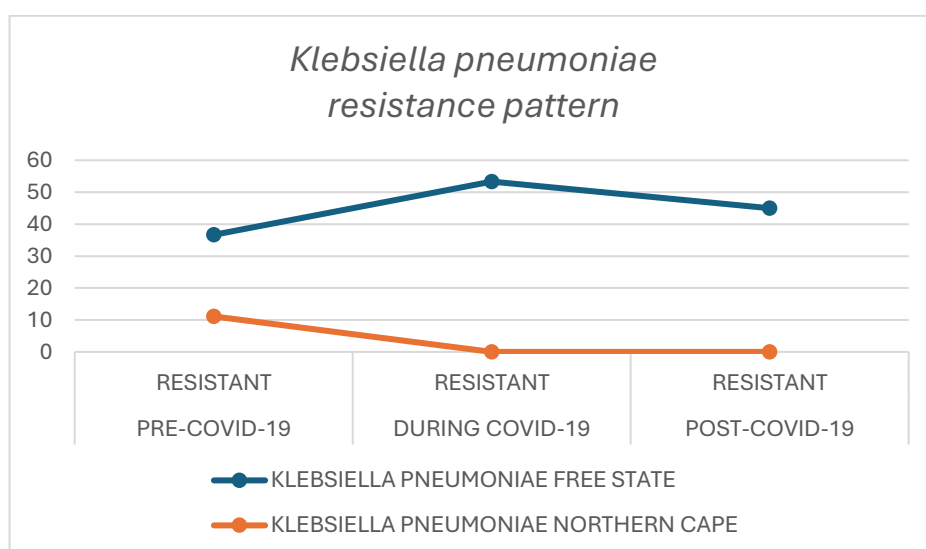


Figure 4 *Klebsiella pneumoniae* Resistance Pattern

In the Free State a sharp increase in resistance could be seen during COVID-19 for *Klebsiella pneumoniae* and then a slight decrease post-COVID-19. The resistance percentage was lower pre-COVID-19 in

the Northern Cape when compared to the Free State. There were no sample during and post-COVID-19 to compare these results with in the Northern Cape.

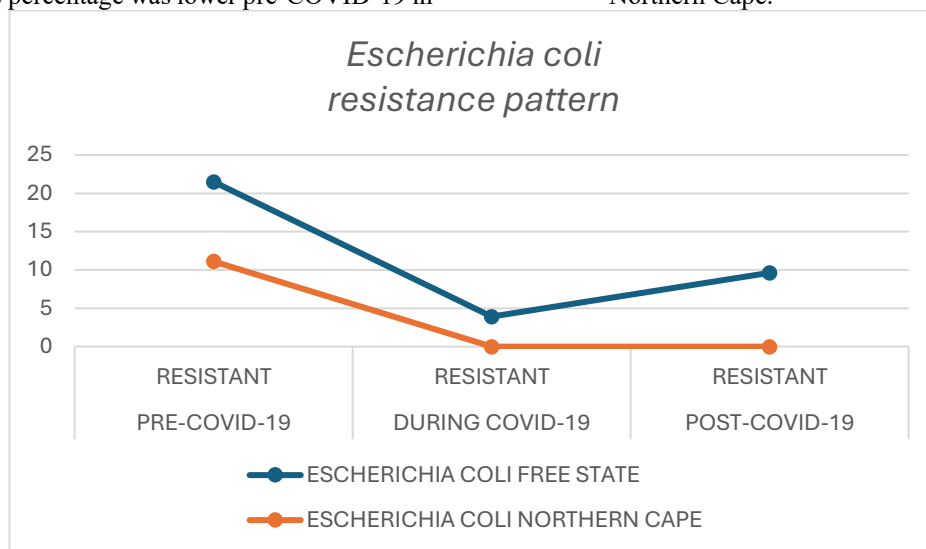


Figure 5 *Escherichia coli* Resistance Pattern

In the Free State a sharp decrease in resistance could be seen during COVID-19 for *Escherichia coli* and then a slight increase post-COVID-19. The resistance percentage was lower pre-COVID-19 in

the Northern Cape when compared to the Free State. There were no sample during and post-COVID-19 to compare these results with in the Northern Cape.

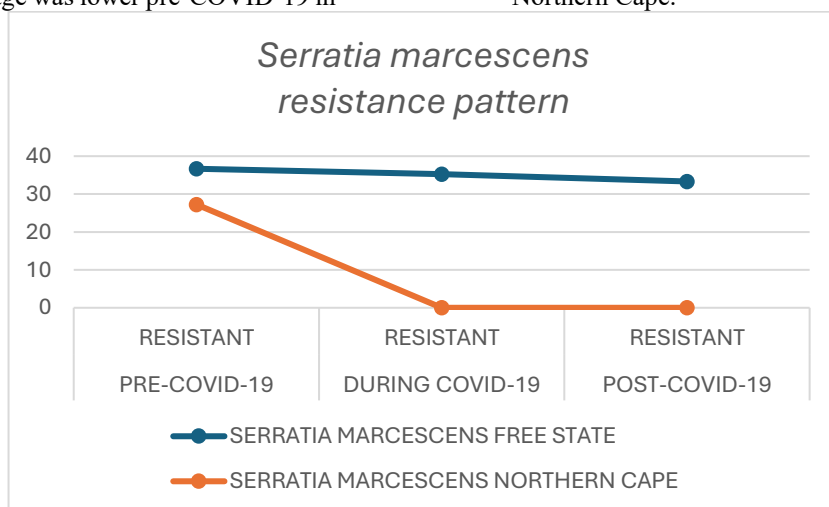


Figure 6 *Serratia marcescens* Resistance Pattern

A steady decrease in resistance could be seen in the Free state for *Serratia marcescens* over the three timeframes. In the Northern Cape the resistance pre-COVID-19 was slightly lower than that of the

Free State. There were no samples during and post-COVID-19 in the Northern Cape to compare these results with.

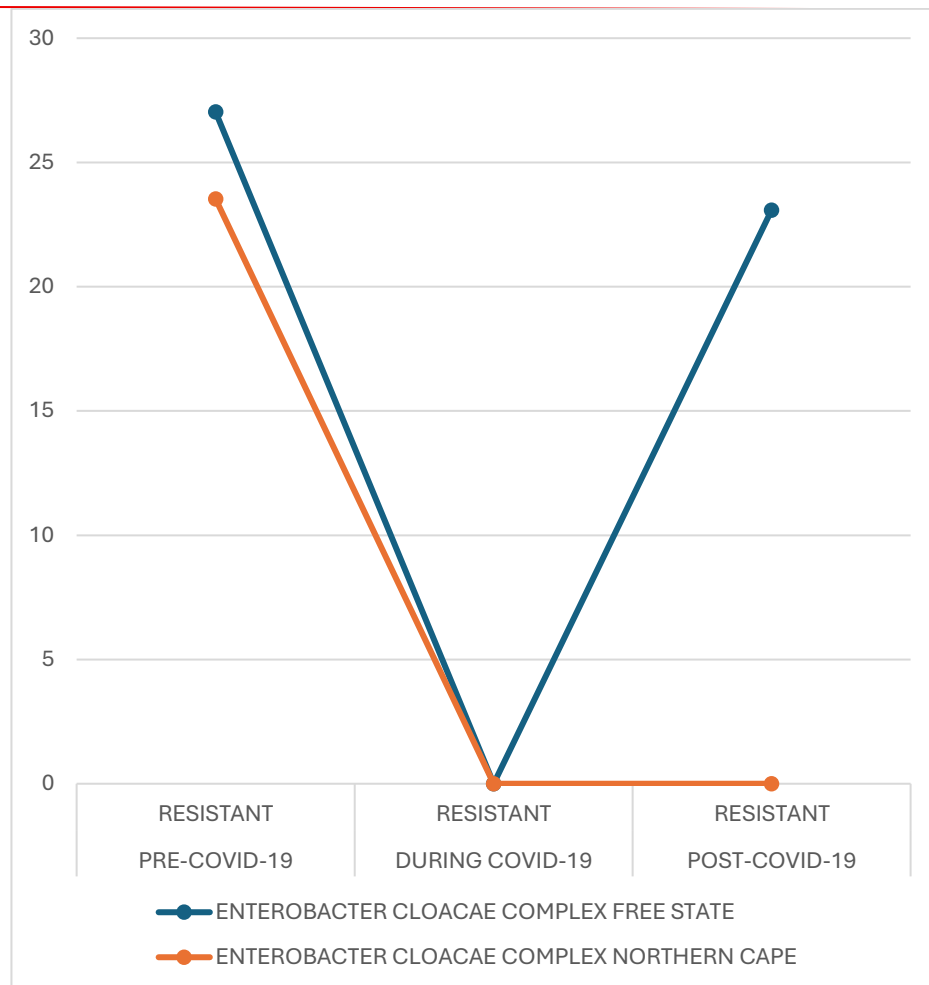


Figure 7 *Enterobacter cloacae* complex Resistance Pattern

The resistance percentage for *Enterobacter cloacae* complex was similar pre-COVID-19 for both provinces. However, there were no samples during COVID-19 to compare these results with for both

provinces. Post-COVID-19 a slightly lower resistance could be seen in the Free State when compared to the pre-COVID-19 percentage.

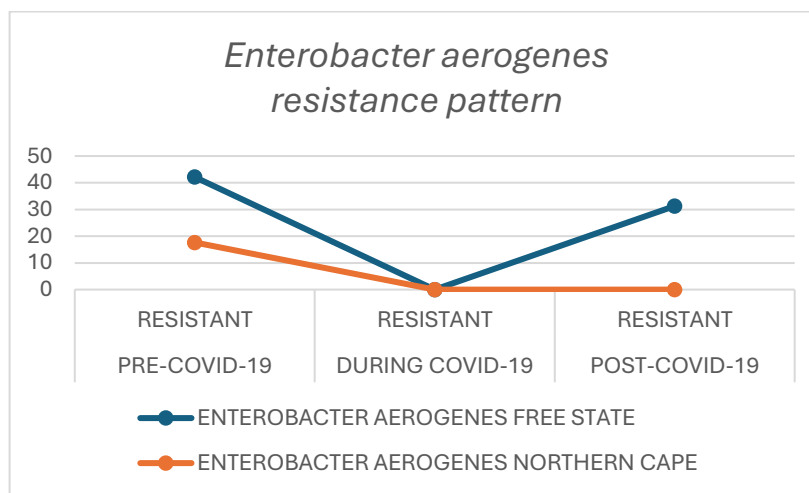


Figure 8 *Enterobacter aerogenes* Resistance Pattern

The resistance percentage for *Enterobacter aerogenes* was lower in the Northern Cape when compared to the Free State pre-COVID-19. However, there were no samples during COVID-19 to compare

these results with for both provinces. Post-COVID-19 a slightly lower resistance could be seen in the Free State when compared to the pre-COVID-19 percentage.

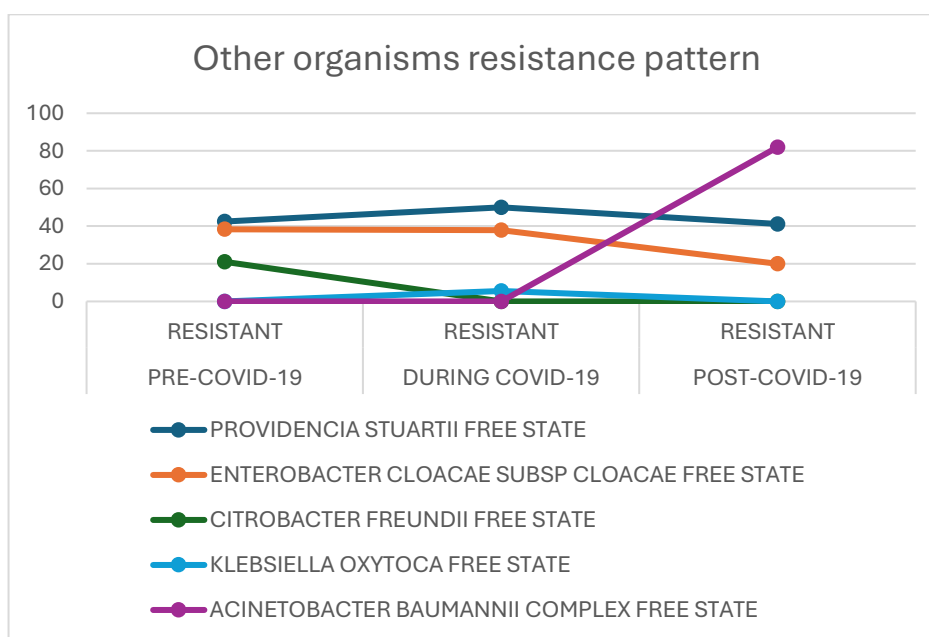


Figure 9 Other Organisms Resistance Pattern

There were no samples that tested positive to any of the organisms in the table above in the Northern Cape. The table is therefore only results of isolates from the Free State. The resistance increased during COVID-19 for *Providencia stuartii* and then decreased post-COVID-19 to levels similar to that of pre-COVID-19. A slight decrease in resistance could be seen during COVID-19 for *Enterobacter*

cloacae and then a sharper decrease in resistance post-COVID-19.

A relatively low percentage of resistance could be seen pre-COVID-19 for *Citrobacter freundii*. There were no samples that tested positive for the isolate during and post-COVID-19. A low percentage of resistance could be seen for *Klebsiella oxytoca*

during COVID-19. There were no samples that tested positive for the isolate pre- and post-COVID-19 to compare these results with. There were no samples that also tested positive for *Acinetobacter baumannii* pre- and during COVID-19 but an alarmingly high level of resistance could be seen post-COVID-19.

Discussion

The findings of this study gave a valuable insight into the trends of Gram-negative isolates and their resistance patterns in the burn units of the hospitals in the Free State and Northern Cape pre, during and post-COVID-19. Table one showed a clear difference in the sample size of the Northern Cape (40) and the Free State (1057). The difference in sample size could be due to a wide range of reasons. The Northern Cape has a much smaller population than the Free State but other factors such as laboratory capacity, patient load and laboratory infrastructure could also influence the sample size. The small sample size of the Northern Cape has made it difficult to identify any trends in sample size and resistance patterns, but the data is still significant since it shows regional variation.

The dominant isolate in both provinces was *Pseudomonas aeruginosa* which accounted for more than half of the total isolates (2435). This finding correlates with research that confirm that *Pseudomonas aeruginosa* is one of the leading causes of burn wound infections due to its acquired and intrinsic resistance mechanisms (Musuroi, et al., 2024). Pre-COVID-19 a total of 12 isolates were seen in the Northern Cape, but during and post-COVID-19 no isolates were found but this could however just be due to a reduced sample collection in these timeframes and not an epidemiological absence of the bacterium. During COVID-19 a decrease was seen in sample size (210 to 139) in the Free State and post-COVID-19 a notable increase (186) could be seen. The decrease and then increase could be due to a decrease in non-critical admissions during the pandemic followed by the resumption of the full hospital operations post-COVID-19 (Jeon, et al., 2022).

Proteus mirabilis is the second most isolated organism overall in both provinces. In the Free State the sample size increased over the three timeframes (30,49,100). This increased prevalence could be due to environmental contamination or even prolonged hospitalization in burn units (Vickers, et al., 2018). In the Northern Cape there was only 9 isolates pre-COVID-19 and one isolate post-COVID-19. The small number of isolates could again just be due to a reduced sample collection in these timeframes and not an epidemiological absence of the bacterium. *Acinetobacter baumannii* showed a small decrease during COVID-19 (32 to 27) in the Free State followed

by a sharp increase in sample size (64) post-COVID-19. This bacterium is known to persist in hospital environments which makes it a particularly difficult bacterium in burn units (Vickers, et al., 2018).

A sharp decrease in isolates could be seen during COVID-19 in the Free State for *Klebsiella pneumoniae* (40 to 13) followed by an increase post-COVID-19 (27). This same trend could be seen for *Serratia marcescens* and *Enterobacter* species during the pandemic. This could be caused by a decrease in specimen submissions during the pandemic since focus was placed on critical COVID-19 patients and samples (Alotaibi, 2022). The rise in sample size that is seen post-COVID-19 could be due to the normalization that is seen in the hospital environments regarding sample testing (Musuroi, et al., 2024).

Table 2 showed the sensitivity results of the Gram-negative bacterial isolates in the Free State. A drastic increase in resistance is seen during COVID-19 (37.39% to 57.49%) in the Free State for *Pseudomonas aeruginosa* followed by a slight decrease post-COVID-19 (49.49%). These findings mimic global findings that suggests that the increased antibiotic consumption during the pandemic lead to elevated resistance levels. Even though a slight recovery in resistance could be seen, the percentage is still high and this highlights the fact that *Pseudomonas aeruginosa* adapts quickly to antimicrobial pressure (Jeon, et al., 2022).

The resistance of *Proteus mirabilis* did not fluctuate over the three timeframes (25.47%, 23.73%, 20.05%). This confirms that the organisms are not that susceptible to rapid resistance formation. *Proteus mirabilis* however showed a decrease in resistance over the three timeframes which indicates that it was less affected by the increased antibiotic use during the pandemic when compared to non-fermenters (Jeon, et al., 2022).

A consistently high percentage of resistance was seen with *Acinetobacter baumannii* with the resistance increasing from 78.79% pre-COVID-19 to 88.89% post-COVID-19. This correlates with global reports that identifies the bacterium as “one of the most difficult-to-treat pathogens” in burn units. This is due to the pathogen's ability to form extensive drug resistance which includes biofilm formation and carbapenemase production (Vickers, et al., 2018). The increased resistance suggests that in South African burn units the spread has not yet been mitigated by the stewardship interventions.

The increased resistance that is seen during COVID-19 in *Klebsiella pneumoniae* (36.65% to 53.33%) could be caused by the increased use of carbapenems and cephalosporins during this timeframe of the pandemic (Alotaibi, 2022). The

resistance dropped slightly post-COVID-19 (45.03%) but it did not go back to pre-COVID-19 levels which suggests the persistence of resistant clones in the burn units (Jeon, et al., 2022).

Escherichia coli showed an atypical resistance pattern. The resistance decreased during COVID-19 (from 21.5% to 3.92%) which then increased post-COVID-19 (9.62%). The reason for this could most likely be due to reduced sampling and the prioritization of COVID-19 samples rather than a true biological reduction (Diop, et al., 2025). The same pattern of resistance could be seen for *Providencia stuartii*. The resistance increased during COVID-19 (42.53% to 50%) and then decreased again to 41.18% post-COVID-19. The temporary increase in resistance that was seen during COVID-19 could be due to the bacterium's ability to acquire integron-mediated resistance (Musuroi, et al., 2024).

Enterobacter cloacae and *Enterobacter aerogenes* showed a gradual decrease across all three timeframes. The resistance decreased slightly during COVID-19 and then decreased even further post-COVID-19. This supports what literature states that the stewardship recovery and stricter infection prevention practices assisted with the decrease in resistance of these organisms (Jeon, et al., 2022); (Ruesegger, Xiao, Naziripour, Brown, & Kanumuambidi, 2022).

Serratia marcescens showed a slight but gradual decrease in resistance (36.7%, 35.3%, 33.3%). These results suggest that there is a gradual restoration of the susceptibility which is due to the bacterium's ability of being less affected by short-term antibiotic surges when compared to other bacteria (Greatorex & Oosthuizen, 2015). *Citrobacter freundii* and *Klebsiella oxytoca* showed infrequent results due to no samples for certain timeframes. This also corresponds with literature which states that these organisms are infrequently isolated in burn wound infections (Kabanangi, et al., 2021).

Table 3 shows the resistance pattern of the Northern Cape, the data that is used in the table is limited due to small sample sizes when compared to the Free State. The following bacteria showed moderate resistance pre-COVID-19; *Pseudomonas aeruginosa* (34.72%), *Klebsiella pneumoniae* (11.11%), *Escherichia coli* (11.11%), *Serratia marcescens* (27.27%), *Enterobacter cloacae complex* (23.53%), *Enterobacter aerogenes* (17.65%). These bacteria had no results during and post-COVID-19 to compare these results of pre-COVID-19 with. These gaps in data could be due to the prioritization of COVID-19 samples during these timeframes. These results correlate with the Free State results of pre-COVID-19 which suggests hospital level

antibiotic pressure (Greatorex & Oosthuizen, 2015).

Proteus mirabilis showed a 31.65% resistance pre-COVID-19 and a 21.05% resistance post-COVID-19 which shows a slight decrease which suggests a decrease in selective pressure. *Acinetobacter baumannii* showed a consistently high resistance of 82.35% pre-COVID-19 and 81.82% during COVID-19 which correlates with literature that suggests that it is a critical pathogen according to the WHO (Vickers, et al., 2018).

Other bacteria such as *Klebsiella pneumoniae*, *E. coli*, and *Serratia marcescens* displayed low pre-pandemic resistance levels (11–27%), but the absence of post-pandemic data makes trend evaluation difficult. Nevertheless, the pattern supports the need for improved microbiological surveillance in under-resourced provinces to ensure early detection of resistance surges (Ruesegger et al., 2022).

Figure 1 showed the resistance pattern of *Pseudomonas aeruginosa* which indicated that there was a sharp increase in the resistance during COVID-19 in the Free State with a post-COVID-19 decline in resistance. The resistance level however did not decrease back to pre-COVID-19 levels. This increase and slight decrease could be due to the overuse of cephalosporins and carbapenems during COVID-19 (Musuroi, et al., 2024). The decrease in resistance that is seen in the Northern Cape is due to there being no samples during and post-COVID-19 for this bacterium.

Figure 2 represents the resistance pattern of *Proteus mirabilis* in both provinces. A gradual decline in resistance could be seen in the Free State. In the Northern Cape there was no samples that tested positive for the bacterium during COVID-19 and that is why a sharp decrease in resistance could be seen on the graph. The resistance pattern before and post-COVID-19 is similar to that of the Free State. This suggests that the bacterium was less impacted by the misuse of antimicrobials during the pandemic than other bacteria (Jeon, et al., 2022).

Figure 3 shows the resistance pattern of *Acinetobacter baumannii* in the Free State and Northern Cape. In the Free State a gradual increase in resistance could be seen across the three timeframes with the resistance being extremely high overall. This correlates with literature that states that *Acinetobacter* rapidly develops resistance to carbapenem and aminoglycosides which was misused during the pandemic (Vickers, et al., 2018). A gradual increase in resistance could also be seen during COVID-19 in the Northern Cape, however there was no samples post-COVID-19 to give a clear result of the resistance pattern.

Figure 4 illustrates the resistance pattern of *Klebsiella pneumoniae* in both provinces. A sharp increase in

resistance could be seen in the Free State during COVID-19 and a slight decrease post-COVID-19. The resistance however did not drop back to pre-COVID-19 levels. The sharp increase in resistance during COVID-19 could be attributed to empirical antibiotic use and the decrease in AMS programmes (Alotaibi , 2022). The slight decrease in resistance post COVID-19 suggests that there is still an ongoing problem with the spread of extended-spectrum-beta-lactamase producing strains (Kabanangi , et al., 2021). In the Northern Cape there was only isolates pre-COVID-19 and the resistance was lower than that of the Free State.

Figure 5 shows the resistance pattern of *Escherichia coli* in the Northern Cape and Free State. In the Free State a sharp decrease in resistance could be seen during COVID-19 followed with a slight increase in resistance post-COVID-19. While the small number of isolates could influence the results, but the increase in resistance post COVID-19 could more likely be due to selective pressure during the lockdown of COVID-19 (Diop, et al., 2025). In the Northern Cape there was only samples pre-COVID-19 and no further samples for the other timeframes to compare these results with.

Figure 6 shows the resistance pattern of *Serratia marcescens* in both provinces. A gradual decrease across all three timeframes could be seen in the Free State and this could possibly be due its lower susceptibility to commonly used antibiotics. Other literature supports this statement since they also found that the bacterium is less multidrug-resistant when compared to other bacteria (Greatest & Oosthuizen , 2015). In the Northern Cape there was only samples that showed resistance pre-COVID-19, but there were no samples for the other timeframes to compare these results with.

Figure 7 represents the resistance pattern of *Enterobacter cloacae* complex in both provinces. In the Free State there was no samples during COVID-19 but when comparing the resistance pre- and post-COVID-19 a slight decrease could be seen. This decrease could be due to the re-implementation of stewardship programs. This could also be due to lower selective pressure after the pandemic (Jeon, et al., 2022). In the Northern Cape there was only samples that showed resistance pre-COVID-19 and no other samples for the other timeframes to compare this with.

Figure 8 shows the resistance pattern of *Enterobacter aerogenes* in both provinces. The results are similar to those of *Enterobacter cloacae* complex. In the Free State there was no samples during COVID-19 but when comparing the resistance pre- and post-COVID-19 a decrease could be seen. This decrease could be due to the re-implementation of stewardship programs and it could also be due to lower selective pressure after the pandemic (Jeon, et al., 2022). In the Northern Cape there was only samples that showed

resistance pre-COVID-19 and no other samples for the other timeframes to compare this with. The bacterium shows a moderate resistance when compared to other organisms such as *Klebsiella* and *Acinetobacter* which suggests that there was a limited exposure to broad-spectrum antibiotics.

Figure 9 included *Providencia stuartii*, *Enterobacter cloacae* subsp *cloacae*, *Acinetobacter baumannii* complex, *Klebsiella oxytoca* and *Citrobacter freundii* in the Free State since there were no samples in the Northern Cape. A slight increase in resistance could be seen for *Providencia stuartii*, *Enterobacter cloacae* subsp *cloacae* and *Klebsiella oxytoca* followed with a sharp decrease in resistance post-COVID-19. *Providencia stuartii* had no samples pre- and during COVID-19, but an extremely high resistance level could be seen post-COVID-19 (81.97%) which is quite concerning due to the persistence of this MDR organism (Musuroi, et al., 2024). *Citrobacter freundii* only had samples pre-COVID-19 and no other samples for to compare these results with.

Conclusion

The overall results that were found in this study correlates with trends that are seen globally during and after COVID-19. The reduced surveillance and IPC together with the increased antimicrobial use during COVID-19 caused a significant increase in MDR Gram-negative infections (Jeon, et al., 2022); (Alotaibi , 2022). In both provinces a consistent high percentage of resistance could be seen for *Acinetobacter* and *Pseudomonas* which highlights the urgency for local stewardship interventions. These findings also emphasize the problem of treating MDR infections in burn wound patients who already have a compromised immunity. *Escherichia coli* and *Proteus mirabilis* showed a positive susceptibility trend and *Klebsiella pneumoniae* showed a partial resistance recovery.

The high resistance rates that are seen post-COVID-19 suggests that COVID-19 had an impact on antimicrobial resistance and that this effect could be long-lasting. Prolonged hospitalization and the use of devices used on these patients create the ideal environment for MDR pathogens to proliferate (Vickers , et al., 2018). These findings suggest that a stricter adherence to IPC guidelines should be followed especially the antibiotic policies. Enhanced laboratory-based surveillance should be done to identify trends in resistance across these provinces to prevent further resistance.

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