Nocardia species epidemiology and susceptibility profiles from 2019 to 2022 in South Africa

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Background. *Nocardia* species cause infections in humans, from localised to disseminated disease. They constitute a public health threat owing to the lack of sufficient information about them. In South Africa (SA), the last publication on this organism was in 2010. Predominant species types and antibiotic susceptibilities may have changed over this period.
Objective. To address the knowledge gap surrounding *Nocardia* species and their antibiotic susceptibilities in SA.
Methods. This was a retrospective and cross-sectional study. Data were collected from the Central Data Warehouse (CDW) of the National Health Laboratory Service (NHLS) on suspected *Nocardia* species from 1 January 2019 to 31 December 2022. Organism speciation was performed using 16S rRNA sequencing and antibiotic susceptibility testing (AST) by the broth microdilution (BMD) method. Data analysis included patient age, sample types from which the organism was cultured, distribution in the various SA provinces, species types and species AST profiles, including a record of trimethoprim-sulfamethoxazole (TMP/SMX) non-susceptibility.
Results. One hundred and sixty-five positive culture results were analysed. The majority of positive cultures (28%, *n*=46) were from the 30 - 39-year age group. The organism was predominantly cultured from pus samples (31%, *n*=51). The top two provinces from which the largest numbers of isolates were submitted were Gauteng (69%, *n*=114) and Western Cape (18%, *n*=30) provinces. Two percent (*n*=4) of isolates were not sequenced, and 18% (*n*=30) of isolates lacked AST results. Twenty-nine percent (*n*=47) of the *Nocardia* species that were sequenced could not be speciated using 16S rRNA sequencing. The top two species country-wide were *N. abscessus* complex (25%, *n*=42) and *N. cyriacigeorgica* (18%, *n*=29). Approximately 90% (*n*=121) of all isolates tested were TMP/SMX susceptible.
Conclusion. The predominant isolation of *Nocardia* species from pus samples suggests that the majority were deep-seated infections. The most common *Nocardia* species types and the AST profiles have changed over time. The study highlights the need for alternative methods for the speciation of this organism.

Keywords: *Nocardia* species, South Africa, epidemiology, antibiotic susceptibility testing

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Nocardia species are Gram-positive, branching aerobic bacilli that have been implicated as a cause of serious human infections, especially in immunocompromised patients.[1] Infections can occur at various sites, such as the skin, lung and central nervous system, or they can be disseminated throughout the body. Sulphonamides were the first agents to be introduced for the treatment of *Nocardia* species, and were associated with a substantial reduction in mortality.[2] Sulphonamides remain the cornerstone of treatment, although sulphonamide-resistant *Nocardia* species have emerged.[3] Combination antibiotic therapy for severe nocardiosis is now considered standard practice.[2] Patients with cutaneous nocardiosis usually have full resolution with antibiotic therapy, and pulmonary nocardiosis is associated with a 90% cure rate. However, disseminated infections, particularly those involving the brain, are associated with 50% mortality.[4] Targeted treatment is based on antibiotic susceptibility profiles, once available.[5] Inadequate treatment is associated with an increase in mortality. Initial empiric treatment for serious infections should consist of combination antibiotic therapy, including sulphonamide.[2] As nocardiosis is rare, clinical trials on best treatment options are limited. The choice of empiric therapy in a geographical area should be based on an understanding of the local *Nocardia* species epidemiology, including species types and antibiotic susceptibility profiles.[6] The introduction of molecular techniques has modified the classification of *Nocardia*, and over 40 species are known to cause human disease.[7,8] Knowledge of the species type is important, as different species have predictable antibiotic susceptibility patterns that can help guide empiric therapy.[9]

Broth microdilution (BMD) testing is the recommended method to determine *Nocardia* species' antibiotic susceptibility profiles, as per the Clinical Laboratory Standards Institute (CLSI).[10] This is a specialised test, and in South Africa (SA), specimens are sent to a single reference laboratory for this to be performed.

Susceptibility patterns change over time, and antibiotic-resistant *Nocardia* species are increasingly being described worldwide.[9,11-14] Susceptibility profiles of the different *Nocardia* species can vary, and within a species, susceptibility can differ in various geographical areas.[15] Susceptibility testing is additionally necessary when there is treatment failure, or when relapse occurs owing to antibiotic resistance. It may also be required if there are concerns about adverse drug reactions to the first-line therapy, sulphonamides.

As *Nocardia* species are slow-growing organisms, susceptibility results are only available after 3 - 5 days of growth. This, combined with the time taken to refer specimens to a specialised laboratory,

contributes to a delay in antibiotic susceptibility results, affecting the timely guidance of treatment. Consequently, empiric therapy is continued for some time before targeted therapy can be prescribed. Therefore, understanding the susceptibility profiles of different *Nocardia* species in SA is important to guide appropriate empiric antibiotic choices.

The last publication from SA describing *Nocardia* species susceptibility was in 2010, which looked at a subset of 39 stored *Nocardia* species isolates from patients in whom antibiotic susceptibility testing (AST) was performed.[16] The study intended to characterise the isolates and to evaluate the performance of the E-test for susceptibility testing of the antibiotic agents in comparison with the BMD method. *N. farcinica*, *N. cyriacigeorgica* and *N. otitidiscaviarum* were the most common isolates at the time. In the study, all of the *Nocardia* species were susceptible to trimethoprim-sulfamethoxazole (TMP/SMX), amikacin and linezolid. An update on the current epidemiology and antibiotic susceptibility profiles of the prominent *Nocardia* species detected in SA is long overdue.

Therefore, the objectives of the study were to:

- report on the population age groups affected by this infection
- report on common sites of *Nocardia* infection in affected patients
- determine which provinces in SA reported the infections
- list the *Nocardia* species causing infections in SA, and
- evaluate the species-specific antibiotic susceptibility profiles to guide empiric antibiotic therapy.

Methods

This was a retrospective and cross-sectional study using data acquired from the Central Data Warehouse (CDW) of the National Health Laboratory Service (NHLS), describing all *Nocardia* isolates cultured in SA from 1 January 2019 to 31 December 2022 and submitted for BMD AST. All patient-identifying information from this database was anonymised. Patient data of interest which were analysed included:

- patient age
- sample type that yielded the positive culture
- SA provinces that yielded positive cultures
- *Nocardia* species isolated based on 16S rRNA sequencing results
- antibiotic susceptibility test results by the BMD method for the isolates.

Nocardia species identification was done through sequencing in one public sector and two private microbiology laboratories. In both settings, 16S rRNA sequencing was performed using the following primers: 4F (TTGGAGAGTTTGATCCTGGCTC) and BAKR (AAGGAGGTGATCCAGCCGCA), for initial amplification using the Qiagen HotStart Taq DNA polymerase (Qiagen) on the T100 thermocycler (BioRad, USA). Purified amplicons were followed by five separate Sanger cycle sequencing reactions using the BigDye terminator version 3.1 cycle sequencing kit on the ABI3730 DNA Sequencer (Applied Biosystems, USA) using one of each of the following primers: 4F, BAKR, 534R (TACCGCGGCTGCTGGCAC), 801R (GGCGTGGACTTCCAGGGTATCT) and 5F (TGGAATTCCTGGTGTAGCGG). Full-length (~1 480bp) 16S rRNA gene sequencing was performed, which allowed for more accurate species identification than traditional 16S rRNA sequencing, which only uses the first ~500bp of sequence information.[17] Sequences were edited in Geneious 11 (BioMatters, USA), and analysed using GenBank BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The CLSI document MM18 2nd edition criteria were used to interpret genus and species level identification of the *Nocardia* species isolates, which recommends a sequence similarity ≥99.0%, and only a few mismatches at positions 160 - 220, 560 - 650 and 970 - 1020.[17] Certain species, notably *N. abscessus*, *N. asiatica* and *N. arthritidis*, shared almost complete sequence identity over the entire 16S rRNA, and were reported as *N. abscessus* complex. Similarly, the *N. nova* complex was reported for the species *N. africana*, *N. aobensis*, *N. cerradoensis*, *N. elegans*, *N. kruczakiae*, *N. mikamii*, *N. nova*, *N. vermiculata* and *N. veterana*, which share almost identical 16S rRNA homology.[17]

All *Nocardia* species susceptibility testing had been conducted by the one public sector laboratory in the country. This was done by the BMD method as per CLSI M24-A2, 2011.[18]

The antibiotics tested included amikacin, amoxicillin-clavulanate (augmentin), ceftriaxone, ciprofloxacin, clarithromycin, imipenem, TMP/SMX and linezolid.

Briefly, as per this method, a 0.5 MacFarland inoculum suspension of the isolate was prepared with sterile deionised water or saline. A volume of 50 µl of this inoculum was added to 10 mL of cation-adjusted Mueller Hinton broth. From this mixture, 100 µl was added to each well in the antibiotic panel. After the antibiotic panel was inoculated, a drop of the suspension was streaked onto a blood agar plate as a purity check. The antibiotic panel was incubated in ambient air at 35°C +/- 2°C for 5 days, and plates were read for the minimum inhibitory concentration for each antibiotic at 48 hours, 72 hours and finalised at 96 hours. Reviewing the plates from 48 hours ensured that early contamination of the panel was timeously picked up. The antibiotic dilutions included: amikacin (0.12 - 64 µg/mL), augmentin (0.06/0.03 - 32/16 µg/mL)), ceftriaxone (0.5 - 256 µg/ml), ciprofloxacin (0.12 - 64 µg/mL), clarithromycin (0.12 - 64 µg/mL), imipenem (0.12 - 64 µg/mL), TMP-SMX (0.015/0.2 - 8/152 µg/mL) and linezolid (0.12 - 64 µg/mL).

Data analysis

The total numbers of the categorical variables, such as age groups, sample types and provinces with positive cultures, as well as the *Nocardia* species cultured, were depicted in bar and pie charts. The antibiotic susceptibility results were presented as percentage susceptibility in bar charts. Excel (Microsoft, USA) was used to create the bar and pie charts.

Ethical approval

Ethics approval for the study was acquired from the Human Research Ethics Committee (Medical) of University of the Witwatersrand on 14 April 2022 (ref. no. W-CBP-220414-01).

Results

A total of 165 positive culture results from both public and private healthcare sectors were analysed from the CDW. The age groups that had the greatest number of positive cultures were between 30 and 39 years (28%, *n*=46) and 40 and 49 years (26%, *n*=43) of age (Fig. 1). Pus (31%, *n*=51) and sputum (29%, *n*=48) samples yielded the most isolates (Fig. 2). Gauteng (69%, *n*=114) and Western Cape (18%, *n*=30) were the two SA provinces where the majority of the isolates originated from (Fig. 3). Four isolates were not sequenced for confirmatory identification. Of the remaining 161 isolates, 87% (*n*=140) were sequenced at a public sector laboratory and 13% (*n*=21) by two private sector laboratories. The top two species country-wide were *N. abscessus* complex (25%, *n*=42) (consists of *N. abscessus*, *N. asiatica*, *N. arthritidis*) and *N. cyriacigeorgica* (18%, *n*=29) (Fig. 4). Thirty-one percent (*n*=51) of isolates could not be speciated, either because 16SrRNA could not speciate further or because

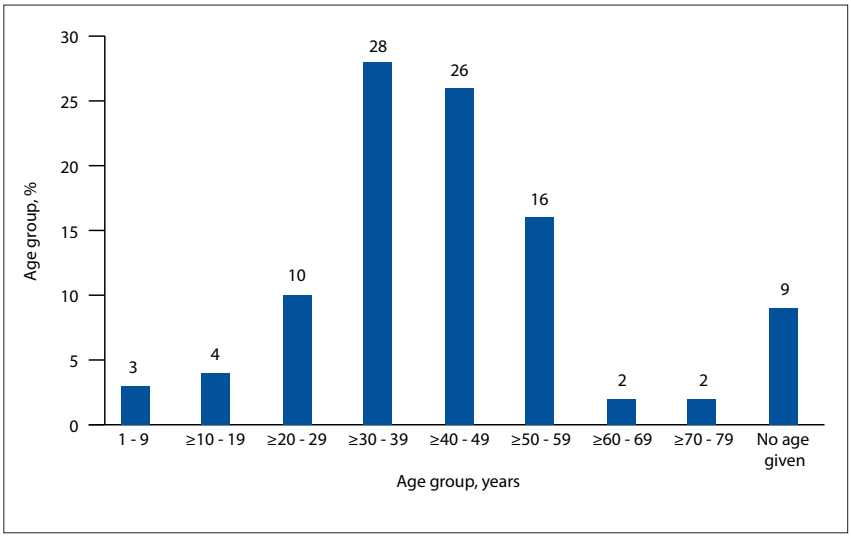

Fig. 1. Percentage of Nocardia *species isolated per age group: 2019 - 2022.*

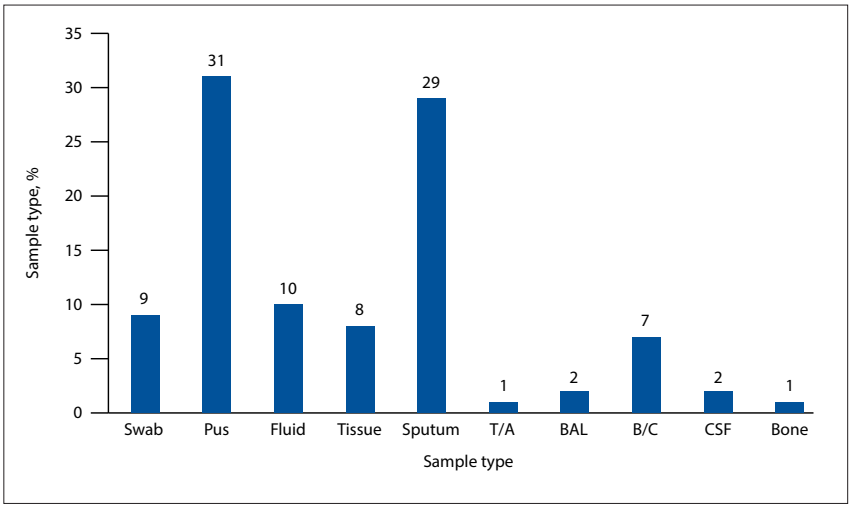

Fig. 2. Percentage of sample types with Nocardia *species isolated: 2019 - 2022. (T/A = tracheal aspirate; BAL = bronchoalveolar lavage; B/C = blood culture; CSF = cerebrospinal fluid.)*

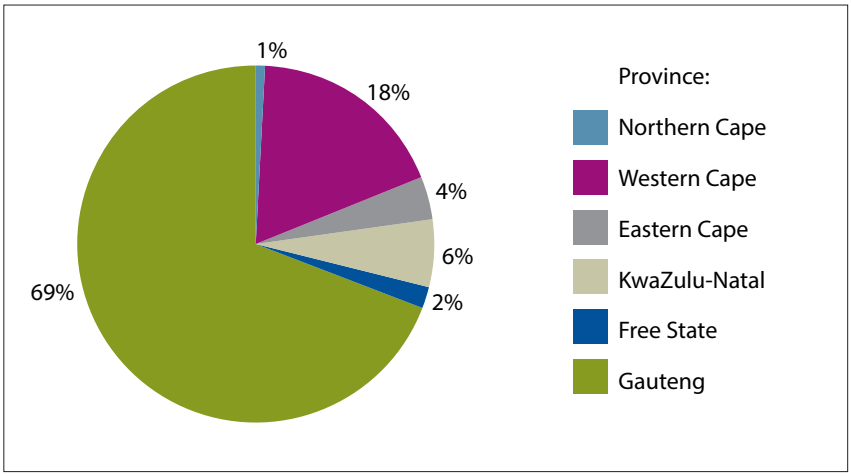

Fig. 3. Percentage of Nocardia *species isolated per province: 2019 -2022.*

the isolates were not sequenced. Eighteen percent (*n*=30) of all isolates had no AST performed at all. Of the isolates with TMP/SMX antibiotic susceptibility results (81%, *n*=134), ~90% (*n*=121) were susceptible to this agent (Fig. 5).

The antibiotic susceptibility results for the top two species (*N. abscessus* complex and *N. cyriacigeorgica*) are depicted separately in Figs 6 and 7.

In Gauteng Province, the top two *Nocardia* species that were identified to species level, in descending order, were *N. abscessus* complex (30%, *n*=34) and *N. cyriacigeorgica* (12%, *n*=14). In Western Cape Province, the order was *N. cyriacigeorgica* (43%, *n*=13) and *N. farcinica* (20%, *n*=6).

TMP/SMX non-susceptibility was seen mainly in three provinces: Western Cape, KwaZulu-Natal and Gauteng. The implicated species were *N. kroppenstedtii* (2%, *n*=3), *N. abscessus* complex (2%, *n*=2), *N. cyriacigeorgica* (2%, *n*=2), *N. brasiliensis* (1%, *n*=1), *N. pseudobrasiliensis* (1%, *n*=1), *N. farcinica* (1%, *n*=1), *N. nova* complex (1%, *n*=1) and in *Nocardia* that could not be speciated (2%, *n*=2).

A summative table of the above results is provided in appendix 1 (http://coding.samedical.org/file/2351).

Discussion

In contrast to the previous study conducted in 2010, this study analysed data collected nationwide over 4 years from both the public and private health sectors. Notably, it included a substantially larger number of clinical isolates. In addition, it is the first SA study to provide age and provincial distribution, along with sample information related to this organism. The age group most affected by infections was 30 - 49 years. A previous study from SA showed that nocardial infection often occurs as a complication of HIV infection, although HIV was not specifically analysed as a risk factor in this study.[1] The predominant isolation of the organisms from pus suggests deep-seated infections. Nocardiosis is known to cause suppurative necrosis with frequent abscess formation at sites of infection.[4] It is not surprising that the second predominant culture source was from sputum samples (lung), as the lung is a common site of *Nocardia* infection.[2]

Gauteng and the Western Cape were the two provinces with the highest number of isolates cultured. This may be because of better awareness of this infection in these provinces due to the presence of more microbiological expertise, along with better healthcare and diagnostic services (including 16SrRNA sequencing for *Nocardia* species and antibiotic susceptibility testing).

Two percent of the total positive cultures were not sequenced for species identification. Of those sequenced (98%), several *Nocardia* species were isolated across the country. The top two species across SA were *N. abscessus* complex (25%) and *N. cyriacigeorgica* (18%).

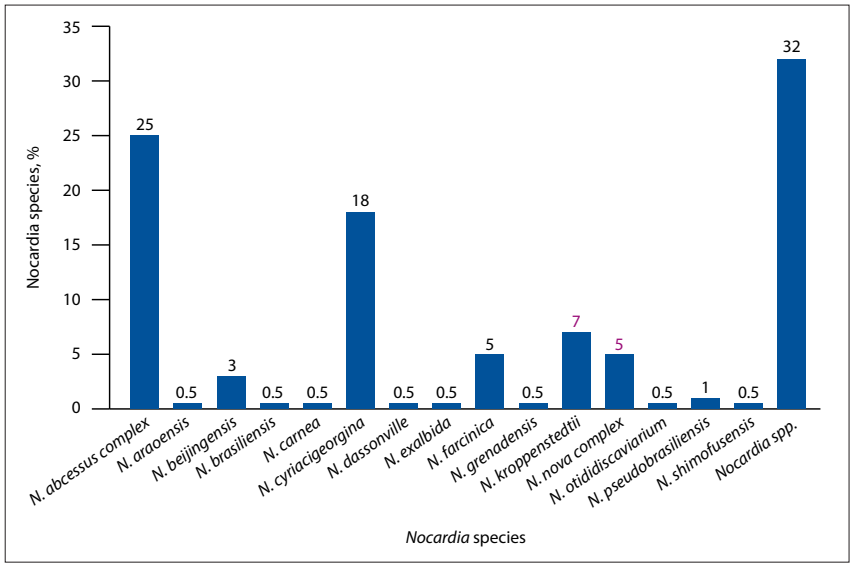

Fig. 4. Percentages of the Nocardia *species isolated: 2019 - 2022.* N. abscessus *complex included* N. abscessus, N. arthritidis *and* N. asiatica*;* N. nova *complex included* N. aobensis, N. nova*, and* N. veterana *isolates from this study.*[17]

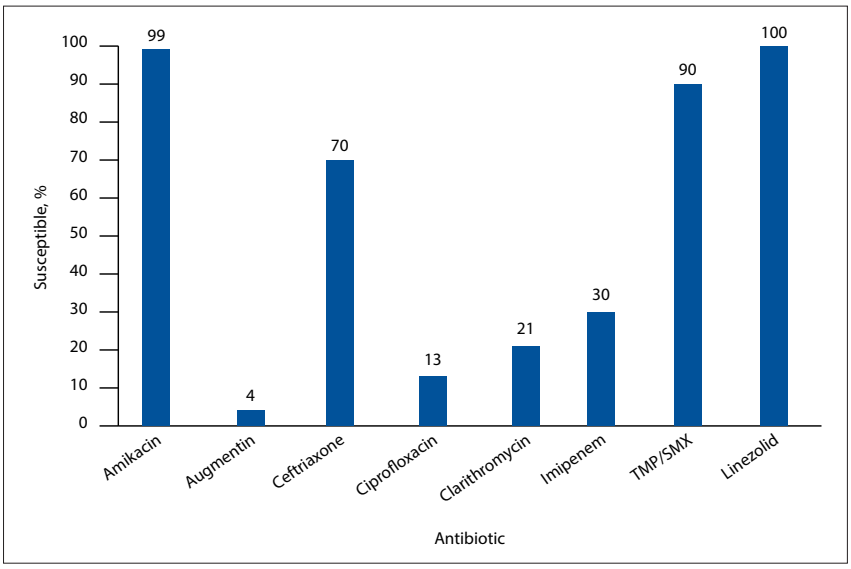

Fig. 5. All species antibiotic susceptibility profile: 2019 - 2022. (TMP/SMX = trimethoprim-sulfamethoxazole.)

N. abscessus complex was found in age groups ranging from 17 to 57 years of age, and in five (Gauteng, Western Cape, Eastern Cape, KwaZulu-Natal, Free State) of the six provinces from which isolates were received. *N. cyriacigeorgica* was found in age groups ranging from 5 to 57 years of age, and in four (Gauteng, Western Cape, Eastern Cape, KwaZulu-Natal) of the provinces. Both organisms were cultured from various specimen types.

N. farcinica (20%) was the second most prominent species in the Western Cape, out of a total of nine isolates of this species identified countrywide. This species was isolated in adults ages ranging from 31 to 63 years old, and predominantly from pus/fluid samples, suggesting deep-seated infections.

Twenty-nine percent of the *Nocardia* species could not be speciated by 16S rRNA sequencing, highlighting the limitations of this method. Novel targets and the combination of known targets to improve species identification have been evaluated. A recent promising novel target is the gene encoding dipeptidyl aminopeptidase BI (*dapb1*), which demonstrated a topology almost identical to genome-based phylogeny.[19] Other investigated targets include *hsp65* (heat shock protein), *gyrB* (the β-subunit of DNA gyrase and a type II DNA topoisomerase), *rpoB* (the β-subunit of DNA-dependent RNA polymerase) and *secA* (essential secretory protein) genes.[20-22]

TMP/SMX, the drug of choice for nocardiosis, was susceptible in 90% of the isolates that were tested. Other antibiotics with good susceptibility profiles included linezolid (100%) and amikacin (99%). Antibiotics with reduced activity included augmentin (4%), ciprofloxacin (13%), clarithromycin (21%), imipenem (30%) and ceftriaxone (70%) (Fig. 5). A similar profile applied to the top two species in the country, *N. abscessus* complex and *N. cyriacigeorgica*, except for ceftriaxone, where susceptibility was better (Figs 6 and 7*)*. TMP/SMX non-susceptibility was observed in 10% of isolates from varied species. These isolates originated from Western Cape, Gauteng and KZN provinces.

In the SA study by Lowman *et al.*[16] conducted in 2010, the most common *Nocardia* species identified were *N. farcinica* and *N. cyriacigeorgica*. This was based on a small sample size of 39 isolates.[16] In our study, the most common *Nocardia* species was *N. abscessus* complex, followed by *N. cyriacigeorgica*. Globally, there is variation in the predominant *Nocardia* species, with geographical variation that may be influenced by differences in soil composition.[23-27] Uniform susceptibility to TMP/SMX was observed in the study by Lowman *et al.*,[16] compared with a 10% overall non-susceptibility rate to TMP/SMX in our study.In an earlier SA study conducted in 2000 by Jones *et al.*,[1] TMP/SMX resistance was documented by BMD testing in five of nine (55%) *Nocardia* species cultured from HIV-infected patients. This study was, however, limited by a very small sample size, making it difficult to estimate the true prevalence of TMP/SMX resistance at the time. In addition, it is worth noting that since the 2010 publication, susceptibility to antibiotics used for managing nocardiosis, such as TMP/SMX, amikacin, augmentin, ciprofloxacin, clarithromycin and imipenem, is decreasing.[16] Globally, resistance rates vary, and are linked to the most prevalent *Nocardia* species isolated. However, in most studies, linezolid and amikacin are reliably susceptible, with overall resistance to TMP/SMX less than 5%.[25-29] The high emerging resistance rates to TMP/SMX in our study are of particular concern, and need to be monitored closely. These high resistance rates may be influenced by the use of TMP/SMX prophylaxis in our HIV-positive population.

Limitations of the study include the fact that risk factors for infection (e.g. HIV infection) were not investigated, and the retrospective nature of the study impacted on accurate data collection, as seen by missing information.

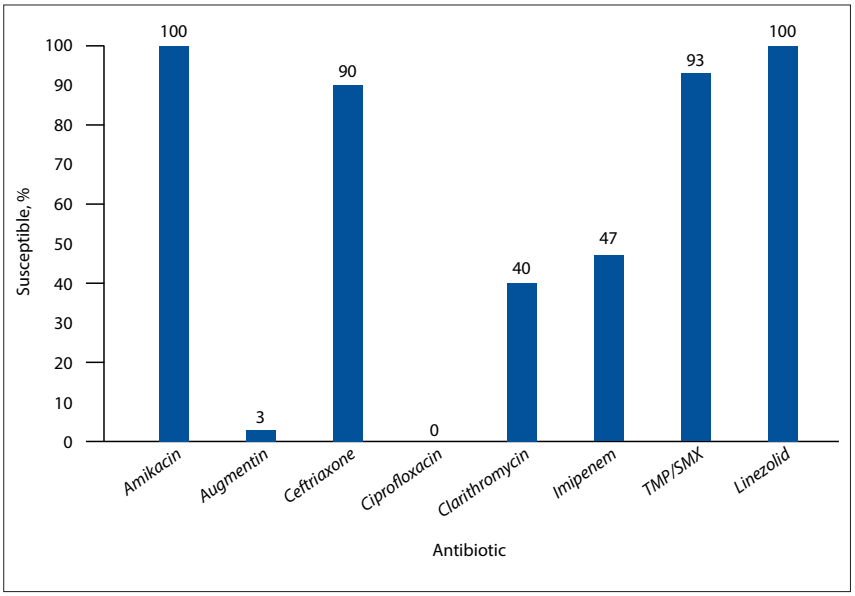

Fig. 6. Nocardia abscessus *complex antibiotic susceptibility profile: 2019 - 2022.*

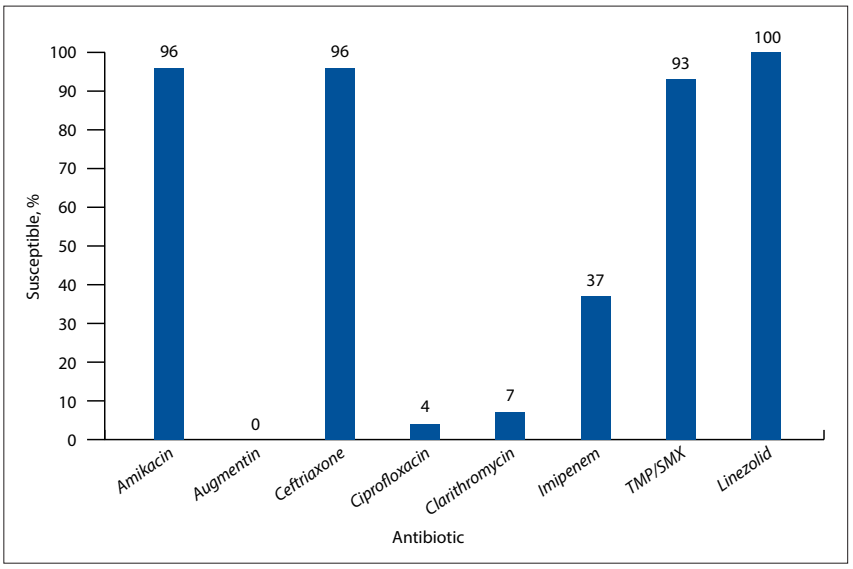

Fig. 7. Nocardia cyriacigeorgica *antibiotic susceptibility profile: 2019 - 2022.*

Conclusion

The study provides an updated perspective on *Nocardia* species epidemiology and susceptibility patterns in SA. The predominant *Nocardia* species types have changed over time and, of concern is the increasing non-susceptibility to TMP/SMX. Therefore, continued monitoring of these trends in the future is vital. Most local laboratories are still using 16S rRNA sequencing for *Nocardia* species identification; however, based on the study findings, it is imperative to investigate alternate targets for speciation.

Data availability. Data will be made available from the authors on request.

Declaration. None.

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Author contributions. TT was responsible for project conceptualisation, data analysis and write up of the article. ML, KL and KS contributed to article write up and provided critical feedback.

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Conflicts of interest. None.

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