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FOREWORD

Odyssean malaria refers to locally acquired-disease transmitted by an infective mosquito inadvertently transported from an endemic to a non-endemic area. This issue details an investigation into four locally-acquired malaria cases that occurred in the Madikwe Game Reserve of North West Province, a non-transmission area of South Africa. A second outbreak report describes gastroenteritis cases amongst learners and teachers of an urban school in Johannesburg who were returning from a school camp in KwaZulu-Natal Province. It is concluded that this outbreak was most likely caused by a waterborne pathogen.

Multidrug-resistant tuberculosis (MDR-TB) is a global public health crisis and seriously affects the treatment of TB in South Africa. This issue details a shorter treatment regimen for the management of drug-resistant TB. Staying with antimicrobial resistance, this issue also contains a report which summarises the preliminary results of surveys conducted in South Africa in 2016 for *Staphylococcus aureus* and carbapenem-resistant Enterobacteriaceae (CRE) bacteraemia and candidaemia. These surveys were conducted under the GERMS-SA umbrella. Lastly, sentinel aetiological surveillance of sexually transmitted infection (STI) syndromes was conducted at primary healthcare facilities in four South African provinces during the period 2014 to 2016, and the results are presented here.

These reports contain highly significant public health information for South Africa, and we trust you will find them interesting and informative. As this is the last issue for 2017, we wish all our readers a safe and joyous holiday season.

Basil Brooke,

Editor

ODYSSEAN MALARIA OUTBREAK AT A BUSH LODGE IN MADIKWE GAME RESERVE, NORTH WEST PROVINCE, SOUTH AFRICA, OCTOBER-NOVEMBER 2015

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Executive summary

In November 2015, the National Institute for Communicable Diseases (NICD) was notified of malaria cases at a lodge in Madikwe Game Reserve, North West Province – usually a non-transmission area in South Africa. Four laboratory-confirmed malaria cases were investigated. A structured questionnaire was used to gather information on demographic, clinical and exposure history. Interviews were conducted with employees and managers and blood samples were collected. Environmental assessment of the residence and immediate surroundings was conducted. Blood smear microscopy and PCR analysis for the detection of malaria parasites were done. All cases were female, employed, and resided in lodge staff accommodation. None of the cases had travelled to malaria-endemic areas. However, history of travel to possible malaria transmission areas was elicited in eight of the 33 staff members interviewed. Staff residences were within 50-60 m of the parking bay for establishment vehicles, including those returning from malaria-endemic areas. There was no evidence of free-standing water that could enable mosquito breeding. No asymptomatic infections were identified: all laboratory investigations for malaria were negative. The outbreak was most likely caused by the accidental introduction of an infected mosquito by a vehicle returning from a malaria transmission area, namely, odyssean malaria.

Background

In South Africa, malaria is endemic in the KwaZulu-Natal, Limpopo and Mpumalanga provinces, especially in the north-eastern lowveld areas.[1,2] Most cases are due to *Plasmodium falciparum*. The disease is seasonal, occurring during the wetter summer months from October to May and typically peaking in January to April each year. Sporadic transmission of malaria during exceptionally wet seasons has also been reported in areas neighbouring the Molopo and Orange Rivers in the North West and Northern Cape provinces.[3] However, cases have been reported in other non-endemic provinces following acquisition in known transmission areas (imported malaria), or due to the phenomenon known as odyssean malaria. This is defined as malaria acquired locally through the bite of an imported *Anopheles* mosquito, in persons whose recent travel history firmly excludes the possibility of exposure to malaria in an endemic area.[4] Most notified cases of malaria in North West Province are imported but odyssean malaria incidents have also been reported; two such cases near Swartruggens were investigated in March 2017.[5]

On 11 November 2015, the Outbreak Response Unit, Division of Public Health Surveillance and Response of the National Institute of Communicable Diseases (NICD), was notified of a malaria case by the North West Province Department of Health. The case originated from a bush lodge establishment in Madikwe Game Reserve, Bojanala District, an area far outside the recognised malaria transmission zone of South Africa (Figure 1). As a few other people at the same establishment were said to have also presented with fever, headache and 'flu-like symptoms, a preliminary investigation to determine the possible cause of the illness/es was undertaken by the provincial Department of Health. These investigations revealed that four employees of the same establishment had been diagnosed with laboratory-confirmed malaria. None of the four case-patients had travelled to malaria-endemic areas for months. Following the recognition of local malaria cases in a non-malaria endemic area, a collaborative field investigation was conducted on 9 December 2015 by the NICD, the North West Province Department of Health and the Bojanala District Communicable Disease team. The objectives of the follow-up investigation were: 1) to identify undiagnosed malaria cases amongst employees and management at the establishment; 2) to describe the characteristics of laboratory-confirmed malaria cases; and 3) to establish if there were breeding sites for mosquitoes, collect mosquito larvae and, if possible, to collect and identify mosquito vectors of malaria.

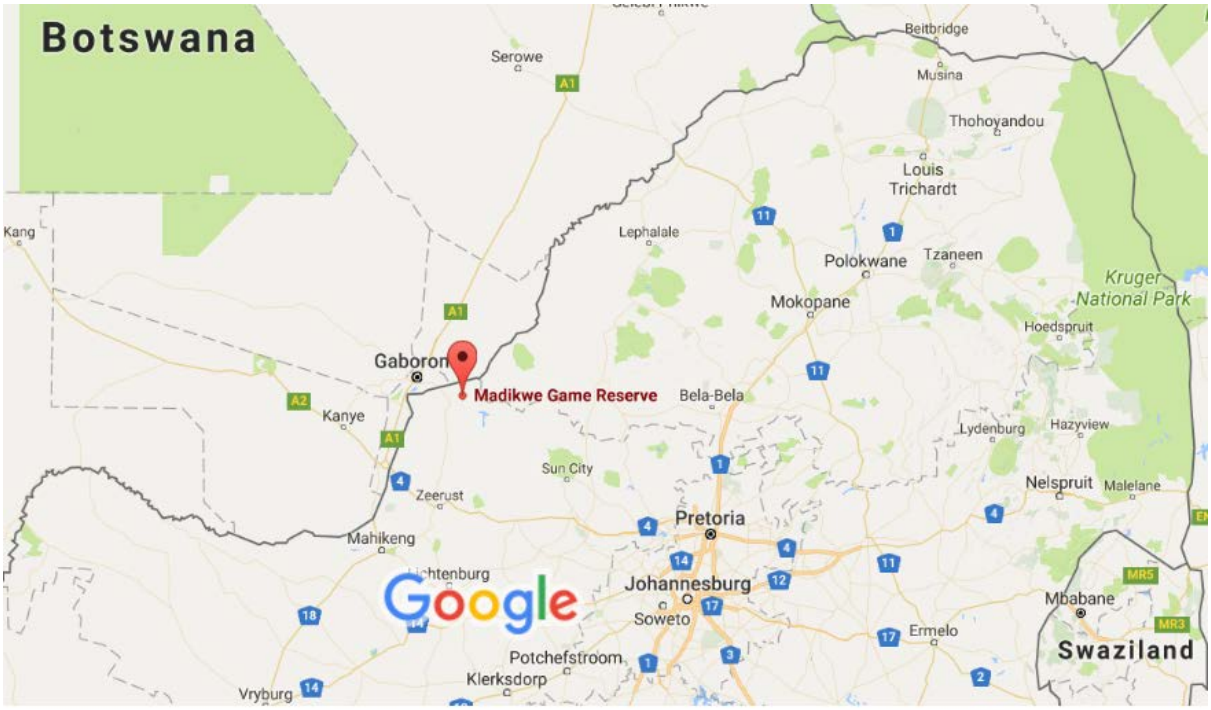

Figure 1. Location of the Madikwe Game Reserve, North West Province, South Africa. The malaria risk areas are adjacent to the north-east and eastern borders of the country (Source: Google Maps)

Methods

The following case definitions were used:

- A suspected case was defined as a person presenting with fever, headache and/or flu-like illness where no obvious cause was evident, during September to November 2015.
- A confirmed case was defined as a suspected case in whose blood smear malaria parasites were observed and/or whose malaria rapid antigen test was positive.
- An asymptomatic infection was defined as the presence of malaria parasites in the blood (tested positive by PCR, smear microscopy or rapid antigen test) in a person with no malaria symptoms at the time of venesection.

To establish the magnitude and possible source/s of the outbreak, the following activities were carried out during the site visit.

- *Epidemiological investigation*: A structured questionnaire/case investigation form was used to gather information on demographic, clinical and exposure history. Where informed consent was granted, face-to-face interviews were conducted with employees who were present on the day of the investigation. Management team members were also interviewed to gather information about malaria exposure in staff members, and about guests who had visited the area before malaria was diagnosed in the case-patients. A line list of cases was compiled.
- *Laboratory investigation*: To detect possible asymptomatic infection with *P. falciparum* in employees and management, EDTA-anticoagulated blood samples were collected. In addition, blood films were prepared on site. Blood smear microscopy and PCR analysis for the detection of malaria parasites were conducted by the Parasitology Reference Laboratory, Centre for Emerging Zoonotic and Parasitic Diseases at the NICD.
- *Environmental investigation*: To identify possible breeding sites for mosquitoes, an environmental assessment of the residence and immediate surroundings was conducted.
- *Intervention measures* included health education/promotion activities, distribution of information pamphlets about malaria to employees and management at the establishment and education of staff on personal protection against mosquito bites.

Results

Epidemiological and clinical findings

During the period October to November 2015, four laboratory-confirmed *P. falciparum* malaria cases were reported and investigated. In all four case-patients, diagnosis was made during November 2015, although all became ill in October 2015. The first case developed symptoms on 17th October, case 2 on 21st October, and the 3rd and 4th case-patients on 22nd October 2015 (Figure 2). All four case-patients were female, aged 27 to 55 years, and resided at the staff residences in the lodge. Three were housekeepers and one was a chef. Cases 1 and 2 had rooms next to each other, while cases 3 and 4 shared a room. None of these patients had a history of travel to malaria-endemic area/s, and no recent blood transfusions were reported. None had received visitors from outside the province. All four case-patients reported fever, while three reported headaches, dizziness and painful joints. Two patients also had diarrhoea, fatigue, nausea and 'flu-like illness, and one reported vomiting. Two of the patients were admitted to hospital and all four recovered following antimalarial treatment.

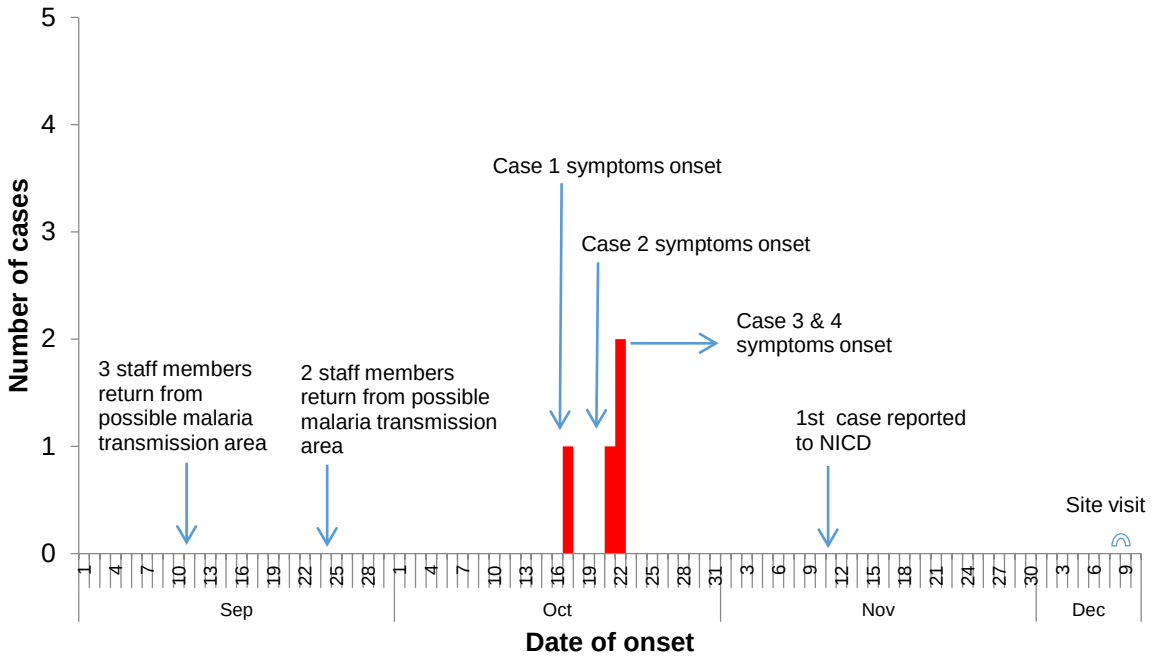

Figure 2. Epidemic curve illustrating number of cases by date of onset of symptoms, affected establishment, North West Province, 1 September to 10 December 2015, with return dates of travel from malaria endemic areas (travellers returning from malaria endemic areas outside likely incubation period not included).

Interviews were conducted with 33 staff members (including three of the confirmed malaria cases). The fourth case-patient was not available as she was on leave. However, information had been obtained during a preliminary investigation while she was hospitalised. Travel to possible malaria endemic areas within the month prior to symptom onset in the case-patients was reported in eight of the 33 staff members: six had travelled to Mpumalanga Province ((Bushbuckridge (n=4) and Kruger National Park (n=2)) and two to Zimbabwe (Table 1). It was established that ten groups of guests visited the lodge during the period August to October 2015. One of these visiting groups was from Zimbabwe, six had no travel history to or from malaria endemic areas, and no information was available for the remaining three. The group from Zimbabwe visited the lodge from 11 to 14 October 2015, too close to onset of disease of the first case to be the source of an infected mosquito.

Table 1. Travel history of staff members to possible malaria transmission areas.

Date of Travel	Area travelled to	Number of people
29 August to 11 September 2015	Bushbuckridge	3
13 to 24 September 2015	Kruger National Park	2
27 September to 14 October 2015	Zimbabwe	1
28 September to 16 October	Zimbabwe	1
2 to 15 October 2015	Bushbuckridge	1

Environmental findings: The lodge is immediately adjacent to the Marico River. It was reported that there were mosquitoes inside and outside the residences (both guest and staff residential areas). However, no mosquitoes were observed or collected during the investigation. The staff residences are in close proximity (within 50-60 m) to the parking bay for lodge vehicles, including those used during travel to possible malaria-endemic areas. The lodge managers indicated that they spray a pyrethroid-based insecticide around both the guest and staff residences for nuisance mosquito control. Besides the Marico River and swimming pools in the area, there was no evidence of free-standing water that could enable mosquito breeding.

Laboratory findings: Blood samples were collected from 32 of the 33 staff members available on the day. Laboratory investigations for malaria (microscopy and PCR analysis) were negative on all samples tested.

Discussion and conclusions

We describe a probable odyssean malaria outbreak at a lodge in Madikwe Game Reserve, involving four female case-patients. None of them had a history of travel to any malaria-endemic areas, which led to delays in malaria diagnosis. No additional malaria cases or asymptomatic infections were identified during the follow-up investigation, nor were any reported in the local municipality and district.

Based on the dates of onset of symptoms and usual incubation period for malaria, it is most likely that the four case-patients acquired malaria from the bites of one or more infective *Anopheles* mosquitoes during early to mid-October 2015. The mosquito/es were likely to have been inadvertently translocated from a malaria endemic area in a vehicle (car, bus, taxi) or in an individual's luggage.[6] The parking bay of the lodge is close to the staff residences and feasibly could have been the mosquito release site. In general, malaria vector mosquitoes tend not to disperse more than a few hundred metres, but can fly up to 1.5 km.[7] Adult malaria infective mosquitoes can survive for up to two weeks in a favourable environment, giving them sufficient time to infect several people, even during one night.[8] Therefore, the most likely vehicle to have transported the mosquito/es was the one arriving from the Kruger Park on 24 September 2015. It is likely that the same infected mosquito/es could have infected all case-patients, especially given their close proximity to each other.

We could not conduct a detailed entomological assessment regarding the presence of malaria mosquito vectors in the area, due to non-availability of entomologists. Spraying of interiors of vehicles arriving from, or leaving to, malaria endemic areas using appropriate insecticides would reduce the risk of transporting vector mosquitoes, but would be difficult to apply or enforce. To avoid mosquito bites, staff members were encouraged to use insect repellents and mosquito coils, and to wear long-sleeved clothing and socks in the evenings. Managers at the lodge and healthcare workers in the area were alerted to the cases in order to increase their awareness of malaria as a differential diagnosis in individuals presenting with fever, headache and 'flu-like illness, where no obvious cause is evident and in whom no history of recent travel to a malaria transmission area is forthcoming. It was further advised that potential mosquito breeding sites should be minimised or treated with an appropriate larvicide.

Acknowledgements

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A GASTROENTERITIS OUTBREAK INVESTIGATION AMONGST LEARNERS AND TEACHERS FROM A CAMP TRIP IN KWAZULU-NATAL PROVINCE, JANUARY 2017

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Executive summary

A gastroenteritis outbreak amongst learners and teachers of an urban school in Johannesburg, who were returning from a school camp in KwaZulu-Natal held from 20 to 27 January 2017, was reported through the National Institute for Communicable Diseases (NICD) hotline during the weekend of 28/29 January 2017. A descriptive investigation was conducted to quantify the outbreak, identify the source, and make recommendations to prevent future events. Epidemiologic and environmental investigations were also conducted. Clinical laboratory investigation depended on private laboratories forwarding bacterial culture results or any enteric bacterial pathogens isolates to the Centre for Enteric Diseases, NICD. Ninety people attended the camp and 48.9% (44/90) of them participated in the study. Of these, 42 developed diarrhoea. The median number of days of illness was 7 (IQR: 5-9 days). Approximately 62% (26/42) of the cases sought medical care and two were hospitalised. On the fourth day of camp, there was heavy rainfall and campers fell ill from the day after the storm. The communities neighbouring the valley had no proper sanitation facilities. River and spring-water used by the campers was not treated. Faecal coliforms were identified from river and spring-water samples at higher counts than the standard limits. *Salmonella enterica* subspecies *diarizonae* was isolated from a stool sample of one of the cases. This is an animal species-specific isolate suggesting the infection was through exposure to a contaminated environment. Although not proven epidemiologically, the outbreak was most likely due to a waterborne infection. In order to prevent future outbreaks, treatment of water from natural sources is recommended as is provision of proper sanitation facilities to the surrounding communities. Outbreak investigations would be facilitated by collection of stool specimens from all patients presenting with diarrhoea and full participation of both affected and unaffected persons in the investigation of public health events.

Introduction

Gastrointestinal infections are of enormous public health importance, killing approximately 2.2 million people globally per year.[1] Clinical symptoms of gastroenteritis are fever, headache, diarrhoea and abdominal pain with or without vomiting. Waterborne and foodborne gastroenteritis outbreaks have been reported before and are mostly associated with pathogens such as *Salmonella*, diarrhoeagenic *Escherichia coli* (*E. coli*), *Shigella*, *Vibrio cholerae* O1, *Campylobacter*, norovirus, rotavirus and *Cryptosporidium*.[1] Infections are acquired

through person-to-person contact due to poor personal hygiene or consumption of food or water contaminated with animal or human faeces. Animal-specific isolates include *Salmonella enterica* subspecies identified in pets, farm animals and wildlife. Water sources previously associated with outbreaks of enteric pathogens include lakes, swimming pools, recreational water, natural springs, rivers, coastal water and drinking water.

A class of grade 7 learners and 12 staff members from a preparatory school in Johannesburg attended an outdoor educational camp in KwaZulu-Natal Province from 20 to 27 January 2017. Outdoor education included water activities such as river studies, swimming in the local dam and free time around the river. One facility was used for food preparation to serve the different groups camping in the valley. In addition, the learners participated in a group activity where they bought food from local retailers, which they had to prepare themselves.

On the evening of 27 January 2017, the day they returned to Johannesburg, some learners and teachers experienced fever, chills, headache, diarrhoea with mucus and vomiting: symptoms suggestive of gastroenteritis. A concerned parent, also a doctor, notified the National Institute for Communicable Diseases (NICD) Outbreak Response Unit (ORU) through the NICD hotline during the weekend of 28/29 January 2017 about her child who attended the camp and developed diarrhoeal symptoms. In response, the Centre for Enteric Diseases (CED), NICD, initiated an outbreak investigation on 2 February 2017. The objectives of the investigation were to quantify the extent of the outbreak, identify the source and provide recommendations to prevent future similar outbreaks.

Methods

A descriptive study was conducted. The study population was a group of learners and teachers from an urban preparatory school in Johannesburg who took a trip to a valley for an educational camp. On 24 January 2017 there were heavy rains, and the river in the valley was in flood. However, only attendees from one of three campsites (A) were affected. A probable case was defined as anyone who went camping in the campsite A from 20 to 27 January 2017 and experienced one or more of the following symptoms: fever, abdominal pain, headache, diarrhoea, or joint pain. A confirmed case definition was a probable case with laboratory confirmation of an enteric pathogen. A tailored gastroenteritis case investigation form (CIF) was issued for self-administration by the learners. The CIF requested the following information from respondents: age, gender, time and date of illness onset, symptoms, duration of illness, healthcare consultation, environmental risk exposures (animals, river and recreational water and sewage disposal), food exposures (for the last three days including day of return), hand washing and laboratory specimen collection. The camp management completed a questionnaire to describe sanitation and water supply systems, geographical distribution of sleeping accommodation and water sources.

A site investigation revealed that there is a river flowing across the valley. The site where the learners were camping (Campsite A) was downstream in the direction of water runoff from the surrounding hills. Upstream were Campsites B and C, a natural spring and human settlements beyond the valley boundaries. The sanitation system comprised of septic tanks and French drains; this was a closed system and no sewage leakages were reported by participants. The water sources for the valley are spring and river-water. Spring-water was fed into tanks and distributed to the kitchens by pipes to fill dispensing containers. Untreated river-water was used for toilets and ablutions.

An environmental health inspection was conducted including an assessment of the human settlement neighbouring the valley campsite. Water samples were collected from five points: office kitchen tap, Campsite

A kitchen, river, Campsite A tent 07 and Campsite B kitchen. The CED-NICD issued a list of all camp attendees (full names and dates of birth) to private laboratories requesting information isolates and/or results of specimens collected from camp attendees. Two stool samples from affected campers were obtained from private laboratories and were tested by the NICD for enteric pathogens using culture and polymerase chain reaction (PCR). Participant characteristics were described using descriptive statistics.

Ethical clearance for this outbreak investigation were covered in terms of the NICD blanket ethical clearance for outbreak investigations (M160667). Informed consent was sought from adult camp attendees and additionally, parental consent and child assent forms in minors less than 18 years of age were obtained.

Results

A total of 78 learners and 12 staff members attended an outdoor educational camp from 20 to 27 January 2017. Of the 90 camp attendees, 54 (60%) consented (assent for minors) and 44 (48.9%) participated in the investigation. Of the 44 participants, 42/44 (95%) were ill (probable cases) and 2/42 were not ill (Table1). The majority were learners (84%) and all were males and their ages ranged from 12 to 13 years. The median age of the teachers was 36 years (age range: 24 to 58 years). The attack rate among the respondents was approximately 95%. The median number of days of illness was 7 (IQR: 5-9 days). Sixty-two percent of the cases sought medical care (Table 1). A learner and female teacher were hospitalised for 5 and 3 days respectively. No deaths were reported from this outbreak.

Table 1. Demographic and clinical characteristics of the participants in a gastroenteritis outbreak investigation, Johannesburg, January 2017.

Characteristics		Frequency (N=44)	
		n	%
Sex	Male	40	91
	Female	4	9
Occupation	Learners	37	84
	Teachers	7	16
Clinical status	Ill	42	95
	Not ill	2	5
GP consultation	Yes	26	62
	No	16	38
Hospital Admissions	Admitted	2	5
	No admission	40	95
Duration of illness	</= 7 days	24	57
	> 7 days	17	41
	Missing	1	2

The epidemiologic curve is shown in Figure 1. The first case reported was a learner aged 12 years, who started feeling ill on 24 January 2017. The duration of illness for the case that fell ill on 25 January was 14 days.

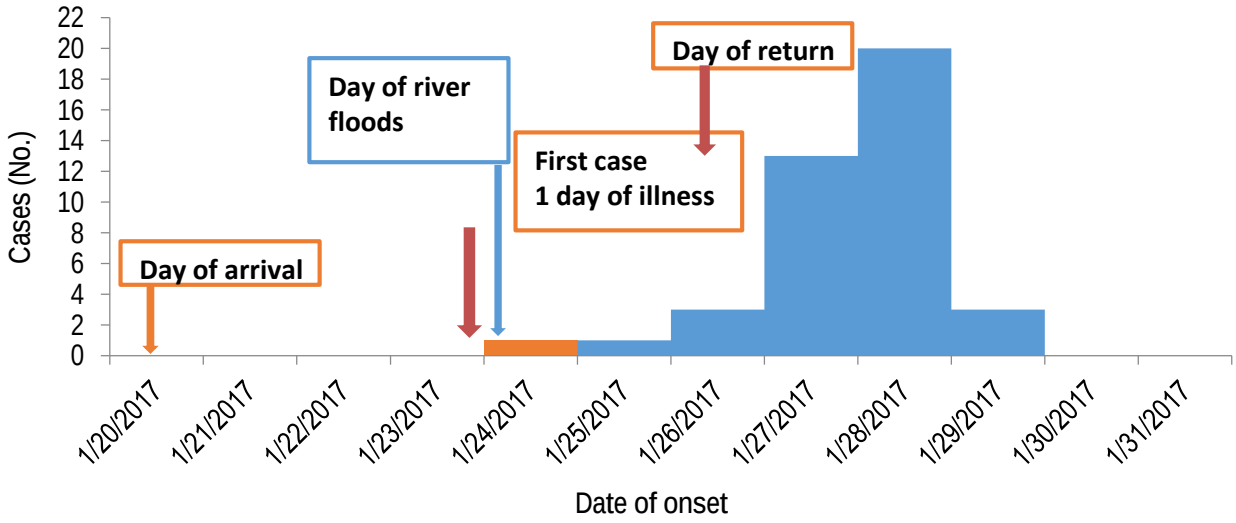
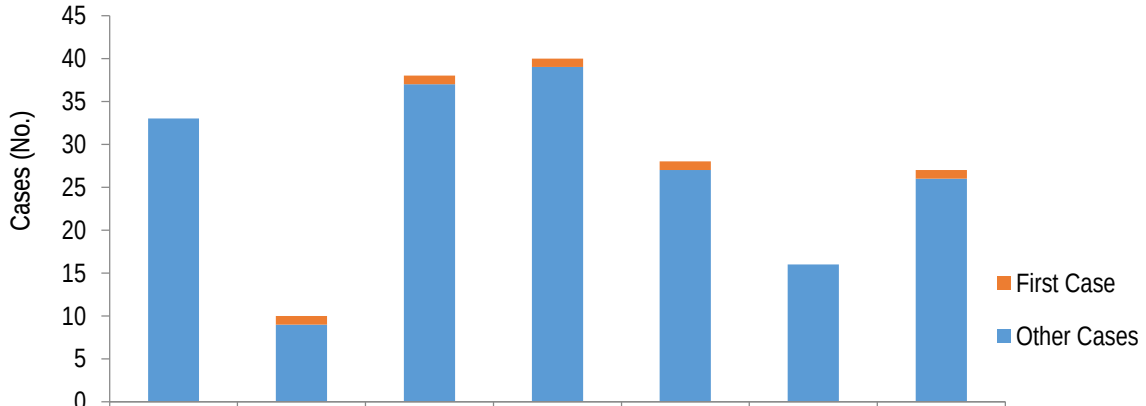


Figure 1. Epidemiologic curve of a gastroenteritis outbreak that occurred at an environmental education facility in KwaZulu-Natal Province, by date of onset of illness, January 2017.

Clinically, the majority of the cases experienced diarrhoea and abdominal pain (Figure 2). Ten cases reported vomiting including the first case with the shortest duration of illness. The second case reported fever, diarrhoea, abdominal pain, joint pain, headache and loss of appetite without vomiting. One of the cases that was hospitalised was treated for dehydration and deep vein thrombosis (DVT).



	Fever	Vomiting	Abdominal pain	Diarrhoea	Headache	Joint pain	Loss of appetite
First Case	0	1	1	1	1	0	1
Other Cases	33	9	37	39	27	16	26

Figure 2. Frequency distribution of symptoms among gastroenteritis cases, Johannesburg, January 2017.

Participants reported multiple food and environmental exposures. Some meals were consumed onsite and lunch packs were prepared to consume offsite when they went on tours. Eight of 44 (18%) respondents indicated exposure to animals including dung beetles, spiders, a puppy from the local village, frogs, crabs, fish, cows, goats and locusts. All the attendees used the river and spring-water sources in the valley for recreation and ablutions. Just over half of the cases (23/44) engaged in river activities after the floods and 57.1% (24/42) reported that they did not wash their hands after camp activities. Hand-washing was done in a shared tub containing water with disinfecting soap. The first case reported was not exposed to animals, but had mud from the river thrown in his face and mouth before the river flooded.

For one of the exercises, the learners were divided into 2 main workgroups to purchase and prepare their own foods; each group took part on a different day. Each group was further sub-divided into smaller groups and prepared different meals for themselves. The attendees were grouped according to responses to the workgroup member list provided by the respondents (Table 2). All groups had at least 1 member who got ill. Based on this variety of food exposures (Table 2), the food purchased for this exercise could be excluded as sources of infection. However, this is challenged by under-representation of most groups by the respondents. The common factor was utilization of river water to cook and boil their foods. Those that did not get ill also consumed food prepared during this exercise.

Table 2. Distribution of cases among workgroups related to a gastroenteritis outbreak, Johannesburg, January 2017.

Groups	Respondents Ill (%)	Respondents not ill (%)	Non-Respondents (%)	Total
1	5 (83.3)	0(0.0)	1(16.7)	6
2	4(80.0)	0(0.0)	1(20.0)	5
3	3(50.0)	0(0.0)	3(50.0)	6
4	2(33.3)	0(0.0)	4(66.7)	6
5	4(57.1)	1(14.3)	2(28.6)	7
6	2(33.3)	0(0.0)	4(66.7)	6
7	4(50.0)	0(0.0)	4(50.0)	8
8	1(13.7)	0(0.0)	5(83.3)	6
9	2(33.3)	0(0.0)	4(66.7)	6
10	3(42.9)	0(0.0)	4(57.1)	7
11	4(66.7)	0(0.0)	2(33.3)	6
12	3(50.0)	1(16.7)	2(33.3)	6
Unknown*	-	-	3	3
Total	**37 (47.4)**	**2(2.6)**	**39 50)**	**78**

*Unknown: Attendees not listed by any of their group members.

The standard of personal hygiene of the kitchen staff was reported as satisfactory by local health officials. Most of the residents in neighbouring settlements had no proper sanitation facilities. Total coliform and *E. coli* counts in river and spring-water were above standard limits according to the essential microbiological criteria of the South African National Standards (SANS) 241. Total coliform count was 241.9/ml in river water and >20/ml in spring water (SANS 241: <=10/ml). *Escherichia coli* count was 770/100ml in river-water and

between 20/ml and 60/ml in spring-water (for drinking water, the South African National Standard (SANS) 241 is nil/ml). These results are suggestive of faecal contamination of river and spring-water. Comments by participants on water quality included identification of black particles in drinking water, murky/yellowish water from taps and toilets and in a swimming pool, and hand-washing in a shared tub of brown-looking water.

Eleven cases had specimens collected for laboratory investigation. The specimens were 9 stools, 2 blood cultures and 1 rectal swab. Ten of these were collected before antibiotic treatment. CED confirmed on serotyping that *Salmonella enterica* subspecies *diarizonae* was identified from a stool specimen of a patient aged 13 years. It should be noted that the report of *Salmonella* Enteritidis, a pathogen that is typically associated with eating contaminated eggs or chicken, from a private laboratory was incorrect and could have misled those involved in a public health intervention. A diffusely adherent *E. coli* was identified from a rectal swab of a 12-year-old learner who presented with dysentery (diarrhoea with blood and mucous). The results for the remaining specimens are either unknown or culture negative for enteric pathogens. No stools submitted to private laboratories thus far were tested for viral enteric pathogens.

Discussion

The investigation studied 44 people and the attack rate among the respondents was 95%, although this is affected by the lack of responses from those camp attendees who did not get ill. Gastroenteritis in this outbreak was severe, with 61% of the respondents seeking medical treatment, and persistent, with 41% of patients being ill for more than a week. The epidemic curve suggests a common source exposure. It is hypothesised that water runoff from heavy rains introduced contaminants from human settlements with poor sanitation systems and the valley environment. The attendees started presenting with gastroenteritis illness after the heavy rains. However, there was limited evidence to epidemiologically link bacteria from water samples to that isolated from stool samples. This made it hard to conclusively identify the source of the outbreak.

At least one patient presented with *Salmonella*, which is a Gram-negative rod-shaped (bacillus) bacterium and has been identified in contaminated riverwater.[2] The first patient developed diarrhoea the same day of heavy rainfall and the river flood. However, this learner fell ill in the morning before the rainfall. His duration of illness was one day and may not be related to the major outbreak in which duration of illness ranged from 2 to 15 days. Although identified in one case, *Salmonella enterica* subspecies *diarizonae* is an environmental isolate and is not associated with exposure to any particular foodstuff or domesticated animals. The reported *Salmonella enterica* subspecies is mostly associated with reptiles, and was previously identified in riverwater after flood events.[3] High counts of total coliform and *E. coli* have been identified previously in springwater due to contamination by animal and human waste disposal. Stormwater runoff is a conduit for the transmission of large diversity of species to surface water.[3] The identified diffusely adherent *E. coli* was not a contributing factor for dysentery in the learner in whom this was identified. This pathogen is primarily associated with diarrhoea, in children under five years of age, and the patient received antibiotics before stool specimen collection.

This study was too limited by lack of food samples, including foods purchased and prepared by the learners, to exclude food items as a source of the outbreak. Another limitation was a low response rate by non-cases; this was a missed opportunity to identify a control group to measure association between exposures and gastroenteritis. Stool specimens should have been requested from all patients presenting with diarrhoea; a missed opportunity to identify a causal agent in this outbreak. Stool specimen testing should have included testing for both viral and bacterial pathogens.

In conclusion, although not proven epidemiologically, the outbreak was most likely waterborne due to contamination of the local water sources to which the campers were exposed by faecal bacteria and possibly viruses. This does not dismiss other potential exposures - analysis of which was limited. With a large proportion of cases seeking health care, the low response rate by the camp attendees may overestimate the severity of illness or underestimate the extent of the outbreak.

Actions taken

Health education was offered to food handlers in the valley on food labelling, personal hygiene and storage of food. However, for patient management, most of the cases consulted health practitioners for treatment in their personal capacity. No secondary cases were reported.

Recommendations

By the time of reporting to stakeholders, it was recommended that treatment of water used for personal care and drinking in the valley adheres to SANS for continuous monitoring of water quality with respect to bacteria and chemicals. To prevent future outbreaks, the valley management made an initiative to engage with the district municipality to assist with water treatment. Furthermore, it is recommended that the communities in the surrounding areas be provided with proper sanitation facilities. In the same light, the communities should be engaged on awareness of water pollution and its economic impact. It is further recommended that both public and private institutions encourage the participation of people who were not affected by a public health event to improve investigation outcomes. Healthcare Practitioners should be encouraged to send stool specimens from patients presenting with diarrhoea prior to antimicrobial treatment, particularly if the diarrhoea is potentially outbreak-associated, and to report potential outbreaks as soon as these are identified. Laboratory results need to be issued with caution as incorrect results may misdirect the outbreak investigation.

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INTRODUCTION OF A NEW RAPID DIAGNOSTIC TOOL AND SHORT-COURSE REGIMEN FOR DRUG-RESISTANT TUBERCULOSIS IN SOUTH AFRICA

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Executive summary

Multidrug-resistant tuberculosis (MDR-TB) was declared a public health crisis by the World Health Organization (WHO) and has been characterised by prolonged therapies and diagnostic delays in drug resistance detection, both leading to poor outcomes. New WHO recommendations state that in patients with rifampicin-resistant or multidrug-resistant TB who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents has been excluded, or is considered highly unlikely, a shorter MDR-TB regimen of 9-12 months may be used instead of the conventional regimen. The WHO also recommends the use of the GenoType MTBDR*sl* (SL-LPA) for patients with confirmed rifampicin-resistant TB or MDR-TB as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing. The high TB-HIV co-infection rates and background second-line TB resistance rates in South Africa could potentially undermine the effectiveness (success and relapse rates) of the shorter regimen if it is not introduced prudently. Use of rapid molecular technologies is essential to the introduction of the shorter regimen, including both the SL-LPA and FL-LPA. The latter is required for triaging the use of high-dose isoniazid and ethionamide in the shorter regimen. Any evidence of resistance to both is an additionally-recommended exclusion criterion for use of the shorter regimen apart from any fluoroquinolone or second-line injectable drug resistance detected. The introduction of the shorter regimen and SL-LPA no doubt improves the detection and management of drug-resistant TB in SA and complements gains being made with the use of newer therapeutic agents in the programme.

Background

Multidrug-resistant tuberculosis (MDR-TB) has been declared a global public health crisis by WHO and in the latest global report an estimated 580 000 incident cases occurred in 2015 alone. South Africa is one of 30 high MDR-TB-burden countries, contributing an estimated 20 000 cases in 2015.[1]

Conventional MDR-TB therapy is prolonged – treatment lasts at least 18 to 24 months and includes an intensive phase with an injectable agent for up to 8 months. Although the time to diagnosis of first-line resistance using molecular tools has reduced from months to days, this is not the case for second-line resistance, which requires slower phenotypic methods. Treatment outcomes for MDR-TB are poor, with only 50% of cases achieving successful outcomes. This reduces to only 20% for extremely drug-resistant TB (XDR-TB).[1] These poor outcomes may be attributed to the long duration of therapy and the intolerability of

drugs included in the treatment regimen. Both contribute to higher 'lost to follow-up' and treatment interruption rates. Additionally, delays in diagnosis, which often depends on microbiological confirmation, is an aggravating factor.

In May 2016, the World Health Organization (WHO) issued recommendations for a shortened MDR-TB treatment regimen (SR)[2] based on observational studies from 10 countries including Bangladesh (n=493), Swaziland (n=24), Uzbekistan (n=65) and seven other sub-Saharan African countries (n=408). Among those with MDR-TB and without previous second-line therapy, successful patient outcomes for those on the 9-12 month regimen were higher than those on the conventional long regimen - 84% (95% CI 79–87%) versus 62% (95% CI 53–70%), respectively.[2] Of the 39% of patients followed up after 12-18 months of treatment completion on the SR, none had a relapse and all were culture negative. These positive findings led to the latest WHO guidelines recommending the use of the SR. The WHO recommendation states that in patients with rifampicin-resistant or multidrug-resistant TB, who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones (FLQ) and second-line injectable agents (SLID) has been excluded or is considered highly unlikely, a shorter MDR-TB regimen of 9-12 months may be used instead of the conventional regimen.[2] This SR comprises an intensive phase of 4-6 months followed by a continuation phase of 5 months. The treatment regimen comprises 7 drugs during the intensive phase, also known as the injectable phase: kanamycin, moxifloxacin, ethionamide, ethambutol, clofazamine, pyrazinamide and high-dose isoniazid. The continuation phase comprises 4 drugs i.e. moxifloxacin, clofazamine, pyrazinamide and ethambutol.

An important prerequisite for the implementation of the shortened regimen is the need for rapid second-line drug susceptibility testing to exclude resistance to the two core agents (FLQ and SLID). The turn-around time for current phenotypic drug susceptibility results is extremely lengthy, requiring 6 – 8 weeks or longer for results to be available, and limits its use for early regimen selection. The need for early regimen triaging is of even greater importance in light of emerging evidence of cross-resistance between clofazimine – a core drug in the SR – and bedaquiline (BDQ), an increasingly-used drug for pre-XDR and XDR-TB cases. Thus delays in the diagnosis of these more resistant forms of TB with exposure to clofazimine in the SR could compromise the next-level BDQ-based regimens used to treat such cases. A new version of the GenoType MTBDR*sl* line probe assay Version 2.0 (Hain Life Sciences, Nehren, Germany) was released in 2015 and offers a potential to address the need of rapid detection of pre-XDR and XDR, being able to identify resistance to FLQ and SLID in days for smear-positive cases, while smear-negative cases may need repeat testing on culture-positive isolates. The genetic targets for resistance determination are *gyr*A and *gyr*B for the FLQ class, and the *rrs* and *eis* promoter for the SLID class. The *eis* promoter target in the latest version of the assay provides improved sensitivity for detecting kanamycin resistance – a drug which is widely used in South Africa for RR/MDR-TB treatment. In 2016 the WHO reviewed data available for the new assay and endorsed the GenoType MTBDR*sl* line probe assay Version 2.0 (Hain Life Sciences, Nehren, Germany) as a rapid initial test to be performed on patients with confirmed RR/MDR-TB in place of second-line DST to detect resistance to FLQ and SLID.[3]

This test can be performed on clinical isolates or directly on sputum samples, eliminating the delays associated with culture. The sensitivity and specificity on smear-positive samples was determined to be 93% and 98.3% for FLQ and 88.9% and 91.7% for SLID.[4] The WHO recommends the use of the SL-LPA for patients with confirmed rifampicin-resistant TB or MDR-TB as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing (DST).

Critical review and significance

The WHO recommendations are an important advancement in the current management of rifampicin-resistant (RR) and MDR-TB and will likely reduce problems concerning adherence. However, there are several issues that need to be considered and a critical review of the data needs to be performed to inform application of these recommendations in the South African context. The successful drug-resistant TB treatment outcomes reported in the non-intervention arms of the studies have been higher than that reported routinely in South Africa (62% versus 48%). This is probably related to the definitions applied, as pre-XDR cases are usually included amongst MDR-TB cohorts for WHO reporting, while these studies have been applied in patients without fluoroquinolone or injectable resistance. The low prevalence of HIV in these studies may have also resulted in better outcomes and low relapse rates. Additionally, the results from the South Africa TB Drug Resistance Survey 2012-14 (DRS) showed high levels of resistance to second-line agents, raising further concerns. The prevalence of resistance among MDR-TB for pyrazinamide was 59% (49.0%-69.1%) and for ethambutol was 44.1% (30.2%-58.0%). Both drugs are included in the continuation phase with moxifloxacin and clofazimine. Ethionamide, having similarly high resistance levels to ethambutol, is added in the intensive phase. It should be noted that the DRS prevalence data among MDR-TB includes the subsets of pre-XDR/XDR, and if these cases were excluded the prevalence would be lower. Clofazimine was not tested but prevalence rates are expected to be low as this drug has been primarily reserved for use in treating pre-XDR/XDR cases, which are excluded from the SR.

The high prevalence of HIV and TB drug resistance raises questions as to the applicability of the SR in South Africa. The inclusion of Swaziland data, which shares a very similar epidemiology to South Africa, only contributed less than 3% of the sample analyzed. More information has however become available since the WHO announcement, with data from close to 100 patients now available (unpublished) with, encouragingly, similarly good outcomes to those of other countries. In the local context, the choice of continued use of high-dose isoniazid and ethionamide for the full duration of treatment is strongly recommended. At least 4 effective TB drugs are required to treat MDR-TB and, in the current SR when used in combination with screening for FLQ and SLID resistance, these two drugs, with clofazimine being the third, will have a high likelihood of effect. The choice of a 4th effective drug is expected from ethambutol, high-dose isoniazid or ethionamide. Pyrazanimide is an important sterilizing agent, is known to have a positive contribution to shortened regimens, and is included irrespective of resistance. The latter is also often a challenge to accurately determine *in vitro* and as such, is best included.

The high specificity of the SL-LPA assay implies that results of resistance testing can be acted upon and this is also the WHO recommendation. There are, however, certain limitations as the test provides resistance determination by drug class and not for individual drugs. In the case of FLQ, this is based on ofloxacin and has shown good correlation. However, moxifloxacin, a newer generation fluoroquinolone, which is widely used for treatment, is likely to have some effect against strains with mutations that confer lower levels of resistance, and could thus potentially be used with effect by applying a higher dosage. Despite this limitation with the SL-LPA over-estimating low-level resistance, it does provide a conservative approach by excluding these patients from SR. For the SLID, correlation with kanamycin resistance, which is the core drug for adult treatment, was also very high and would appropriately exclude such cases with resistance. On the opposite end are concerns around the sensitivity of the assay in identifying resistance, which has been shown to be variable. The assay could miss approximately 15% of each class of resistance and the implications are that some of these patients may be started on an SR. Although this appears to be a high proportion of cases, based on the most recent DRS, the prevalence of resistance to each of these drug classes among MDR-TB patients was 13%, meaning that the vast majority of cases would be susceptible and of those resistant (13%), only a subset (15% of the 13%) would be missed. Testing all cases phenotypically to identify such a small proportion may not be feasible, and it would thus be prudent to minimize this group by also including prior

second-line drug exposure history, especially those with unsuccessful outcomes in that episode, as an exclusion criterion. Lastly, clinical response and culture conversion will also serve as an important indicator for subsequent phenotypic resistance testing.

Public health significance and applicability

Following the WHO recommendations, a series of consultations followed with relevant stakeholders and a revised laboratory-clinical algorithm was developed (Table 1) while the new revised guideline is still in development. An important requirement for the implementation was the need for close alignment between the laboratory tools and treatment decisions. Furthermore, current algorithms in place, including the use of bedaquiline (a new agent for pre-XDR and XDR-TB patients, as well as selected MDR-TB patients) and FL-LPA mutation profiles used to guide therapeutic decisions, needed to be incorporated. Patients with *katG* mutations which are likely to have high levels of isoniazid resistance, and high-dose isoniazid may not be effective in some individuals, based on their host genetics. Thus, due consideration should be given to exclude this drug in the regimen. However, the possibility of *in vivo* synergy between isoniazid and clofazamine has been suggested previously and is another consideration to continue high-dose INH despite the presence of a *katG* mutation. Patients with an *inhA* mutation are likely to have ethionamide resistance, and would not have this agent included in their regimen, which apart from the resistance, is further justified due to its poor patient tolerability profile. The new algorithm incorporates these elements as well as the new WHO recommendations. Thus, in the latest iteration of the algorithm, patients with any FLQ or SLID resistance, and those with mutations in both *katG* and *inhA* would be excluded from SR. The latter criterion was important, as a double mutation would imply the loss of two agents thus compromising the SR. The loss of one of the latter two agents is not an exclusion and is different from the WHO recommendations. The rationale for this is that the absence of either one of these mutants implies susceptibility to the drug (i.e. high-dose INH or ethionamide) and thus at least four active drugs are available to complete therapy. All other patients would be referred to the next level of care for a decision on an individualised regimen and the use of BDQ. As testing accuracy is not absolute it was agreed that patients with prior multidrug-resistant therapy with line probe assay results indicating eligibility for the Short MDR Regimen, would need phenotypic DST and the case closely followed up, though it was appreciated that such cases are likely to be uncommon.

Table 1. Conceptual framework for the revised rifampicin-resistant/multidrug-resistant TB algorithm incorporating second-line line-probe assay

Step	Patient Status	Action
1	Rifampicin-resistant TB	Consider patient for SR, submit sample for DR-TB reflex test and complete baseline assessments
2	Is patient eligible with no contraindications present	If yes, start SR and follow-up LPA results
3	LPA – second-line result	
	3.1. Resistant to fluoroquinolones	Stop SR and refer to next level of care
	3.2. Resistant to injectable agent	Stop SR and refer to next level of care
4	LPA - first-line result	
	4.1. Isoniazid-resistant	
	katG mutation present	Continued use of high-dose isoniazid uncertain
	inhA mutation present	Stop ethionamide and continue SR
	Both *katG* and *inhA* mutations present	Stop SR and refer to next level of care
	4.2. Isoniazid-sensitive	Continue SR

SR: Short regimen, LPA: Line probe assay

Operational consideration included both clinical and laboratory indicators. The SL-LPA uses the same infrastructure as for the LPA first line (FL-LPA), which is widely used (dating back to 2009) and makes the implementation easier. The reduction in the use of the FL-LPA since the introduction of GXP in 2011 resulted in underutilization and this spare capacity is available for the introduction of the new assay. Testing using the new SL-LPA began on 1st of January 2017 and by the end of April 2017, 4282 samples were tested. Uptake has been good with almost all districts having patient samples tested and volumes increased month on month since January. Testing is now linked to a 'super-set' of tests for baseline assessment of all new RR/MDR-TB patients starting the SR, which includes smear microscopy, culture and both first- and second-line LPAs. In addition, if any resistance to FLQ or SLID is detected, the designated laboratories for second-line TB testing would proceed to perform phenotypic DSTs to moxifloxacin at two concentrations, capreomycin, and linezolid to aid formulation of an individualised regimen.

An important issue for clinical implementation is the drug availability of clofazimine and the additional clinical assessments (e.g. audiology, ECG etc.). For the latter, these are well established in the historic DR-TB initiation sites but are now being expanded to decentralised sites across the country and are at variable stages of implementation. The issue around clofazamine accessibility is, however, a bigger one as it is a core drug for the SR and is thought to have sterilizing activity that has led to success in reducing treatment duration. To date the drug has been accessed through the Section 21 regulatory process on a named-patient basis. This is not sustainable long-term and efforts are underway to address the challenge. Clofazimine is currently not officially registered for MDR-TB therapy in South Africa and due to the low uptake and associated costs, this has not materialised. The WHO has recently added the drug to the Essential Medicines List (EML) but the local registration through the Medicines Control Council still needs to be completed by the manufacturer. In the interim, several provinces have already procured stock through the existing procedure and have begun initiating patients on SR. The roll-out of the SR has been accompanied by a package of training of doctors and nurse initiators at the decentralised sites that are now in excess of 600 nationally.

There are some important gaps not fully addressed in the guidelines which are still being considered for the local context. Pregnant women and patients with extra-pulmonary TB (EPTB) would not normally be eligible for SR, but there is a rationale to consider uncomplicated EPTB for SR with a longer duration as is done for drug-sensitive TB. For pregnant women, efficacious drug substitution for the injectable may be justified in the SR as is already practiced for the current standard MDR regimen. Cases where testing is not performed or testing fails, and patients are put on SR, will require careful consideration in the final guidelines. Furthermore, patients on SR that have failed or are lost to follow-up are another group for which guidance needs to be developed. The SR and SL-LPA are new innovations and close monitoring with regular reviews will be essential to chart the best course for these emergent issues.

Conclusion

The introduction of the SR and SL-LPA no doubt provides important steps to improving the detection and management of drug-resistant TB in South Africa, and complements gains being made with the use of newer therapeutic agents. Anecdotally, responses from laboratorians and clinicians has been very positive with pre-XDR and XDR cases now being identified in under a week, which has revolutionised diagnostics in the DR-TB program. Although the clinical efficacy of SR may not be dramatic, the public health benefits to patients with a regimen whose duration is similar to the current one for susceptible TB is likely to reduce loss to follow-up rates, and improve overall outcomes. Furthermore, this will now set a new standard for DR-TB management with shorter regimens in the future. It is still early but the expectation is that these new changes will help take South Africa closer to achieving the goals of the END TB Strategy.

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GERMS-SA LABORATORY-BASED SURVEILLANCE FOR ANTIMICROBIAL-RESISTANT BACTERIAL AND FUNGAL BLOODSTREAM INFECTIONS, 2016

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Introduction

The Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses (CHARM) at the National Institute for Communicable Diseases (NICD) uses the GERMS-SA laboratory-based surveillance platform to conduct intermittent surveys for antimicrobial-resistant bacterial and fungal bloodstream infections. The primary objective is to describe the epidemiology of key bloodstream infections, including antimicrobial resistance trends. The GERMS-SA platform facilitates a more detailed epidemiological description of laboratory-confirmed cases through enhanced sentinel surveillance. This report summarises preliminary results of national or sentinel surveys conducted in 2016 for *Staphylococcus aureus* and carbapenem-resistant Enterobacteriaceae (CRE) bacteraemia and candidaemia (compared to the preceding year's results where applicable).

Enhanced sentinel surveillance for *Staphylococcus aureus* bacteraemia in Gauteng and Western Cape provinces

The epidemiology of *S. aureus* bacteraemia has been poorly described in low- and middle-income countries (LMICs) compared to high-income countries (HICs).[1,2] Historically, methicillin-resistant *S. aureus* (MRSA) isolates that are resistant to β-lactam antibiotics were confined to healthcare facilities. However, in the mid-1990s, the emergence of community-associated MRSA (CA-MRSA) strains, which cause infections among patients with no previous exposure to the healthcare environment, resulted in a considerable shift to CA-MRSA-associated disease in HICs in North America, Asia, Europe and Australia, but less so in LMICs. These strains may be distinguished by molecular characterization of the staphylococcal cassette chromosome *mec* (SCC*mec*) with HA-MRSA strains typically carrying a large SCC*mec* type I-III and CA-MRSA strains carrying the smaller SCC*mec* elements IV-V. The aim of this project was therefore to determine the prevalence of antimicrobial resistance and molecular epidemiology of *S. aureus* bacteraemia at enhanced surveillance sentinel sites in two provinces for guideline/ policy formulation and antimicrobial stewardship activities.

Methods

NICD-CHARM initiated laboratory-based enhanced surveillance for *S. aureus* bacteraemia in September 2012 in Gauteng and Western Cape provinces. The results for the period 1 January 2015 through to 31 December 2016 are reported here. A case was defined as an individual diagnosed with culture-confirmed *S. aureus* bacteraemia at any sentinel site. A new episode was defined if an individual had a positive culture >21 days after the first culture. Quarterly audits of the NHLS Corporate Data Warehouse (CDW) were

conducted to confirm completeness of reporting. At sentinel public-sector hospitals, nurse surveillance officers collected clinical data from admitted case patients by interview or chart review. Corresponding bloodstream isolates were submitted to CHARM for confirmation of identification using a matrix-assisted laser desorption/ionization time of flight instrument (MALDI Biotyper System, Bruker Diagnostics, Bremen, Germany). Antimicrobial susceptibility testing was performed using commercially-available microbroth dilution panels MIC Panel Type 33 (MicroScan, Beckman Coulter, USA). Minimum inhibitory concentrations were interpreted using Clinical and Laboratory Standards Institute (CLSI) M100-S27 breakpoints.

Results

In 2016, 955 cases of *S. aureus* bacteraemia were detected (Table 1). The majority of cases were detected from sentinel sites in Johannesburg and Pretoria (560; 59%). 586 (61%) patients were male. Adults aged ≥18 years accounted for 548 (57%) cases. *Staphylococcus aureus* isolates were available for 78% (746/955) of case patients. The proportion of MRSA cases decreased from 32% (242/748) in 2015 to 25% (188/746) in 2016 (p=0.002) (Figure 1). SCC*mec* typing was performed for 187 *mec*A-positive *S. aureus* isolates in 2016. There was a predominance of type III SCC*mec* in Gauteng (73/187; 39%) and type IV in the Western Cape (38/187; 20%) (Figure 2). Among 746 viable *S. aureus* isolates, 200 (73%) were non-susceptible to clindamycin. All isolates were susceptible to vancomycin and daptomycin in 2016. A total of 731 (95%) isolates were susceptible to mupirocin (Table 2 and Figure 2). Among 955 patients, 273 (29%) died.

Table 1. Numbers and percentages of cases of *Staphylococcus aureus* bacteraemia reported to GERMS-SA sentinel sites by province, South Africa, 2015 (n=927) and 2016 (n=955) (including audit cases).

Province	2015		2016		Total	
	n	%	n	%	n	%
Gauteng	516	56	560	59	1076	57
Western Cape	395	44	395	41	806	43
Total	**927**	**100**	**955**	**100**	**1882**	**100**

Table 2. Numbers and percentages (parentheses) of viable *Staphylococcus aureus* isolates susceptible to various antimicrobial agents, 2016, n=746.

Province	Oxacillin	Clindamycin	Vancomycin	Mupirocin	Daptomycin
	n=746	n=746	n=746	n=746	n=743
Gauteng	305 (75)	292 (72)	406 (100)	401 (99)	406 (100)
Western Cape	253 (74)	254 (75)	340 (100)	330 (97)	337 (100)
Total	**558 (75)**	**546 (73)**	**746 (100)**	**731 (98)**	**743 (100)**

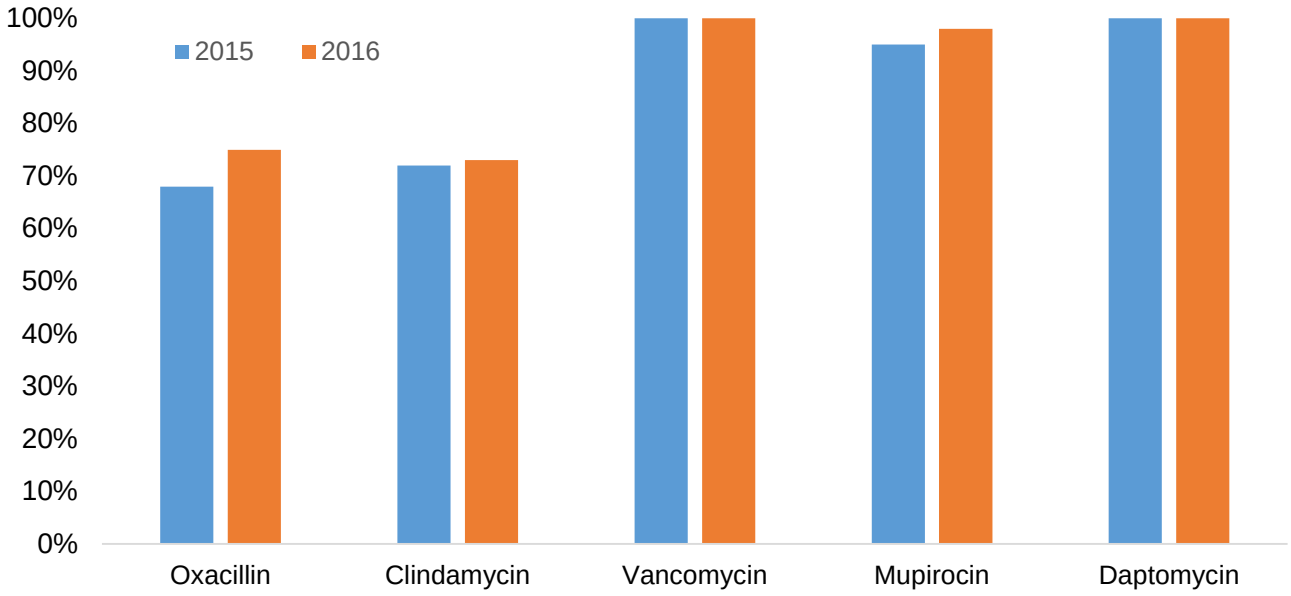

Figure 1. Proportion of cases of laboratory-confirmed *Staphylococcus aureus* bacteraemia with isolates susceptible to various anti-microbial agents reported to GERMS-SA in Gauteng Province, 2015 and 2016, n=1494.

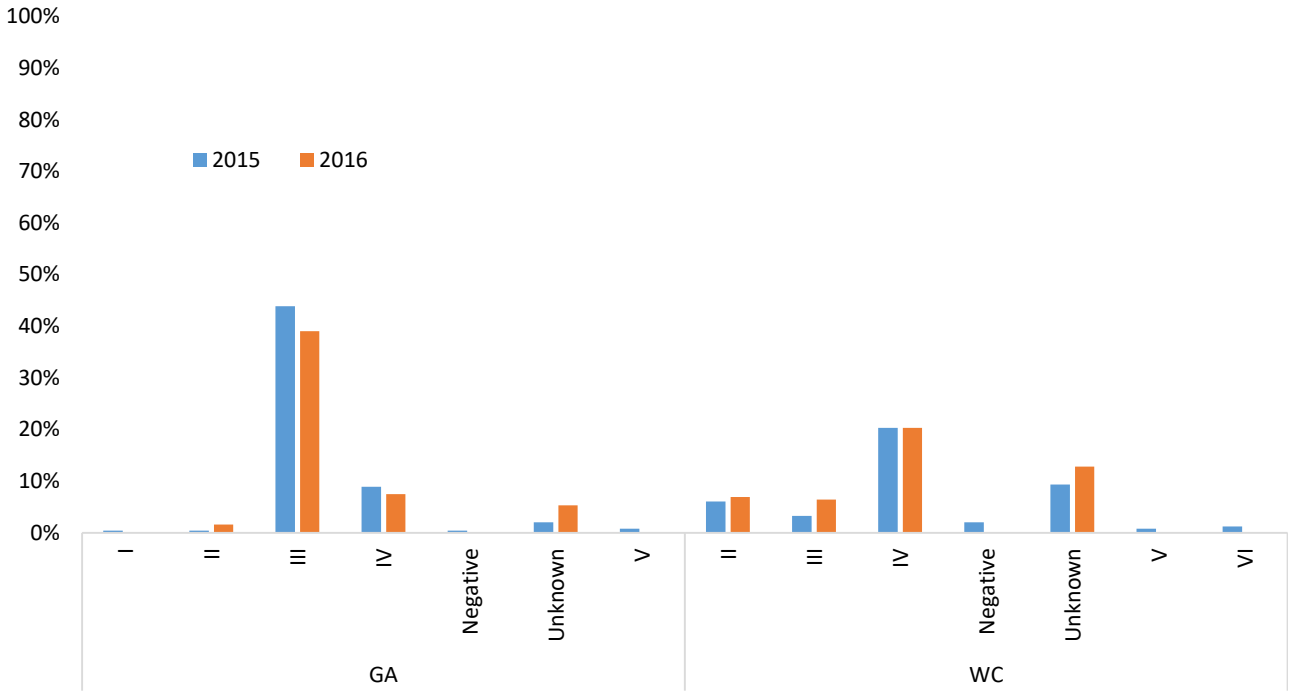

Figure 2. Staphylococcal cassette chromosome *mec* (SCC*mec*) distribution for laboratory-confirmed cases of *Staphylococcus aureus* bacteraemia reported to GERMS-SA by province, 2015 and 2016, n=433. GA = Gauteng Province; WC = Western Cape Province.

Discussion

There was a significant decrease in the proportion of cases of MRSA bacteraemia in 2016 compared to 2015. Overall, SCC*mec* type III predominated and was more common in Gauteng while type IV was dominant in the Western Cape. A similar proportion of isolates was resistant to clindamycin and oxacillin. As expected, no vancomycin or daptomycin non-susceptible isolates were identified. Other than a reduction in MRSA cases, there was no change in the susceptibility pattern of bloodstream *S. aureus* isolates over the reporting period.

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Enhanced sentinel surveillance for Carbapenem-resistant Enterobacteriaceae (CRE) bacteraemia in four provinces

The past few decades have seen the rapid emergence and spread of antimicrobial resistance with clinicians having to rely on the carbapenem class of antibiotics to treat resistant bacterial infections.[1] However, carbapenem resistance is increasingly being reported. The mechanism of resistance to carbapenems in the Enterobacteriaceae is complex and mediated by several different mechanisms such as over-production of ampC enzymes, extended-spectrum β-lactamases (ESBLs), carbapenemases, efflux pumps and loss of porin channels.[2-4] There are two main subsets of carbapenemase-producing Enterobacteriaceae (CPEs), producing either non-metallo-enzymes including *Klebsiella pneumoniae* carbapenemases (KPC), Guiana extended-spectrum β-lactamases (GES), and oxacillinase-type carbapenemases (OXA-48) and their derivatives; or metallo-β-lactamases (MBLs) including imipenemases (IMP), Verona integrated metallo-beta-lactamases (VIM) and New Delhi metallo-beta-lactamase (NDM-1/2). Genes encoding these enzymes can be detected using molecular methods. Infections caused by CPEs are associated with increased patient morbidity and mortality owing to limited treatment options rather than the expression of specific virulence characteristics.[5,6] There is potential for widespread transmission of carbapenem resistance owing to easily-transmissible resistance genes.[5,6] CPE are thus a high-priority group of pathogens (versus other mechanisms of resistance). The aim of this project was therefore to estimate the burden of laboratory-confirmed CRE bacteraemia at sentinel sites in South Africa and to describe the epidemiological characteristics to support development of guidelines for antimicrobial use.

Methods

NICD-CHARM initiated laboratory-based enhanced surveillance for CRE bacteraemia on 1 July 2015 at 12 sentinel public-sector hospitals in four South African provinces. The results for the period 1 July 2015 through 31 December 2016 are reported here. A case was defined as an individual diagnosed with bacteraemia at a surveillance site with Enterobacteriaceae isolates resistant to any of the carbapenems (ertapenem, imipenem, meropenem and/or doripenem) at a diagnostic laboratory based on the disk diffusion method, Etest, automated systems (Vitek 2 or MicroScan) or modified Hodge test (MHT). *Enterobacter* species showing borderline resistance to ertapenem but susceptibility to other carbapenems were excluded. A new episode was defined if an individual had a positive culture >21 days after the first culture. Quarterly audits of the NHLS CDW were conducted to confirm completeness of reporting. At sentinel hospitals, nurse surveillance officers collected clinical data from admitted case patients by interview or chart review.

Corresponding bloodstream isolates were submitted to the Centre for confirmation of identification using the MALDI Biotyper System. Antimicrobial susceptibility testing was performed using commercially-available microbroth dilution panels MIC Panel Type 44 (MicroScan). Minimum inhibitory concentrations were interpreted using CLSI M100-S27 breakpoints. Molecular tests were performed for confirmation of CPE genes. After DNA extraction by using a crude boiling method at 95°C for 25 minutes for cell lysis, the supernatant was harvested and screened for *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{OXA-48}$ and its variants (OXA-162, 163, 244 245, 247, 181, 204, 232), *bla*$_{GES}$ (GES-1-9, 11), *bla*$_{IMP}$ (IMP-9, 16, 18, 22, 25) and *bla*$_{VIM}$ (VIM-1-36) using a multiplex real-time polymerase chain reaction (PCR) (LightCycler 480 II, Roche Applied Science, LightCycler 480 Probes Master kit and the individual LightMix Modular kits (Roche Diagnostics, IN, USA).

Results

There were 440 cases of CRE bacteraemia (as detected by a diagnostic laboratory) reported to GERMS-SA from July 2015 through to December 2016 (Table 3). Half (n=220) were male and the majority (233; 53%) were adults aged 16-55 years. The majority of cases were detected from sentinel sites in Gauteng (298; 68%) followed by KwaZulu-Natal (105; 24%) (Table 3). CRE isolates were available for 67% (294/440) of patients and submitted to NICD for antimicrobial susceptibility testing (Table 4). *Klebsiella pneumoniae* was the commonest organism (217; 74% of cases) followed by *Enterobacter cloacae* (28; 10%), *Serratia* (19; 7%) and *Escherichia coli* (17; 6%) (Figure 3). Most cases occurred in adult medical wards (Figure 4). Among all isolates, 87% (256) were non-susceptible to ertapenem, 57% (168) non-susceptible to imipenem and 58% (171) non-susceptible to meropenem and doripenem (Figure 5). Carbapenemase genes were confirmed in 81% (238/294) of isolates including NDM (109/238; 45%) and OXA-48 or variants (111/238; 47%) (Figure 6). 23 (8%) isolates were susceptible to ertapenem with an MIC ≤ 0.5 mg/L but were OXA-48 positive. Over the surveillance period, there was a shift towards CRE mediated by OXA-48 & variants (Figure 6). Among viable isolates, 76% were susceptible to tigecycline (Table 4). Of all patients with CRE bacteraemia, 158 (36%) died.

Table 3. Numbers of cases of carbapenem-resistant Enterobacteriaceae (CRE) bacteraemia reported to GERMS-SA by province, July 2015 to December 2016, n=440 (including audit cases).

Province	2015		2016		Total	
	n	%	n	%	n	%
Free State	1	1	3	1	4	1
Gauteng	80	68	218	67	298	68
KwaZulu-Natal	32	27	73	23	105	24
Western Cape	4	4	29	9	33	7
Total	**117**	**100**	**323**	**100**	**440**	**100**

Table 4. Numbers and percentages of carbapenem-resistant Enterobacteriaceae (CRE) bloodstream isolates reported to GERMS-SA susceptible to antimicrobial agents by province, 2015-2016, n=294.

Province	Antimicrobial agents							
	Tigecycline		Ceftazidime		Ciprofloxacin		Doripenem	
	S	%	S	%	S	%	S	%
Free State	2	100	0	0	0	0	1	50
Gauteng	149	75	21	11	25	13	112	56
KwaZulu-Natal	60	76	1	1	6	8	4	5
Western Cape	12	80	0	0	2	13	7	47
Total	**223**	**76**	**22**	**7**	**33**	**11**	**124**	**42**

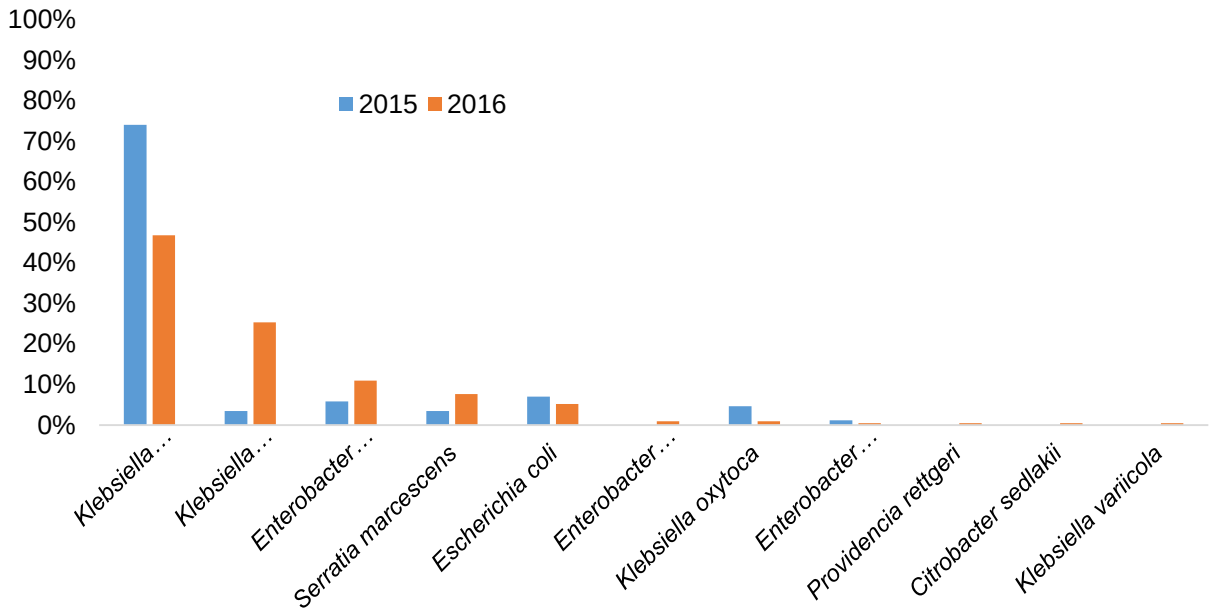

Figure 3. Species distribution of Enterobacteriaciae submitted for carbapenem-resistant Enterobacteriaceae (CRE) bacteraemia surveillance to GERMS-SA, n=294.

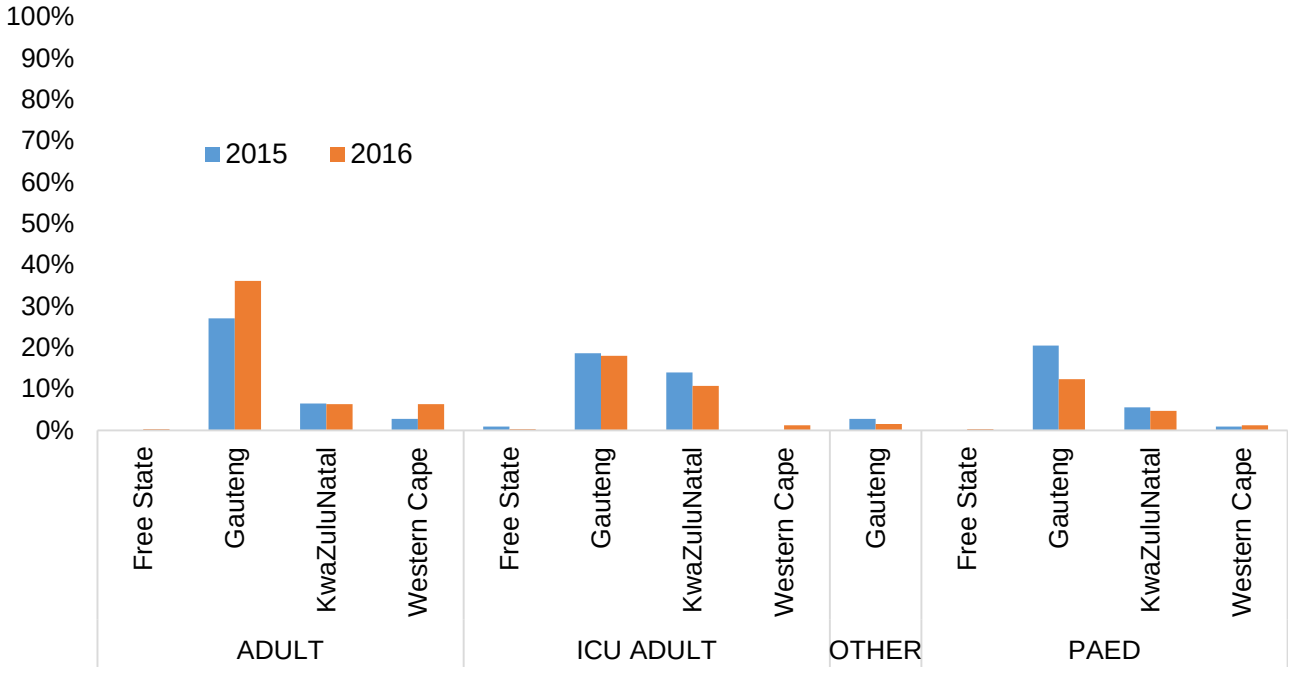

Figure 4. Distribution of cases of carbapenem-resistant Enterobacteriaceae (CRE) bacteraemia by hospital ward type, n=440.

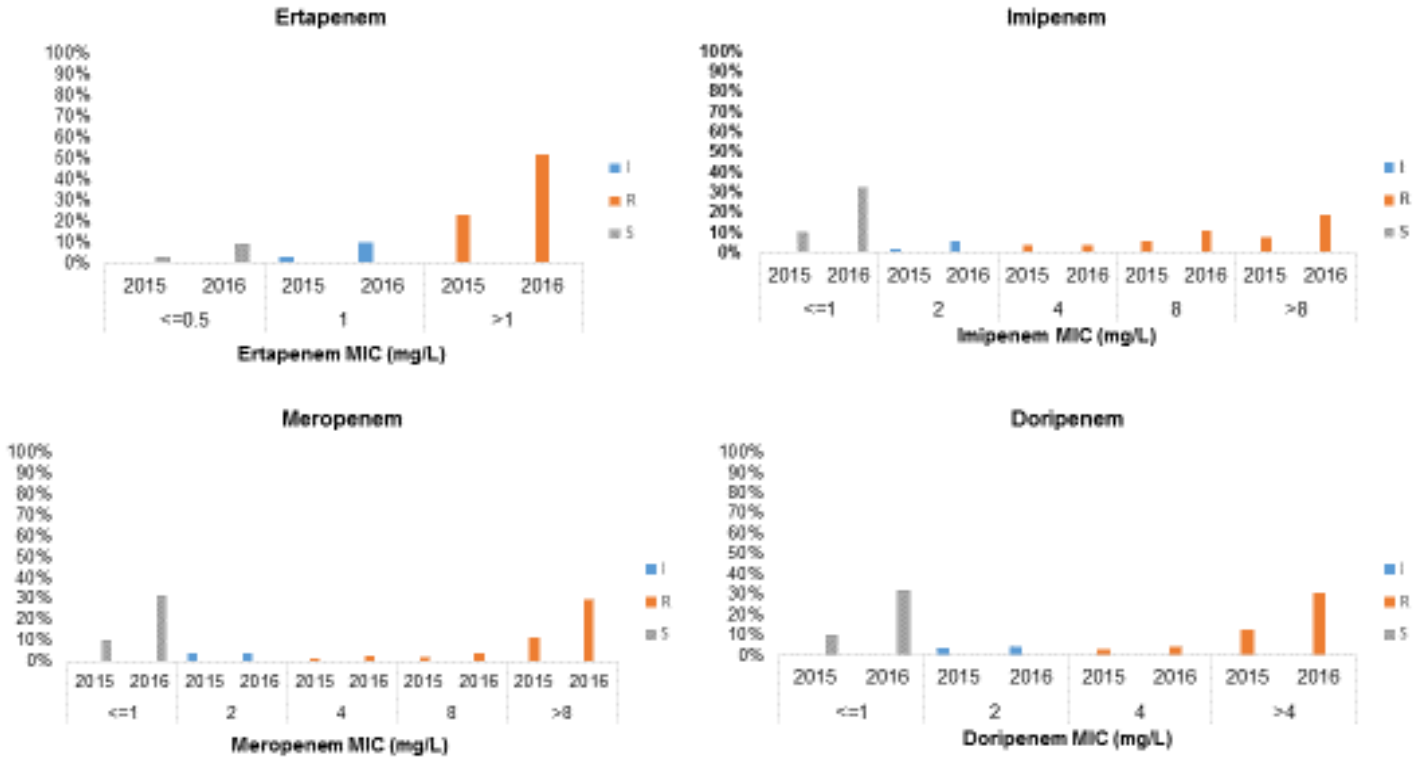

Figure 5. Antimicrobial susceptibility results for Enterobacteriaceae bloodstream isolates, n=294. I=intermediate; R=resistant; S=susceptible

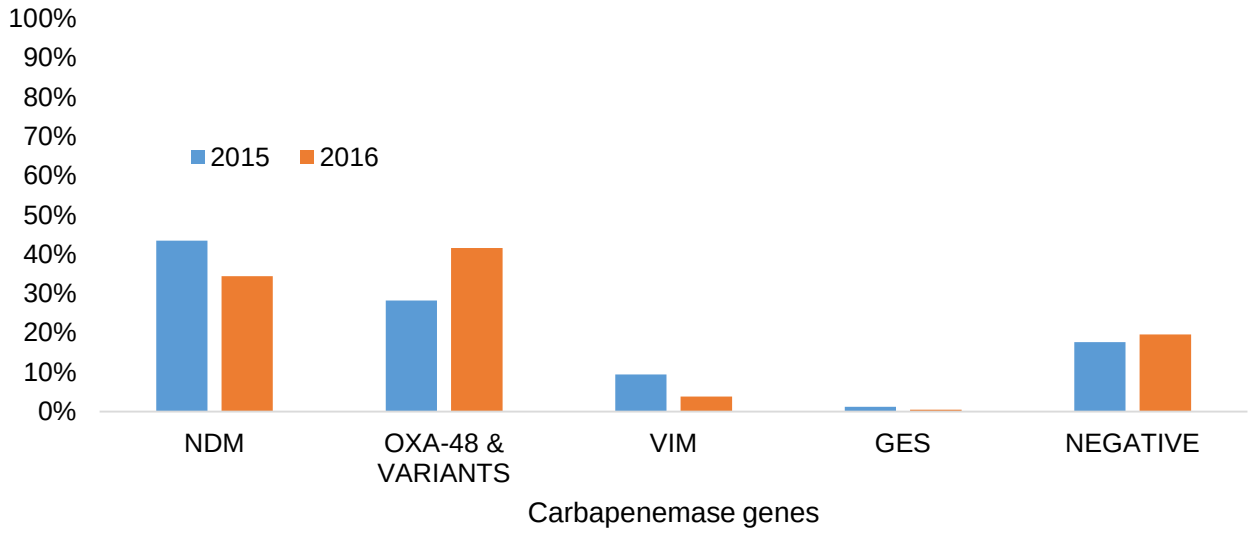

Figure 6. Carbapenemase gene detection in 238 (81%) of 294 Enterobacteriaceae bloodstream isolates.

Discussion

The number of CRE bacteraemia cases detected over the surveillance period is relatively small but these highly-resistant organisms have an impact on the public-sector health system in terms of patient outcomes and healthcare costs. Most cases were detected in Gauteng and KwaZulu-Natal. A shift to CPE mediated by OXA-48 & variants was noted even though these enzymes are not easily detected in the laboratory. In addition, the OXA genes are located on a very efficient transposon with the potential for point mutations.

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National and enhanced sentinel surveillance for candidaemia

In 2009 and 2010, the first national surveillance for candidaemia was conducted which described the species distribution and baseline antifungal susceptibility profiles of the most common *Candida* species in the public and private sectors.[1] In that survey, *Candida albicans* was the most common species isolated from 906 cases in the public sector followed by *C. parapsilosis, C. glabrata, C. tropicalis, C. krusei* and other *Candida* species. In contrast, *C. parapsilosis* was the dominant species among private-sector cases, accounting for 53% of all reported cases. In 2014, the initial emergence of *Candida auris* in South Africa was described.[2] Five years on from the first survey, national surveillance with the aim of describing changes to the epidemiology of candidaemia was re-initiated. In addition, enhanced surveillance at all GERMS sentinel hospitals and for the first time, at three private facilities in Johannesburg and Pretoria to determine the clinical epidemiology of disease, was set up.

Methods

NICD-CHARM conducted national laboratory-based surveillance for candidaemia from 1 January through 31 December 2016. A case was defined as a person diagnosed with candidaemia at any public- or private-sector laboratory in South Africa by culture of any *Candida* species from blood. A new episode was arbitrarily defined if a person had a positive culture >30 days after the first culture. Quarterly audits of the NHLS CDW were conducted to ensure completeness of reporting. Similar audits were not performed in the private sector. At sentinel public- or private-sector hospitals, nurse surveillance officers collected clinical data from admitted case patients by interview or chart review. Corresponding bloodstream isolates were submitted to the Centre for confirmation of species-level identification using the MALDI Biotyper System (Bruker) or sequencing of the internal transcribed spacer region of the multi-copy fungal ribosomal gene. Antifungal susceptibility testing

was performed for anidulafungin, fluconazole and voriconazole using commercially-available microbroth dilution panels containing Alamar Blue (Thermo Fisher Scientific, Cleveland, Ohio, USA) and for amphotericin B using the Etest method (bioMérieux, Marcy l'Etoile, France). Minimum inhibitory concentrations were interpreted using CLSI M27-S4 species-specific breakpoints.

Results

In 2016, 1760 cases of candidaemia were detected, 1127 (64%) of which were diagnosed in Gauteng Province. Of all cases, 473 (27%) were reported from the private sector. The age of cases was significantly lower in the public vs. the private sector (median, 3 years [IQR, 7 months to 46 years] vs. median, 56 years [IQR, 37 to 68 years]; p<0.001). Where sex was known, 54% (939/1732) of patients were male. Clinical case report forms were completed for 979 (55%) patients, including 75 cases at 3 private facilities in Gauteng Province. The overall crude case-fatality ratio was high (408/964; 42%) and varied significantly by species (*C. albicans*, 49%; *C. parapsilosis*, 35%; *C. glabrata*, 48%; *C. tropicalis*, 33% and *C. auris*, 48%; p=0.02) and age category (infants <1 year, 36%; children 1-17 years, 27%; adults 18-44 years, 50%; adults 45-64 years, 54% and adults ≥65 years, 69%; p<0.001). HIV infection was not an independent risk factor for candidaemia; however, 23% (127/542) of patients were HIV-infected, all but 3 in the public sector. A significantly higher proportion of patients was admitted to an intensive care unit in the private vs. public sector (68/74 [92%] vs. 633/875 [72%]; p<0.001). At least one viable isolate was identified to species level for 1408 (80%) cases of candidaemia. Overall, *C. parapsilosis* was the commonest species followed by *C. albicans;* the species distribution differed significantly by sector (p<0.001) (Table 5; Figure 7). Of particular concern, *C. auris* accounted for 9% (126/1372) of cases and was the second commonest species in the private sector and the fourth commonest in the public sector. All *Candida* isolates had an amphotericin B minimum inhibitory concentration (MIC) ≤2 µg/ml (apart from 4 *C. krusei*, 2 *C. parapsilosis* and 1 *C. albicans* isolate). Susceptibility results for five commonest *Candida* species, including *C. auris*, and three antifungal agents are summarised in Table 6. Anidulafungin MICs are presented as a proxy for susceptibility to the echinocandin class.

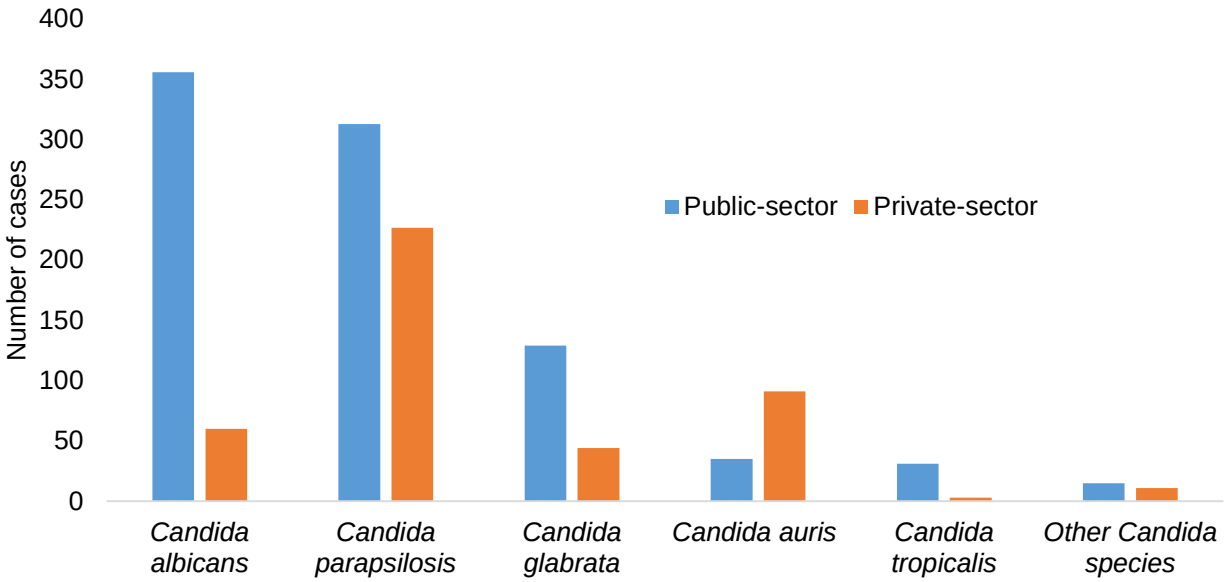

Figure 7. Species distribution of cases of candidaemia with a viable bloodstream isolate by health sector, South Africa, 2016, n=1366.

Table 5. *Candida* species distribution for cases of candidaemia with a viable bloodstream isolates by health sector and province, South Africa, 2016, n=1366.

Species	n (%):									
	EC	FS	GA	KZ	LP	MP	NC	NW	WC	Overall
Public-sector facilities										
Candida albicans	21 (46)	34 (37)	165 (33)	49 (42)	12 (55)	7 (70)	7 (54)	5 (38)	56 (46)	356 (38)
Candida parapsilosis	8 (17)	47 (51)	188 (38)	37 (32)	1 (5)	0 (0)	4 (31)	4 (31)	24 (20)	313 (34)
Candida auris	0 (0)	0 (0)	32 (6)	0 (0)	2 (9)	0 (0)	0 (0)	0 (0)	1 (1)	35 (4)
Candida glabrata	11 (24)	7 (7)	56 (11)	15 (13)	4 (18)	2 (20)	2 (15)	3 (23)	29 (24)	129 (14)
Candida tropicalis	3 (7)	0 (0)	11 (2)	10 (9)	0 (0)	0 (0)	0 (0)	1 (8)	6 (5)	31 (3)
Other *Candida* species	3 (7)	4 (4)	42 (9)	6 (5)	3 (14)	1 (10)	0 (0)	0 (0)	7 (6)	15 (2)
Sub-total	46	92	494	117	22	10	13	13	123	930
Private-sector facilities										
Candida albicans	0 (0)	0 (0)	48 (13)	2 (22)	1 (100)	3 (19)	0 (0)	2 (33)	4 (13)	60 (14)
Candida parapsilosis	0 (0)	0 (0)	192 (52)	5 (56)	0 (0)	9 (56)	0 (0)	1 (17)	20 (61)	227 (52)
Candida auris	0 (0)	1 (100)	83 (22)	2 (22)	0 (0)	4 (25)	0 (0)	0 (0)	1 (3)	91 (21)
Candida glabrata	2 (100)	0 (0)	35 (10)	0 (0)	0 (0)	0 (0)	0 (0)	3 (50)	4 (12)	44 (10)
Candida tropicalis	0 (0)	0 (0)	3 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1)
Other *Candida* species	0 (0)	0 (0)	7 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (12)	11 (3)
Sub-total	2	1	368	9	1	16	0	6	33	436
Total	**48**	**93**	**862**	**126**	**23**	**26**	**13**	**19**	**156**	**1366**

EC: Eastern Cape, FS: Free State, GA: Gauteng, KZ: KwaZulu-Natal, LP: Limpopo, MP: Mpumalanga, NC: Northern Cape, NW: North West

Table 6. Numbers and percentages of *Candida* bloodstream isolates (five commonest species only) susceptible[a] to fluconazole, voriconazole and anidulafungin by health sector, South Africa, 2016, n=1288.

Antifungal agent	Number (%) of isolates susceptible to:				
	C. albicans	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*	*C. auris*
Public-sector facilities	n=354	n=316	n=130	n=31	n=33
Fluconazole	345 (97)	101 (32)	0 (0)	30 (97)	No breakpoints or ECV[b]
Voriconazole	349 (99)	170 (54)	No breakpoints	27 (87)	No breakpoints or ECV[c]
Anidulafungin	354 (100)	316 (100)	128 (98)	30 (97)	No breakpoints or ECV[d]
Private-sector facilities	n=60	n=227	n=44	n=3	n=90
Fluconazole	60 (100)	31 (14)	0 (0)	3 (100)	No breakpoints or ECV[b]
Voriconazole	60 (100)	68 (30)	No breakpoints	3 (100)	No breakpoints or ECV[c]
Anidulafungin	60 (100)	227 (100)	43 (98)	3 (100)	No breakpoints or ECV[d]

[a]Based on CLSI M27-S4 species-specific breakpoints for susceptibility; [b]98% of isolates with an MIC ≥8 mg/L; [c]44% of isolates with an MIC ≥1 mg/L; [d]3 isolates with an MIC ≥1 mg/L; ECV: epidemiologic cut-off value

Discussion

The epidemiology of culture-confirmed candidaemia has changed since a national survey was last conducted in 2009 and 2010, with the emergence of *C. auris* as a major pathogen. There continue to be differences in epidemiology between the public and private sector, with some variation by province. In 2016, candidaemia was diagnosed far more commonly among young children, predominantly neonates, in the public sector and among older adults in the private sector. Overall, more than a third of patients with candidaemia, many of whom were critically ill, died in hospital. A large majority of bloodstream *C. parapsilosis* isolates were resistant to fluconazole. *Candida auris*, an emerging pathogen, is also fluconazole resistant, with very few exceptions. Azole-resistant strains of *C. parapsilosis* and *C. auris* now dominate in the private sector, particularly in Gauteng Province. Fluconazole prophylaxis should thus be discouraged in this setting, even in high-incidence hospital units. Knowledge of local hospital or hospital unit epidemiology should guide empiric treatment choices. Conventional amphotericin B remains the empiric antifungal agent of choice for candidaemia in the public sector because of the high prevalence of azole-resistant *C. parapsilosis* isolates. Caspofungin, micafungin or anidulafungin are also good choices for empiric treatment in all settings where these agents are available.

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AETIOLOGICAL SURVEILLANCE OF SEXUALLY TRANSMITTED INFECTION SYNDROMES AT SENTINEL SITES: GERMS-SA 2014-2016

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Executive Summary

Sentinel aetiological surveillance of sexually transmitted infection (STI) syndromes was conducted at primary healthcare facilities in four South African provinces during the period 2014-2016. *Neisseria gonorrhoeae* was the predominant cause of male urethritis syndrome (MUS), and syndromic management with dual antimicrobial therapy that also covers *Chlamydia trachomatis*, the second most common pathogen, is necessary. Continued surveillance for antimicrobial resistance in *N. gonorrhoeae* is essential. Herpes simplex virus was the commonest detectable cause of genital ulceration, supporting the continued use of acyclovir in syndromic management. The syndromic management of vaginal discharge syndrome (VDS) remains complex because the commonest causes - bacterial vaginosis and candidiasis - are not considered to be STIs. However, a significant proportion of patients with either condition were co-infected with STI pathogens. The HIV seroprevalence among STI patients was high, underlining the importance of linkage to universal HIV counselling and testing in primary healthcare settings.

Background

In South Africa, sexually transmitted infections (STIs) are managed principally at primary healthcare facilities (PHCs) using standard syndromic management guidelines.[1] National clinical STI syndrome surveillance is conducted by the National Department of Health (NDoH) at 270 surveillance sites across the country. Clinical surveillance data on the distribution of STI syndromes in Gauteng Province public health clinics (PHCs) during the period 2000 to 2007 revealed that male urethritis syndrome (MUS), vaginal discharge syndrome (VDS) and genital ulcer syndrome (GUS) combined constitute nearly 80% of all syndromes seen.[2]

Periodic aetiological surveillance of the three main STI syndromes is critical in terms of validating the existing treatment algorithms. The STI Section of the National Institute for Communicable Diseases (NICD) has conducted regular aetiological and antimicrobial resistance surveillance at sentinel PHCs since 2007. During 2014 to 2016, STI aetiological surveillance was conducted in the following provinces: Gauteng (Alexandra Health Centre), Mpumalanga (Kabokweni and Hluvukani Clinics), KwaZulu-Natal (Eastboom Community Health Centre in Pietermaritzburg and Phoenix Clinic in Durban) and Eastern Cape (Gqebera Clinic).

The primary objectives of this surveillance were to determine the aetiologies of the three major STI syndromes (MUS, GUS, VDS) and the antimicrobial susceptibility profiles of *Neisseria gonorrhoeae* isolates. Secondary objectives were to determine syphilis, herpes simplex type 2 (HSV-2) and HIV co-infections among patients presenting with STI syndromes.

Methods

Consecutive consenting patients presenting with MUS, VDS or GUS at the selected PHCs between January 2014 and December 2016 were included in the surveillance. Inclusion criteria were STI patients aged 18 years and above with a new episode of clinically confirmed MUS, VDS and/or GUS. The target sample size per site was as follows: 100 cases each of MUS and GUS and approximately 150-200 cases of MUS (in order to obtain at least 100 viable gonococcal isolates from each site). Following eligibility and informed consent procedures, a nurse-administered questionnaire was used to document demographic and clinical information. Swabs were used for the sampling of genital discharge (vaginal, endocervical, urethral) and genital ulcers. Additionally, a 10 ml specimen of venous blood was collected from each participant. Laboratory testing was performed using the diagnostic assays shown in Table 1. Data from a survey-specific database were imported into STATA 14® [Stata Corporation, College Texas] for analysis.

Table 1. Specimen types and laboratory testing by sexually transmitted infection (STI) syndrome, South Africa, 2014 – 2016.

Syndrome	Specimen	Test
Male urethritis syndrome (MUS)	Endourethral smear	Gram stain for Gram-negative diplococci
	Endourethral swab (Dacron)	In-house multiplex real-time (RT) PCR for discharge pathogens
	Endourethral swab in Amies transport medium	*Neisseria gonorrhoeae* culture and antimicrobial susceptibility. E-test MIC (bioMerieux): cefixime, ceftriaxone, ciprofloxacin Agar dilution MIC: penicillin, tetracycline, azithromycin
Vaginal discharge syndrome (VDS)	Vaginal smear	Gram stain: Nugent score (bacterial vaginosis); yeast (candidiasis)
	Endocervical swab	In-house multiplex RT PCR for discharge pathogens
Genital ulcer syndrome (GUS)	Ulcer smear	Giemsa stain for *Klebsiella granulomatis* (granuloma inguinale)
	Ulcer swab	In-house multiplex RT PCR for ulcer pathogens Commercial PCR (Sacace Biotechnologies) for HSV-1 & 2 subtyping LGV-specific in-house RT PCR for *Chlamydia trachomatis* L1-3 serovars
All participants	10 ml venous blood for serology	HSV-2 (Focus HerpeSelect 2 IgG) HIV (Trinity Biotech Unigold; Determine Alere diagnostics) RPR (Immutrep Omega diagnostics)

Results

Patient demographic and clinical characteristics

Of 1824 participants, 962 (52.6%) were male (Table 2). Median age of participants was 27 years (IQR 23-32 years) and the majority were of black African ethnicity (99.4%) and of heterosexual orientation (98.9%). With respect to high risk sexual behaviours, median age at sexual debut was 17 years (IQR 16-19 years)

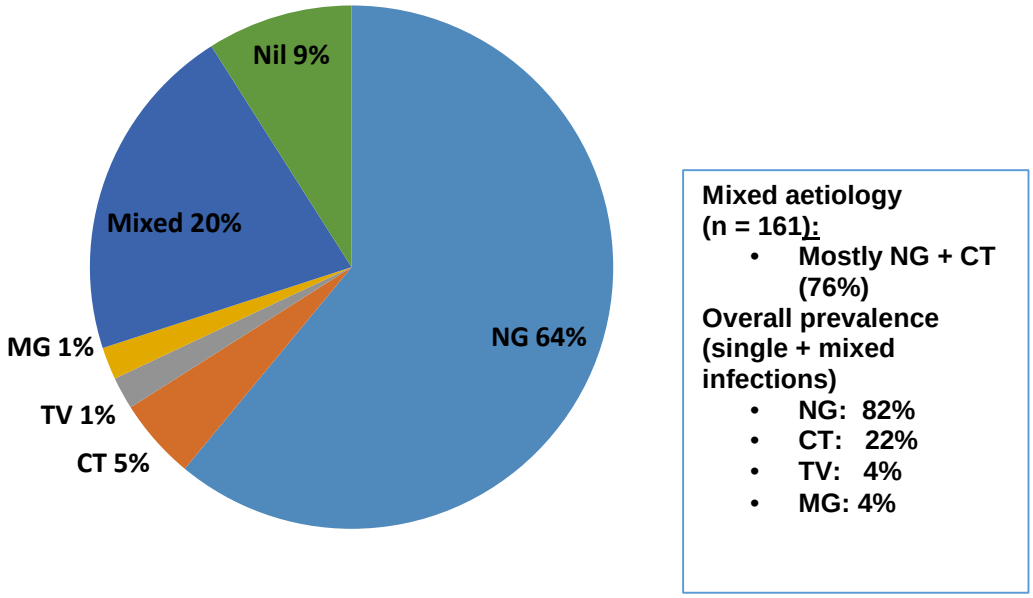
and self-reported condom use at last sexual encounter was low (17.6%). Almost one third of participants (28.7%) had been diagnosed with an STI syndrome within the preceding 12-month period.

Table 2. Demographic and clinical characteristics of participants enrolled into national sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016.

Variable	All (N = 1824)
N	962 (52.7)
Current age median (IQR)	27 (23- 32)
Black Africans	1813 (99.4)
(n, %)	
Age at sexual debut	17 (16- 19)
Median (IQR)	
Condom use	322 (17.6)
(n, %)	
Sex with someone outside province	292 (16.0)
(n, %)	
Sex with someone outside country	214 (11.7)
(n, %)	
STI syndrome diagnosed in the past 12 months	523 (28.7)
(n, %)	
Heterosexual orientation	1803 (98.9)
(n, %)	
Main syndrome diagnosed:	
Male urethritis syndrome	808 (44.3)
Vaginal discharge syndrome	757 (41.5)
Genital ulcer syndrome	366 (20.1)
>=2 syndromes	107 (5.9)

STI Syndrome aetiologies

Male urethritis syndrome: Among 808 patients presenting with MUS, *N. gonorrhoeae* was the commonest cause (666, 82.4%; 95% CI 79.6 – 84.9) followed by *Chlamydia trachomatis* (178, 22.0%; 95% CI 19.3 - 25) (Figure 1). The majority of patients (578, 71.5%; 95% CI 68.3 – 74.5) had infections caused by single agents. *Trichomonas vaginalis* and *Mycoplasma genitalium* accounted for less than 5% of MUS. Multiple pathogens were detected in approximately 20% (161; 95% CI 17.3 – 22.8). The majority of these mixed infections (150, 93.2%) were caused by *N. gonorrhoeae* together with one or more STI pathogens, mostly *C. trachomatis* (123,76.4%). An STI pathogen was detected in approximately 91% of specimens (739; 95%CI 89.3-93.2) and less than 10% (69; 95% CI 6.8 – 10.7) had no identifiable STI aetiology.



Neisseria gonorrhoeae (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG)

Figure 1. Relative prevalence of sexually transmitted infection (STI) pathogens in patients presenting with male urethritis syndrome (MUS), South Africa, 2014 – 2016, (N = 808).

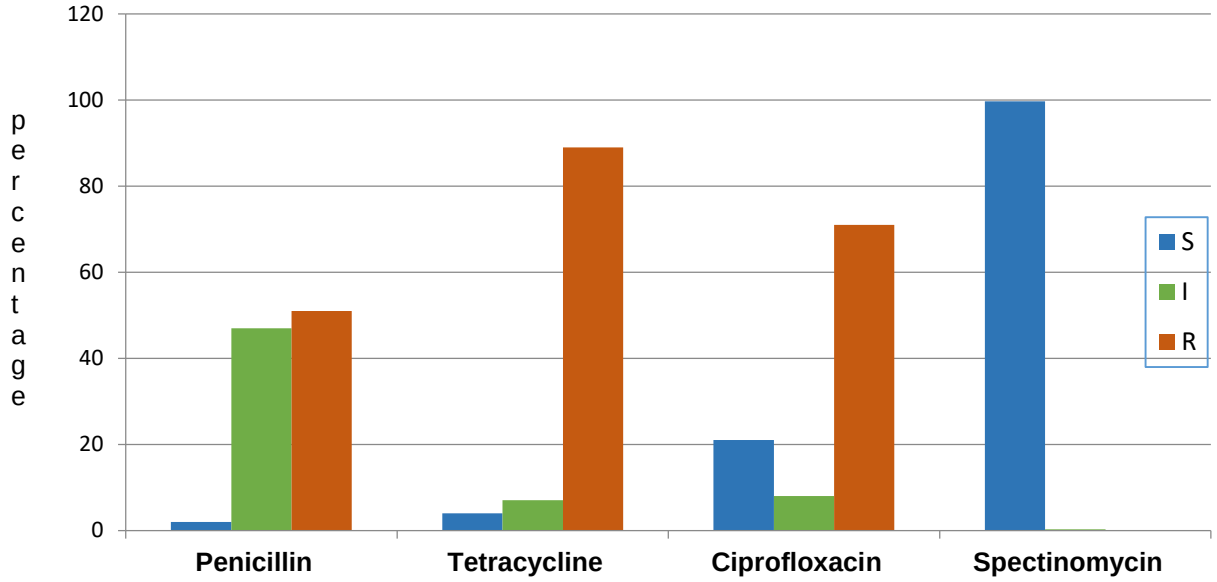
Neisseria gonorrhoeae antimicrobial susceptibility profiles: *Neisseria gonorrhoeae* minimum inhibitory concentrations (MICs) to extended-spectrum cephalosporins and azithromycin were available for 339 viable culture isolates from male urethral discharge specimens collected in 2016 (Table 3). All isolates demonstrated low ES-cephalosporin MICs that were within the susceptible range. MICs to azithromycin showed that 99.4% isolates were susceptible to azithromycin with only two (0.6%) isolates from Mpumalanga and KwaZulu-Natal provinces, respectively, exhibiting intermediate resistance (MIC = 0.5 µg/ml). The susceptibility profiles to the other antimicrobials tested are shown in Figure 2. High-level resistance rates were as follows: 51% for penicillin; 88% for tetracycline and 70% for ciprofloxacin. All isolates, except for one that displayed an intermediately-resistant MIC of 64 µg/ml, were susceptible to spectinomycin.

Table 3. *Neisseria gonorrhoeae* minimum inhibitory concentrations (MICs) to extended-spectrum cephalosporins and azithromycin. National sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016, (n = 339).

Antimicrobial	MIC	MIC	Maximum MIC	% with MIC = 0.125	% with MIC = 0.25	% WITH MIC ≥ 0.5
Cefixime	< 0.016	< 0.016	0.016	0	0	0
Ceftriaxone	0.003	0.006	0.032	0	0	0

Antimicrobial	MIC	MIC	Maximum MIC	% with MIC ≤ 0.25	% with MIC = 0.5	% with MIC ≥ 1
Azithromycin	0.128	0.25	0.5	99.4	0.6	0

Interpretive criteria used: CLSI for extended-spectrum cephalosporins; EUCAST for azithromycin; MICs in µg/ml



Interpretive criteria used: CLSI. S = sensitive; IR = intermediately-resistant; R = resistant

Figure 2. Neisseria gonorrhoeae antimicrobial susceptibility profiles, national sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016, (n = 330).

Vaginal discharge syndrome: Among 756 women with VDS (Figure 3), less than 50% had a detectable STI pathogen in single or mixed infections (330; 95% CI 40.1 – 47.1). The commonest STI aetiology was *N. gonorrhoeae* (140, 18.5%; 95%CI 15.9 – 21.4), followed by *C. trachomatis* (134, 17.7%; 95% CI 15.2 – 20.6). *T. vaginalis* accounted for less than 15% of infections, and *M. genitalium* less than 10%. Overall, single STI pathogens were detected in 234 VDS cases (31%; 95% CI 27.7 – 34.3); and mixed infections with multiple (two or more) STI pathogens in 96 (13%; 95% CI 10.5 – 15.3).

Most VDS cases were attributed to conditions that are not traditionally considered to be STIs. Bacterial vaginosis (BV) was identified in 427/752 (56%; 95% CI 52.8 – 59.9) cases, and vulvovaginal candidiasis (CA) accounted for 167 (22%; 95% CI 19.2 – 25.1) cases. An identifiable pathogen or cause was not found for 144 (19%; 95% CI = 16.4 - 22) of VDS cases.

A significant proportion of VDS patients had co-infection with STI and non-STI aetiologies. Only 98/752 (13%) of VDS cases tested for all causes had a sole STI aetiology. The rest (232/752, 31%) had an STI plus BV and/or CA.

Overall, 205 VDS cases (27%) had BV-STI co-infections, and sixty-five VDS cases (8.5%) had CA-STI co-infections. Therefore, 205/427 patients with BV (48%; 95% CI 43.3 – 52.8) and 65/167 patients with CA (39%; 95% CI 31.8 – 46.6) had STI co-infections.

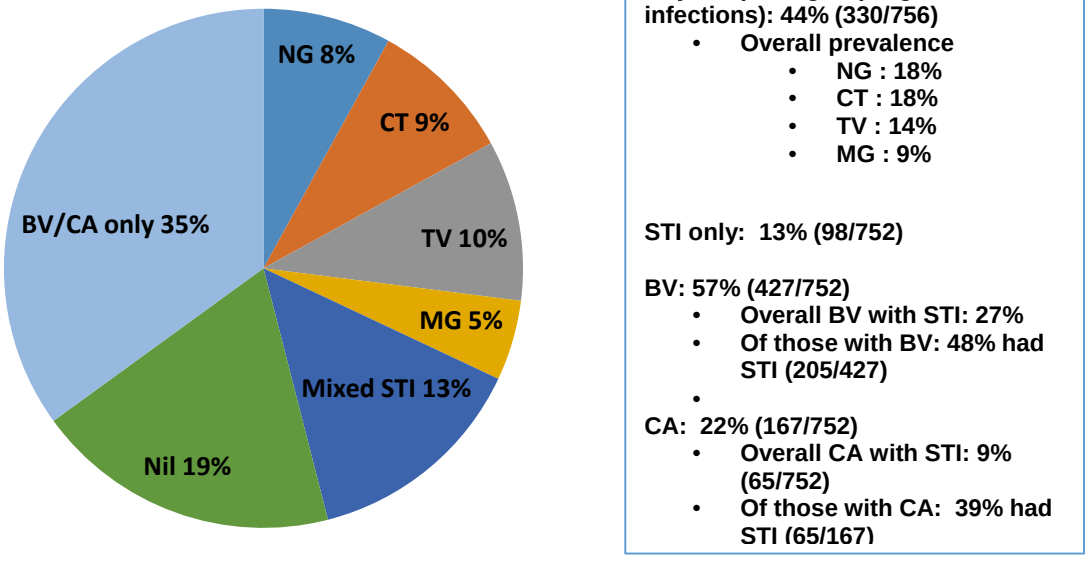
The median age (and IQR) of women with non-STI causes of VDS was 27 years (22-33), and that of women harbouring one or more STI pathogens was also 27 years (23-31).

The relative prevalence of STI pathogens detected in co-infections is presented in Table 4. The commonest STI pathogen in BV co-infected patients was *N. gonorrhoeae* (93/205, 45%). The commonest STI pathogen in CA-STI co-infected patients was *C. trachomatis* (29/65, 45%).

Table 4. Prevalence of sexually transmitted infection (STI) pathogens among vaginal discharge syndrome (VDS) patients with bacterial vaginosis (BV) and vulvovaginal candidiasis (CA), national STI syndrome surveillance, South Africa, 2014 – 2016.

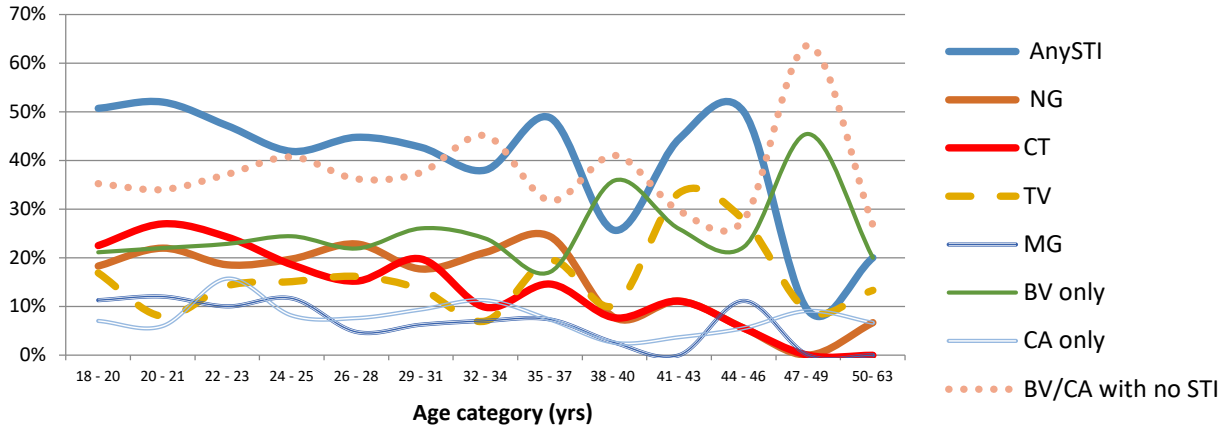
Infection	n	*Neisseria gonorrhoeae* (%)	*Chlamydia trachomatis* (%)	*Trichomonas vaginalis* (%)	*Mycoplasma genitalium* (%)
BV with STI	205	93 (45)	88 (43)	55 (27)	44 (21)
CA with STI	65	23 (35)	29 (45)	19 (29)	15 (23)

Microbial aetiology of VDS and STI pathogen prevalence, stratified by age, shows that age is not an accurate predictor of infection with STI pathogens, including *N. gonorrhoeae*, or with non-STI related conditions such as bacterial vaginosis or candidiasis (Figure 4). There was a significant sustained downward trend in the prevalence of *C. trachomatis* with increasing age in which the prevalence declined significantly in those aged 35 years and older.



Key: *Neisseria gonorrhoeae* (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); bacterial vaginosis (BV); vulvovaginal candidiasis (CA)

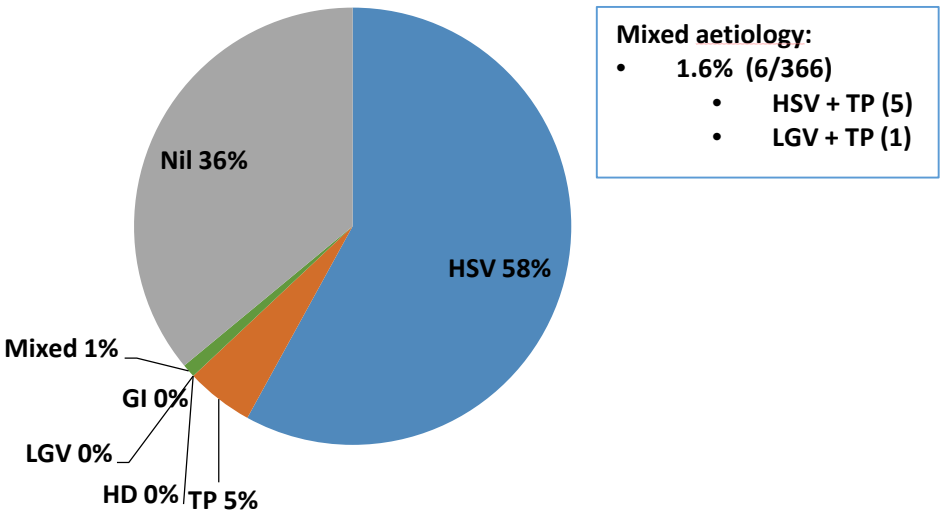
Figure 3. Relative prevalence of vaginal discharge syndrome (VDS) aetiologies, national sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016, (N = 752).



Neisseria gonorrhoeae (NG); *Chlamydia trachomatis* (CT); *Trichomonas vaginalis* (TV); *Mycoplasma genitalium* (MG); bacterial vaginosis (BV); vulvovaginal candidiasis (CA)

Figure 4. Distribution of vaginal discharge syndrome (VDS) aetiologies by age. National sexually transmitted infection (STI) syndrome surveillance, South Africa, 2014 – 2016.

Genital ulcer syndrome: Among 366 GUS cases (Figure 5), the major cause was herpes simplex virus (HSV) in 59.3% (217/366; 95% CI 54 - 64), followed by *Treponema pallidum* (TP) in 6% (22/366; 95% CI 4 - 9) of cases. Type-specific PCR revealed that 99.0% (215/217) HSV infections were caused by HSV-2. Of two HSV-1 infected ulcers from Gauteng and Mpumalanga provinces, one was co-infected with HSV-2. There was only 1 case each (0.6%) of *Haemophilus ducreyi* (HD) causing chancroid (Eastern Cape) and lymphogranuloma venereum (LGV) caused by *C. trachomatis* L1-L3 (Mpumalanga). No cases of granuloma inguinale were detected in any of the provinces. Most pathogen-detectable cases had a single aetiology (228/366, 62.3%). Only 6 cases had mixed aetiology. They were all were co-infected with HSV and one other pathogen, namely TP (5), HD and LGV.[1] An ulcer-derived pathogen was not identified in 36.1% of GUS cases (132; 95% CI 31.3 – 41.1).



Herpes simplex virus (HSV); *Treponema pallidum* (TP); lymphogranuloma venereum (LGV); granuloma inguinale (GI)

Figure 5. Relative prevalence of sexually transmitted infection (STI) pathogens in patients presenting with genital ulcer syndrome (GUS), South Africa, 2014 – 2016.

Serological results

Syphilis (RPR) seroprevalence was highest at 10.2% among GUS patients (37/364; 95% CI 7.4 – 13.7), followed by 2.9% in MUS (23/791; 95% CI 1.9 – 4.3) and 3% (22/742; 95% CI 2.0 – 4.5) in VDS patients. Active syphilis, defined by an RPR titre ≥ 4, was identified in 7.4% of GUS (95% CI 5.1 – 10.6), 2.4 % of MUS (95% CI 1.5 – 2.7) and 2.2% (95% CI 1.3 – 3.4) of VDS cases. Among the 22 GUS patients whose ulcers were attributed to primary syphilis, 19 (86%; 95% CI 62.8 – 96.0) had positive RPR results and 15 (68%; 95% CI 44.7 – 85.0) had RPR titres of $\underline{\geq}$ 4. The sero-prevalence of anti-HSV-2 antibodies among the GUS patients whose ulcers were caused by HSV-2 was 82% (178/217; 95% CI 76.3 – 86.6). HIV co-infection rates were as follows: 57.3% (208/363; 95% CI 52.1 – 62.3) in GUS; 47.2% (350/742; 95% CI 43.6 – 50.8) in VDS and 26.6% (211/794; 95% CI 23.6 – 29.8) in MUS. There was a significant association between HIV seropositivity and all STI syndromes ($p<0.001$).

Discussion and Conclusions

This surveillance study provides a snapshot of STI syndrome aetiologies across several South African provinces during the period 2014 to 2016. Overall, the study found that the majority of participants enrolled with STI syndromes were young and reported high-risk sexual behaviour, such as young age at sexual debut and unprotected sex at last sexual encounter.

Neisseria gonorrhoeae was the predominant cause of male urethritis syndrome. Based on these data, syndromic management for MUS in the South African public health sector should include cover for the two leading causes, *N. gonorrhoeae* and *C. trachomatis*. In 2015, the national STI syndromic management guidelines were formally revised in response to the increase in *N. gonorrhoeae* antimicrobial resistance observed worldwide, as well as reports of cefixime resistance in South Africa.[3] A pre-emptive strategy of dual antimicrobial therapy was incorporated to curb the emergence of resistance in *N. gonorrhoeae* to extended-spectrum cephalosporins. Specifically, oral cefixime was replaced with single doses of injectable ceftriaxone and oral azithromycin. Azithromycin used in dual therapy for *N. gonorrhoeae* also provides empiric cover for *C. trachomatis* infection. *Trichomonas vaginalis* was detected in less than 5% of men presenting with urethritis. These findings support the current syndromic approach to reserve metronidazole treatment for those whose partners report vaginal discharge, or for those whose symptoms persist following first-line treatment for *N. gonorrhoeae* and *C. trachomatis*.

Bacterial vaginosis was the leading cause of VDS and was prevalent in over 50% of females. Although the condition, which is associated with dysbiosis of the vaginal microbiome, is not considered to be a traditional STI, systematic review and meta-analysis of sexual risk factors have revealed that the epidemiological profile of BV is similar to that of established STIs.[4] The current VDS management algorithm has an age cut-off of 35 years and older for treatment of BV and *Candida* only, with exclusion of antimicrobial therapy for *N. gonorrhoeae* and *C. trachomatis* in older patients. These data reveal that there is no significant difference in median age of women infected with STI pathogens and those having BV or candidiasis. Further analyses of those infected only with *N. gonorrhoeae* or *C. trachomatis* revealed that there appears to be no appropriate age cut-off for therapy directed solely against these two infections in management guidelines. A significant proportion of women with BV were co-infected with one or more STI pathogens. These findings suggest that BV is associated with risk factors for traditional STI infections, and that the management algorithm for VDS should be reconfigured to remove non-specific variables such as age and include specific sexual risk characteristics that increase the predictive value of the algorithm for STI pathogens.

Herpes simplex virus-2 remains the leading cause of pathogen-detectable GUD in Gauteng, and this supports the use of anti-viral therapy in the syndromic management guidelines. A change in epidemiology to HSV-1 has not been observed. Approximately 80% of genital herpes cases were HSV-2 antibody positive and represented clinically apparent reactivation disease. Primary syphilis and LGV are relatively uncommon causes of GUD in the predominantly heterosexual populations accessing STI services in South African primary healthcare centres. In keeping with epidemiological trends worldwide, chancroid, a predominant cause of GUD in South Africa in the late 20th century (responsible for up to 70% of genital ulceration), is now only detected sporadically.[5] The significant proportion of cases without an identifiable ulcer-derived aetiology requires further research.

The HIV prevalence among patients presenting with STI syndromes is significantly higher than the UNAIDS 2015 estimated prevalence of 19.1% for adults aged 15-49 years in the general South African population. This underscores the importance of linkage to universal HIV testing and treatment for STI patients and supports the recently adopted national policy of early ARV initiation for those who are HIV-infected.

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