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FOREWORD

This issue is about certain bacterial pathogens and the diseases they cause. *Brucella abortus* and, to a lesser extent, *B. melitensis* cause the zoonotic disease brucellosis, which can be acquired from infected livestock, especially cattle, goats and sheep. Humans are most likely to acquire an infection by drinking unpasteurised milk. The incidence, pathology, diagnosis, treatment and control of brucellosis in South Africa is reviewed in this issue.

Typhoid fever, caused by *Salmonella enterica* subspecies *enterica* serotype Typhi, is endemic in South Africa, although incidence is generally low. The risk of transmission is associated with poor sanitation, unsafe water, and unsafe food production and handling processes. A recent typhoid outbreak in Sekhukhune District, Limpopo Province, is described in this issue, which also contains a report of antimicrobial resistance surveillance in *Neisseria gonorrhoeae*, the causative agent of the sexually transmitted infection gonorrhoea. This report shows that the currently-recommended dual therapeutic regimen of ceftriaxone and azithromycin is appropriate for use in infection management in South Africa and that ongoing surveillance is essential for the potential occurrence of extensively drug-resistant (XDR) gonorrhoea that has acquired resistance to the extended-spectrum cephalosporins.

All participating laboratories and contributors are thanked for their inputs. This is the final issue for 2018 and we wish all our readers and contributors a safe and joyous holiday season.

Basil Brooke, Editor

BRUCELLOSIS IN SOUTH AFRICA – A NOTIFIABLE MEDICAL CONDITION

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Executive summary

Brucellosis is an important zoonotic condition in many regions of the world. In South Africa brucellosis is a controlled animal disease and is a notifiable medical condition in humans. The predominant pathogen is *Brucella abortus*, with far fewer reported *B. melitensis* infections in animals and humans. The predominant clinical manifestation in animals is abortion. Transmission from animals to humans is mainly through consumption of unpasteurised milk. Occupational exposure typically occurs in farm, abattoir, veterinary and laboratory situations. Bovine brucellosis (*B. abortus*) occurs across all nine provinces, but most infected cattle herds occur in the central and Highveld provinces. Clinically, human brucellosis is highly variable in presentation, with *B. melitensis* more likely to produce severe or complicated disease. Treatment generally requires use of antibiotic combinations for prolonged periods. In animals, one of the control measures for brucellosis involves the use of attenuated live vaccines, which sometimes cause accidental human infections. Dairy products are rendered safe for human consumption through pasteurisation. Bovine brucellosis control in cattle is currently being prioritised under the South African Veterinary Strategy (2016-2026).

Introduction

Brucellosis is a bacterial disease of animals, and is transmissible to humans. *Brucella abortus* causes the majority of bovine and human brucellosis cases in South Africa, whereas the usual reservoirs of *B. melitensis* are goats and sheep. Pregnant livestock may abort, with *Brucella* bacteria being shed in the birth fluids and potentially in their milk. Brucellosis has also been documented in certain wildlife in the country.

Brucellosis is a controlled animal disease in South Africa, and human brucellosis is a notifiable medical condition. Persons working in occupations where contact with livestock and livestock tissues frequently occur are at highest risk of brucellosis (farmers and farm workers, abattoir workers, veterinarians, animal health technicians, etc.). Humans acquire *Brucella* infection by three routes:

- Direct contact with infected animal tissues or secretions through skin cuts/abrasions or conjunctival splashes;
- Inhalation of contaminated aerosols (uncommon);
- Consumption of unpasteurised dairy products (incl. milk, yoghurt, cheese).

It is important to note that pasteurised or adequately boiled milk or milk products, and cooked meat from infected animals, are safe to consume and do not transmit infection. Accidental self-injection with *Brucella* vaccine is a risk for farmers, vets and animal health technicians, and laboratory staff may also be accidentally exposed when dealing with *Brucella* cultures (discussed later).

Bovine brucellosis – a cattle herd disease

Brucella abortus is primarily a cattle disease but may affect most other mammals, including humans.[1] The disease in cattle typically presents with abortion, reduced fertility, orchitis, joint problems, a drop in milk production and decreased general production.[2-4] Currently, most cattle farmers in South Africa are at risk of acquiring brucellosis-positive cattle in their herds, as there is generally poor compliance with brucellosis vaccination and testing.[5] In South Africa, cattle are serologically screened using the Rose Bengal test (serum) or milk ring test (milk) followed by the complement fixation test (serum) on screen-positive samples.[6] Culture is considered the gold standard diagnostic.[6] Test results are interpreted at herd level and an entire epidemiological herd is placed under quarantine if positive cattle are identified, owing to the long incubation period of the disease. Testing individual animals before movement is considered a high-risk practice. Whole epidemiological herds should preferably be tested regularly to establish their brucellosis status.

Bovine brucellosis occurs in all nine provinces of South Africa's provinces, but is especially concentrated in the central and Highveld regions. Figure 1 shows the *B. abortus* outbreaks from January 2015 to May 2018 as reported to the Directorate Animal Health of the Department of Agriculture, Forestry and Fisheries (DAFF).

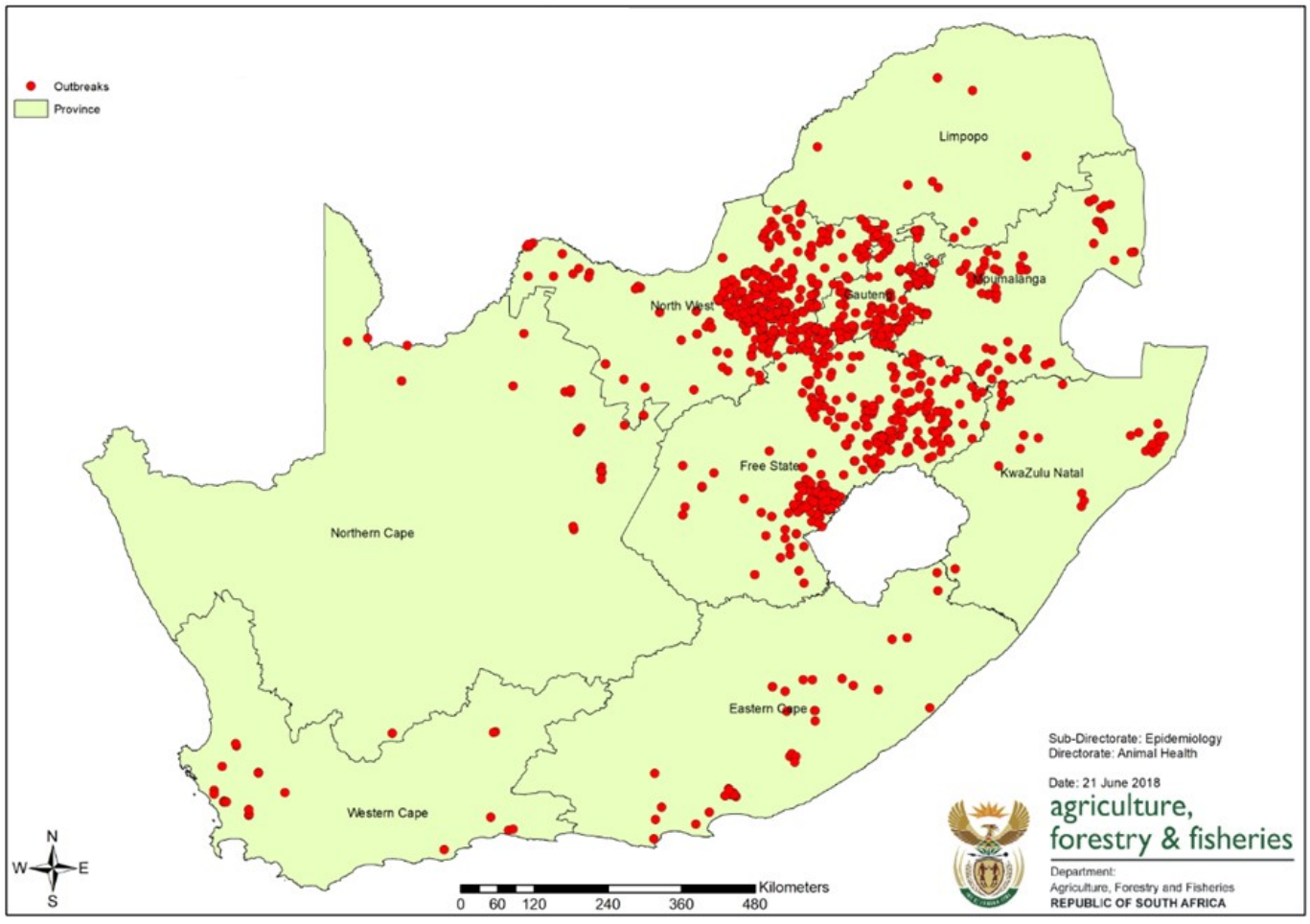

Figure 1. Reported *Brucella abortus* outbreaks in animals from January 2015 to May 2018 across all nine provinces of South Africa. Image courtesy of the Sub-Directorate: Epidemiology of the Directorate Animal Health, Department of Agriculture, Forestry and Fisheries (DAFF).

DAFF brucellosis policy development

As part of the South African Veterinary Strategy (2016-2026), bovine brucellosis was selected to be used as a 'model disease' by which to approach future livestock disease control efforts.[7] Review of the bovine brucellosis control policy in cattle is underway to facilitate implementable, cost-effective and sustainable control of the disease.

Several shortcomings in the Bovine Brucellosis Scheme R.2483 of 9 Dec 1988 have been identified, including the voluntary nature of the scheme, testing programmes that are not all herd based, movement of test-negative animals from quarantined farms, and capacity concerns to handle infected herds.[5] The first step in the Brucellosis Policy review was the drafting of the 'Discussion Paper on the Review of Bovine Brucellosis Control in South Africa' that was published for public comment in the Government Gazette on 5 May 2017.[5] Several challenges and key points were discussed and highlighted in the document. Several inputs and ideas where received from various stakeholders that will be considered going forward.

Numerous underlying brucellosis-related matters are constantly being addressed with the aim of building a more solid foundation for policy implementation. This includes, but is not limited to, public and farmer awareness, training of veterinarians and animal health technicians, promoting vaccination of heifer calves, SANAS accreditation and DAFF approval of laboratories, investigating potential incentive strategies, addressing abattoir-related matters and investigating authorizations to increase manpower. The second step of policy drafting is being addressed by the Bovine Brucellosis Working Group (which reports to the Ministerial Technical Committee of Veterinary Services).

Provincial Veterinary Services' initiatives

Several provinces are increasing their efforts at heifer calf vaccination. The Free State Province rolled out vaccination in 2017, focusing on high-risk herds, positive herds and farms neighbouring positive herds (mostly commercial sector). The vaccination efforts in the North West Province commenced in November 2017. To date, over 45 000 doses of vaccine have been acquired and over 20 000 heifers have been given initial and booster vaccine doses (mostly in the communal sector).

Human brucellosis in South Africa

Globally, brucellosis is considered one of the most common zoonoses with an estimated 500 000 new cases diagnosed annually. In Africa, human brucellosis incidence is largely unknown with the estimated burden of the disease varying widely from <0.09 to >8.43 per 100 000 population.[8-10] In South Africa, brucellosis is considered a priority zoonotic disease and despite being a notifiable medical condition, the true incidence of the disease is unknown. The last formally published incidence rate of >0.2 per 100 000 population was based on a survey conducted between 1956 to 1959.[11] A Department of Health analysis of national notifications between 1977 and 1984 showed annual incidence rates between <0.1 and 0.3 per 100 000 population,[12] and there has not been a subsequent update on the national incidence rate. There is currently no national human brucellosis surveillance program and over the recent past only a limited number of studies have been conducted in acute febrile illness patients and at-risk populations (e.g. farming community, abattoir workers and veterinary professionals; not published). Some sporadic human infections and related laboratory exposures have been reported to the National Institute for Communicable Diseases (NICD).[13-16] The case definitions for brucellosis notification in South Africa are provided as an addendum to this article.

Clinical disease

Owing to its ability to cause a wide spectrum of clinical manifestations with a tendency towards chronicity and persistence, brucellosis is one of the three 'great imitators', along with TB and syphilis. It evolves into a granulomatous disease capable of

affecting any organ system. The clinical features depend on the stage of disease as well as the organ/s involved. Fever is the most common feature, followed by osteoarticular involvement, sweating and constitutional symptoms. Hepatosplenomegaly is evident in a third of patients, and lymphadenopathy in 10%. Osteoarticular manifestations (sacroiliitis, spondylitis, peripheral arthritis and osteomyelitis) account for over half of the focal complications, while pulmonary disease may be evident in up to 16% of complicated cases, and genitourinary complications can be found in 10% of patients. Neurological involvement may be evident in about 6% of cases, with protean nervous system manifestations.

Human brucellosis diagnosis

Laboratory diagnosis of human *Brucella* infection is complicated and none of the currently available diagnostic tools can be used in solo to reliably detect the causative agent. Definitive laboratory diagnosis of human brucellosis is based on the isolation of bacteria from clinical samples (blood, bone marrow or other tissues). However, cultures give a low yield as *Brucella* is fastidious and the number of bacteria in clinical samples may vary widely. The isolation of *Brucella* is highly dependent on the stage of disease (acute versus chronic), antibiotic treatment, availability of appropriate clinical sample and the culturing method used.[17] Advances in automated blood culture systems (e.g. BACTEC™ and BacT/Alert™) have decreased the culture time and increased the recovery rate from sterile body fluids.[18] In recent years, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been shown to be an effective tool to identify *Brucella* spp. directly, from both culture plates and blood culture bottles.[19-21] However, this method is not routinely used as it requires access to a specialised protein profile database.

Serological testing is preferred in routine clinical practice as it is non-hazardous, faster and more sensitive than bacterial culture. Many serological assays are available for the diagnosis of human brucellosis. The most commonly used assays include the serum agglutination test (SAT), the Coombs anti-*Brucella* test, the Rose Bengal test, complement fixation and more recently enzyme-linked immunosorbent assay (ELISA). However, interpretation of these assays is often difficult and several factors should be considered when interpreting serological results: i) many patients are seronegative in the acute phase of the disease, which necessitates the serological testing of paired sera or performing more than one serological test, ii) a high proportion of the population in endemic regions may have persistent antibody titres due to ongoing exposure to *Brucella*, and appropriate cut-off values should be determined, iii) antibodies can remain detectable despite successful therapy, and iv) cross-reaction with other lipopolysaccharide (LPS) containing Gram-negative bacteria (e.g. *Salmonella*, *Yersinia*) may occur.[19] Serology results should be interpreted in combination with clinical signs and symptoms. Serological assays available at public and private pathology laboratories in South Africa include SAT and ELISA.

Various polymerase chain reaction (PCR) assays targeting different gene loci have been developed for the diagnosis of human brucellosis from pure cultures and clinical specimens (i.e. serum, whole blood, urine samples, various tissues, etc.).[19] Independent of the disease stage, PCR is more sensitive than blood cultures and more specific than serological tests.

Treatment

Treatment of human brucellosis is often complicated by treatment failures and relapses. Meta-analysis has shown that dual or triple regimens including an aminoglycoside (doxycycline-streptomycin/gentamicin or doxycyline-rifampicin-streptomycin/ gentamicin) significantly reduce treatment failure and relapse rates, and are currently recommended as first-line treatment regimens. Duration of treatment is at least six weeks for doxycycline and rifampicin, and up to two weeks for aminoglycoside

therapy (daily intramuscular injections) - see Tables 1 and 2 for details. Patients require prolonged follow-up to monitor for further complications or relapse.

Table 1. Treatment regimens for brucellosis.[22]

Form of brucellosis infection		Recommended antibiotic regimen	Duration
Uncomplicated - adults		Doxycycline plus streptomycin or gentamicin (preferred regimen)	6 weeks 1 - 2 weeks
		OR doxycycline plus rifampicin	6 weeks
- children	<8y	Cotrimoxazole plus rifampicin	4 - 6 weeks
	≥8y	Doxycycline plus rifampicin	
Focal - adults			
Spondylitis		Doxycycline plus streptomycin or gentamicin	12 weeks 2 weeks
		OR doxycycline plus rifampicin	12 weeks
		OR doxycycline plus ciprofloxacin	12 weeks
Neurobrucellosis		Doxycycline plus rifampicin plus (ceftriaxone OR cotrimoxazole)	Prolonged, until CSF normalises
Endocarditis		Doxycycline plus rifampicin plus streptomycin or gentamicin Surgery if indicated	6 weeks to 6 months, depending on clinical response
Focal – children	<8y	Cotrimoxazole plus streptomycin or gentamicin	6 weeks at least 2 weeks
	≥8y	Doxycycline plus streptomycin or gentamicin	6 weeks at least 2 weeks
		Rifampicin can be added to either regimen	6 weeks at least
Brucellosis in pregnancy		Rifampicin with/without cotrimoxazole (avoid in last week before delivery: risk of kernicterus)	6 weeks
Complex focal, relapsed or refractory infection, or antibiotic toxicity/resistance		Consider adding quinolone or cotrimoxazole as second-line to doxycycline or rifampicin; triple therapy has better cure rates	

Table 2. Antibiotic dosages for brucellosis treatment.

Cotrimoxazole	Trimethoprim 10 mg/kg/d (max. 480 mg/d), sulfamethoxazole 50 mg/kg/d (max. 2 g/d)	In 2 doses/day
Doxycycline	2-4 mg/kg/d (max. 200 mg/d)	In 2 doses/day
Rifampicin	15-20 mg/kg/d (max. 2 g/d)	In 1 or 2 doses/day
Gentamicin	5 mg/kg/d	In 1 to 3 doses/day
Streptomycin	20-40 mg/kg/d (max. 1 /d)	In 2 doses/day
Ciprofloxacin	1 g/d	In 2 doses/day
Ofloxacin	400 mg/d	In 2 doses/day

Brucella vaccine exposure in humans

Two vaccines are used for immunising animals against *B. abortus* in South Africa: S19 (widely available through farmers' co-ops) and RB51 (veterinarian-prescribed). *Brucella melitensis* Rev 1 vaccine is used for sheep and goats. These are live attenuated vaccines and are potentially able to cause infection in humans; S19 being more likely to do so than RB51.[23] Accidental occupational exposure to *Brucella* vaccine is well described, and is usually via injection. Accidental spray of vaccine into the conjunctiva and open wounds has also occurred, and should be managed as for needlestick inoculation. Accidental exposures to *Brucella* vaccines should be managed as follows:

- For S19 exposure, take a blood sample for baseline serological testing and storage of serum. There is no serological test available for RB51.
- For S19, recommended post-exposure antibiotic prophylaxis (PEP) is doxycycline (or doxycycline plus rifampicin) for at least 3 weeks; RB51 is resistant to rifampicin, so doxycycline (or doxycycline plus cotrimoxazole or a fluoroquinolone) is used, for at least 3 weeks.[26]
- Those with contraindications to doxycycline (e.g. pregnancy, or attempting to become pregnant,) should use cotrimoxazole for at least 3 weeks.
- Symptoms and daily temperature should be actively monitored for at least 4 weeks, and passive reporting of symptoms should continue for 6 months.
- Any raised temperature and/or symptoms of infection (sweating, fever, chills, arthralgia, myalgia, anorexia, etc) should trigger clinical examination and laboratory investigation (repeat serological testing to check for seroconversion, blood culture, full blood count, inflammatory markers, etc). If infection is confirmed, appropriate antibiotic combination therapy as for any other invasive brucellosis disease must be initiated.

Laboratory exposure to *Brucella* species

Laboratory-acquired *Brucella* infections and outbreaks are well known.[24,25] Live cultures are easily aerosolised, and the infective dose is very low. Additionally, it is an uncommon isolate in most clinical microbiology laboratories, and unidentified *Brucella* samples and cultures may arrive without warning. High-risk exposures for laboratory staff are if they work (or are within 1.5 m of others who work) with *Brucella* cultures on an open bench, or if they open or sniff culture plates, or if they come into direct skin or mucous membrane contact with cultures, suspensions or aerosols of the organism. Working with *Brucella* in a class II biosafety cabinet without additional BSL-3 protection (gown, gloves, mask, goggles) is also high-risk exposure. Certain procedures involving *Brucella* are high risk for all persons in a laboratory, because they potentially produce aerosols: centrifugation without using sealed containers, vortexing, sonicating, shaking, diluting, and accidental spilling or splashing of infected material. Low-risk laboratory exposure is working more than 1.5 m away from manipulations of cultures on an open bench or a class II biosafety cabinet, but without the high-risk aerosol-generating procedures described above. Laboratory exposures should be managed as follows:

- All staff exposures in the laboratory at the time should be risk-assessed, as defined above.
- High-risk exposures: PEP is doxycycline for 3 weeks, alternatively cotrimoxazole, as described above for live vaccine exposure. The US CDC recommends doxycycline plus rifampicin[24] but the combination does not appear to provide increased protection and there is more chance of adverse effects and reduced compliance.
- Low-risk exposures: employees should be offered the option of PEP. All immunocompromised and pregnant workers should be considered for PEP.

- High- and low-risk exposures: blood samples for baseline serological testing should be taken and the serum stored, and employees should be under active medical surveillance. Symptoms and daily temperatures should be monitored for 6 weeks, and passive reporting of symptoms continued for 6 months.
- Any raised temperature and/or symptoms of infection (sweating, fever, chills, arthralgia, myalgia, anorexia, etc) should trigger clinical examination and laboratory investigation (repeat serological testing to check for seroconversion, blood culture, full blood count, inflammatory markers, etc). If infection is confirmed, appropriate antibiotic combination therapy as for any other invasive brucellosis disease must be initiated.
- A full incident investigation should be done and extended to other potential sites if necessary (e.g. referring laboratories).

References

1. Corbel MJ. Brucellosis: an overview. *Emerg Infect Dis* 1997; 3(2): 213.
2. World Health Organization. The control of neglected zoonotic diseases: a route to poverty alleviation. Geneva: WHO, 2006.
3. Olsen S, Tatum F. Bovine brucellosis. *Vet Clin North Am Food Anim Pract* 2010; 26(1): 15-27.
4. Godfroid J, Bosman P, Herr S, Bishop G. Bovine Brucellosis. In: Coetzer J, Tustin R, eds. Infectious Diseases of Livestock 3. 2nd ed. Cape Town: Oxford University Press Southern Africa, 2004. p. 1510-27.
5. Department of Agriculture Forestry & Fisheries. Discussion Paper on the Review of Bovine Brucellosis Control in South Africa. Pretoria: DAFF, 2017.
6. Department of Agriculture Forestry & Fisheries. Bovine Brucellosis Manual. Pretoria: DAFF, 2016.
7. Department of Agriculture Forestry & Fisheries. South African Veterinary Strategy (2016-2026). Pretoria: DAFF, 2016.
8. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: a re-emerging zoonosis. *Vet Microbiol* 2010; 140 (3): 392-8.
9. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. *Lancet infect Dis* 2006; 6 (2): 91-9.
10. Taleski V, Zerva L, Kantardjiev T, et al. An overview of the epidemiology and epizootology of brucellosis in selected countries of Central and Southeast Europe. *Vet Microbiol* 2002, 90, 147-55.
11. Schrire L. Human brucellosis in South Africa. *S Afr Med J* 1962; 36 (5), 342-8.
12. Küstner HGV. Brucellosis in the eastern Orange Free State. Epidemiological Comments 1985; 12(3): 2-23.
13. Anonymous. Case of brucellosis: laboratory diagnosis and safety. *NICD Communicable Diseases Communique* 2015; 14 (8): 6-7.
14. Anonymous. Brucellosis in a child in the Western Cape Province. *NICD Communicable Diseases Communique* 2016; 15(6): 3.
15. Anonymous. Brucellosis: case report and request for increased clinician awareness. *NICD Communicable Diseases Communique* 2016; 15(9): 3.
16. Anonymous. A case of human brucellosis in Mpumalanga Province. *NICD Communicable Diseases Communique* 2016; 15 (11): 3.
17. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis: a review of the literature. Part I: techniques for direct detection and identification of Brucella spp. *Clin Lab* 2003; 49(9-10); 487-505.
18. Cetin ES, Kaya S, Demirci M, Aridogan BC. Comparison of the BACTEC blood culture system versus conventional methods for culture of normally sterile body fluids. *Adv Ther* 2007; 24(6): 1271-7.
19. Al Dahouk S, Sprague LD, Neubauer H. New developments in the diagnostic procedures for zoonotic brucellosis in humans. *Rev Sci Tech* 2013; 32(1): 177-88.

20. Ferreira L, Vega Castaño S, Sánchez-Juanes F, et al. Identification of *Brucella* by MALDI-TOF mass spectrometry. Fast and reliable identification from agar plates and blood cultures. *PLoS ONE* 2010; 5(12); 14235.
21. Lista F, Reubsaet FA, De Santis R, et al. Reliable identification at the species level of *Brucella* isolates with MALDI-TOF-MS. *BMC Microbiol* 2011; 11, 267.
22. Bosilkovski M. Clinical manifestations, diagnosis, and treatment of brucellosis. UpToDate 2015. Available at: www.uptodate.com
23. Heymann DL (ed). Control of Communicable Diseases Manual. 18th ed. Washington: American Public Health Association, 2004.
24. Traxler RM, Guerra MA, Morrow MG, et al. Review of brucellosis cases from laboratory exposures in the United States in 2008 to 2011 and improved strategies for disease prevention. *J Clin Microbiol* 2013; 51: 3132-6.
25. Wojno JM, Moodley C, Pienaar J, et al. Human brucellosis in South Africa: Public health and diagnostic pitfalls. *S Afr Med J* 2016; 106(9): 883-5.
26. Centers for Disease Control and Prevention, 2017. Exposure to RB51: How to reduce risk of infection. Available at: http:// www.cdc.gov/brucellosis/veterinarians/rb51-reduce-risk.html (accessed 26 September 2018).

Addendum. Case definitions for notification of human brucellosis

Brucellosis (*Brucella* spp.)

Clinical Description

An illness characterized by acute or insidious onset of fever and one or more of the following: night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly).

Laboratory Criteria for Diagnosis

Definitive:

- Culture and identification of *Brucella* spp. from clinical specimens
- Evidence of a fourfold or greater rise in *Brucella* antibody titer between acute- and convalescent-phase serum specimens obtained greater than or equal to 2 weeks apart

Presumptive:

- *Brucella* total antibody titer of greater than or equal to 160 by standard tube agglutination test (SAT) or *Brucella* microagglutination test (BMAT) in one or more serum specimens obtained after onset of symptoms
- Detection of *Brucella* DNA in a clinical specimen by PCR assay

Case Classification

Probable:

A clinically compatible illness with at least one of the following:

Epidemiologically linked to a confirmed human or animal brucellosis case

Presumptive laboratory evidence, but without definitive laboratory evidence, of *Brucella* infection

Confirmed:

A clinically compatible illness with definitive laboratory evidence of *Brucella* infection

TYPHOID FEVER OUTBREAK INVESTIGATION IN SEKHUKHUNE DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA, NOVEMBER 2017 TO JANUARY 2018

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Executive Summary

Typhoid fever remains an endemic disease in South Africa, and the risk of transmission is associated with poor sanitation, unsafe water, and unsafe food production and handling processes. In South Africa, laboratory-based surveillance for typhoid fever is co-ordinated by the National Institute for Communicable Diseases (NICD). Fewer than 130 cases were reported annually since 2012, indicating low endemicity. The findings of a typhoid outbreak investigation in Sekhukhune District, Limpopo Province, from November 2017 to January 2018 are reported here.

An investigation was conducted to quantify the outbreak, identify the source, and make recommendations to stop the outbreak and prevent future events. Epidemiologic, laboratory and environmental investigations were conducted. *Salmonella enterica* subspecies *enterica* serotype Typhi (*S*. Typhi) isolates were submitted to the Centre for Enteric Diseases (CED), NICD, and whole genome sequencing (WGS) was conducted by the Sequencing Core Facility, NICD. Polymerase chain reaction (PCR) testing for *Salmonella* species in water samples was conducted by the Council for Scientific and Industrial Research (CSIR). Location and distribution of cases and water sources were mapped using a geographic information system (GIS) mapping tool. Amongst 122 cases with a median age of 11 years (interquartile range, 2 to 83 years), 66/122 (54%) were female and 7/122 (6%) were laboratory confirmed. *Salmonella* species were detected in 10/27 (37%) water samples collected in the district. GIS mapping showed clustering of cases in Tswaing-Kgwaripe and Vlakplaas villages, with 58% laboratory-confirmed cases and 68% of probable cases from the former. WGS results indicated that isolates for cases from Tswaing-Kgwaripe and Strydkraal villages (5/6) were genetically highly related (<22 single nucleotide polymorphisms).

The molecular epidemiology of available isolates suggests a common source outbreak, supported by the detection of *Salmonella* spp. in samples from multiple water sources in the affected district. Access to safe and clean water, an inter-

sectoral health, water and sanitation committee, and continuous community health education were recommended.

Introduction

Typhoid fever is a systemic bacterial illness caused by *Salmonella enterica* subspecies *enterica* serotype Typhi (*S.* Typhi),[1] characterised by prolonged fever, nausea, headache, loss of appetite and gastrointestinal symptoms, including abdominal pain, constipation or diarrhoea.[2,3] Severe disease can lead to life-threatening complications including intestinal perforation, intestinal haemorrhage and encephalopathy with haemodynamic shock. The incubation period ranges from 3 to 60 days, but is usually 7 to 14 days.[2] *S.* Typhi is transmitted through ingestion of food or water contaminated by faeces or urine of symptomatic cases and asymptomatic carriers.[2,4]

Humans are the only reservoir and a carrier state may follow acute illness or subclinical infection.[2] Typhoid fever occurs mostly in areas with poor sanitation and lack of potable water. According to the most recent World Health Organization estimates (published in 2014), approximately 21 million cases and 222 000 typhoid-related deaths occur annually worldwide.[4] *Salmonella* Paratyphi A, B or C cause paratyphoid fever, which is clinically indistinguishable from typhoid fever. Typhoid fever and paratyphoid fever are collectively termed enteric fever.

As typhoid fever is clinically indistinguishable from a wide range of other common febrile illnesses, laboratory testing is recommended in all patients presenting with clinically-compatible disease. The diagnosis of typhoid fever requires the isolation of *S.* Typhi from blood, bone marrow, stool or other tissue specimens. Serological tests such as the Widal test are not recommended for screening or diagnosis of typhoid fever due to variable host antibody responses and cross-reactivity with other enteric bacteria.

Typhoid fever is endemic in South Africa. However, the number of cases has declined over the last 22 years from an estimated 6 000 in 1985 to 200 cases in 2002.[7] Large outbreaks of typhoid fever occurred in Delmas, Mpumalanga Province in 1993 and 2005, when over 600 cases were reported.[5-6] Low numbers of cases continued to be observed between 2013 and 2015 (Figure 1) through a national, active laboratory-based surveillance system at the National Institute for Communicable Diseases (NICD).[7]

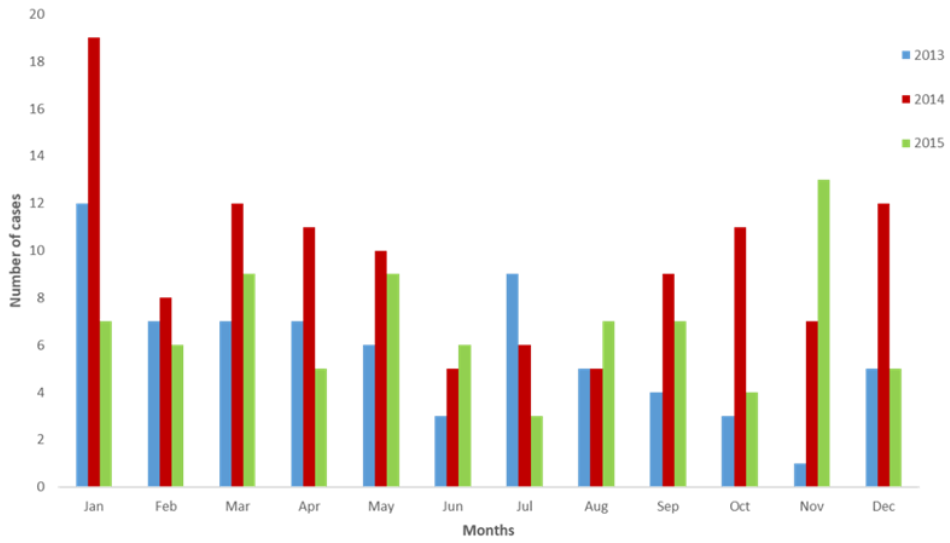

Figure 1. Numbers of laboratory-confirmed *Salmonella* Typhi cases by month in South Africa, 2013 – 2015.

In 2016, case numbers were comparable with previous non-outbreak years, with 123 cases recorded. Molecular epidemiological analysis of cases revealed both endemic strains and strains with identical patterns to those responsible for the typhoid outbreak in Zimbabwe during 2010.[8]

While 83% of South African households have water provided by municipal structures, 43% of households in Limpopo Province experienced water interruptions of any duration during 2016.[9] Sekhukhune District, approximately 150 km from Polokwane city in Limpopo Province, consists of four local sub-district municipalities (Figure 2). The district lies in the south-eastern part of the province and it is mostly rural, with almost 605 villages and an estimated 5% of the population living in urban areas. Potable water supply by the municipality is inconsistent. Residents in villages rely on private boreholes and untreated drinking water reservoirs including ground wells, rivers, irrigation furrows, boreholes, rainwater, and large plastic storage (Jojo) tanks.

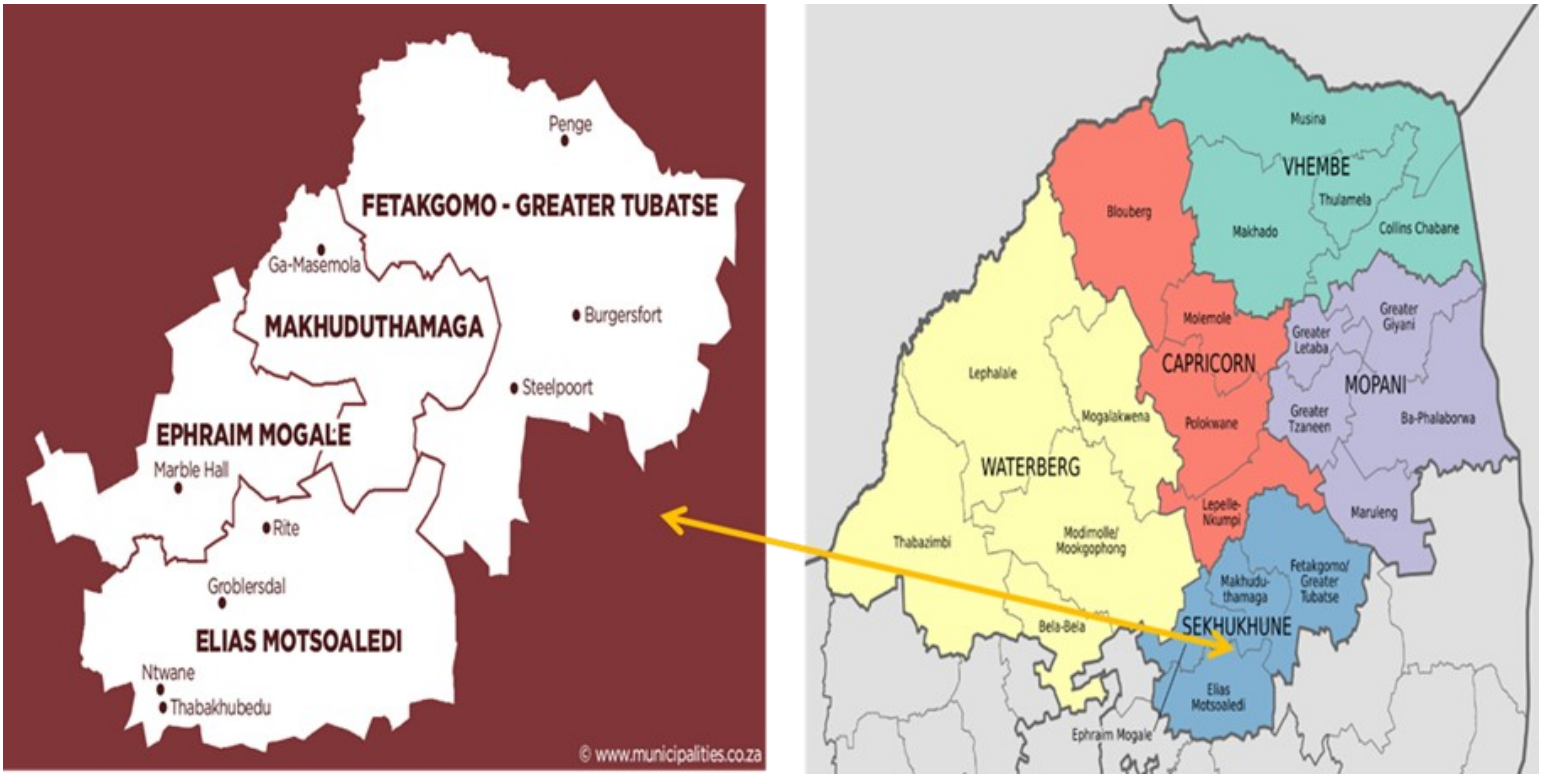

Figure 2. Right: Limpopo Province, South Africa, showing location of Sekhukhune District. Left: Sekhukhune District showing sub-districts.

On 15 November 2017, Sekhukhune District Department of Health (DoH) received a notification about a laboratory-confirmed case of typhoid fever initially admitted to Pietersburg Tertiary Hospital, then transferred back to the district hospital. The Outbreak Response Unit (ORU) at NICD was alerted of a suspected typhoid fever outbreak in Limpopo Province on 22 November 2017. Collaborative actions and investigations to support response interventions were commenced. The findings from an investigation of a typhoid outbreak in Sekhukhune District, Limpopo Province, from November 2017 to January 2018 are here described.

Methods

Investigating and response teams

Following the notification, the district epidemic preparedness and response (EPR) team was activated and met to co-ordinate response activities. The district EPR team consists of the Sekhukhune Provincial and District Communicable Disease Control team (Department of Health), Deputy Director Health Promotion, hospital environmental health practitioners (EHPs), Provincial Field Epidemiologist and sub-district mobile teams. On two occasions (25 January 2018, and 31 January - 2 February 2018), a team of medical epidemiologists, field epidemiology training programme resident, public health registrars, and a geographic information system GIS specialist from the Outbreak Response Unit, NICD conducted site visits to provide feedback on investigations, training of health practitioners, and to conduct further investigations.

Case definitions

The following case definitions were formulated from the national typhoid guidelines:[3]

- A confirmed typhoid case: any person from Sekhukhune District with isolation of *S.* Typhi, or *S.* Paratyphi A, B or C from a clinical specimen in the presence of symptoms compatible with enteric fever from 6 November 2017 to January 2018.
- A probable typhoid case: any person from Sekhukhune District with symptoms compatible with enteric fever who is epidemiologically linked to a confirmed case, from 6 November 2017 to January 2018, with negative malaria rapid test to exclude malaria.
- A typhoid carrier (convalescent or chronic carrier):
    - ◊ A convalescent carrier: any person in Sekhukhune District who is still excreting *S.* Typhi or *S.* Paratyphi A,B or C after receiving two courses of appropriate antibiotic therapy.
    - ◊ A chronic carrier: any person in Sekhukhune District who continues to excrete *S.* Typhi or *S.* Paratyphi 12 months after receiving appropriate antibiotic therapy.

Epidemiological and clinical investigations

A retrospective descriptive cross-sectional study was conducted using data collected from case-patient interviews.

Case finding and sources of data

Attending clinicians at district hospitals identified and investigated persons with suspected typhoid fever based on the case definitions above. The provincial epidemiologist together with the Communicable Disease Control team coordinated the investigation of cases and completion of case investigation forms (CIFs) by the three district hospitals in Sekhukhune District where case-patients were admitted. Limpopo Province is a malaria-endemic area, therefore the clinical investigation of typhoid cases presenting with fever included a malaria rapid diagnostic test to exclude malaria. The CIF used to interview case-patients and contacts focused on respondent demographics, clinical presentation, and potential exposure risk factors such as water sources and type of toilet use. A line list to record all cases was developed. Additional patient data were obtained through the National Health Laboratory Service (NHLS) TrakCare Web results viewer system. Health facilities and mobile teams were responsible for daily reporting to Sekhukhune DoH, public health unit.

Laboratory investigations

Attending clinicians at the three district hospitals where patients presented, submitted clinical specimens for laboratory investigations including full blood count, urea and creatinine, blood and stool or rectal swabs and blood for serology (Widal test)

to the NHLS. The Widal test is not recommended for the diagnosis of typhoid fever; however, due to the unavailability of blood culture bottles in health facilities, serological testing was requested. In cases where *S*. Typhi was identified, the health authorities were notified by the NHLS. The NHLS submitted *S*. Typhi isolates to the Centre for Enteric Diseases (CED), NICD, for confirmatory testing and whole-genome sequencing (WGS). WGS is a widely-used technique for molecular subtyping of bacteria, providing data that enable high-resolution typing for surveillance, and additional data regarding further characterization of emerging clones based on genetic differences.[10]

Environmental investigations

Water samples were collected by the EHPs from several water sources identified through interviews with cases and at community meetings. Food samples were not available for collection. Water samples were sent to the Council for Scientific and Industrial Research (CSIR) laboratory, where a real-time PCR test was performed to detect the presence of *Salmonella* spp. The PCR-based test used by CSIR detects all *Salmonella enterica* subspecies, as well as *Salmonella bongori,* by targeting the *invA* gene that is located on the pathogenicity island 1 of *Salmonella* spp. The methodology is not able to distinguish between typhoidal and non-typhoidal *Salmonella* species.[11-13]

GIS mapping

During the NICD team visits, additional data were collected, including interviews of cases and contacts to complete CIFs, and GIS locations of case-patients' places of residence and open water sources. A GIS tool is a computer system with the capacity to capture, store, analyse and display geographically-referenced information.[14] GIS provides ways of visualizing and analysing epidemiologic data, and helps in identifying disease trends and multi-disease surveillance activities.[14-15] Health organisations can visualize, analyse and interpret geo-location data through the use of GIS tools or mapping applications. Specific diseases and other public health events can be mapped to monitor and manage of epidemics[15]. The GIS mapping was limited to villages with typhoid fever cases and water sources that were sampled for typhoid investigation purposes within Sekhukhune District.

The case-patients' home addresses were obtained from completed CIFs, Tswaing local clinic daily register for patients and the ward-based outreach team (WBOT), who work in collaboration with the clinic. The location of water sources sampled (which included taps, boreholes, furrows and wells) was obtained from the CSIR report. The trained NICD team together with the GIS specialist used mobile phones to determine spatial distribution of cases and water sources. The Collector for ArcGIS (South Africa, Midrand) mapping tool was used for field data collection. Information such as patient demographics and Global Positioning System (GPS) coordinates were collected. We used colour-coded points to indicate cases (confirmed and probable) and water sources tested for *Salmonella* spp.

Data processing and analysis

Data on CIFs were captured into Microsoft (MS) Excel 2016 (Microsoft Corporation, Redmond, Washington, USA). Cleaning of data was done using MS Excel by checking for duplicate records, missing data, etc. Data was analysed using STATA version 15 (StataCorp LP, College Station, Texas, USA) and descriptive statistics (mean, range and percentages) were used. Geographic maps were prepared using Collector for ArcGIS.

Results

Epidemiological and clinical findings

The first laboratory-confirmed case of typhoid fever was reported on 15 November 2017. The patient's initial consultation was the district hospital, and was later transferred to Pietersburg Tertiary Hospital. As of 31 January 2018, 122 confirmed and suspected cases were reported, of which 6% (7/122) were confirmed by blood culture (Tables 1 and 2, Figure 3). The median age of confirmed and suspected was 11 years (IQR, 2 - 83 years). Children of school-going age were mostly affected (64%, 78/122). The most common presenting symptoms were fever, diarrhoea and abdominal cramps/pain (Figure 4). Two local municipalities were affected (Makhudumathaga and Tubatse-Fetakgomo) with the majority of cases from Tswaing (67%, 82/122), Vlakplaas (10%, 12/122) and Strydkraal (9%, 11/122) (Table 2). Figure 5 shows clusters of laboratory-confirmed cases (58%, 4/7) in Tswaing, and clusters of probable cases in Vlakplaas and Strydkraal. Case-patients were treated with intravenous ceftriaxone and/or oral ciprofloxacin according to South African national guidelines.[3] Table 1 outlines the demographic and clinical characteristics of laboratory-confirmed cases and Figure 3 illustrates the number of cases by date of symptom onset.

Table 1. Demographic and clinical characteristics of confirmed typhoid cases, Sekhukhune District, Limpopo Province, South Africa, 6 November to 31 December 2017 (n=7).

Case no.	Age (yrs.)	Sex	Place of residence	Clinical presentation	Specimen types	Date of diagnosis	Type of water source
1	23	F	Strydkraal	Diarrhoea, malaise	Blood culture	15/11/2017	Stream
2	4	F	Tswaing	Fever, diarrhoea, painful legs	Blood culture	22/11/2017	Furrow
3	19	M	Apel Cross	Vomiting, diarrhoea, malaise	Stool, blood culture	23/11/2017	Communal tap
4	9	F	Tswaing	Fever, diarrhoea	Rectal swab, blood culture	23/11/2017	Stream
5	12	F	Tswaing	Fever, abdominal cramps, painful legs	Stool, rectal swab, blood culture	23/11/2017	Furrow
6	15	M	Tswaing	Diarrhoea, malaise, respiratory symptoms, abdominal cramps	Rectal swab, blood culture	23/11/2017	Furrow
7	71	F	Ga-Phaahla (Mamatsekele)	Not known	Stool, blood culture	24/11/2017	Well

Table 2. Typhoid cases characterised by age, gender, location and water sources, Sekhukhune District, Limpopo Province, South Africa, 6 November to 31 December 2017 (n=122).

Variable	Total cases (N=122) n/(%)	Suspected (N=115) n/(%)	Confirmed (N= 7) n/(%)
Age group in years			
≤4	14 (11)	13 (11)	1 (14)
5 - 14	78 (64)	76 (66)	2 (29)
15- 49	25 (21)	22 (19)	3 (43)
≥ 50	5 (4)	4 (4)	1 (14)
Sex			
Female	66 (54)	62 (54)	5 (71)
Male	56 (46)	53 (46)	2 (29)
Villages			
Apel cross	2 (2)	1 (1)	1 (14)
Ga-Masemola	6 (5)	6 (5)	0 (0)
Ga-Phaahla	1 (1)	0 (0)	1 (14)
Tswaing	82 (67)	78 (68)	4 (58)
Vlakplaas	12 (10)	12 (10)	0 (0)
Strydkraal	11 (9)	10 (9)	1 (14)
Other	8 (6)	8 (7)	0 (0)
Water Sources			
Stream	9 (7)	7 (6)	2 (29)
Furrow	70 (57)	67 (58)	3 (43)
Jojo tank	1 (1)	1 (1)	0 (0)
Borehole	2 (2)	2 (2)	0 (0)
Communal taps	2 (2)	1 (1)	1 (14)
Well	1 (1)	0 (0)	1 (14)
Unknown	37 (30)	37 (32)	0 (0)
Type of toilet			
Pit latrine	110 (90)	103 (90)	7 (100)
Unknown	22 (10)	22 (10)	0 (0)

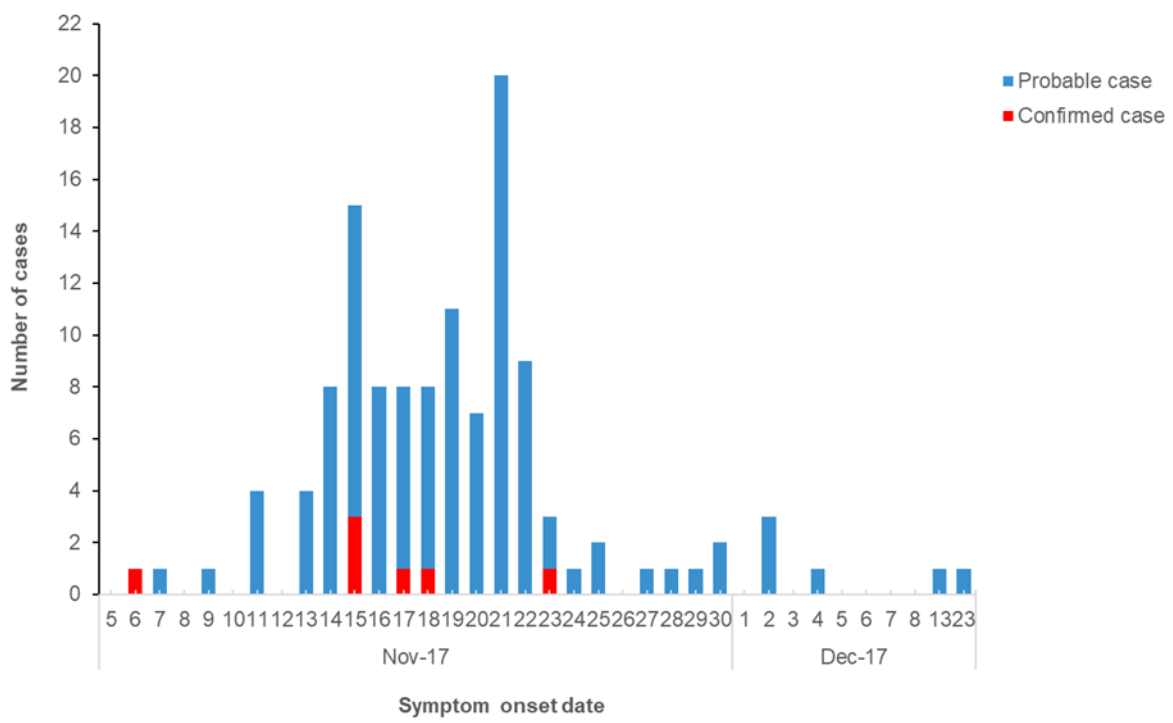

Figure 3. Epidemic curve showing distribution of typhoid cases by date of onset of symptoms, Sekhukhune District, Limpopo Province, South Africa, 6 November to 31 December 2017 (n=122).

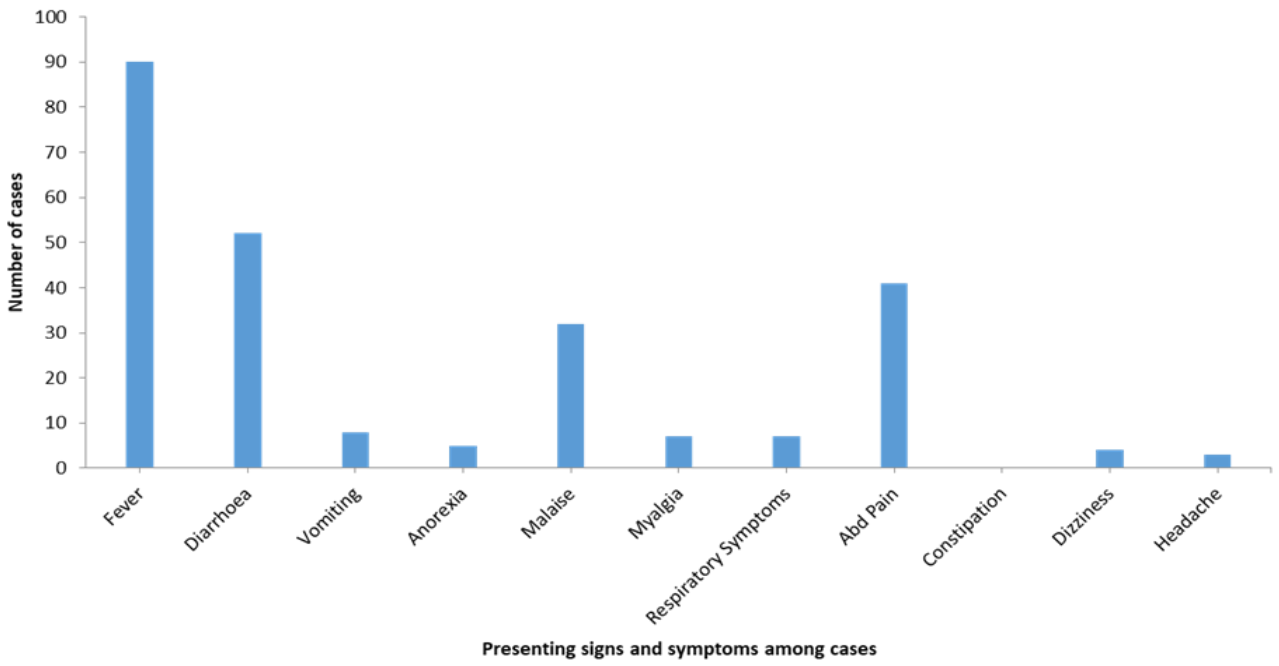

Figure 4. Presenting signs and symptoms among typhoid cases, Sekhukhune District, Limpopo Province, South Africa, 6 November to 31 December 2017.

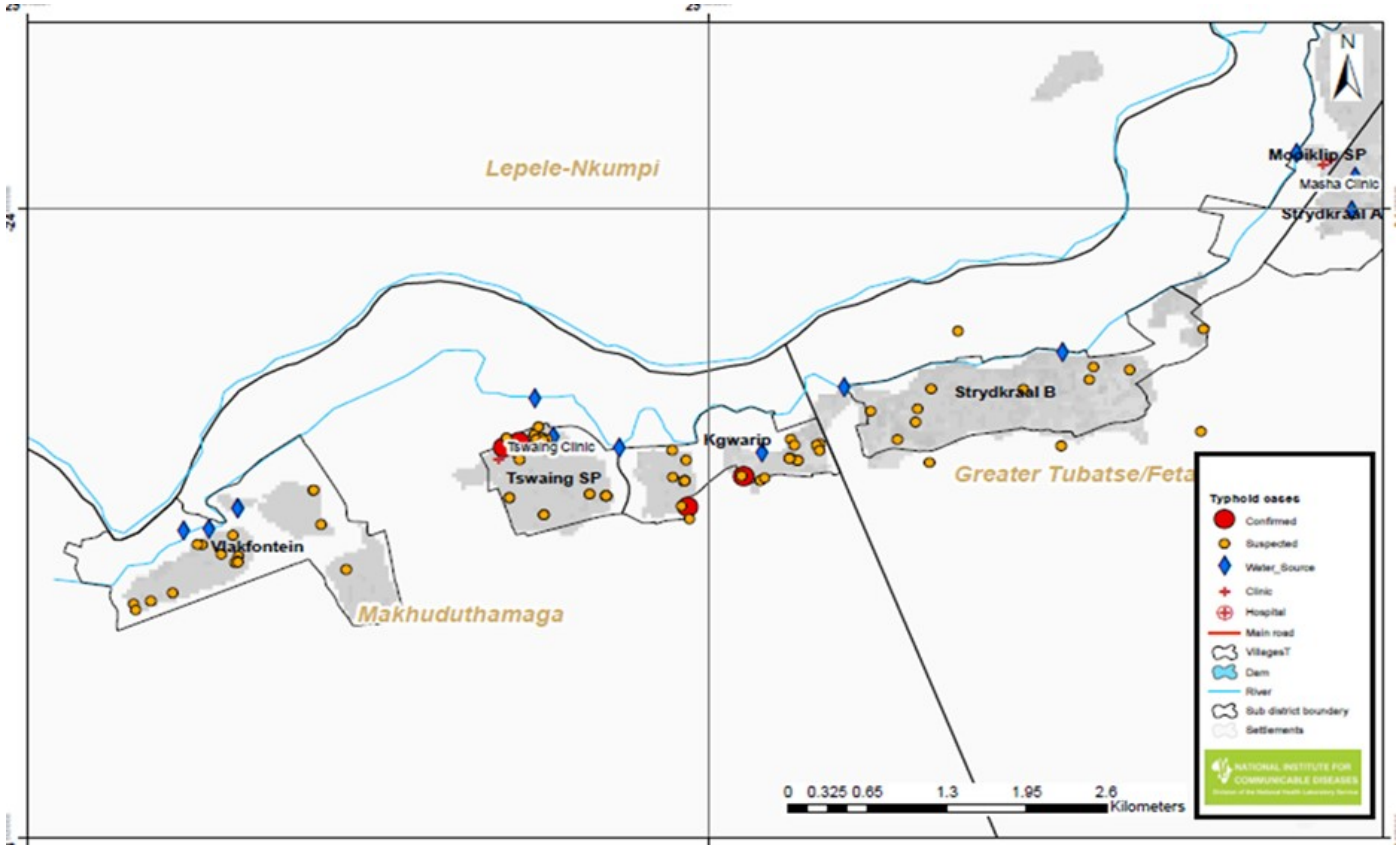

Figure 5. Geographic distribution of typhoid fever cases and water sources used, Sekhukhune District, Limpopo Province, South Africa, 6 November to 31 December 2017.

Laboratory findings

The NHLS received specimens from 106 suspected typhoid fever cases under investigation. This included blood cultures (65/106), stool cultures (6/106), rectal swabs for stool culture (3/106) and serology (Widal test, 32/106). Of the 122 cases reported, 16 (13%) had no record of specimens submitted for culture. For 16 probable cases, it could not be ascertained whether any specimens were collected. Amongst laboratory-confirmed cases, all were positive for *S*. Typhi on blood culture, while five also had stool or rectal swabs culture-positive for *S*. Typhi. WGS results indicated that five of six isolates obtained from cases resident in Tswaing and Strydkraal were genetically highly related (<22 single nucleotide polymorphism) (Figure 6). A single, un-clustered strain was closely related to the 2016 Zimbabwe typhoid outbreak isolates.

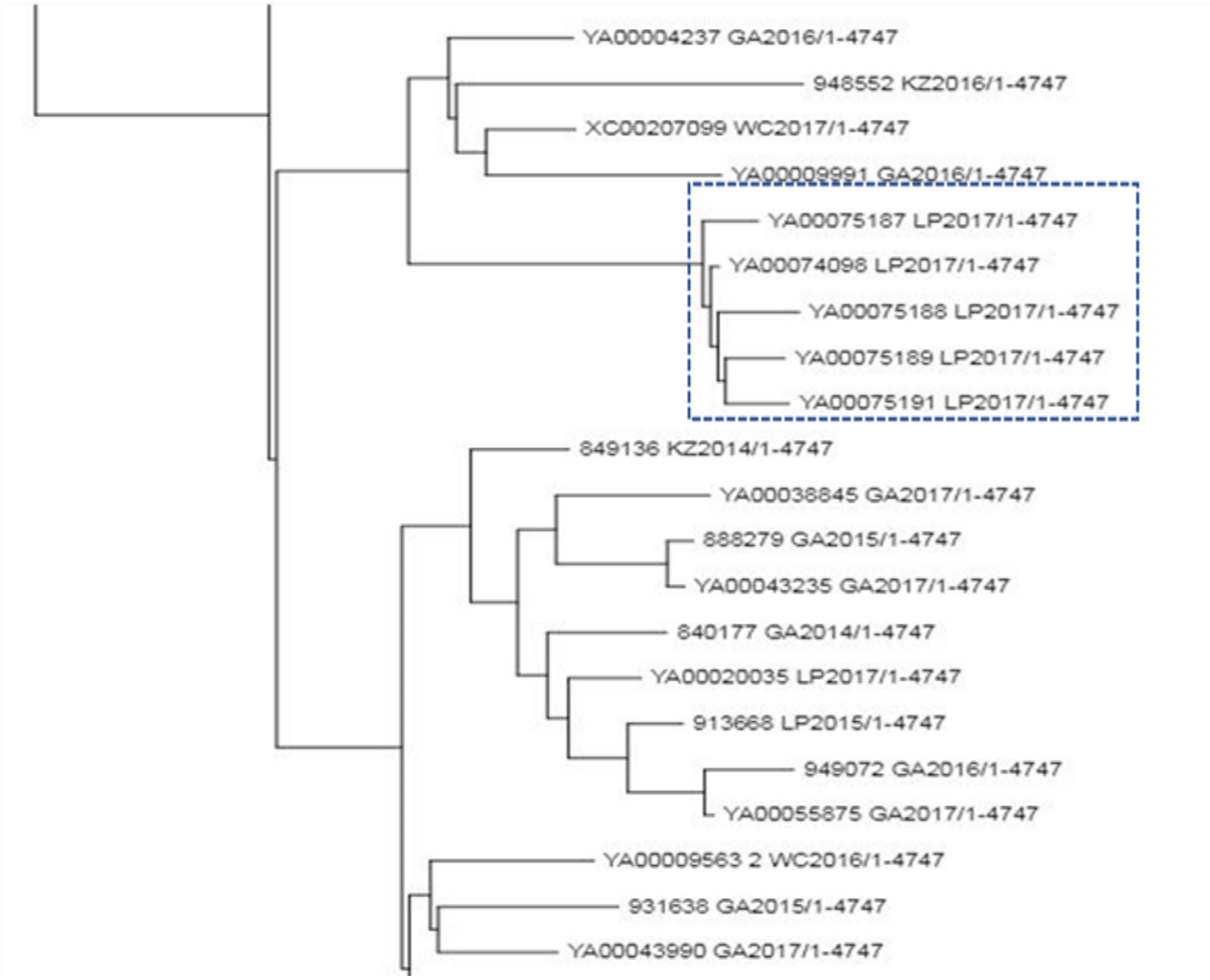

Figure 6. Molecular characterization of *Salmonella enterica* subspecies *enterica* serotype Typhi clinical isolates among confirmed cases, showing a cluster of related isolates, Sekhukhune District, Limpopo Province, South Africa, November to January 2018. The figure shows a snapshot from a maximum likelihood phylogenetic tree drawn using SNP alignments from WGS data of isolates.

Environmental findings

In Sekhukhune District, approximately 65% of cases reported the use of water from untreated open water sources such the furrows, streams and wells due to lack of access to clean water. The furrow that ran parallel to the river and adjacent to villages where cases were clustered was intended for crop irrigation and for the use of domestic animals. Amongst 27 water samples, 37% (10/27) were positive for *Salmonella* spp. on PCR. Of the 27 water sources tested for *Salmonella* spp., 44% (12/27) were linked with villages where case-patients resided. *Salmonella* spp. was identified in 83% (10/12) of the water sources linked with the outbreak villages (Table 3). Of the seven confirmed cases, 86% (6/7) reported obtaining water from the untreated open water sources. Although one confirmed case from Apel Cross (not shown in Figure 6) reported using water from the communal tap, he was a learner in one of the schools located in Tswaing.

Interventions implemented

The Sekhukhune District EPR team was activated on 20 November 2017, and an intervention plan was developed. The district municipality was informed of the detection of *Salmonella* spp. in water samples, and subsequently treated water was distributed by water tankers in the affected villages. In addition, a water treatment plant that was previously erected in Vlakplaas was repaired so that piped potable water provision could resume. The municipality district office procured bleach and distributed it in all affected areas. Environmental health practitioners (EHPs) conducted home visits to monitor sanitation, hygiene and food and water safety in the households.

Multiple community outreach activities, including door-to-door home visits, school visits and outreach through media platforms, were conducted by health officials and community health workers. These included education on water and foodborne illnesses. Community members with symptoms compatible with typhoid fever were encouraged to seek medical care at health facilities.

From 21 November to 31 December 2017, mobile clinic teams conducted contact tracing. All contacts with symptoms compatible with typhoid fever were referred to the nearest healthcare facility for investigation. Clinical samples were only collected from symptomatic contacts. Awareness campaigns about waterborne diseases, the prevention measures and when to seek medical attention was facilitated by ward councillors in the communities.

Training of health professionals and community health workers was conducted at the three district hospitals where patients presented for care. Regular EPR meetings were held for discussions and follow up of executed plans.

Discussion

Based on these clinical, epidemiological and molecular findings, we hypothesise that the typhoid outbreak in Sekhukhune District was caused by contaminated water. This outbreak illustrates the importance of thorough investigation of suspected waterborne illness and the need for uninterrupted supplies of clean, safe water.

Unregulated, untreated open water sources are major contributors to typhoid outbreaks. The association of typhoid outbreaks with contaminated water has been observed in other sub-Saharan African countries including Zambia, Malawi, Uganda and Zimbabwe.[16 - 19] In Zimbabwe, a large typhoid outbreak in Harare in 2011 with over 4 000 cases was associated with drinking water from a well (AOR=5.8; 95% CI 1.9-17.78) and a burst sewage pipe (AOR=1.2, 95% CI 1.10-2.19).[16] The outbreaks of typhoid fever in Delmas, Mpumalanga Province, were associated with unregulated contaminated water supplies.[5-6] A number of recent typhoid cases that occurred in South Africa were associated with imported S. *Typhi*,[7] including strains similar to those

identified in the 2010 Harare outbreak. Our case-patients' isolates were not similar to these strains. Only a single strain, from GaPhaahla, was related to the 2016 Zimbabwe typhoid outbreak strains. However, this case-patient did not live in the villages of interest (Figure 6). Although this case-patient did not report a travel history, healthcare workers did not explore travel history of his/her contacts.

The Constitution of the Republic of South Africa, the National Development Plan (NDP) and the Sustainable Development Goals (SDGs) introduced in 2016 all emphasise that access to sufficient water and adequate sanitation is essential to preserve public health. In Limpopo Province, only 79% of persons have access to piped water, compared with the national average of 89%.[9] Further, 79% of Limpopo households that experienced water interruptions in the previous three months of a survey conducted in 2016 reported that these that lasted longer than two consecutive days.[9] This outbreak illustrates the health consequences of the interruption of safe water supplies, when residents are forced to use other available water sources, which may not be treated, or safe.

This outbreak highlighted challenges in the diagnosis of typhoid cases. The gold standard of typhoid fever diagnosis is culture of the organism from a clinical specimen, preferably blood or bone marrow.[3] A shortage of blood culture bottles at district hospitals within Sekhukhune District meant that initial cases were not identified until they were referred to a tertiary hospital where blood cultures were performed. As an interim measure, clinicians resorted to the Widal test, which is not recommended as a diagnostic test, due to variable host antibody responses and cross-reactivity with other enteric bacteria. When blood culture bottles were made available at the district hospitals, most suspected cases had already received antibiotic treatment. This may have accounted for the low rate of culture-based confirmation amongst case-patients.

Regarding case management, South African national typhoid fever guidelines advise that all typhoid fever cases should have three follow-up stool specimens to confirm clearance of the organism. The stool sample should be collected one week after completion of antibiotics, and two subsequent samples should be collected 48 hours apart. If all follow up stool samples are negative, the case can be released from surveillance.[3] Further, guidelines recommend that contacts of cases submit stool specimens for culture to exclude asymptomatic carriage. These recommendations were not adhered to in the Sekhukhune typhoid outbreak, so there remains a risk of transmission by typhoid carriers.

A number of recommendations were made to provincial and local authorities, including:

- The formation of an inter-sectoral health, water and sanitation committee to facilitate communication between departments and to ensure that challenges pertaining to access to clean water and sanitation are timeously addressed.
- Purification and chlorination of public water supplies should be monitored by the by inter-sectoral health, water and sanitation committee. Chlorination may minimise or limit contamination when possible backflow connections between potable water and open water sources occur.[2]
- Continuous community health education and awareness regarding the importance of handwashing, correct disposal of human faeces, and maintenance of fly-proof latrines should take place.
- Case management should include contact tracing with investigation for asymptomatic carriage, documentation of eradication of carriage, and restrictions on food handling practices amongst laboratory-confirmed cases until at least three consecutive negative stool cultures have been taken.[3]
- Health professionals to complete CIFs to ensure that quality data is collected.

Conclusion

This outbreak investigation suggests that contamination of open water sources and an interruption of municipal water supply led to an outbreak. The investigation highlights the importance of the provision of safe water and sanitation, and highlights the ability of district surveillance systems to identify and contain outbreaks.

Acknowledgements

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References

1. Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. *Salmonella* nomenclature. Journal of Clinical Microbiology. 2000; 38(7):2465-7.
2. Heymann, DL. Control of Communicable Diseases Manual. American Public Health Association; 2008.
3. National Institute for Communicable Disease. Typhoid: recommendations for diagnosis, management and public health response. 2016 [accessed 22 January 2018]. Available from: http://www.nicd.ac.za/assets/files/Guidelines_typhoid_20160125.pdf
4. World Health Organization. Typhoid [accessed 18 February 2018]. Available from: http://www.who.int/immunization/diseases/typhoid/en/
5. Waner S, Kfir R, Idema GK, Coetzee DJ, Rasmussen K, Koornhof HJ, Klugman KP. Waterborne outbreak of typhoid fever in Delmas. Southern African Journal of Epidemiology and Infection. 1998; 13:53-7.
6. Keddy KH, Sooka A, Ismail H, Smith AM, Weber I, Letsoalo ME, Harris BN. Molecular epidemiological investigation of a typhoid fever outbreak in South Africa, 2005: the relationship to a previous epidemic in 1993. Epidemiology & Infection. 2011;139(8):1239-45.
7. National Institute for Communicable Disease. Typhoid fever cases in South Africa and Gauteng Province, 2016 [accessed 22 January 2018]. Available from: https://pmg.org.za/files/160309Typhoid.pptx
8. National Institute for Communicable Disease. GERMS South Africa: Annual report. 2016 [accessed 220 April 2018]. Available from: http://www.nicd.ac.za/wp-content/uploads/2017/03/GERMS-SA-AR-2016-FINAL.pdf
9. Stats SA. The state of basic service delivery in South Africa: In-depth analysis of the Community Survey 2016 data. Report; 2016.
10. Gupta R, Shriram R. Disease surveillance and monitoring using GIS. In: 7th Annual International Conference, India, 2004.
11. Musa GJ, Chiang PH, Sylk T, Bavley R, Keating W, Lakew B, Tsou HC, Hoven CW. Use of GIS mapping as a public health tool–-from cholera to cancer. Health Services Insights. 2013;6: HSI-S10471.
12. Gymoese P, Sørensen G, Litrup E, Olsen JE, Nielsen EM, Torpdahl M. Investigation of outbreaks of *Salmonella enterica* serovar Typhimurium and its monophasic variants using whole-genome sequencing, Denmark. Emerging Infectious Diseases. 2017;23(10):1631.
13. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. Applied and Environmental Microbiology. 2003;69(1):290-6.
14. Kumar S, Balakrishna K, Batra HV. Detection of *Salmonella* enterica serovar Typhi (S. Typhi) by selective amplification of *invA, viaB, fliC-d* and *prt* genes by polymerase chain reaction in mutiplex format. Letters in Applied Microbiology. 2006;42 (2):149-54.

15. Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella* enterica serovars differ. Frontiers in Microbiology. 2014; 5:391.
16. Muti M, Gombe N, Tshimanga M, Takundwa L, Bangure D, Mungofa S, Chonzi P. Typhoid outbreak investigation in Dzivaresekwa, suburb of Harare City, Zimbabwe, 2011. Pan African Medical Journal. 2014;18(1).
17. Murphy JL, Kahler AM, Nansubuga I, Nanyunja EM, Kaplan B, Jothikumar N, Routh J, Gómez GA, Mintz ED, Hill VR. Environmental survey of drinking water sources in Kampala, Uganda, during a typhoid fever outbreak. Applied and Environmental Microbiology. 2017;83(23): e01706-17.
18. Kariuki S. Typhoid fever in sub-Saharan Africa: challenges of diagnosis and management of infections. The Journal of Infection in Developing Countries. 2008;2(06):443-7.
19. Feasey NA, Archer BN, Heyderman RS, Sooka A, Dennis B, Gordon MA, Keddy KH. Typhoid fever and invasive nontyphoid salmonellosis, Malawi and South Africa. Emerging Infectious Diseases. 2010;16(9):1448.

NEISSERIA GONORRHOEAE ANTIMICROBIAL RESISTANCE SURVEILLANCE: NICD GERMS-SA 2017

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Executive summary

Gonorrhoea, a sexually transmitted infection, is associated with increased HIV transmission and reproductive health complications if left untreated. The causative agent, *Neisseria gonorrhoeae*, has acquired resistance to all sequential first-line antimicrobial agents. *Neisseria gonorrhoeae* resistance profiles from the National Institute for Communicable Diseases (NICD) GERMS-SA sentinel surveillance, conducted in 2017 in four of South Africa's provinces, show that the currently-recommended dual therapeutic regimen of ceftriaxone and azithromycin for gonorrhoea is appropriate for use in STI syndromic management guidelines for the largely heterosexual populations accessing primary healthcare services. Ceftriaxone-resistant gonorrhoea was listed as a notifiable medical condition in December 2017. Cefixime-resistant urogenital gonorrhoea has previously been detected in men-who-have-sex-with-men (MSM) presenting to general practitioners in Gauteng, Cape Town and the Eastern Cape. Based on local and global epidemiology, enhanced national culture-based surveillance, particularly in key populations such as MSM, is essential to detect the emergence of extensively-drug resistant (XDR) gonorrhoea that has acquired resistance to the extended-spectrum cephalosporins.

Introduction

Gonorrhoea is a sexually transmitted infection that is of major public health concern worldwide. There is a high transmission efficiency, and infection is associated with a fivefold increase in HIV transmission and complications such as pelvic inflammatory disease and infertility, which compound the global health burden.[1] In 2017, the South African general population prevalence and incidence estimates for gonorrhoea in males was 3.5 % (1.7 – 6.1%) corresponding to 2.2 (1.1 – 3.8) million cases, respectively.[2] In females, in whom the average duration of infection is longer, the prevalence estimate was higher at 6.6% and incidence was estimated at 2.3 million cases.

Neisseria gonorrhoeae, an obligate human pathogen, has acquired resistance, through genetic mechanisms (both chromosomal and plasmid-mediated), to all sequential first-line antimicrobial agents.[3] Resistance does not appear to impose a fitness cost as resistant strains continue to predominate following withdrawal of the affected antimicrobial agent from clinical use.[4]

In South Africa, sexually transmitted infections (STIs) are managed syndromically, which ensures that treatment is given for the major causative pathogens based on clinical manifestations. The Centre for HIV and STIs (CHIVSTI) at the National Institute for Communicable Diseases (NICD) in Johannesburg has co-ordinated microbiological surveillance in patients presenting to sentinel primary healthcare clinics (PHCs) since 2007. Results from aetiological surveillance of STI syndromes indicate that

N. gonorrhoeae is the predominant cause of male urethritis syndrome (MUS) at a prevalence of 70-85%, and causes 10-20% of symptomatic vaginal discharge syndrome (VDS) cases.[5] *Neisseria gonorrhoeae* antimicrobial resistance surveillance from Gauteng during the period 2008 – 2015 revealed high prevalence resistance to penicillin, tetracycline and ciprofloxacin[6], obviating the incorporation of these antibiotics for treatment of *N. gonorrhoeae* in future genital discharge syndrome management algorithms. The currently recommended first-line treatment for gonorrhoea includes single doses of injectable ceftriaxone (250mg IM) and oral azithromycin (1g PO).[7]

Neisseria gonorrhoeae resistance profiles from NICD GERMS-SA sentinel surveillance, conducted in 2017 in four of South Africa's provinces, are described here.

Methods

In the surveillance year 2017, consecutive males presenting with visible urethral discharge were enrolled at NICD STI sentinel surveillance sites (primary healthcare centres) in the Gauteng (Alexandra Health Centre), Eastern Cape (Zwide Clinic), Free State (Heidedal Clinic) and Western Cape (Khayelitsha Site B Clinic) provinces. Written informed consent was followed by a short nurse-administered questionnaire which collected demographic and behavioural information of participants. Each participant was given a unique survey number which was delinked from personal identifiers.

Sample collection

Urethral discharge specimens were collected using Rayon swabs or e-swabs (Copan Diagnostics). They were either inoculated directly on New York City agar (Diagnostic Media Products, National Health Laboratory Service), and placed in a holding candle jar prior to same-day transfer to the STI Reference laboratory at the NICD, or placed in Amies transport medium for transport on ice for culture in the reference laboratory.

Laboratory procedures

Neisseria gonorrhoeae culture isolates were tested for susceptibility to antimicrobials by E-test™ (bioMérieux, Marcy-l'Étoile, France) for cefixime, ceftriaxone and gentamicin; or agar dilution for azithromycin and spectinomycin, according to established standard operating protocols. Minimum inhibitory concentrations (MICs) were interpreted according to criteria recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[8], and for spectinomycin susceptibility according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[9] Clinical breakpoints for gentamicin susceptibility testing have not yet been established, and previously published interpretive criteria were used in this analysis.[10] For purposes of quality control a panel of 2016 WHO reference strains of *N. gonorrhoeae* was included in every batch of clinical isolates tested.

Data management and statistical analysis

Data from clinical questionnaires and results of gonococcal antimicrobial susceptibility testing were merged into a survey specific Access© [Microsoft, Seattle Washington] database and exported into STATA 14.2® [Stata Corporation, College Texas] for analysis. The enrolled participants were described using frequencies and proportions for categorical data, and medians and interquartile ranges (IQRs) for continuous variables. Analysis of susceptibility in *N. gonorrhoeae* involved determination of the relative prevalence of susceptibility and resistance. For antimicrobials that are recommended for use in current guidelines, such as extended-spectrum cephalosporins and azithromycin, MIC_{50} (minimum concentration needed to inhibit 50% of isolates); MIC_{90} (minimum concentration needed to inhibit 90% of isolates); and maximum MICs were determined.

Results

Characteristics of participants

In total, *N. gonorrhoeae* isolates from 315 men presenting with urethral discharge were tested in 2017. Demographic and clinical characteristics of these participants are given in Table 1. All participants were black African, and the vast majority identified as heterosexual (99.7%). Although only one participant self-reported homosexual orientation, on further enquiry another 5 (2%) confirmed that they had sex with men only; and 11 (4%) reported having receptive anal sex. Type of sex act was vaginal sex only in the majority (77%), with oral sex was practised by approximately 20%. Median age of participants was 27 years (IQR 23-32). Median age of sexual debut was 17 years. Nearly 60% of men reported sex with at least one casual (i.e. non-regular) partner in the preceding 3 months; only 11% had used a condom at last sex; and 26% gave a history of STI syndrome within the preceding year, which was a previous episode of MUS in the majority. In the 3 months prior to presentation, over 20% of men reported having a sex with a partner residing/living in another province; and over 10% with someone residing in another country. Six percent (19 participants) had been treated for male urethritis syndrome (MUS) without success in the preceding 3-month period. Of these, 50% had received treatment by a pharmacist or a private practitioner; and the majority (14/18; 78%) had received only tablets as part of their syndromic management. Approximately one-fifth (64/315; 20.3%) of all participants were HIV-infected, and less than 20% were medically circumcised.

Table 1. Demographic and clinical characteristics of male urethritis syndrome (MUS) participants with *Neisseria gonorrheoae* isolated on culture, GERMS-SA sentinel surveillance in the Gauteng (Alexandra Health Centre), Eastern Cape (Zwide Clinic), Free State (Heidedal Clinic) and Western Cape (Khayelitsha Site B Clinic) provinces, South Africa, 2017.

Characteristic	N=315
Age (median, IQR)	27 (23- 32)
Ethnic group (black African)	315 (100)
History of STI syndrome in past 12 months	83 (26.3)
MUS among those with history of STI syndrome	81 (97.6)
Heterosexual orientation (self-reported)	314 (99.7)
Type of sex partner	
Women only	309 (98.1)
Men only	6 (1.9)
Type of sex act	
Vaginal only	241 (76.5)
Vaginal and oral	59 (18.7)
Receptive anal +/- other	11 (3.5)
Oral only	2 (0.6)
Sex with non-regular sexual partner in the past 3 months	184 (58.4)
Condom use at last sexual encounter	36 (11.4)
Treated for MUS with no success in the past 3 months	19 (6.0)*
Treated by a pharmacist	4 (22.2)
Treated by a private practitioner	5 (27.8)
Treated at a PHC	9 (50.0
Treatment received	
Injection and tablets	4 (22.2)
Tablets only	14 (77.8)
Diagnosed with an STI syndrome in the past 12 months	83 (26.4)
Sex with someone living in another province in the past 3 months	70 (22.2)
Sex with someone living outside the country in the past 3months	41 (13.0)
Medically circumcised	49 (15.6)
HIV infected	64 (20.3)
Facility	
EC	76 (24.1)
FS	49 (15.6)
GP	128 (40.6)
WC	62 (19.7)

*Additional data available for 18/19 participants. EC = Eastern Cape Province, FS = Free State Province, GP = Gauteng Province, \WC = Western Cape Province

Neisseria gonorrhoeae antimicrobial resistance profiles

The majority of *N. gonorrhoeae* isolates tested were susceptible to gentamicin (88.6%); 11% (36/315) showed intermediate resistance, and there were no fully-resistant isolates (Figure 1). All isolates were uniformly susceptible to spectinomycin.

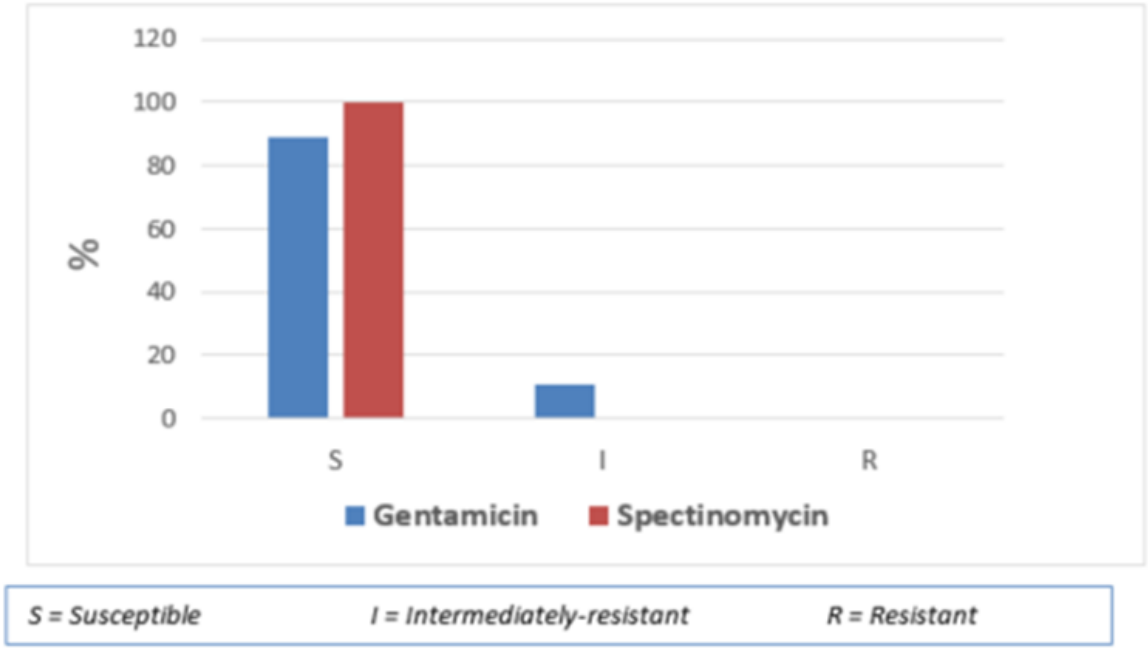

Figure 1. *Neisseria gonorrhoeae* antimicrobial resistance profiles, GERMS-SA sentinel surveillance in the Gauteng, Eastern Cape, Free State and Western Cape provinces, South Africa, 2017; n = 315.

Resistance to extended-spectrum cephalosporins (MIC ≥0.25 µg/ml) was not observed using EUCAST breakpoint interpretive criteria. All isolates were susceptible to ceftriaxone (Table 2). All isolates were also sensitive to cefixime with very low MICs, with the exception of one isolate from Johannesburg that had an MIC (0.125 g/ml) that was one dilution below the resistant breakpoint.

Full resistance to azithromycin (MIC ≥1 µg/ml) was not seen among the isolates tested; and less than 5% showed intermediate azithromycin resistance (MIC 0.5 µg/ml) (Table 3).

Table 2. Extended-spectrum cephalosporin minimum inhibitory concentrations (MIC) for *Neisseria gonorrhoeae* isolates, GERMS-SA sentinel surveillance in the Gauteng, Eastern Cape, Free State and Western Cape provinces, South Africa, 2017; n = 315.

Antimicrobial	MIC_{50}	MIC_{90}	Minimum MIC	Maximum MIC	% with MIC = 0.125	% with MIC = 0.25	% with MIC >/= 0.5
Cefixime	<0.016	<0.016	< 0.016	0.125	0.3 (n = 1)	0	0
Ceftriaxone	0.003	0.006	<0.002	0.032	0	0	0

Table 3. Azithromycin MICs for *Neisseria gonorrhoeae* isolates, GERMS-SA sentinel surveillance in the Gauteng, Eastern Cape, Free State and Western Cape provinces, South Africa, 2017; n = 302.

Antimicrobial	MIC_{50}	MIC_{90}	Minimum MIC	Maximum MIC	% with MIC = 0.25	% with MIC = 0.5	% with MIC $\geq$ 1
Azithromycin	0.128	0.25	0.032	0.5	20 (n = 61)	2.3 (n = 7)	0

Discussion

This surveillance report describes the 2017 resistance profiles to currently used antimicrobials and to those that may be used in future treatment failure algorithms for gonorrhoea. The male participants presenting with symptomatic urethritis were mostly young adults exhibiting high-risk sexual behaviour. Uptake of STI/HIV preventive measures such as condom use and male medical circumcision were uniformly low; and a significant proportion were migrant workers who had partners in other regions of the country or continent.

Surveillance reveals that the currently-used dual therapeutic regimen of ceftriaxone and azithromycin for gonorrhoea is appropriate for continued use in MUS syndromic management guidelines at PHC level in the largely heterosexual populations accessing clinic services. Ceftriaxone is the mainstay of gonorrhoea treatment, and extensively-drug resistant (XDR) isolates are characterised by resistance to extended-spectrum cephalosporins. Dual therapy with azithromycin is recommended to limit the emergence of XDR *N. gonorrhoeae*, particularly in settings with limited surveillance capacity.

In South Africa in 2012, the first two cases of resistance associated with cefixime treatment failure were described in two patients presenting with persistent urethral discharge.[11] Genotypic testing of the two isolates using *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) and multi-locus sequence typing (MLST), revealed that they were identical sequence types (NG-MAST ST4822 and MLST ST1901) belong to a multi-drug resistant clone associated with cefixime treatment failure and global spread. Both patients were men-who-have-sex-with-men (MSM) who had possible links to sexual networks in Europe and North America.

There are two factors that could lead to the spread of resistance in this key population: high risk sexual behaviour and participation in international sexual networks; and the presence of pharyngeal gonorrhoea, which is typically asymptomatic. Gonococci residing in the pharynx are at a survival advantage due to differential concentrations of antimicrobials at this site, presenting an opportunity for genetic exchange with oropharyngeal commensal *Neisseria* species. An additional two cases of cefixime resistance were identified in MSM residing in Cape Town and East London, respectively (D. Lewis, unpublished data). Therefore, there is a need to establish sustained gonococcal antimicrobial resistance surveillance in key populations such as men-who-have-sex-with-men (MSM), that have specific risk factors for XDR gonorrhoea. Interestingly, these data reveal that although only a small minority of men presenting with urethritis to the PHC identified as homosexual; on further enquiry an additional number reported sex with only male partners and receptive anal sex. Approximately 20% reported recent oral sex. This highlights the importance of thorough history-taking to ascertain sexual behaviour and assess risk for infection at extragenital sites.

Only low-prevalence intermediate-resistance to azithromycin (MIC 0.5µg/ml) among gonococcal isolates has been identified in recent years. Although clinical effectiveness of azithromycin for urethral and endocervical infections has been estimated to be >95%[12], it is recommended only in dual therapy due to the ease of resistance development to macrolide monotherapy. Successful and sustained spread of a high-level azithromycin resistant (MIC ≥256 µg/ml) clone of *N. gonorrhoeae* has been described in England.[13] Whole genome sequencing has raised concerns that high-level resistance may develop stepwise from low-level resistance, especially in the setting of azithromycin selection pressure.

Failure of dual ceftriaxone-azithromycin therapy has been described in the United Kingdom, with persistence of asymptomatic pharyngeal gonorrhoea in a heterosexual man treated for urogenital symptoms in 2014.[14] The patient was infected with an XDR strain, which had acquired multiple resistance mechanisms to both ceftriaxone and azithromycin. Molecular epidemiology established links to an XDR genogroup that is spreading in Japan. More recently, Public Health England issued a report of a heterosexual man with a test-of-cure pharyngeal gonococcal isolate showing resistance to ceftriaxone (MIC 0.5 µg/ml) and high-level resistance to azithromycin (MIC >256 µg/ml), with possible links to a female sexual contact in South East Asia.[15] Although a small proportion of men in this survey reported unsuccessful treatment for urethritis in the preceding three months, they are likely to have received inappropriate therapy that did not include injectable ceftriaxone for the first episode of urethral discharge. It is essential that national STI syndromic management guidelines are circulated widely among healthcare practitioners, and training strengthened with the use of continuing medical education activities to ensure correct prescription practices that are standardised across all health sectors.

There is a need to remain vigilant and establish additional surveillance activities for XDR gonorrhoea. In December 2017, ceftriaxone-resistant gonorrhoea was added to the list of Notifiable Medical Conditions (Category 3) in South Africa, mandating all laboratories, both private and public sector, to notify the Department of Health following the identification of such isolates.[16] These ceftriaxone-resistant isolates should also be referred to CHIVSTI, NICD, for confirmation of resistance by additional phenotypic and molecular testing. It is important for laboratories to standardise the testing and reporting of antimicrobial resistance in *N. gonorrhoeae*. Clinicians should have a heightened awareness of the possibility of XDR *N. gonorrhoeae* infection in cases of persistent MUS, with non-resolution of symptoms 7 days after administration of standard first-line syndromic therapy. The next revision of the National Department of Health Adult Hospital Level Guidelines will include a chapter outlining the appropriate laboratory investigation and management of such cases. Sexual contact tracing and partner management would be an important component of public health action.

Combination gentamicin and high dose azithromycin therapy is a therapeutic option for confirmed ceftriaxone-resistant gonorrhoea cases. A small proportion of isolates showed intermediate resistance to gentamicin; however, these data must be interpreted together with results of clinical effectiveness studies, and standardized susceptibility testing guidelines.

Additional early warning surveillance activities would include sustained antimicrobial surveys in key populations, incorporating extra-genital sampling and test-of-cure for pharyngeal gonorrhoea. The development and implementation of accurate rapid diagnostics for gonorrhoea would further facilitate screening for asymptomatic and extra-genital infection in high-risk populations, and expedite control efforts such as specific pathogen-directed partner treatment.

Conclusion

Surveillance of men presenting with urethritis to primary healthcare facilities in four provinces has not identified XDR *N. gonorrhoeae* resistance to the extended-spectrum cephalosporins. The prevalence of low-level azithromycin resistance is <5%. These data support the continued use of both ceftriaxone and azithromycin in dual therapy for gonorrhoea. Based on local and global epidemiology, enhanced national culture-based surveillance, particularly in key populations such as MSM, is essential to detect the emergence of XDR gonorrhoea.

Key Messages

- The extent of antimicrobial resistance in *N. gonorrhoeae* is increasing worldwide
- Extensively-drug resistant (XDR) *N. gonorrhoeae* strains are characterised by resistance to extended-spectrum cephalosporins
- Based on antimicrobial resistance surveillance in South Africa, dual therapy with injectable ceftriaxone and azithromycin for gonorrhoea is appropriate for use in syndromic management guidelines
- Standard treatment guidelines for male urethritis should be implemented across all healthcare sectors
- Enhanced culture-based surveillance is essential to detect emerging XDR gonorrhoea, including specimen collection and microbiological testing for cases of persistent MUS unresponsive to standard first-line syndromic treatment
- Ceftriaxone-resistant gonorrhoea is a Notifiable Medical Condition (Category 3)

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References

1. Emergence of multi-drug resistant *Neisseria gonorrhoeae.* World Health Organization; 2012.
2. Kularatne RS, Niit R, Rowley J, Kufa-Chakezha T, Peters RPH, Taylor MM, et al. Adult gonorrhea, chlamydia and syphilis prevalence, incidence, treatment and syndromic case reporting in South Africa: Estimates using the Spectrum-STI model, 1990-2017. *PloS One*. 2018;13(10):e0205863.
3. Goire N, Lahra MM, Chen M, Donovan B, Fairley CK, Guy R, et al. Molecular approaches to enhance surveillance of gonococcal antimicrobial resistance. *Nature Reviews Microbiology*. 2014;12(3):223-9.
4. Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clinical microbiology reviews*. 2014;27(3):587-613.
5. Kularatne R. Aetiological surveillance of sexually transmitted infection syndromes at sentinel sites: GERMS-SA 2014-2016. National Instutute for Communicable Diseases; 2017.
6. Kularatne R, Maseko V, Gumede L, Kufa T. Trends in *Neisseria gonorrhoeae* antimicrobial resistance over a ten-year surveillance period, Johannesburg, South Africa, 2008(-)2017. *Antibiotics* (Basel). 2018;7(3).
7. Primary Healthcare Standard Treatment Guideline and Essential Medicine List. 6th ed. . Essential Drugs Programme. Republic of South Africa: National Department of Health; 2018.

8. Breakpoint tables for interpretation of MICs and zone diameters. European Committee on Antimicrobial Susceptbility Testing; 2016. p. 56-60.
9. Performance standards for antimicrobial susceptibility testing. Clinical Laboratory Standards Institute; 2016. p. 90-2.
10. Brown LB, Krysiak R, Kamanga G, Mapanje C, Kanyamula H, Banda B, et al. *Neisseria gonorrhoeae* antimicrobial susceptibility in Lilongwe, Malawi, 2007. *Sexually Transmitted Diseases*. 2010;37(3):169-72.
11. Lewis DA, Sriruttan C, Muller EE, Golparian D, Gumede L, Fick D, et al. Phenotypic and genetic characterization of the first two cases of extended-spectrum-cephalosporin-resistant *Neisseria gonorrhoeae* infection in South Africa and association with cefixime treatment failure. *Journal of antimicrobial chemotherapy*. 2013;68(6):1267-70.
12. Bignell C, Garley J. Azithromycin in the treatment of infection with *Neisseria gonorrhoeae*. *Sexually Transmitted Infections*. 2010;86(6):422-6.
13. Fifer H, Cole M, Hughes G, Padfield S, Smolarchuk C, Woodford N, et al. Sustained transmission of high-level azithromycin-resistant *Neisseria gonorrhoeae* in England: an observational study. *Lancet Infect Dis*. 2018.
14. Fifer H, Cole M, Hughes G, Padfield S, Smolarchuk C, Woodford N, et al. Sustained transmission of high-level azithromycin-resistant *Neisseria gonorrhoeae* in England: an observational study. *Lancet Infect Dis*. 2018;18(5):573-81.
15. Smolarchuk C, Wensley A, Padfield S, Fifer H, Lee A, Hughes G. Persistence of an outbreak of gonorrhoea with high-level resistance to azithromycin in England, November 2014 - May 2018. Euro Surveillance: bulletin Europeen sur les maladies transmissibles. *European Communicable Disease Bulletin*. 2018;23(23).
16. National Health Act, 2003 (Act No 61 of 2003): Regulations relating to the surveillance and the control of Notifiable Medical Conditions. National Department of Health; 2017.

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