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FOREWORD

Three important surveillance reports for South Africa are presented in this issue. Firstly, the occurrence of multi drug resistant TB (MDR–TB) and extensively drug resistant TB (XDR-TB) has long been recognized in South Africa. A recent survey covering the period 2012 to 2014 shows that high levels of drug resistance and the loss of key drugs among pre-XDR and XDR cases necessitates the use of a new regimen incorporating newly introduced drugs. Secondly, surveillance statistics for 2014 and 2015 show that since the introduction of the rotavirus vaccine into South Africa's national immunization program there has been a sustained reduction in both rotavirus and all-cause diarrhoeal disease in children <5 years. Thirdly, human papillomavirus (HPV) is one of the most common sexually transmitted infections and is a major risk factor for cervical cancer. Surveillance data for the period 2014 to 2016 in South Africa show that the high prevalence of HPV types targeted by current vaccines encourages continued vaccination of young women. Lastly, guidelines for the diagnosis, management and public health response to Legionnaires' disease are given in this issue.

All participating laboratories and contributors are thanked for their inputs. This is the final issue for 2016 and we wish all our readers and contributors a safe and joyous holiday season.

Basil Brooke, Editor

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DRUG RESISTANT TUBERCULOSIS IN SOUTH AFRICA: FINDINGS FROM A NATIONWIDE SURVEY, 2012-2014

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Executive summary

South Africa is one of the 22 highest tuberculosis (TB) burdened countries globally and the occurrence of laboratory-confirmed multi drug resistant TB (MDR–TB) and extensively drug resistant TB (XDR-TB) has long been recognized in South Africa. A drug resistance survey (2012-2014) to quantify and delineate the extent of drug resistance in new and retreatment TB patients nationally and provincially in South Africa, as well as to compare findings with the previous survey (2001-2002) was undertaken based on WHO guidelines. The prevalence of MDR-TB nationally in the latest survey was measured at 2.1% in new cases and 4.6% in retreatment cases with an overall, MDR-TB estimate of 2.8%. Compared to the previous survey, the MDR-TB prevalence has remained relatively stable over the ten-year period. The highest rate observed was in Mpumalanga province with an overall rate of 5.1%. Contrasted to the MDR-TB prevalence nationally, the rate of any rifampicin-resistance prevalence has increased since the previous survey, primarily seen among new cases, and almost doubling from 1.8% to 3.4%, highlighting the likely role of transmission. Second -line drug resistance prevalence among MDR-TB cases was for the first time evaluated in this survey and the findings are concerning. The prevalence of resistance to ethionamide and pyrazinamide, both used empirically in the treatment of MDR-TB, was found to be high at 44.7%% and 59.1% respectively. Additionally, resistance levels to the key drug classes, fluoroquinolones and injectable agents, were both 13%, highlighting the relatively high frequency of pre-extensively drug-resistant tuberculosis cases among those with MDR-TB. These findings highlight emerging threats to drug resistant TB control requiring urgent intervention.

Introduction

South Africa is one of the 22 highest tuberculosis (TB) burdened countries globally and has the second highest TB incidence rate in the world.[1] The first national survey of TB drug resistance in South Africa was undertaken between 2001 and 2002[2]. The study reported an overall multi drug resistant TB (MDR-TB) rate in South Africa of 1.6% (95% CI: 1.1%-2.1%) in new cases and 6.6% ((95% CI: 4.9%-8.2%) in retreatment cases.

Although the MDR-TB prevalence appears to be low among primary TB cases, this needs to be interpreted in the context of a high incidence of TB in South Africa. In the *WHO Global TB Report 2015*, South Africa had the second highest absolute number of notified rifampicin-resistant (RR)/MDR cases globally (18 734)[1], with India ranked number one (25 748) but the latter having a population 20 times that of South Africa. The occurrence of laboratory-confirmed extensively drug resistant TB (XDR-TB), a more resistant form of MDR–TB, has long been recognized in South Africa, and was managed as difficult-to-treat MDR-TB cases. An outbreak of XDR-TB was reported in 2005 at the Church of Scotland Hospital in Tugela Ferry, KwaZulu-Natal province and was followed by a report of the emergence of "totally drug-resistant" TB in Eastern Cape province based on strains collected during the period 2008-2009.[3]

Routine notification data has shown that the treatment success rate is approximately 50% in MDR-TB cases and 20% in XDR-TB patients.[4] Furthermore, many of these unsuccessfully treated patients die. The situation has however improved with the introduction of bedaquiline for which early programme data suggests improved outcomes.[5] The current WHO recommendation is to conduct a TB drug resistance survey every five years[6] and a new survey was long overdue to quantify and delineate the extent of drug resistance in new and retreatment TB patients nationally and provincially in South Africa, as well as to compare findings with the previous survey.

Methods

A survey aimed at providing MDR–TB estimates for each province and nationally was designed using a population-based cross-sectional study according to WHO guidelines.[6] Clusters were randomly selected and were either individual healthcare facilities or a combination of facilities. Patients were eligible for inclusion in the survey if they were older than 18 and presented as a presumptive TB case, according to WHO/ International Union against Tuberculosis and Lung Disease (IUATLD) definitions.

All consecutive presumptive TB cases, who provided informed consent at selected facilities during the survey period, had a case report form (CRF) completed through direct patient interview by a healthcare worker at the health facility and in addition had a survey-specific sputum sample collected, were included. The CRF with the corresponding sample was sent to the Centre for Tuberculosis at the National Institute for Communicable Diseases in Johannesburg, where smear microscopy, liquid mycobacterial culture and HIV testing on sputum was performed. This was followed by drug susceptibility testing against a panel of first-line and second-line anti-TB drugs on *Mycobacterium tuberculosis*-confirmed isolates. Data from the CRF and the laboratory testing process were collated and analyzed.

The survey received ethical approval from the University of Witwatersrand Research Ethics Committee on the 26/11/2010 (Ethics clearance No. M081022). Clearance was also received from the Centers for Disease Control and Prevention, Atlanta, USA. The survey was initiated after consultation and approval from the respective provinces and the South African National TB Control Programme.

Results

The South African Tuberculosis Drug-Resistant Survey (DRS) of 2012-2014 was the largest TB DRS conducted

in the country to date with 200 358 persons screened from 464 randomly selected facilities in all nine provinces. A total of 10 044 culture confirmed TB cases was identified. These underwent both first- and second-line drug susceptibility testing. Nationally, 22% of culture positive TB cases reported prior treatment for TB and the highest incidence was in the Western Cape at 35%. HIV co-infection was 63.2% nationally and ranged between 47.4% (Western Cape) and 76.8% (Mpumalanga).

The prevalence of MDR-TB nationally was measured at 2.1% (95% CI: 1.5%-2.7%) in new cases and 4.6% (CI 95%: 3.2%-6.0%) in retreatment cases with an overall MDR-TB estimate of 2.8% (95%CI: 2.0%-3.6%) (Table 1). Compared to the previous survey of 2001-2002, the prevalence of MDR-TB has remained relatively stable over the ten-year period with the overall MDR-TB rate in the previous survey being 2.9% (95% CI: 2.4%-3.5%).

Provincial MDR-TB prevalence varied with six of nine provinces showing MDR-TB rates below 2% among new cases in the current survey. The highest rate observed was in Mpumalanga province with an overall rate of 5.1% (95% CI: 3.7%-7.0%), including both new and previously treated cases, which was higher than the national rate (2.8%; 95% CI: 2.0%-3.6%). This is of particular concern requiring urgent intervention.

Contrasting with the MDR-TB prevalence nationally, rifampicin-resistance prevalence has increased since the previous survey, with the overall prevalence at 4.6% (95% CI: 3.5%-5.7%) nationally in the current survey, compared to 3.4% (95% CI: 2.8%-3.9%) in the previous survey. The increase was primarily seen among new cases, almost doubling from 1.8% (95% CI: 1.3%-2.3%) to 3.4% (95% CI: 2.5%-4.3%), highlighting the likely role of transmission. Rifampicin mono-resistance (RMR), which showed a low prevalence in the previous survey, has emerged as a concern. It was below 0.5% overall in the previous survey but has increased to 1.7% in the current survey. Provincial variation was observed in RMR-TB cases with several provinces showing similar prevalence rates of MDR and RMR-TB cases while Limpopo province showed higher RMR-TB prevalence than MDR-TB. The reason for the emergence of RMR-TB in the context of standardized combination therapy is unclear and should be further investigated. The prevalence of isoniazid resistance (9.3%; 95% CI: 7.9%-10.7%) was higher than that of rifampicin resistance (4.6%; 95% CI: 3.5%-5.7%). A notable increase in isoniazid mono-resistance (IMR) was observed between the current survey (4.9%; 95% CI: 4.1%-5.8%) and the previous survey (2.7%; 95% CI: 2.2%-3.2%).

Second-line drug resistance prevalence among MDR-TB cases was evaluated for the first time in this survey and the findings are concerning (Table 2). The prevalence of resistance to ethionamide and pyrazinamide, both used empirically in the treatment of MDR-TB, was found to be high at 44.7%% (95% CI: 25.9%-63.6%) and 59.1% (95% CI: 49.0%-69.1%) respectively. This compromises the effectiveness of the standard MDR-TB regimen and could lead to further selection of resistance to other drugs. Additionally, resistance levels to the key drug classes - fluoroquinolones and injectable anti-TB agents - were both 13% (95% CI: 5%-21%), highlighting the relatively high frequency of pre-extensively drug-resistant TB (XDR) cases among those with MDR-TB confirmation, and the need to identify these cases early.

Table 1: National first-line drug resistance estimates among new and previously treated TB cases, 2012-14 survey, South Africa.

TB resistance	New (%, 95% CI)	Previously treated (%, 95% CI)	Overall (%, 95% CI)
MDR	2.1 (1.5-2.7)	4.6 (3.2-6.0)	2.8 (2.0-3.6)
Any rifampicin	3.4 (2.5-4.3)*	7.1 (4.8-9.5)	4.6 (3.5-5.7)
Rifampicin mono†	1.4 (0.9-1.8)	2.5 (1.2-3.7)	1.7 (1.1-2.2)
Rifampicin mono (strict)[1]	0.9 (0.5-1.3)*	1.8 (0.7-2.9)	1.1 (0.6-1.7)*
Rifampicin mono (other)[2]	0.4 (0.1-0.7)*	0.7 (0.2-1.2)	0.5 (0.2-0.8)*
Any isoniazid††	7.6 (6.4-8.7)	11.1 (9.1-13.1)	9.3 (7.9-10.7)
Isoniazid mono	5.5 (4.6-6.5)	6.5 (5.1-7.9)	6.1 (5.1-7.1)
Isoniazid mono (strict)[1]	4.5 (3.6-5.3)*	5.5 (4.3-6.8)*	4.9 (4.1-5.8)*
Isoniazid mono (other)[2]	1.1 (0.3-1.8)	1.0 (0.4-1.6)	1.1 (0.4-1.7)
Ethambutol	2.0 (1.2-2.8)*	3.5 (2.2-4.8)	2.5 (1.7-3.3)*
Streptomycin	3.9 (2.8-5.1)	5.1 (3.8-6.5)*	4.5 (3.5-5.5)*
Pyrazinamide	2.9 (2.2-3.6)	5.2 (3.8-6.7)	3.7 (2.9-4.5)

† rifampicin-resistant & isoniazid susceptible
†† rifampicin susceptible & isoniazid resistant
[1] strict (without resistance to another first line drug: streptomycin/ethambutol)
[2] other (with resistance to another first line drug: streptomycin/ethambutol)
*non-overlapping 95% confidence intervals between 2012-14 and 2001-2

Table 2: National second-line drug resistance among MDR-TB cases, 2012-14 survey, South Africa.

Drug	Overall (%, 95% CI)
Pyrazinamide	59.1 (49.0-69.1)
Ethambutol	44.1 (30.2-58.0)
Streptomycin	63.0 (52.8-73.2)
Ethionamide	44.7 (25.9-63.6)
P-aminosalicylic acid	5.3 (2.2-8.3)
Second-line injectable	13.0 (5.0-20.9)
Ofloxacin	13.0 (5.0-21.0)
XDR-TB	4.9 (1.0-8.8)

Discussion

South Africa has experienced a stable MDR-TB epidemic spanning a ten-year period. However, resistance to individual drugs is on the increase. The concerning increase in rifampicin mono-resistance, primarily among new cases, is suggestive of transmission although the underlying reasons for its occurrence may relate to sub-optimal dosing of rifampicin, the bioavailability of rifampicin being affected by drug interactions, and intermittent compliance with treatment.[7,8]

The use of Xpert MTB/RIF as the primary diagnostic tool[9] will be important for detecting those cases with rifampicin resistance early, together with rapid initiation of therapy to halt further transmission.

The increased occurrence of isoniazid mono-resistance is also of concern and can be missed with the current national diagnostic algorithm. Although its impact on patient outcomes is poorly defined, rifampicin mono-resistance could potentially impact MDR-TB levels in the future as undetected cases may effectively continue to receive rifampicin mono-therapy. Strengthening the continuation phase regimen needs consideration and the potential role of isoniazid preventative therapy (IPT) as a driver of this increase in the South African context needs to be investigated.[10] Furthermore, the effectiveness of IPT could be reduced as the prevalence of any isoniazid resistance is almost 10% which makes it essential to conduct a risk-benefit assessment.

The province of greatest concern is Mpumalanga which shows higher MDR-TB rates than the national average, as was also observed in the previous survey. This province shares a border with Swaziland, the country with the highest MDR-TB prevalence in the region[11] and with well recognized chronic health system issues. Although the RR/MDR-TB prevalence in Mpumalanga was higher, the rate of isoniazid mono-resistance was similar to that of other provinces.

Rates of resistance to fluoroquinolones and pyrazinamide, both considered companion drugs for new regimens for TB treatment, have shown to be low among TB cases, rendering these regimens suitable for implementation within South Africa. Contrasted with this are the high rates of resistance to ethionamide and pyrazinamide among MDR–TB cases, which may be contributory factors to the poor outcomes seen in these cases. XDR-TB rates nationally were below 5% among MDR-TB cases and lower than the global average, indicating that the problem is not widespread across the country. Taking into consideration the high pre-existent levels of second-line drug resistance and the loss of one or both key drugs among pre-XDR and XDR cases, achieving improved outcomes is likely to require the use of a new regimen incorporating newly introduced drugs.

Recommendations

The findings from the South African TB DRS 2012-14 provide important information which could potentially guide future planning and address the current poor outcomes among drug-resistant TB cases. The following recommendations are made based on the findings of the survey:

- Urgent implementation of interventions in Mpumalanga:
  - ⇒ Identify potential risk factors for targeted interventions.
  - ⇒ Improve cross-border cooperation with Swaziland and Mozambique, utilising existing agreements achieved through the SADC declaration.
  - ⇒ Conduct further research to fully define drivers of resistance in the province.
- Develop interventions to curb IMR and its secondary effects:

- ⇒ Strengthen the current first-line regimen for continuation phase by adding ethambutol with or without pyrazinamide (RHE or RHZE), or institute appropriate measures for early identification of IMR.
- ⇒ Assess the contribution and effectiveness of IPT in the light of increasing cases of resistance.

- Monitor transmission of RMR, research underlying reasons for RMR and institute appropriate interventions:
  - ⇒ Regularly review transmission data from the surveillance system.
  - ⇒ Review current rifampicin dosing and conduct rifampicin bioavailability studies in the four- and two-drug combination with and without antiretroviral therapies (ARTs) in areas with high RMR occurrence.
  - ⇒ Undertake close monitoring of the quality of drugs used in the standard regimen.
- Conduct randomised control trials (RCTs) and review existing standard of care data to assess the effectiveness of existing first and second-line regimens.
- Monitor use of the Xpert MTB/Rif assay for early detection of rifampicin resistance and improve early detection of second-line drug resistance.
- Optimise the existing MDR-TB regimen and consider shortening the MDR-TB regimen with triage algorithm for appropriate patient selection.
- Design an appropriate regimen for pre-XDR/XDR patients using a combination of new drugs.
- Maintain and enhance the routine surveillance system for monitoring existing and new drug resistance and reduce the proportion of diagnosed cases not started on treatment.

The full survey report is available at http://www.nicd.ac.za/assets/files/K-12750%20NICD%20National%20Survey%20Report_Dev_V11-LR.pdf

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ROTAVIRUS SURVEILLANCE REPORT, SOUTH AFRICA, 2014-2015: A COMPARISON WITH PREVIOUS ROTAVIRUS SEASONS

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Executive summary

Since the introduction of the rotavirus vaccine into the national immunization program in August 2009, there has been a sustained reduction in both rotavirus and all-cause diarrhoeal disease in children <5 years in South Africa. Diarrhoeal surveillance at selected sentinel sites in 2014 and 2015 showed lower rotavirus prevalence and reduced absolute numbers of hospitalized diarrhoea cases in children <5 years compared to 2013. The genotypes circulating in 2014 included G1P[8] and G2P [4] and in 2015, G9P[8] and G3P[8] with no strain replacement evident. Surveillance also showed that genotypes circulating in the Western Cape consistently differed from the predominant types in the rest of the country. Despite the success of the rotavirus vaccine, protection is not complete and annual rotavirus seasons from May-September, affecting mostly children <2 years, should be expected. Health care providers are encouraged to prepare for the annual rotavirus season by ensuring adequate supplies of oral rehydration solution and intravenous fluids, and educating mothers on vaccination and signs of dehydration.

Introduction

Since South Africa introduced the rotavirus vaccine into the national immunization program in August 2009, the Centre for Enteric Diseases (CED) at the National Institute for Communicable Diseases (NICD) has been monitoring the impact of the vaccine at selected sentinel sites. In addition to describing the annual rotavirus

season and prevalence of rotavirus disease, the surveillance system reports on the circulating genotypes each year.

Early rotavirus vaccine impact studies conducted at three of the sentinel sites between 2009 and 2011 indicated that the vaccine reduced rotavirus hospitalizations in children <5 years by 54% - 58% and lowered all-cause diarrhoea hospitalization by one-third.[1] Data from a vaccine effectiveness case-control study conducted between 2010 and 2012 showed that the vaccine was 40% effective after one dose and 57% effective after two doses in preventing rotavirus diarrhoea.[2] The study also revealed that vaccine effectiveness was similar in HIV-exposed versus HIV-unexposed children.[2] A further study looking at the temporal association of the introduction of the rotavirus vaccine and all-cause childhood diarrhoea hospitalizations at Chris Hani Baragwanath Academic Hospital revealed a 45-66% reduction in diarrhoea incidence in children <1 year and a 40-50% reduction in diarrhoea incidence in children in their second year of life.[3] This study also showed that reductions were observed in both HIV-infected and HIV-uninfected children.[3]

This report describes the timing, age distribution and circulating genotypes for the 2014 and 2015 rotavirus seasons in South Africa. The report compares the 2014 and 2015 seasons to the 2013 season to assess rotavirus prevalence per site, magnitude of the season and age distribution of cases. In addition, the timing of the rotavirus seasons and the genotypes circulating in each sentinel site were assessed between 2009 and 2015 to examine any potential changes that may have occurred since the introduction of the rotavirus vaccine.

Methods

During the period 2013 to 2015, the programme enrolled children <5 years of age admitted to sentinel hospitals with symptoms of three or more loose stools within a 24 hour period, following informed consent. The sentinel hospitals included Chris Hani Baragwanath Academic Hospital, Mapulaneng Hospital, Matikwane Hospital, Dr George Mukhari Hospital, Edendale Hospital and Red Cross Children's Hospital. Kimberley Hospital was included as a sentinel site in September 2014 and Pelonomi Hospital was established in April 2015. Case investigation forms including patient demographic, socioeconomic and clinical information, were completed by surveillance officers. A stool specimen was collected from each case for rotavirus screening.

Testing of stool samples was performed at the Centre for Enteric Diseases (CED), NICD, and at the MRC - Diarrhoeal Pathogens Research Unit (MRC-DPRU), Sefako Makgatho Health Sciences University. The stool samples were screened with the ProSpecT™ Rotavirus Microplate Assay (Oxoid, Basingstoke, UK).

Rotavirus positive samples were further characterized to determine the G and P genotype of each strain. Rotavirus dsRNA was extracted from each stool sample using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and genotyped using standardized RT-PCR methods and primers for G-specific (G1, G2, G3, G4, G8, G9, G10, G12) and P-specific (P[4], P[6], P[8], P[9], P[10], P[11], P[14]) genotypes.[4]

The start of the rotavirus season was defined as a rotavirus detection rate of above 20% for two consecutive weeks. The end of the season was defined as a rotavirus detection rate of below 20% for two consecutive weeks. The rotavirus prevalence per site, magnitude of the season and age distribution of the cases was determined for the 2014 and 2015 rotavirus seasons. The 2013 season was also included in this analysis for comparison. The timing and duration of the

rotavirus seasons as well as the predominant genotypes per site were compared between 2009 and 2015 to determine changes (if any) since the introduction of the rotavirus vaccine in August 2009.

Results

A total of 954 stool specimens was collected in 2014 with a further 838 collected in 2015 (Table 1). There was insufficient stool collected from 12% (256/2048) of diarrhoea cases preventing laboratory screening of them. Rotavirus was detected in 23% (217/954) of cases in 2014 and in 20% (170/838) of cases in 2015.

The start of the rotavirus season is usually in May although the season can start as early as March (Table 2, Figure 1). The end of the rotavirus season is usually in September although the season may terminate as early as August and as late as October (Table 2, Figure 1). The median duration of the rotavirus season is 20 weeks and ranges from 28 weeks in 2013 to 15 weeks in 2012 (Table 2). The maximum detection rate has decreased from 82% in 2009 to 53% in 2015 with the peak week of detection in June. The peak week of detection (week 35, 24 Aug) was late in 2015 compared

Table 1: Total numbers of stools collected and rotavirus results per surveillance site, South Africa, 2014 and 2015. (The 2013 rotavirus season has been included for comparison.)

Site	Rotavirus positive (%)		
	2015	2014	2013
Chris Hani Baragwanath	57/256 (22)	94/337 (28)	87/267 (33)
Mapumaleng	9/41 (22)	19/68 (28)	16/77 (21)
Matikwane	19/65 (29)	1/46 (2)*	29/114 (25)
Dr George Mukhari	22/108 (20)	28/115 (24)	26/134 (19)
Edendale	12/40 (30)	22/56 (39)	23/73 (32)
Red Cross Children's	19/128 (19)	51/304 (17)	149/434 (34)
Kimberley	13/55 (24)	2/28 (7)§	N/A
Polokwane	2/32 (6)#	N/A	N/A
Pelonomi	17/113 (15)#	N/A	N/A
Total	170/838 (20)	217/954 (23)	330/1099 (30)

* No surveillance officer between June and December 2014
§ Surveillance from September to December 2014
# Surveillance started in April/May 2015

Table 2: Characteristics of rotavirus seasons in South Africa between 2009 and 2015.

Year	Start week	End Week	Duration	Maximum detection rate	Peak week	Prevalence
2009	16 (14 Apr)	40 (4 Oct)	25	82% (37/45)	21 (18 May)	47% (428/917)
2010	20 (17 May)	36 (12 Sep)	17	60% (18/30)	24 (14 Jun)	25% (323/1317)
2011	19 (9 May)	39 (2 Oct)	21	72% (18/25)	25 (20 Jun)	27% (339/1246)
2012	21 (21May)	35 (2 Sep)	15	63% (15/24)	25 (18 Jun)	21% (202/963)
2013	12 (18 Mar)	39 (23 Sep)	28	61% (22/36)	30 (22 Jul)	30% (330/1099)
2014	16 (14 Apr)	34 (24 Aug)	19	65% (30/44)	24 (30 Jun)	23% (217/954)
2015	20 (11 May)	39 (27 Sep)	20	53% (9/16)	35 (24 Aug)	20% (170/838)

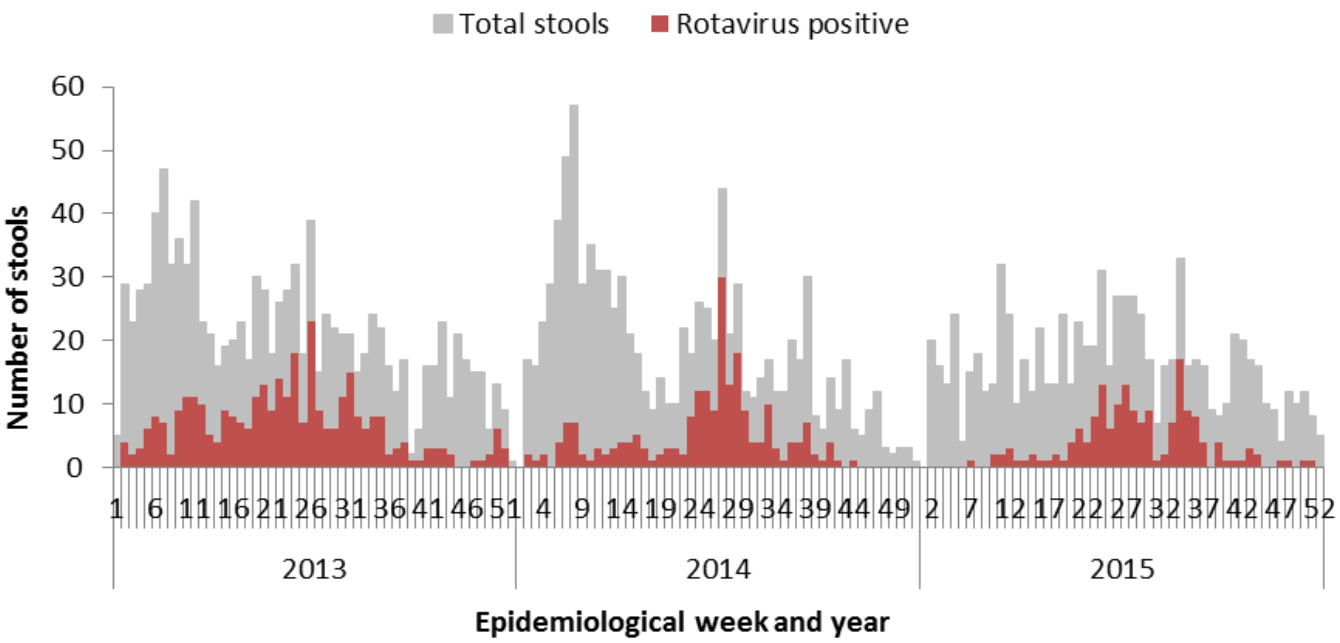

Figure 1: Numbers of stool specimens screened and rotavirus cases by epidemiological week between 2013 and 2015, South Africa.

The ages of children infected by rotavirus have remained relatively constant with children in the 7-9 month age group primarily affected (Table 3). The genotyping of the rotavirus strains revealed that G1P[8] (29%, 64/217) and G2P[4] (24%, 51/217) strains were predominant (Table 4) in 2014. In 2015, these strains were replaced by G9P[8] (67%, 99/148) and G3P[8] (16%, 24/148; Table 5). Analyses of genotype distribution by site between 2009 and 2015 showed that genotypes circulating in the Western Cape sentinel site differed from the rest of the country (Table 6). Similar observations were noted for sites in the Northern Cape and Free State provinces for 2015 (Table 6).

Table 3: Age distribution of children with rotavirus infections in 2014 and 2015, South Africa. The 2013 season has been included for comparison.

Age range (in months)	Rotavirus positive (%)		
	2015	2014	2013
0-3	11/83 (13)	36/153 (24)	35/150 (23)
4-6	30/ 133 (23)	27/134 (20)	70/198 (35)
7-9	37/125 (30)	51/154 (33)	69/185 (37)
10-12	26/103 (25)	40/133 (30)	57/172 (33)
13-18	26/132 (20)	30/156 (19)	61/183 (33)
19-24	8/50 (16)	20/101 (20)	20/95 (21)
>24	9/100 (9)	10/118 (8)	18/116 (16)
Unknown	23/112 (21)*	3/5 (60)	0 (0)
Total	170/838 (20)	217/954 (23)	330/1099 (30)
Predominant genotypes	G9P[8] G3P[8]	G1P[8] G2P[4]	G2P[4] G9P[8]

*Age data for Dr George Mukhari Hospital missing for 2015

Table 4: Rotavirus strains (G and P genotypes) detected at sentinel sites in South Africa in 2014. The predominant strains in each site are shaded grey.

Genotype	CHBAH		MP		DGM		EdH		RCCH		Total
	n	%	n	%	n	%	n	%	n	%	
Rotavirus strains covered by the monovalent vaccine											
G1P[8]	36	38	7	37	16	57	2	9	3	6	64
G3P[8]	10	11	3	16	0	0	1	5	5	10	19
G9P[8]	19	20	0	0	2	7	0	0	0	0	21
G12P[8]	1	1	0	0	0	0	1	5	0	0	2
Total	66	70	10	53	18	64	4	18	8	16	106
Rotavirus strains not covered by the monovalent vaccine											
G2P[4]	2	2	2	11	1	4	8	36	38	75	51
G2P[6]	17	18	6	32	4	14	6	27	1	2	34
G9P[4]	1	1	0	0	0	0	0	0	0	0	1
G9P[6]	0	0	1	5	3	11	0	0	0	0	4
Total	20	21	9	47	8	29	14	64	39	76	90
Mixed and non-typeable rotavirus strains											
Mixed	4	4	0	0	2	7	0	0	2	4	8
Not typed	4	4	1	5	0	0	4	18	2	4	11
Total	8	9	1	5	2	7	4	18	4	8	19
Grand total	94	43	20	9	28	13	22	10	51	24	215*

CHBAH = Chris Hani Baragwanath Academic Hospital, MP = Mapulaneng and Matikwane Hospitals, DGM = Dr. George Mukhari, EdH = Edendale Hospital and RCCH = Red Cross Children's Hospital.
*Two rotavirus-positive specimens from Kimberley Hospital were typed as G1P[8] and G2P[6].

Table 5: Rotavirus strains (G and P genotypes) detected at sentinel sites in South Africa in 2015. The predominant strains in each site are shaded grey.

Genotype	CHBAH		MP		EdH		RCCH		KBH		PLH		Total
	n	%	n	%	n	%	n	%	n	%	n	%	
Rotavirus strains covered by the monovalent vaccine													
G1P[8]	0	0	0	0	3	25	0	0	0	0	0	0	3
G3P[8]	0	0	1	4	0	0	7	37	8	62	8	47	24
G9P[8]	54	95	20	71	8	67	5	26	3	23	7	41	97
G12P[8]	1	2	6	21	0	0	0	0	0	0	0	0	7
Total	55	96	27	96	11	92	12	63	11	85	15	88	131
Rotavirus strains not covered by the monovalent vaccine													
G2P[4]	1	2	0	0	0	0	6	32	2	15	2	12	11
G2P[6]	0	0	1	4	0	0	1	5	0	0	0	0	2
Total	1	2	1	4	0	0	7	37	2	15	2	12	13
Mixed and non-typeable rotavirus strains													
Mixed	1	2	0	0	0	0	0	0	0	0	0	0	1
Not typed	0	0	0	0	1	8	0	0	0	0	0	0	1
Total	1	2	0	0	1	8	0	0	0	0	0		2
Grand total	57		28		12		19		13		17		146*

CHBAH = Chris Hani Baragwanath Academic Hospital, MP = Mapulaneng and Matikwana Hospitals, EdH = Edendale Hospital, RCCH = Red Cross Children's Hospital, KBH = Kimberley Hospital and Pelonomi Hospital.
*Genotyping data from Dr George Mukhari Hospital is missing for 2015 (n=22).
*Two rotavirus-positive specimens from Polokwane Hospital were genotyped G9P[8]

Table 6: Annual predominant rotavirus genotype compared to the predominant genotypes circulating in each site by year. The strains in each site that differ from the predominant genotype are shaded grey.

Year	2009	2010	2011	2012	2013	2014	2015
Predominant genotype	G1P[8] 46%	G1P[8] 22%	G12P[8] 48%	G12P[8] 41%	G2P[4] 54%	G1P[8] 30%	G9P[8] 67%
Site							
CHBAH	G1P[8] 36%	G1P[8] 35%	G12P[8] 42%	G12P[8] 27%	G2P[4] 70%	G1P[8] 38%	G9P[8] 95%
MP+MK	G1P[8] 55%	G2P[4] 28%	G12P[8] 47%	G12P[8] 47%	G2P[4] 84%	G1P[8] 35%	G9P[8] 71%
DGM	G1P[8] 58%	G2P[4] 30%	G9P[8] 36%	G12P[8] 67%	G2P[4] 88%	G1P[8] 57%	ND
EDH	ND	G1P[8] 21%	G12P[8] 65%	G8P[4] 50%	G2P[4] 83%	G2P[4] 36%	G9P[8] 67%
RCCH	ND	G12P[8] 40%	G12P[8] 54%	G2P[4] 62%	G9P[8] 77%	G2P[4] 75%	G3P[8] 37%
KBH	ND	ND	ND	ND	ND	ND	G3P[8] 62%
PLH	ND	ND	ND	ND	ND	ND	G3P[8] 47%

CHBAH = Chris Hani Baragwanath Academic Hospital, MP = Mapulaneng and Matikwana Hospitals, EdH = Edendale Hospital, RCCH = Red Cross Children's Hospital, KBH = Kimberley Hospital and Pelonomi Hospital.

Discussion

The South African rotavirus seasons in 2014 and 2015 were lower than the comparatively high season of 2013. These annual fluctuations are considered normal after rotavirus vaccine introduction. In fact, the average rotavirus detection rates in the US vary between 10% in high years and 4% in low years.[5] In addition, certain regions in the US have reported differences in rotavirus prevalence between high and low years of up to 12%.[5] These increases are, however, substantially below pre-vaccine rotavirus levels and the absolute numbers of hospitalized rotaviruses cases has decreased.

Analyses of the timing of the rotavirus seasons suggest that the start of the season shifts later in four year cycles, returning to an earlier start during a comparatively high year. Furthermore, rotavirus seasons are shorter (15-21 weeks; 2010-2012, 2014, 2015) in low seasons compared to high seasons (25-28 weeks; 2009 and 2013).

The age group with the highest detection rate for rotavirus amongst diarrhoea patients <5 years remained in the children 7-9 months old age group, even in low years although the absolute numbers of cases tends to decrease. Children within this age group should have been vaccinated and factors including reduced vaccine effectiveness, incomplete protection against certain genotypes and the presence of concomitant enteric pathogens may contribute to the development of diarrhoeal disease. Despite the success of the rotavirus vaccine in reducing diarrhoea in children <2 years, protection is not complete and healthcare providers are encouraged to prepare for the annual rotavirus season by educating mothers with children < 2 years of age on the signs of dehydration in children with diarrhoea and ensuring adequate supplies of oral rehydration solution and intravenous fluids.

The current genotyping results continue to demonstrate that strain replacement after the introduction of the rotavirus vaccine is not present. Various rotavirus genotypes continue to circulate in an annual or biannual manner with an inability to predict the predominant genotype from one year to the next. An interesting observation was that the genotypes circulating in the

Western Cape site are often different from those circulating in the rest of the country. The season in the Western Cape also starts earlier (March/April) compared to the rest of the country (May). These results indicate that the predominant genotype may not always be circulating across South Africa and a proportion of rotavirus-positive specimens from a site without sentinel surveillance should be genotyped to detect any challenges associated with incomplete protection.

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SENTINEL SURVEILLANCE OF HUMAN PAPILLOMAVIRUS GENOTYPES AMONG PATIENTS ATTENDING PUBLIC HEALTHCARE FACILITIES IN SOUTH AFRICA, 2014-2016

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Executive summary

Cervical cancer is the third most common cancer in South African women. Human papillomavirus **(**HPV) is one of the most common sexually transmitted infections and the mucosal types are grouped into high-risk, probable high-risk and low-risk according to their link with cancer. HPV-16 and -18 are associated with approximately 70% of cervical cancer cases. The objectives of this surveillance programme are to determine the prevalence of HPV infection and to identify individual HPV genotypes including genotypes targeted by HPV vaccines among young women attending public health care facilities in South Africa. For the period 2014-2016, single or multiple HPV infections were detected in approximately half of participants, a third of whom were characterised as high risk for cancer. HPV infection was found to be significantly higher among women who reported sexual debut at ≤16 years compared to those >16 years at sexual debut. The overall high HPV prevalence among young women, in particular HPV-16 and HPV-18 which are associated with majority of cervical cancers, is of concern. The high prevalence of HPV types targeted by Gardasil-9 HPV encourages use of this vaccine as it targets a larger number of HR-HPV types that cause cancer and genital warts. The high prevalence of HPV types targeted by current HPV vaccines suggests that young South African women would greatly benefit from these vaccinations.

Introduction

Cervical cancer is the third most common cancer in South African women, with age standardized incidence of 21.67 per 100 000 (95% CI: 21.06-22.27) for 2011 according to the National Cancer Registry which collects statistics for histologically diagnosed cancers in South Africa. Human papillomavirus (HPV) is one of the most common sexually transmitted infections and in women its prevalence peaks during adolescence, soon after sexual debut, and decreases with age. HPV mucosal types are grouped into high-risk (HR), probable high-risk and low-risk (LR) according to their link with cancer. HPV-6 and -11 are associated with genital warts, while HPV-16, -18, -31, -33, -52, -56 and -58 are associated with cervical cancer. Both HPV-16 and -18 are associated with approximately 70% cervical cancer cases.[1]

There are currently three vaccines registered to prevent HPV infection, namely Cervarix targeting HPV-16 and -18; Gardasil targeting HPV-6, -11, -16 and -18; and Gardasil-9 targeting HPV-6, -11, -16, -18, -31, -33, -52, -56 and -58. In South Africa, the National school-based HPV vaccination programme in public schools uses a two dose Cervarix schedule. As part of South Africa's HPV vaccination strategy it is important to have baseline data on HPV in teenagers and young women so that the impact of vaccination in the long term can be assessed.[2] The objectives of this surveillance programme are to

determine the prevalence of HPV infection and to identify individual HPV genotypes including genotypes targeted by HPV vaccines among young women attending public health care facilities in South Africa.

Results

Study population

Study participants were recruited from public health clinics in Gauteng (Alexandra clinic), Mpumalanga (Hluvukani and Kabokweni clinic), KwaZulu Natal (Phoenix and East boom clinic), Eastern Cape (Gqebera clinic) and North West province (Jouberton clinic). The median age at first sex for the study participants was found to be 17 years (IQR, 16-18 years). All participants were heterosexually active and the majority were African. The use of condoms during their last sexual act was reported by 44.7% women. Vaginal discharge, lower abdominal pain or genital ulcers were reported in 30.9%, 15.3% and 6.6% of these women respectively.

Overall HPV prevalence

Overall HPV infection was detected in 57.6% (190/330) of participants. Of these, single HPV infection was detected in 23.0% (76/330) while multiple (2-14) HPV infection was detected in 34.5% (114/330). HR-HPV infection was detected in 37.9% (125/330) women, probable HR-HPV infection in 15.5% (15/330) and LR-HPV infection in 40.0% (132/330, Table 1). HPV infection was found to be significantly higher among women who reported sexual debut at ≤16 years compared to those >16 years at sexual debut (68.5% 76/111; 52.9% 92/174 P=0.001 respectively).

Prevalence of HPV types targeted by HPV vaccines

The genotype distribution was as follows: 5.5% women were infected with HPV-6, 3.9% with HPV-11, 7.0% with HPV-16, 6.1% with HPV-18, 2.1% with HPV-31, 1.2% with HPV-33, 3.0% with HPV-52, 3.0% with HPV-56 and 7.6% with HPV-58 (Figure 1). A proportion of 11.5% (38/330) were infected with one or more HPV types targeted by Cervarix HPV vaccine (HPV-16/18), 19.1% (63/330) with one or more HPV type(s) targeted by Gardasil HPV vaccine (HPV-6/11/16/18) and 29.4% (97/330) with one or more HPV type(s) targeted by Gardasil-9 HPV vaccine (HPV-6/11/16/18/31/33/52/56/58, Figure 2).

Table 1: Human papillomavirus (HPV) prevalence in young women (N=330) attending family planning clinics in South Africa, 2014-2016. Risk groups refer to probable links to cancer.

HPV infection	n	%
Any HPV	190	57.6
Single HPV infection	76	23.0
Multiple HPV infections	114	34.5
High-risk HPV infection	125	37.9
Probable high-risk HPV infection	51	15.5
Low-risk HPV infection	132	40.0

Any HPV infection: HPV infection with any of the 37 HPV types detected by Roche Linear Array HPV genotyping assay.
Single HPV infection: Infection with one HPV type.
Multiple HPV infections: infection with more than one HPV types.
High-risk HPV infection: infection with HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58 or -59.
Probably high-risk HPV infection: Infection with HPV-26, -53, -66, -67, -68, -70, -73 or -82.
Low-Risk HPV infection: Infection with HPV-6, -11, -40, -42, -54, -55, -61, -62, -64, -69, -71, -72, -81, -83, -84, -89 (HPV-CP6108) or -IS39.

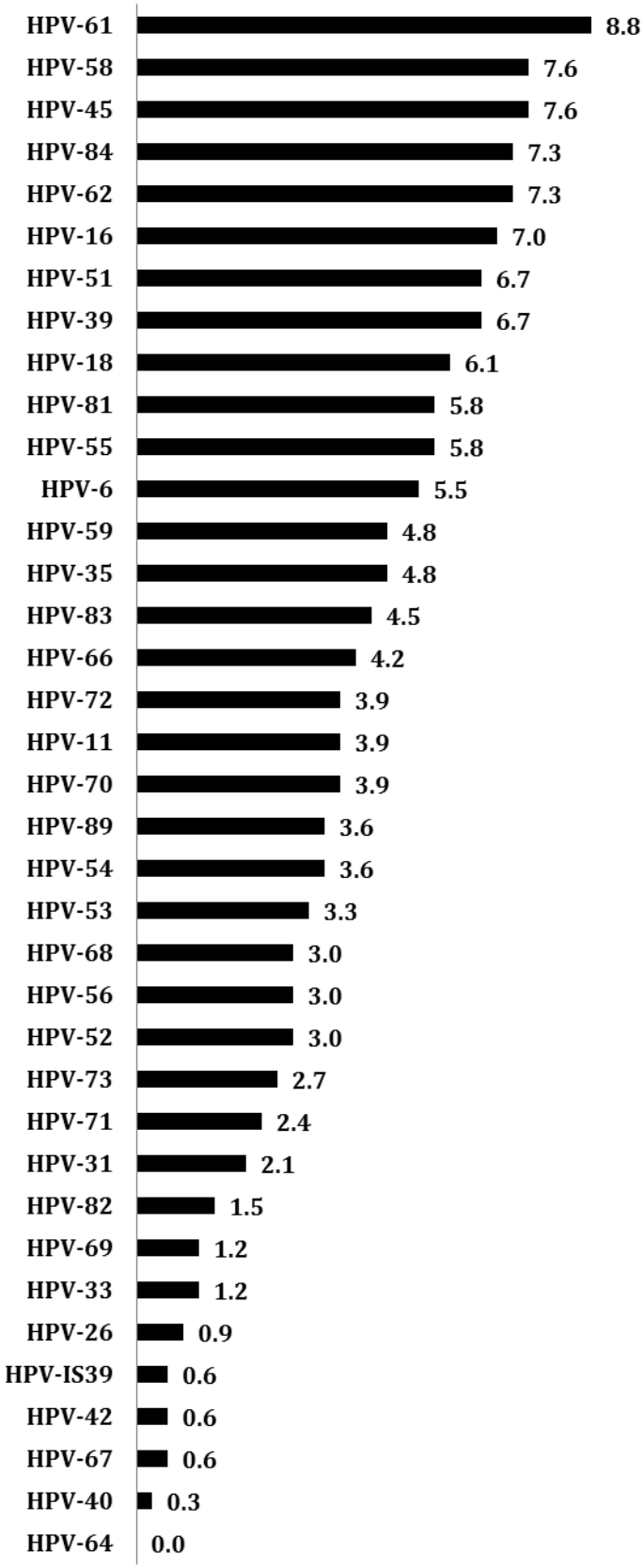

Figure 1: The prevalence of individual human papillomavirus (HPV) genotypes in young women attending family planning clinics in South Africa, 2014-2016.

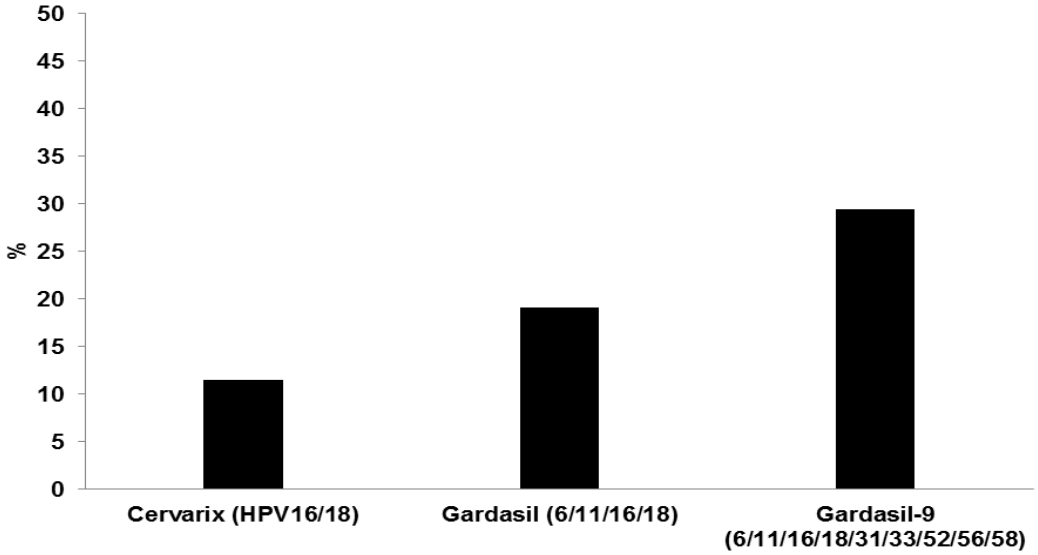

Figure 2: The prevalence (percentage) of human papillomavirus (HPV) types targeted by the Cervarix, Gardasil and Gardasil-9 vaccines in women attending family planning clinics in South Africa, 2014-2016.

Discussion and conclusions

The overall high HPV prevalence (58.2%) among young women attending family planning clinics, and in particular HPV-16 and HPV-18 which are associated with majority of cervical cancers, is of concern. The observed high HPV prevalence among women who reported sexual debut at ≤16 years confirms the importance of HPV vaccination in younger age groups in order to protect against acquisition of infection.

The high prevalence of HPV types targeted by Gardasil-9 HPV encourages use of this vaccine as it targets a larger number of HR-HPV types that cause cancer and genital warts. With high vaccine coverage, Gardasil-9 may protect against approximately 90% of cervical cancer cases. Coverage of more than 90% was achieved in 2014 when the South Africa National Department of Health introduced a national school-based HPV vaccination programme in public schools for girls aged 9-10 years. These findings encourage continued large scale roll-out of HPV vaccination to South African girls and the setting up of catch-up vaccinations in older age groups in the hope of reducing HPV prevalence and associated disease in South Africa. The high prevalence of HPV types targeted by current HPV vaccines suggests that young South African women would greatly benefit from these vaccinations.

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LEGIONNAIRES' DISEASE: NICD RECOMMENDATIONS FOR DIAGNOSIS, MANAGEMENT AND PUBLIC HEALTH RESPONSE

Compiled by the Centre for Respiratory Diseases and Meningitis,
National Institute for Communicable Diseases (NICD) of the
National Health laboratory Service (NHLS)

Version 1.2, 9th March 2016

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Guideline review:
Version 1.1: Submitted to NHLS Microbiology expert working group and Legionella Action Group

Quick Reference Guide – Legionellosis

Legionellosis case definitions:

A confirmed case of Legionnaires' disease:

- Any person with clinical/radiological evidence of pneumonia **AND** isolation of *Legionella* spp. from a clinical specimen, detection of *L. pneumophila* serogroup 1 antigen in urine, or *L. pneumophila* serogroup 1 specific antibody response.

A probable case of Legionnaires' disease:

- Any person with clinical/radiological evidence of pneumonia **AND** detection of *Legionella* spp. nucleic acid in a clinical specimen, or *L. pneumophila* non-serogroup 1 or other *Legionella* spp. specific antibody response.

Diagnosis of Legionnaire's disease:

For a patient with suspected Legionnaires' disease the following specimens should be collected: Urine and sputum or other lower respiratory tract specimen (bronchoalveolar lavage, tracheal aspirate, pleural fluid or lung tissue).

Diagnostic tests include:

1. Urinary antigen test (urine specimen) – detects *L. pneumophila* serogroup 1
2. Culture (sputum or respiratory specimen) – detects all *Legionella* spp.
3. Polymerase chain reaction (sputum or respiratory specimen) – detects all *Legionella* spp.

Treatment of Legionnaire's disease

Patients with Legionnaires' disease require early treatment with a macrolide or fluoroquinolone antibiotic.

- Recommended duration for antimicrobial therapy is 7 to 10 days, and up to 21 days for immunosuppressed patients.
- Beta-lactam antibiotics are not effective.

Environmental assessment for Legionella *spp*.

An environmental assessment includes environmental sample collection for laboratory testing.

- Emergency and long-term remediation measures may be recommended.
- Continuous, thorough and routine maintenance and treatment is required to prevent growth of the Legionella bacteria.

Public health response to Legionnaire's disease

Legionellosis is a notifiable condition. If a confirmed **OR** probable case is detected:

1. The clinician must notify the District CDC, and complete Form GW 17/5
2. The District CDC must investigate the case through completion of a case-investigation form (CIF).
3. The District CDC should inform the NICD and forward all available documentation
4. The diagnostic laboratory or microbiologist should inform the attending clinician and NICD and submit specimens and isolates to NICD.

If a cluster (≥2) of cases of Legionnaires' disease with epidemiological links is identified during a 12 month period, an outbreak investigation and environmental assessment will be conducted.

Notification of cases and additional support:

- Laboratory support:

  National Institute for Communicable Diseases, Centre for Respiratory Diseases and Meningitis: Nicole Wolter
  011-555-0352 / nicolew@nicd.ac.za ,

  or after-hours,
  NICD doctor-on-call 082 883 9920:

- Public health support and notification of cases:

  Notify the Provincial Communicable Diseases Control Officer,

  or the NICD Outbreak Response unit
  011-555-0542 / outbreak@nicd.ac.za.

Introduction

Legionellosis, or disease caused by bacteria from the genus *Legionella* is a notifiable condition in South Africa. Infection is acquired from inhalation of contaminated aerosols. Infection with *Legionella* commonly may present with a spectrum of illness ranging from asymptomatic, to severe pneumonia (Legionnaire's Disease (LD)), often requiring hospitalization. The disease has a case-fatality ratio of 10-15%[1].

These guidelines have been drawn up to assist with the diagnosis, management and public health response to Legionnaires' disease in South Africa.

Microbiology

Legionnaires' disease is caused by the gram-negative bacterium *Legionella*. More than 50 species of *Legionella* have been described, however only approximately 20 have been associated with disease in humans[1]. *Legionella pneumophila* serogroup 1 accounts for the majority of clinical cases, causing up to 90% of laboratory-diagnosed cases in the US and Europe[1;2]. Whereas in other parts of the world, such as Australia, *L. longbeachae* (found in compost and potting soil) is predominant. Data on the prevalence of *Legionella* species are limited in South Africa. *Legionella* bacteria are ubiquitous and exist in natural water sources such as lakes and streams, although transmission is predominantly associated with warm man-made water systems which provide the 3 conditions needed for transmission: heat (20˚C to 45˚C), stasis and aerosolisation. Potential sources of infection include[3]:

- Hot and cold water systems
- Cooling towers and evaporative condensers
- Spa pools / natural pools / thermal springs
- Fountains / sprinklers
- Respiratory therapy equipment
- Potting soil / compost
- Car washes
- Water-cooled machine tools

Epidemiology of Legionnaires' disease

Legionnaires' disease may present in three epidemiological scenarios[3]: 1) as an outbreak of 2 or more cases following a spatial and temporal exposure to a single source, 2) as a series of independent cases in an area in which it is highly endemic, or, 3) as sporadic cases without any obvious temporal or geographical grouping. The majority of cases of LD are isolated and sporadic. Illness can occur any time of the year, however it occurs more commonly in the summer and early autumn seasons.

LD may be community-acquired, however it is more commonly associated with nosocomial transmission (hospital-associated LD) and travel.

Immunocompromised patients in health-care settings are at increased risk of developing Legionnaires' disease if exposed to contaminated water, whereas the complex water systems of large buildings are more prone to *Legionella* contamination.

Globally, *Legionella* spp. account for 2-5% of community -acquired pneumonia (CAP) cases in adults and are rarely detected in children[1;2]. However, Legionnaires' disease is considerably underdiagnosed and underreported. It is estimated that less than 5% of cases are reported to public health authorities through passive surveillance. *Legionella* spp. are more commonly associated with sporadic disease, however may cause outbreaks. In the United States (US), between 8,000 and 18,000 people are hospitalized with Legionnaires' disease each year. This, however, is likely to be an underestimate as most infections are not diagnosed or reported[4].

Data on the epidemiology of Legionnaires' disease in South Africa are limited. In a recent study of syndromic pneumonia surveillance at two sentinel sites in South Africa from June 2012 through September 2014, *Legionella* spp. were detected in 21 (1.2%) of 1805 cases. This study reported that community-acquired LD in South Africa occurs predominantly in chronically ill adults with HIV and/or TB infection, and the majority of cases are not diagnosed and are sub-optimally treated[5]. Hospital-acquired and travel-associated cases of Legionnaires' disease have been reported in South Africa. As in other parts of the world, the prevalence of Legionnaires' disease in South Africa is underestimated due to a lack of clinical index of suspicion and request for testing by clinicians who generally treat empirically for CAP, inadequate diagnostic tests and limited surveillance programs[1].

Legionnaires' disease may be classified into the following three categories based on the source of exposure[6]:

- Travel-associated case: a case that has a history of spending at least one night away from home, either in the same or different country, in the two weeks before onset of illness
- Nosocomial case: a case that stayed or spent time (e.g. as an outpatient) in a hospital or healthcare facility in the two weeks before onset of illness
- Community-acquired case: a case with no history of overnight stays outside of the home or hospital admission or association with a healthcare facility in the two weeks before onset of illness

Pathogenesis, pathology and transmission

Legionella pneumophila is a facultative intracellular bacterium that can invade human macrophages and can also replicate inside amoebae, which can serve as a reservoir for *L. pneumophila*, as well as provide protection from environmental stresses, such as chlorination[1;7].

Legionnaires' disease is usually acquired through the respiratory system by the inhalation of air droplets that contain *Legionella* bacteria. An aerosol is formed from tiny droplets that can be generated by spraying the water or bubbling air into it. More rarely, aspiration of contaminated water has been the cause of disease. Human-to human transmission is not common, and only one probable case has recently been reported[8]. After inhalation, symptoms usually commence within 2 to 10 days, but may commence up to 3 weeks after exposure.

Although *Legionella* bacteria are ubiquitous in the environment, they rarely cause disease. A combination of factors are required for disease to develop: (i) presence of a virulent strain in a water source (or soil in the case of *L. longbeachae*), (ii) means for dissemination (aerosolisation) of the bacteria, (iii) environmental conditions allowing the survival an inhalation of an infectious dose of the bacteria, (iv) a susceptible host[1;2]. Once the bacteria enter the lung, they are phagocytosed by alveolar macrophages, multiply within the macrophage which leads to death of the macrophage and releases large numbers of bacteria into the extracellular environment. These bacteria are then re-phagocytosed by macrophages, resulting in intracellular multiplication of the bacteria within the alveoli of the lung[1;7] .

Clinical presentation and risk factors

Host risk factors for LD include those that result in decreased local or systemic cellular immunity and those that increase the chances of exposure to an infectious aerosol or microaspiration of contaminated water[1]. Recognised personal risk factors include the following: Older age (≥50 years), male gender, chronic underlying disease including diabetes and heart or lung disease,

HIV and TB, high alcohol intake, current or past history of heavy smoking, immunosuppression / immune system disorders such as organ transplant recipients or persons receiving chemotherapy.

Environmental risk factors include activities that increase the chances of exposure to contaminated water include recent overnight travel, use of well water in the home, recent plumbing work within the home, disruptions of water supply resulting in "brown" water at the tap, using an electric water heater, use of or proximity to a spa pool, living in close proximity to a cooling tower, or being near decorative fountains[1;7]. In addition, nosocomial exposures include delivery of *Legionella*-contaminated water (through tap water filled or rinsed nebulizers, humidifiers, ventilator tubing, nasogastric feedings or lavages) into the respiratory tract.

Legionellosis is associated with two clinically and epidemiologically distinct illnesses; Legionnaires' disease (LD) and Pontiac fever. Legionnaires' disease is a relatively uncommon form of pneumonia, which has a high case-fatality rate of 10-15% (up to 30%). Symptoms include flu-like illness (high fever, muscle aches, headaches), followed by a dry cough and progression to pneumonia[7]. Approximately 20-50% of people with LD may also present with diarrhoea, and approximately 50% may show signs of mental confusion. If not treated, the symptoms normally worsen rapidly and may result in respiratory failure, shock, multi -organ failure and death. Situations suggesting LD include[1;7]: 1) Gram's stains of respiratory samples revealing many polymorphonuclear leukocytes with few or no organisms; 2) the presence of hyponatremia, 3) pneumonia with prominent extrapulmonary manifestations (eg. diarrhoea, confusion, other neurologic symptoms), 4) failure to respond to administration of beta-lactams, aminoglycoside antibiotics, or both.

Pontiac fever is a non-pneumonic illness also caused by *Legionella* bacteria[1;7]. It has a shorter incubation period of 12-48 hours, presents as a mild flu-like illness, and lasts up to a few days. The illness is self-limiting, and no antibiotic treatment is necessary for this illness.

Laboratory diagnosis of Legionnaires' disease

Legionnaires' disease presents with an acute consolidating pneumonia, which can be radiologically and clinically indistinguishable from other aetiological causes of pneumonia[1;7]. Therefore laboratory investigations must be carried out to obtain a diagnosis. The following patients should be tested for LD[4]:

1) Patients with pneumonia who have failed empiric antibiotic therapy; 2) Patients with severe pneumonia, in particular those requiring intensive care; 3) Immunocompromised individuals with pneumonia; 4) Patients with pneumonia in the setting of a legionellosis outbreak; 5) Patients who have travelled away from their home within two weeks before the onset of illness; 6) Patients suspected of health-care associated pneumonia.

Specimen collection

For a patient with suspected Legionnaires' disease the following specimens should be collected:

- Urine specimen for antigen testing
- Sputum specimen (as this disease presents with a dry cough, the sputum may need to be induced), or
- Other respiratory samples such as, bronchoalveolar lavage, tracheal aspirates, pleural fluid or lung tissue (trans-bronchial biopsy) for detection of the organism by culture or PCR.

A nasopharyngeal specimen (in transport medium such as Cary Blair, Universal transport medium, or

Primestore molecular transport medium) may be collected if a sputum specimen cannot be obtained, although this is not recommended. If the NP specimen tests positive for *Legionella* spp., the result will confirm the diagnosis however, due to the low sensitivity of the specimen type a negative test result does not exclude *Legionella* infection. Oropharyngeal swabs are not recommended for the diagnosis of LD.

Urine and respiratory specimens should be collected in a sterile container. Specimens should be immediately refrigerated at 2-8˚C after collection and transported to the laboratory on ice or ice-packs in a cooler box. If possible for lower respiratory tract specimens, freeze the specimens after collection and transport to the laboratory on dry-ice.

Diagnostic tests and specimen types

Table 1 lists diagnostic tests for LD, and the appropriate specimen types on which they should be performed[1;7]. The most commonly used diagnostic test for LD is the detection of *Legionella* antigen in a urine specimen during the acute phase of illness. The urinary antigen test (UAT – *in vitro* rapid immunochromatographic assay) is rapid and inexpensive, although it only detects the most common strain of *Legionella*, *Legionella pneumophila* serogroup 1. As the urinary antigen test only detects *Legionella pneumophila* serogroup 1, a negative test does not exclude Legionnaires' disease.

The "gold standard" diagnostic method remains culture from a respiratory specimen, which enables strain characterisation[1]. Culture is an important test as it allows for comparison of strains from environmental and clinical sources, as well as the identification of less common strains[4]. Investigations of outbreaks of Legionnaires' disease rely on a comparison of environmental and clinical isolates. For culture, the specimen should be cultured on buffered charcoal yeast extract (BCYE) agar containing 0.1% α-ketoglutarate with L-cysteine and incubated at 35˚C in a humidified (sealed plastic bag), 2.5% CO_2 atmosphere. Most isolates grow within 3-5 days. However, a negative result is only released after 7-10 days of incubation.

More recently, real-time PCR on respiratory specimens is also used. PCR is able to detect all species of *Legionella*. However the disadvantage is that no culture isolate is available for comparison with environmental strains.

Table 1: Diagnostic tests and specimen types for the diagnosis of Legionnaire's disease

Test	Specimen	Species identified	NHLS and private laboratories offering testing
Urinary antigen	Urine	*Legionella pneumophila* serogroup 1	NHLS Groote Schuur CRDM, NICD NHLS Infection Control Lab Some private laboratories
Culture	Sputum /Other lower respiratory tract samples	*Legionella pneumophila* serogroup 1 *Legionella pneumophila* serogroups 2-14 *Legionella* spp.	NHLS Infection Control Lab CRDM, NICD
PCR	Sputum / Other lower respiratory tract samples	*Legionella* spp. *Legionella pneumophila* serogroup 1 *Legionella longbeachae*	CRDM, NICD Some private laboratories

Case definitions

In order to appropriately investigate a case, or outbreak of LD, the following case definitions are proposed for the South African setting. Clinical and laboratory criteria are listed in Table 2[3;4;6].

A probable case of LD: Any person meeting the clinical criteria AND at least one laboratory criteria for a probable case

A confirmed case of LD: Any person meeting the clinical criteria AND at least one laboratory criteria for a confirmed case

Table 2: Clinical and laboratory criteria for the diagnosis of Legionnaire's disease.

Clinical criteria for the diagnosis of LD:	Clinical or radiological evidence of pneumonia
Laboratory criteria for the diagnosis of LD:	
A confirmed case of LD requires at least one of the following:	• Isolation of *Legionella* spp. from a respiratory specimen or any normally sterile site • Detection of *Legionella pneumophila* serogroup 1 antigen in urine • *Legionella pneumophila* serogroup 1 specific antibody response (fourfold or greater rise in specific serum antibody titer)
A probable case of LD requires at least one of the following:	• Detection of *Legionella* spp. nucleic acid in a clinical specimen • *Legionella pneumophila* non-serogroup 1 or other *Legionella* spp. specific antibody response (fourfold or greater rise in specific serum antibody titer)

Treatment of Legionnaire's disease

According to the South African guidelines for community -acquired pneumonia (CAP), empiric antibiotic therapy for CAP patients is amoxicillin, which should be replaced with co-amoxiclav if there is structural lung disease or recent antibiotic use[9]. A macrolide (usually azithromycin) should be added to beta-lactam therapy in patients with severe CAP. *Legionella* infections, which are intracellular pathogens, do not respond to β-lactam antibiotics like penicillins and cephalosporins and therefore will not be covered by empiric therapy for CAP in South Africa. Patients with Legionnaires' disease require early treatment from an appropriate range of antibiotics which can penetrate the cells such as macrolide or fluoroquinolone antibiotics[1;7]. Therefore it is important for clinician's to have a high index of suspicion for *Legionella* infection and request appropriate diagnostic tests. Recommended duration for antimicrobial therapy is 7 to 10 days but some authors recommend up to 21 days for immunosuppressed patients.

Prevention of Legionnaire's disease

The proper design, maintenance and temperature of potable water systems are the most important method for preventing the amplification of *Legionella*[7]. Hot water should be stored above 60˚C and delivered to taps above 50˚C. Cold water should be stored below 20˚C, and dead legs or low flow areas eliminated. There are currently no vaccines available for the prevention of Legionnaires' disease, and prior infection does not necessarily prevent reinfection[1].

Public health response to Legionnaires' disease

The majority of Legionnaires' disease cases are isolated and sporadic. Outbreaks are commonly associated with buildings or structures that have complex water systems, such as hotels, hospitals and cruise ships. The most likely sources of infection include water used for drinking and showering and air-conditioning cooling towers.

Prompt notification of public health authorities of any suspected or confirmed case is critically important for

detecting epidemics of the disease. Legionnaires' disease is a notifiable condition in South Africa.

Response to a single case of Legionnaires' disease

If a confirmed or probable case is detected the following steps should be taken at least within 3 days of diagnosis:

1. The clinician must notify the District Communicable Diseases Co-ordinator (CDC), and complete Form GW 17/5
2. The District CDC must investigate the case through completion of a case-investigation form (CIF). This will require an interview with the patient or close relative to identify potential sources of infection. The CIF can be found on the NICD web-site.
3. The District CDC should inform the NICD (Dr Kerrigan McCarthy: kerriganm@nicd.ac.za or Dr Nicole Wolter: nicolew@nicd.ac.za) as soon possible and forward all available documentation (lab results, notification, CIF) by email.
4. The diagnostic laboratory or microbiologist should inform the attending clinician and NICD (Kerrigan McCarthy: kerriganm@nicd.ac.za or Nicole Wolter: nicolew@nicd.ac.za) and submit specimens and isolates as soon as possible to the Centre for Respiratory Diseases and Meningitis (CRDM) bacteriology laboratory, National Institute for Communicable Diseases (NICD), 1 Modderfontein Road, Sandringham, Johannesburg, 2131.

It may not be necessary to conduct an environmental assessment when a single, isolated case of LD has been identified. However, the facility (the putative source of infection – the hotel or hospital or other institution) should be notified of the case to raise awareness of the risk of legionellosis so that preventative measures can be strengthened. All potential nosocomially-acquired cases of infection will be further investigated to confirm/exclude the hospital as the source of infection, and environmental sampling conducted when considered necessary.

Case definitions for nosocomial Legionnaires disease[6;7]:

- Definite nosocomial - Legionnaires' disease in a person who was in hospital or other healthcare facility for at least 10 days before the onset of symptoms.
- Probable nosocomial - Legionnaires' disease in a person who stayed or spent time (e.g. as an outpatient or healthcare worker) in a hospital or other healthcare facility for 1-9 of the 10 days before the onset of symptoms, and either became ill in a hospital associated with one or more previous cases of Legionnaires' disease, or yielded an isolate that was indistinguishable from the hospital water system at about the same time.
- Possible nosocomial - Legionnaires' disease in a person who was stayed or spent time (e.g. as an outpatient or healthcare worker) in a hospital or other healthcare facility for 1-9 of the 10 days before the onset of symptoms, in a hospital not previously known to be associated with any case of legionnaires' disease, and where no microbiological link has been established between the infection and the hospital.

Response to a cluster of cases of Legionnaires' disease

If a cluster (≥2) of cases of Legionnaires' disease with epidemiological links to a specific location, such as a hotel or health facility, is identified during a 12-month period, it becomes necessary to conduct a full environmental assessment of the specific location. The steps listed above should be followed for each case identified. The Department of Health will request an outbreak investigation which should be initiated as soon as possible (ideally within 24 hours) after the notification of 2 or more probable/confirmed cases. This may include an environmental assessment as well as environmental sample collection for laboratory testing. The number and type of samples depend on the size and complexity of the facility, as well as the location of

the reported cases. It may also be necessary to establish if additional persons who are currently or were resident at the facility have developed or are at risk for LD. A template letter in Appendix 1 may be used to inform persons of their risk.

Environmental sampling (Appendix 2) includes 1L water collection of water sources in sterile plastic bottles, as well as swabs of biofilms. At the time of collection water temperature is monitored and recorded. Samples are transported immediately to the NHLS Infection Control laboratory in Johannesburg for culture and testing. If the culture is positive, *Legionella* serogroup 1 or *Legionella* serogroups 2-14 OR *Legionella* spp. can be identified & quantified.

Based on the findings of the investigation, a final report with recommendations for remediation and control measures will be provided. Both emergency and long-term remediation measures are recommended. Emergency remediation may include heat disinfection or chemical disinfection (hyperchlorination), and should be carried out as soon as the cluster has been identified but not before samples have been collected. Remediation should be conducted in consultation with an accredited water treatment company.

There are however no permanent solutions and ongoing maintenance of water systems is necessary. Continuous, thorough and routine maintenance and treatment is required to prevent re-growth of the *Legionella* bacteria. Further follow-up environmental sampling may be recommended.

Additional information:

If you require additional information, please contact the NICD:

Kerrigan McCarthy
011-555-0542 / kerriganm@nicd.ac.za

or

Nicole Wolter
011-555-0352 / nicolew@nicd.ac.za,

or

After-hours
NICD doctor-on-call 082 883 9920.

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APPENDICES

APPENDIX 1: TEMPLATE LETTER TO INFORM INDIVIDUALS OF POTENTIAL EXPOSURE FOLLOWING RECOGNITION OF AN OUTBREAK OF LEGIONNAIRE'S DISEASE AT AN IDENTIFIED LOCATION

[Address of sender]

[Contact details of sender]

[Date of letter]

[Address of recipient]

Dear [Name of person resident in implicated facility]

Re: *Legionella* infections in [Name of institution / hotel / facility]

Legionnaires' disease has been diagnosed in a number of individuals that have previously visited **[Name of institution / hotel / facility]**

We are writing to you because you have been resident at [Name of institution / hotel / facility] and there is a chance that you may have been infected with this disease. Legionnaire's disease is an uncommon form of pneumonia caused by a type of bacterium that is found in the environment. It causes disease when it is spread through the air as a spray or vapour from a water source and droplets are inhaled. Spread from one person to another is uncommon.

The symptoms of Legionnaires' disease include a 'flu-like' illness with muscle aches, tiredness, headaches, dry cough and fever, leading on to pneumonia. Sometimes diarrhoea occurs and patients may suffer from confusion. It can be treated with antibiotics. The period between infection and symptoms developing (the incubation period) ranges from 2 to 19 days. In rare cases some people may develop symptoms as late as three weeks after exposure.

If you experience the symptoms outlined above please contact your doctor and take a copy of this letter with you.

If you require further information please see the Frequently Asked Questions document on the NICD website (www.nicd.ac.za).
Please contact us using the contact details provided below. The following international websites may be helpful

http://www.cdc.gov/legionella/index.html
http://ecdc.europa.eu/en/healthtopics/legionnaires_disease/Pages/basic_facts.aspx

Yours sincerely,

[Name of Sender]

[Email and telephone numbers of the Sender]

APPENDIX 2: PROCEDURES FOR COLLECTING WATER SAMPLES FOR LEGIONELLA DETECTION IN A HOSPITAL, HOTEL OR LARGE BUILDING

Introduction

Legionella may be found in the various water systems of a large building and the following are principles for guidance in effective sampling. Please liaise with Mr Rob Stewart (Rob.Stewart@nhls.ac.za, 011 489 8578) or Dr Teena Thomas (teena.thomas@nhls.ac.za, 011 489 9181) at the NHLS Infection Control laboratory before collecting specimens for Legionella testing.

Planning

The first task is to map the water systems in the building with the maintenance manager or a person with a working knowledge of the plumbing and air conditioning systems.
Representative samples should be taken so that the different systems in the building are all sampled.
Hot and cold water systems as well as open air conditioning systems should be sampled.
The number and frequency of sampling will be affected by the budget so it is best to start with high-risk areas first.

Sampling

A one litre sample should be taken from each sample site.
The sample should be taken immediately as the tap is opened. An immediate sample is most representative of the colonization of the outlet and gives the best indication of risk to the user.
Samples may be transported at room temperature to the testing lab, provided they will be delivered within 48 hours. If there is a likelihood of prolonged delay in transit then the samples should be placed in a cooler box with cooler bricks.

Sampling for hotels and large buildings

Take hot & cold water samples from the following areas:

- High risk areas defined as areas where one or more Legionella cases have been confirmed or areas where many people are potentially exposed.
- The tap most distant in the building from the mains inlet (cold).
- Representative samples from each wing
- Representative samples from each floor
- Cold water holding tank /s (roof tank /s)
- Central hot water tank /s or representative samples from geysers
- The tap most distant from the hot water boiler unit
- Separate buildings
- Hot water taps where the temperature does not reach 50^{o}C within 30 seconds
- Hot water taps where the flow is very slow
- Cold water taps where the temperature is too high (lukewarm)
- Showers and taps that are seldom used may be tested in a high risk area but ideally they should be flushed on a regular basis (weekly)

- Taps that have thermostatic mixing valves to regulate the temperature
- New areas of the building not utilized yet
- Any rooms or floors that have not been in use (stagnation of water)
- Decorative water features inside or outside the building
- Cooling towers from the Cooling tower pond
- Air conditioning sumps
- Jacuzzi
- Swimming pools
- Sauna
- Gym
- Ice machines
- Water stations (25 l water bottles)
- Misting devices
- Natural thermal springs and water distribution system
- Heat exchangers
- Chillers
- Pumps
- Feed tanks
- Humidifiers
- Irrigation systems
- Cistern of toilets and especially from disabled toilets

Sampling for hospitals

Take hot and cold water samples from the following areas:

- High risk areas such as: bone marrow and other transplant units, oncology & surgical unit, ICU, Renal unit, Neonatal ICU, Theatre
- The tap most distant in the building from the mains inlet (cold).
- Representative samples from each wing
- Representative samples from each floor
- Cold water holding tank (roof tank)
- Central hot water tank / representative samples from geysers
- Separate buildings
- Hot water taps where the temperature does not reach 50^{o}C within 30 seconds
- Hot water taps where the flow is very slow
- Cold water taps where the temperature is too high (lukewarm)

- Taps that have thermostatic mixing valves to regulate the temperature
- Showers and taps that are seldom used may be tested in a high risk area but ideally they should be flushed on a regular basis (weekly)
- New areas not utilized yet
- Decorative water features inside the building
- Cooling towers
- Air conditioning sumps

Temperatures

It is important to take temperatures of hot and cold water at all the sites where samples are taken. The general guideline is that cold water temperatures ≥ 20 ^{o} C and hot water temperatures ≤ 50 ^{o} C indicates conditions favourable for the amplification of Legionella organisms.

A thermometer that has been calibrated or validated against a calibrated thermometer should be used.

The tap temperature should be taken after the water has run for 60 seconds.

Beware of cross-contaminating water samples with the thermometer probe. Use 70% alcohol to disinfect the probe between samples.

Testing of environmental samples at NHLS Infection Control Laboratoty

Environmental samples may be sent to NHLS Infection Control Laboratory, Charlotte Maxeke Hospital, Johannesburg, for Legionella testing. Please contact Mr Rob Stewart (Rob.Stewart@nhls.ac.za, 011 489 8578) or Dr Teena Thomas (teena.thomas@nhls.ac.za, 011 489 9181) for more information.

Note the following guidelines:

1. The required sample size for Legionella analysis is one litre of water.
2. Sample bottles (new, unused plastic containers are suitable) may be obtained from the NHLS Infection Control services laboratory. Please do not use glass sample bottles as these are prone to breakage during transit.
3. Specimen bottles must be clearly labelled with marker pen. If a number is placed on the lid it must also be on the side of the sample container.
4. Please send a list of samples that have been dispatched in the box of samples. We cannot process samples without a request form (available on request).
5. If possible, samples should not be dispatched on a Friday or before a Public holiday or long weekend to avoid delays in transit. It is preferable if you send samples as early in the week as possible.
6. The best practice is to process samples as soon after they are taken as possible so please avoid delays in dispatching samples to us.
7. Samples should be kept at room temperature if they will arrive at the lab within 24 hours of being taken. If longer delays are anticipated or if the samples will be subjected to high temperatures during transit, then they must be transported in a cooler box with ice bricks.
8. It will take 10 – 14 days for your results to be ready.
9. Please supply the email address to which results must be sent to.
10. The name of the sender plus all contact details such as landline, cell number and email address are required on the request form.

Notifiable Medical Conditions (NMC) table coming soon (2017)

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