ENHANCED SURVEILLANCE FOR ADDITIONAL RESPIRATORY PATHOGENS, 2012-2013

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Introduction

The National Institute for Communicable Diseases (NICD) has been conducting active, prospective, hospital-based sentinel surveillance for severe acute respiratory illness (SARI) since February 2009 in three of South Africa's provinces (Chris Hani-Baragwanath Hospital (CHBH), Gauteng Province; Edendale Hospital, KwaZulu-Natal Province and Matikwana and Mapulaneng Hospitals, Mpumalanga Province. Patients were enrolled based on a standardised clinical case definition. The programme initially focused on the detection of influenza, but included testing for other respiratory viruses and *Streptococcus pneumoniae*. In June 2010, Klerksdorp-Tshepong Hospital Complex (KTHC), North West Province, was included as a new site and the case definition was expanded to include

cases with a more chronic presentation of severe respiratory illness (SRI), and patients with a clinician admission diagnosis of suspected tuberculosis (TB). In 2012, the surveillance was further enhanced at two sites (Edendale and KTHC) to include expanded testing of specimens (naso- and oropharyngeal swabs and aspirates) and collection of additional specimens (induced sputum and oral washes) from patients with SRI. Respiratory samples were tested for the following pathogens: *Haemophilus influenzae*, *Streptococcus pneumoniae, Bordetella pertussis, Mycoplasma pneumoniae*, *Chlamydophila (Chlamydia) pneumoniae*, *Legionella* species and *Pneumocystis jirovecii*. Induced sputum and oral washes were tested for the following pathogens: *P. jirovecii*, *Mycobacterium tuberculosis*, *H. influenzae*, *S. pneumoniae, B. pertussis, M. pneumoniae*, *C. pneumoniae* and *Legionella* species. In addition, influenza-like illness (ILI) surveillance at two primary health care clinics serviced by the these two enhanced sites (Edendale and KTHC) was started in 2012, and 4 additional clinics were added in 2013. A sample of individuals asymptomatic for respiratory disease was enrolled at ILI sites.

The primary objectives of the surveillance for additional respiratory pathogens were:

- To estimate the prevalence and proportion of patients with *P. jirovecii*, *M. tuberculosis*, *S. pneumoniae, B. pertussis, H. influenzae* and atypical bacterial causes of pneumonia (*Legionella species, C. pneumoniae* and *M. pneumoniae*) in HIV-infected and HIV-uninfected adults and paediatric patients hospitalised with SRI, and to describe the factors associated with positivity for these infections
- To describe the burden and aetiology of outpatient influenza-like illness in children and adults in selected sites in South Africa, in HIV-infected and HIV-uninfected populations

This report details preliminary results from the enhanced surveillance for additional respiratory pathogens from Edendale Hospital and KTHC and the associated ILI sites. From ILI surveillance sites only ILI cases are included in this report.

Methods

Hospitalised patients meeting the clinical case definition for SRI, outpatient cases meeting the case definition for ILI and healthy controls (asymptomatic individuals) were prospectively enrolled from May 2012 to June 2013. Clinical and epidemiological data were collected using standardised questionnaires. Information on in-hospital management and outcome was also collected.

Sample collection and processing

Upper respiratory tract samples (oropharyngeal and nasopharyngeal swabs in patients ≥5 years or nasopharyngeal aspirates in patients <5 years of age) were collected from hospitalised patients (SRI) and outpatients (ILI and healthy controls). Induced sputum, blood and oral washes were collected from hospitalised patients only. In patients ≥5 years, induced sputum and oropharyngeal mouth rinse were collected. In those patients where TB testing was not conducted as part of clinical care, an expectorated sputum or second induced sputum sample (in patients who could not expectorate), was collected and tested at the local laboratory for TB. In patients <5 years, the first induced sputum was tested at the surveillance site laboratory for *M. tuberculosis* using GeneXpert, and a second sample was tested at the NICD for *P. jirovecii*, *M. tuberculosis* and bacterial pathogens. Collections of induced sputum started in June 2012 and November 2012 for adult and paediatric patients respectively.

Collected upper respiratory specimens were placed in 4 ml cryovials containing virus transport medium. Oral washes and sputum were collected in universal containers. Whole blood samples were collected in EDTA-containing vacutainer tubes within 24 hours of hospital admission.

Following collection, respiratory and blood samples were kept at 4°C at the local laboratory, and were transported to the NICD on ice within 72 hours post-collection. At the start of the programme, sputum samples were transported together with the oropharyngeal/nasopharyngeal samples. From July 2013 sputum samples were stored separately at -20^{0}C at the local laboratory and transported to the NICD on dry ice on a weekly basis.

Laboratory procedures

DNA was extracted from the clinical specimens and tested for bacterial pathogens and *P. jirovecii* by real-time polymerase chain reaction (PCR).

Detection of bacterial pathogens

Induced sputum and nasopharyngeal samples were tested for *M. pneumoniae*, *C. pneumoniae, Legionella* spp. and *B. pertussis.* A specimen was considered positive for *M. pneumoniae* if the *MP181* target was detected (Ct<45), *C. pneumoniae* if the *CP-Arg* target was detected (Ct<45) and *Legionella* spp. if the Pan-Leg target was detected (Ct<45).[1] This multiplex real-time PCR assay is only able to identify *Legionella* spp., but further assays are required to identify samples to species level. Any specimen that was positive for the *MP181*, *CP-Arg* or Pan-Leg targets was DNA re-extracted and the PCR was repeated in duplicate. If there was an insufficient amount of primary specimen, the initial DNA extract was repeated in duplicate. A specimen was only reported as a positive if the PCR result was positive in at least 2 of the 3 reactions i.e. identified through two extracts. A positive result for pertussis was obtained when a specimen was positive for *IS481* and/or *ptxS*1 genes.[2] A specimen was considered negative if the organism-specific targets (*MP181*, *CP-Arg* and Pan-Leg)[1] were not detected (Ct>45) and the *RNAse P* target was positive (Ct ≤45).

Blood specimens were tested using quantitative real-time PCR for the presence of pneumococcal DNA (*lyt*A gene), and for *H. influenzae* targeting *IgA, bexA* and region II of the *cap* locus of *H. influenzae.* For lytA testing, specimens with a lytA Ct-value <40 were considered positive.[3]

Determination of TB status

TB testing at the local laboratory or at the NICD was based on the GeneXpert System (Cepheid, Sunnyvale, CA) using the cartridge-based Xpert MTB/RIF (Xpert) assay. TB microscopy for acid-fast bacilli was conducted for some patients. All induced sputum specimens were also tested for *M. tuberculosis* by culture in liquid media using BD Bactec MGIT 960 at the NICD. Positive cultures were identified as *M. tuberculosis* complex using Ziehl-Neelsen staining and antigen testing. Genotypic resistance to isoniazid and rifampicin was tested using the Hain MTBDR*plus* v2 assay. A laboratory-confirmed TB case was defined as an individual with a positive result for *M. tuberculosis* on microscopy, culture or PCR from the GeneXpert MTB/RIF test at either the local hospital or the NICD TB Laboratory.

Detection of P. jirovecii

Induced sputum, nasopharyngeal and oral wash samples were tested for the mitochondrial gene coding for the large ribosomal subunit (mtLSU) of *P. jirovecii*. Polymerase chain reaction for *P. jirovecii* detects the organism at much lower levels than do staining techniques and allows for less invasive specimens to be used.[4] However, *P. jirovecii* DNA has been found in patients with no clinical symptoms or signs of pneumonia.[5] Further studies are required to distinguish between colonisation and disease when using PCR. For our study, a specimen was considered positive if *P. jirovecii* DNA was positive on PCR. No distinction was made between true infection and colonisation.

Determination of HIV status

HIV status data was obtained from two data sources. Firstly, for some patients HIV testing was requested by admitting physicians as part of clinical management. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen for patients ≥18 months of age, and qualitative HIV PCR testing for confirmation of HIV-infection status in children <18 months of age. Secondly, for consenting patients, linked anonymous HIV PCR testing for children <18 months of age or ELISA for patients ≥18 months of age was performed using a dried blood spot or whole blood specimen.

Data management

Data management was centralised at the NICD where laboratory, clinical and demographic data from enrolled patients were recorded on a Microsoft Access database.

Ethical considerations

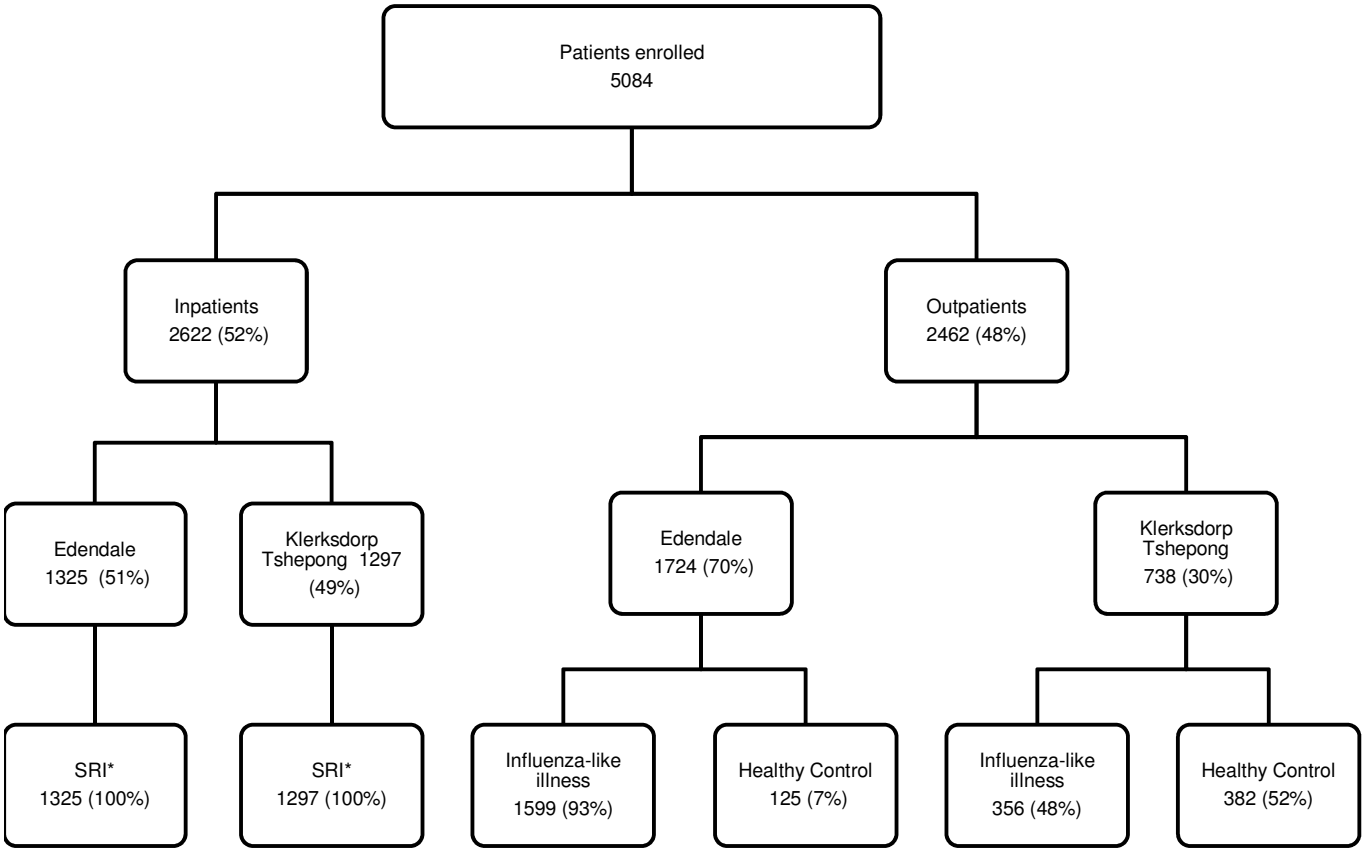
The protocol was approved by the Research Ethics Committees of the University of the Witwatersrand and University of KwaZulu-Natal.

Results

Enhanced surveillance patient enrollment

For the period May 2012 to June 2013 a total of 5084 subjects was enrolled at the two enhanced sites. Of these, 52% (2622/5084) and 48% (2462/5084) were hospitalised patients and outpatients respectively. Similar proportions of patients were enrolled from the two hospital sites, 51% (1325/2622) from Edendale Hospital and 49% (1297/2622) from KTHC. For ILI, the majority of patients (65%; 1599/2462) were enrolled from clinics that refer to Edendale Hospital (figure 1). Of the 5084 enrolled subjects, 10% (507) were healthy controls and these were not included for further analysis.

Figure 1: Numbers and proportions of patients and controls enrolled at enhanced sites for surveillance of additional respiratory pathogens, May 2012 - June 2013.



*SRI= severe respiratory illness.

Characteristics of cases with severe respiratory illness and influenza-like illness enrolled at enhanced surveillance sites

For the period May 2012 through June 2013, 4577 patients meeting the criteria for SRI and ILI were enrolled at the enhanced sites i.e. 2622 (57%) and 1955 (43%) for SRI and ILI respectively (table 1). Patient enrollment varied by age group with the highest proportion (34%, 1538/4577) drawn from the 24-44 year age group. 57% (2577/4521) of enrolled patients were female. HIV status was available for 85% (2220/2622) of patients hospitalised with SRI, of which 56% (1226/2200) were HIV infected. HIV infection varied by age group with the highest proportion that tested HIV positive (89%, 692/770) drawn from the 25-44 year age group. Of the 2425 patients admitted with SRI that had outcome data, 208 (9%) died.

Table 1: Characteristics of cases enrolled with severe respiratory infection and influenza-like illness at enhanced sites, May 2012 - June 2013, South Africa.

Characteristic	Influenza-like illness Number (%)	Severe respiratory illness Number (%)
Age Group (years)		
0-4	406/1955 (21)	878/2622 (33)
5-24	610/1955 (31)	242/2622 (9)
25-44	659/1955 (34)	879/2622 (34)
45-64	233/1955 (12)	480/2622 (18)
≥65	47/1955 (2)	146/2622(6)
Gender		
Female	1225/1905 (64)	1352/2616 (52)
Hospital/Clinic name		
Edendale Hospital		1325/2622 (51)
Klerksdorp-Tshepong Hospital Complex		1297/2622(49)
Imbalenhle/Edendale Gateway clinic	1599/1955 (82)	
Jouberton/Tshepong Gateway clinic	356/1955 (18)	
Underlying illness		
No	1910/1941 (98)	2302/2616 (88)
Outcome		
Died	0/1955(0)	208/2425 (9)

Bacterial pathogens

Bordetella pertussis

Among the 3664 patients with severe respiratory infection and influenza-like illness who were tested for bacterial pathogens, 42 (1%) were positive for *B. pertussis* of which 31 (74%) presented with SRI and 11 (26%) with ILI. The majority of cases occurred in the winter and spring months (figure 2), and cases occurred at all study sites (figure 3). The highest detection rates of *B. pertussis* were in the 25-44 (17/1230, 1.4%) and 45-64 (10/549, 1.8%) year age groups (figure 4). Cases of *B. pertussis* were detected either in nasopharyngeal specimens (30/42, 71%), or induced sputa (8/42, 19%) or in both specimen types (4/42, 10%).

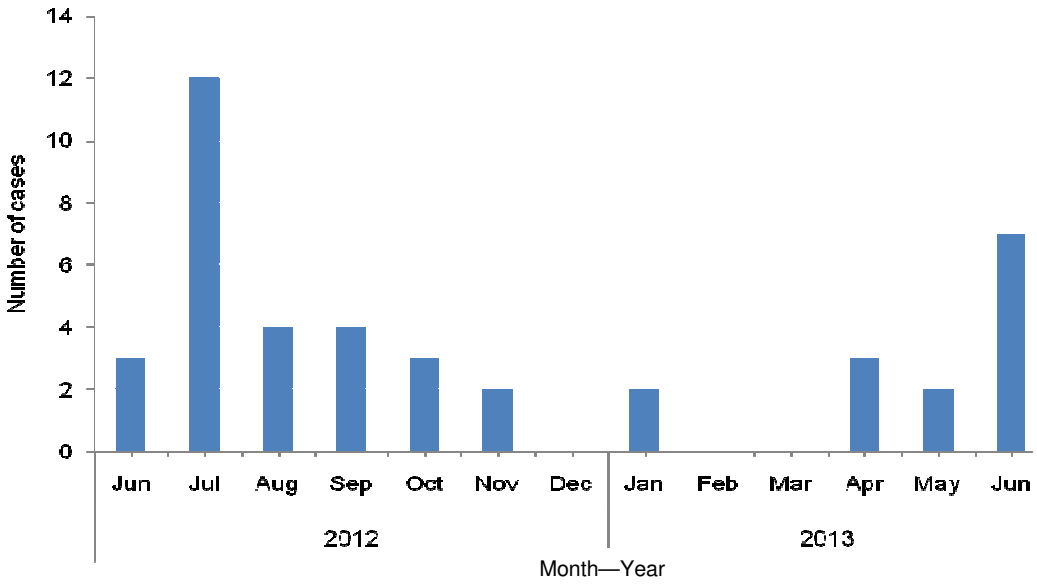


Figure 2: Numbers of cases of *Bordetella pertussis* among patients with severe respiratory infection and influenza-like illness by month and year, May 2012 - June 2013. N=42.

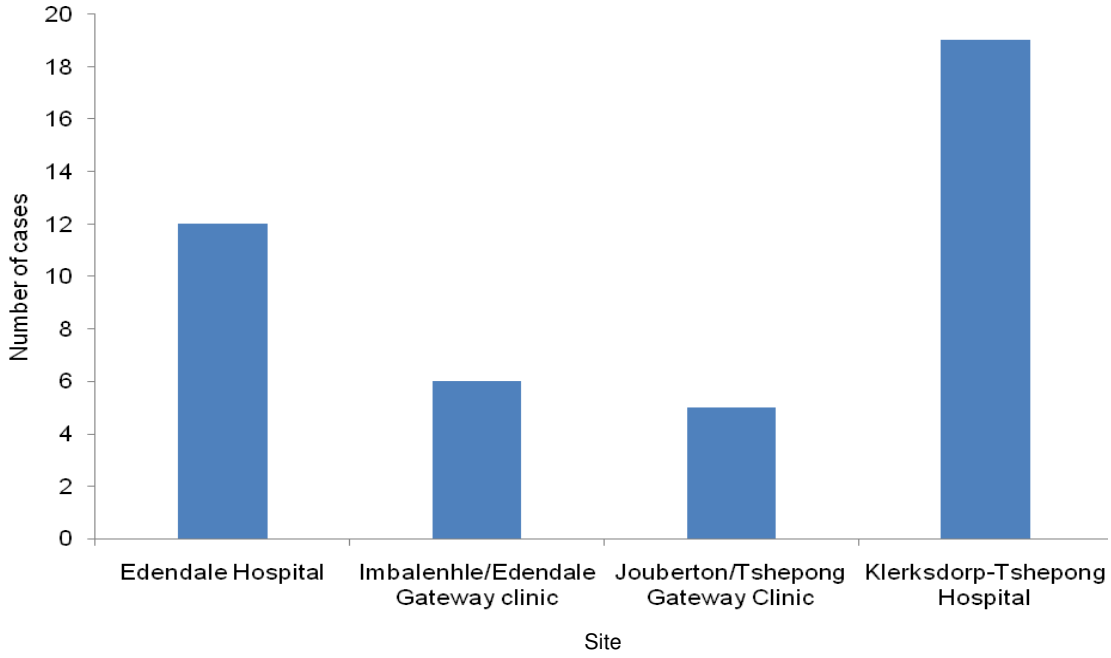


Figure 3: Numbers of cases of *Bordetella pertussis* among patients with severe respiratory infection and influenza-like illness by study site, May 2012 - June 2013. N=42.

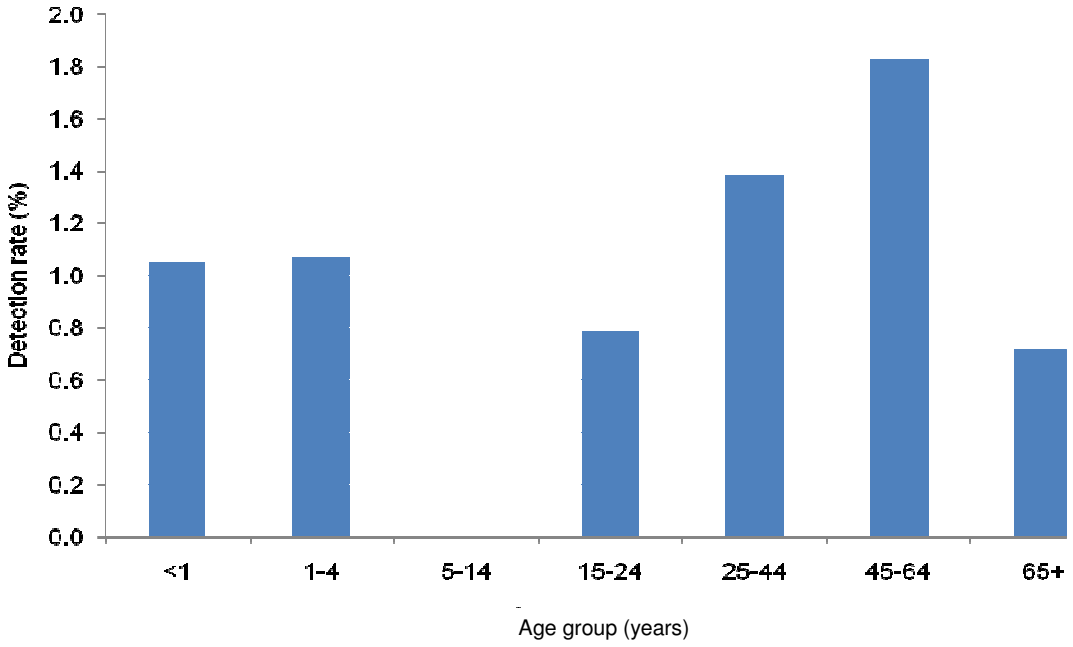


Figure 4: Detection rate of *Bordetella pertussis* among patients with severe respiratory infection and influenza-like illness by age group, May 2012 - June 2013. N=3664.

Atypical bacteria

Among SRI cases, 1964 (75%) were tested for atypical pneumonia causing bacteria. Of these, 33 (2%) were positive for *M. pneumoniae*, 18 (1%) were positive for *Legionella* spp. and 4 (0.2%) were positive for *C. pneumoniae* (figure 5). The majority of *M. pneumoniae cases* were detected at Edendale Hospital (30/33, 91%), whereas the majority of *Legionella* spp. cases were detected at Klerksdorp-Tshepong Hospital (13/18, 72%) (figure 6). The overall detection rate of *M. pneumoniae* was 2% (33/1964), with the highest detection rate in the 1-4 year age group (9/233, 4%) (figure 7).

Cases of *M. pneumoniae* were identified either in naso-pharyngeal specimens (24/33, 73%), or induced sputa (5/33, 15%) or in both specimen types (4/33, 12%). All cases of *Legionella* spp. (18/18) were detected in adult patients, 15-64 years of age, of which 17 (94%) were detected in induced sputa and 1 (6%) was detected in a nasopharyngeal specimen. For patients with induced sputum tested, the detection rate of *Legionella* spp. was 2% (17/718), with the highest detection rates in the 15-24 (2/53, 4%) and 45-64 (7/190, 4%) year age groups (figure 7).

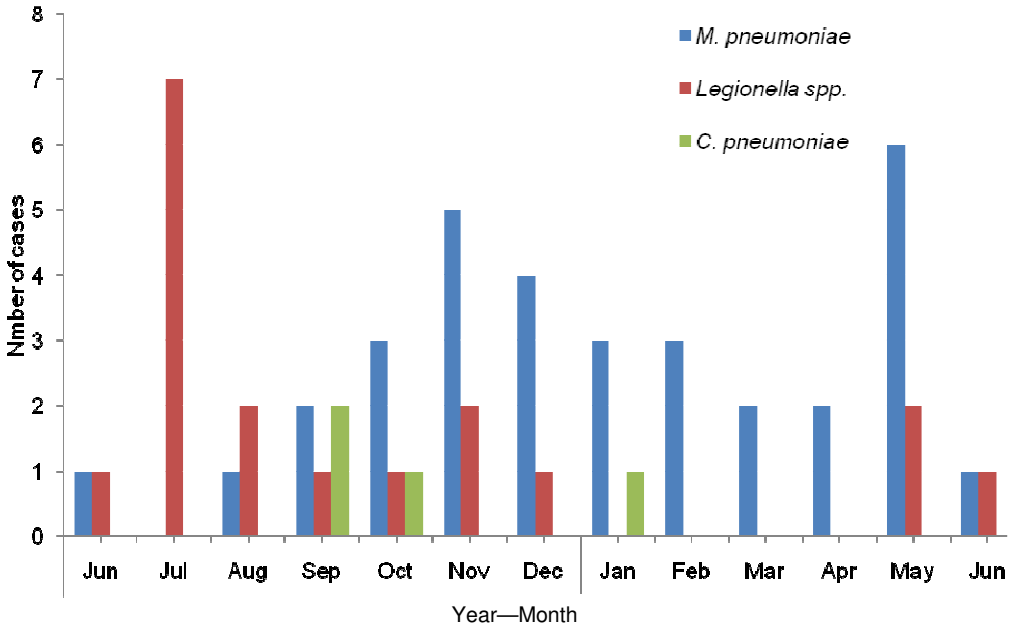


Figure 5: Numbers of cases of *Mycoplasma pneumoniae, Legionella* spp. and *Chlamydophila pneumoniae* among patients with severe respiratory infection by month and year, May 2012 - June 2013.

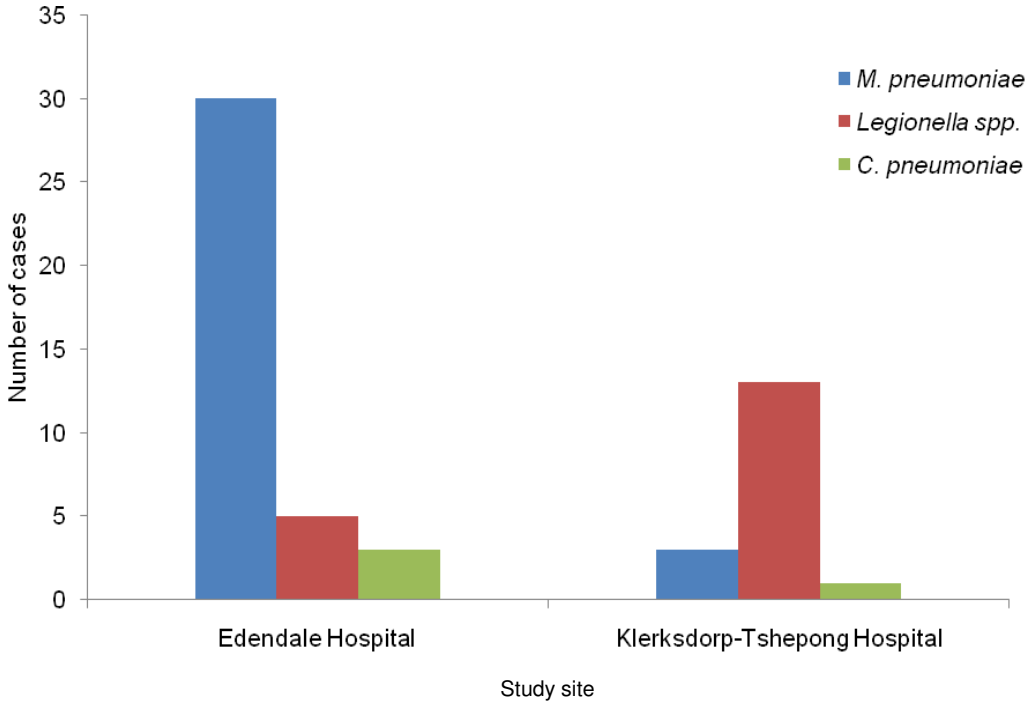
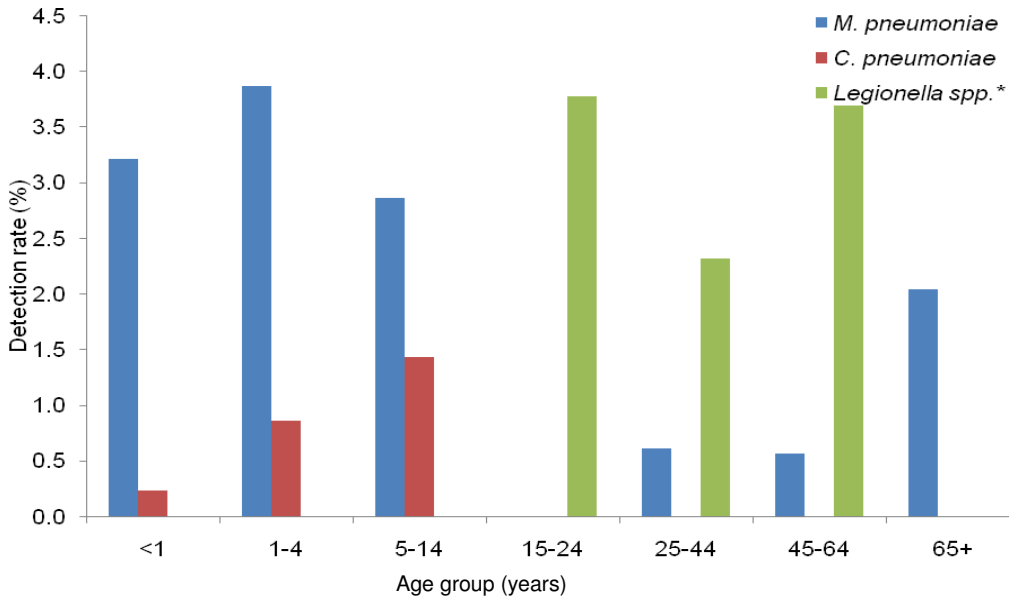


Figure 6: Numbers of cases of *Mycoplasma pneumoniae, Legionella* spp. and *Chlamydophila pneumoniae* among patients with severe respiratory infection by study site, May 2012 - June 2013.

Figure 7: Detection rate of *Mycoplasma pneumonia* (N=1964), *Legionella* spp. (N=718) and *Chlamydophila pneumoniae* (N=1964) among patients with severe respiratory infection by age group, May 2012 - June 2013.



*Detection rate for *Legionella* spp. calculated only for patients with an induced sputum specimen tested.

Streptococcus pneumoniae and *Haemophilus influenzae*

Blood specimens were tested for *S. pneumoniae* for 85% (2141/2522) of SRI patients enrolled from May 2012 to June 2013 and 202 (9%) were positive for *S. pneumoniae*. Pneumococcal infection was detected throughout the year with peaks in the winter and spring months (figure 8), and cases were distributed between the two study sites (figure 9). The overall detection rate of *S. pneumoniae* was 9% (202/2141), which ranged from 16% (13/81) in the 5-14 year age group to 8% (10/130) in the 15-24 year age group (figure 10). Of the 2160 blood specimens tested for *H. influenzae,* 47 (2%) were positive. Of these, 4 (9%) were *H. influenzae* sero-type b with 1 case in the <1 age group,1 case in the 5-14 year age group and 2 cases in the 25-44 year age group. Cases of *H. influenzae* were not seasonal (figure 8), and were detected at both study sites (figure 9). The overall detection rate of *H. influenzae* was 2% (47/2160), with the highest detection rate in the 5-14 year age group (6/85, 7%) (figure 10).

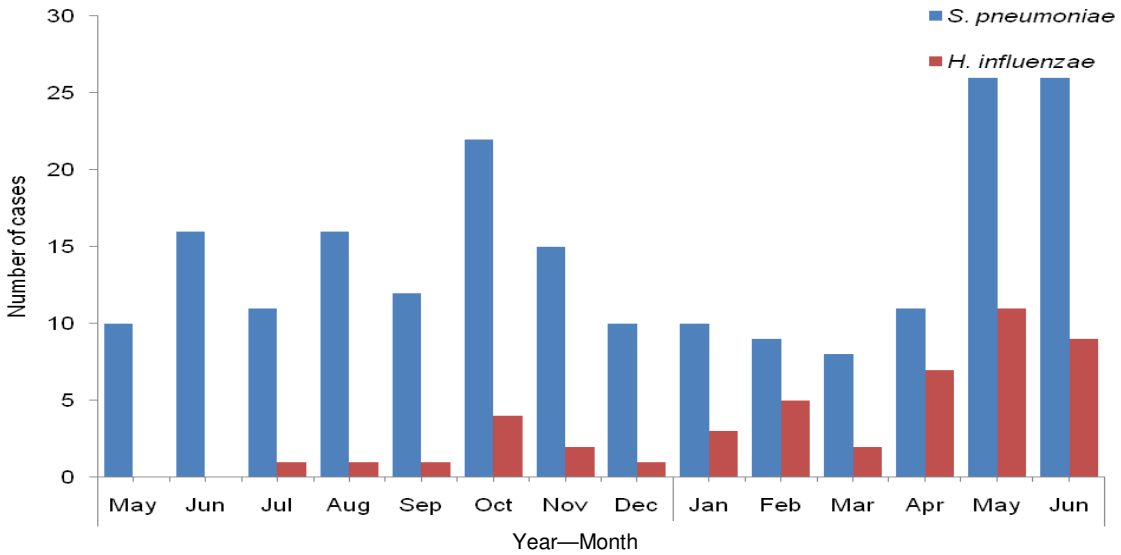


Figure 8: Numbers of cases of *Streptococcus pneumoniae* and *Haemophilus influenzae* from patients with severe respiratory infection by month and year, May 2012 - June 2013.

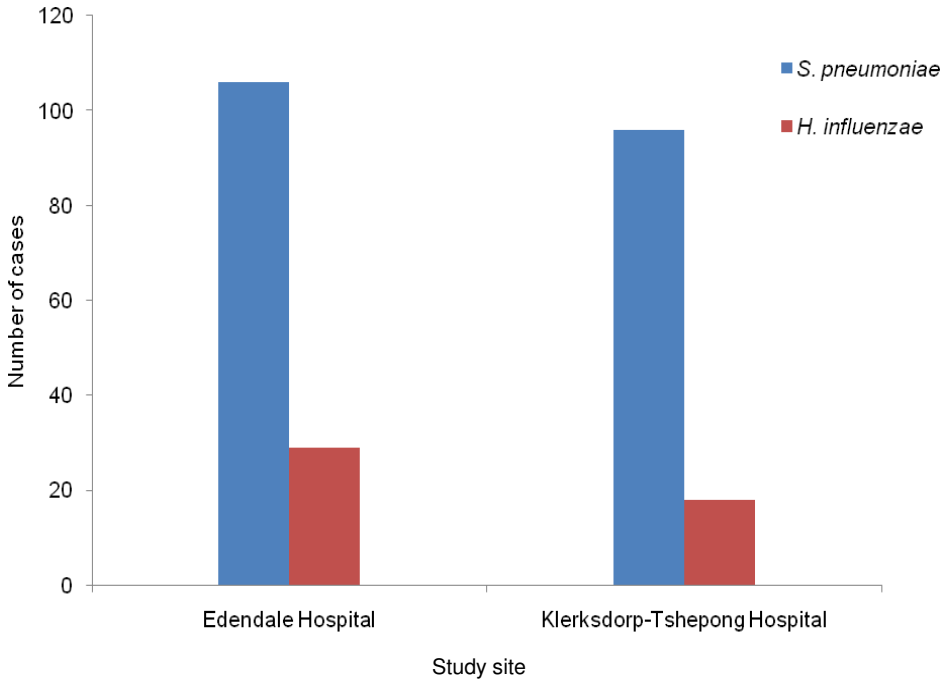


Figure 9: Numbers of cases of *Streptococcus pneumoniae* and *Haemophilus influenzae* among patients with severe respiratory infection by study site, May 2012 - June 2013.

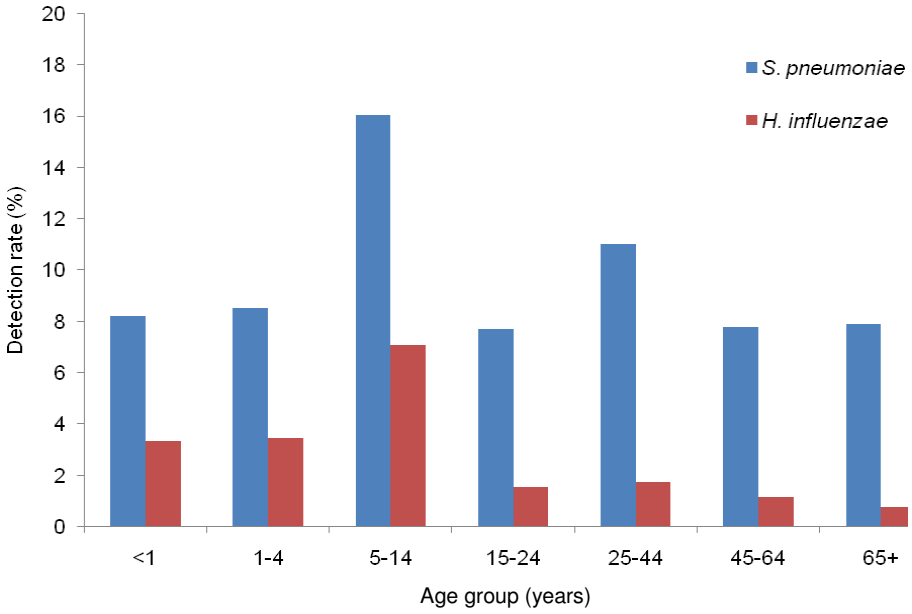


Figure 10: Detection rate of *Streptococcus pneumoniae* (N=2141) and *Haemophilus influenzae* (N=2160) among patients with severe respiratory infection by age group, May 2012 - June 2013.

Tuberculosis

For the period May 2012 through June 2013 59% (1555/2622) of patients admitted with SRI were tested for TB. Testing for TB varied by age group, with the highest proportion tested among the 25-44 years age group (693/879, 79%, p<0.001) (figure 11). Testing for TB also varied by hospital: KTHC (880/1297, 68%) and Edendale (675/1325, 51%). Onsite testing for TB was conducted for 56% (1380/2447) of SRI cases and contributed to 89% (1380/1555) of the total number of cases tested. KTHC tested a higher proportion of cases onsite (783/1200; 65%) than did Edendale Hospital

(597/1247; 48%). Of the total cases tested for TB, 69% (1073) were tested at the NICD. HIV results were available for 88% (1370/1555) of the patients tested for TB of which 66% (908) were HIV infected.

Of the 1555 patients tested for TB 25% (391) were positive. The highest percentage (217/693; 31%) that tested *M. tuberculosis* positive was in the 25-44 years age group. The detection rate for TB was higher in KTHC (254/880; 29%) than in Edendale (137/675; 20%) (figure 12). The HIV prevalence in TB positive cases was 76% (table 2).

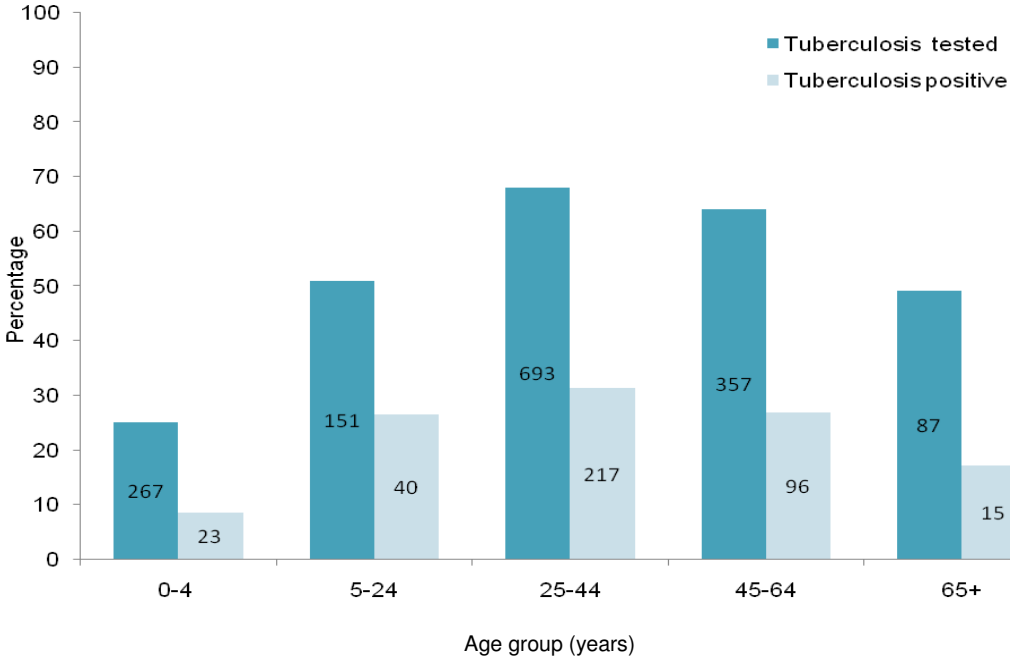


Figure 11: Proportions tested and detection rate for *Mycobacterium tuberculosis* in patients admitted with severe respiratory illness by age group, May 2012 - June 2013.

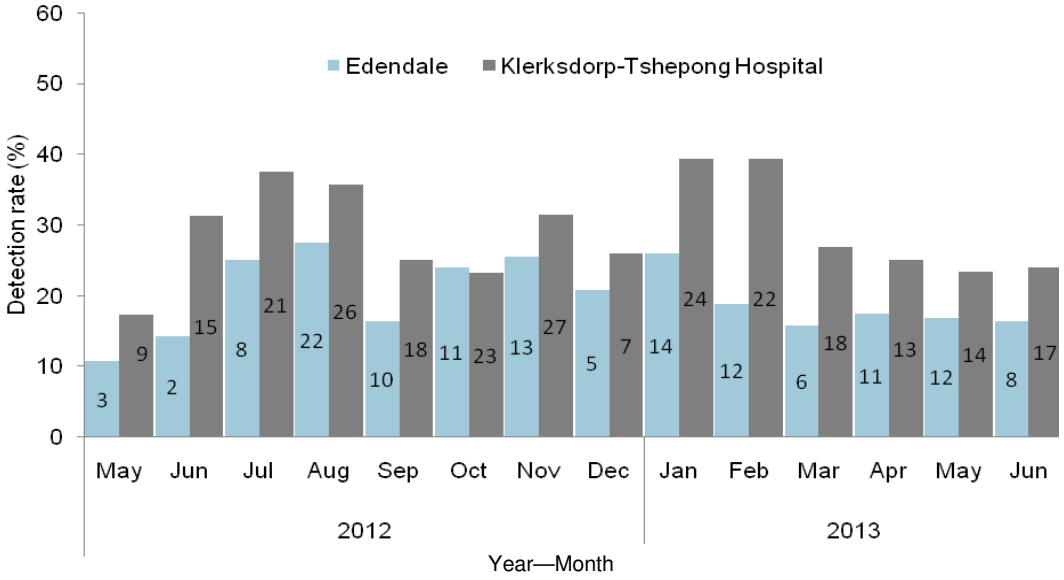


Figure 12: Detection rate of *Mycobacterium tuberculosis* in patients admitted with severe respiratory illness by site, month and year, May 2012 - June 2013.

Table 2: Characteristics of patients with severe respiratory illness who tested positive for *M. tuberculosis*, May 2012 - June 2013.

Characteristics		Laboratory confirmed tuberculosis n/N (%)
Age (years)	<5	23/391 (6)
	5-24	40/391 (10)
	25-44	217/391 (55)
	45-64	96/391 (25)
	≥65	15/391 (4)
Sites	Edendale Hospital	137/391 (35)
	Klerksdorp-Tshepong	254/391 (65)
Gender	Female	192/391 (49)
HIV status	HIV-infected	261/339 (76)
Outcome	Died	40/361 (11)

Pneumocystis jirovecii

For the period June 2012 to June 2013, 4330 specimens (oral rinse, nasopharyngeal and induced sputum) from 2233 SRI patients were tested for *P. jirovecii* (figure 13). Of the patients tested, 329 (15%) were positive for *P. jirovecii* DNA. Nasopharyngeal specimens were the most common specimen type received (2098/4330, 48%). However, induced sputum samples gave the highest detection rate (175/1060, 17%) compared to nasopharyngeal (186/2098, 9%) and oral rinse (63/1172, 5%) specimens (figure 14). The majority (126/329, 38%) of *P. jirovecii*-positive cases was in the 25-44 year age group. Among cases positive for *P. jirovecii*, the HIV prevalence was 68% (199/293) and more than two-thirds (88%, 266/302) survived (table 3).

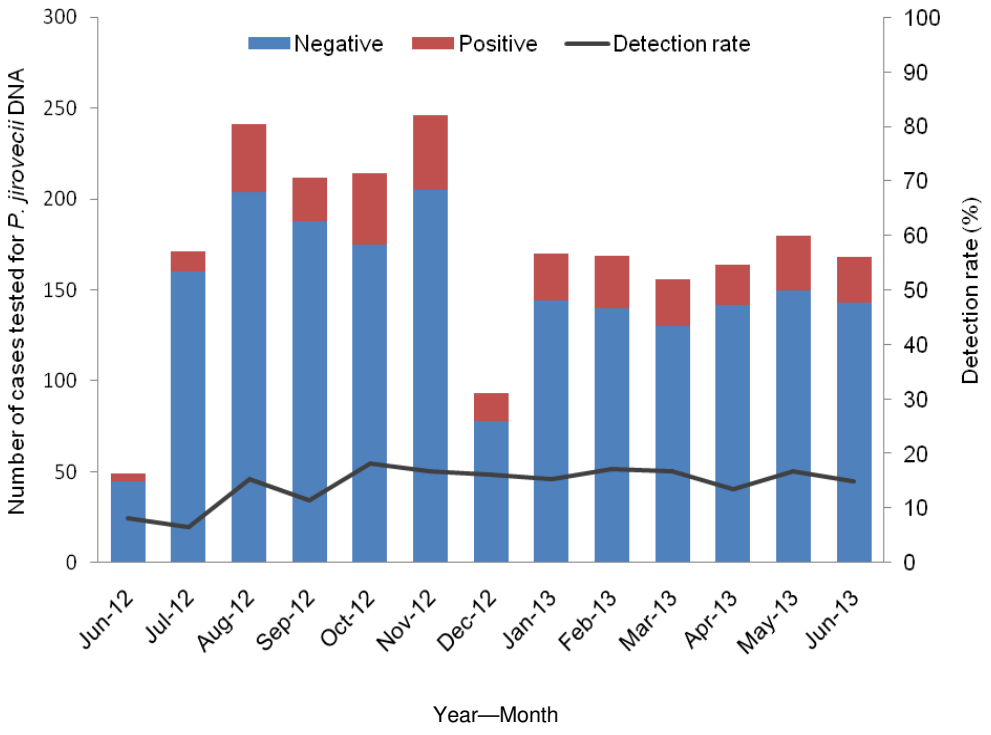


Figure 13: Numbers of cases tested for *Pneumocystis jirovecii* and detection rate by month, June 2012 – June 2013. N=2233.

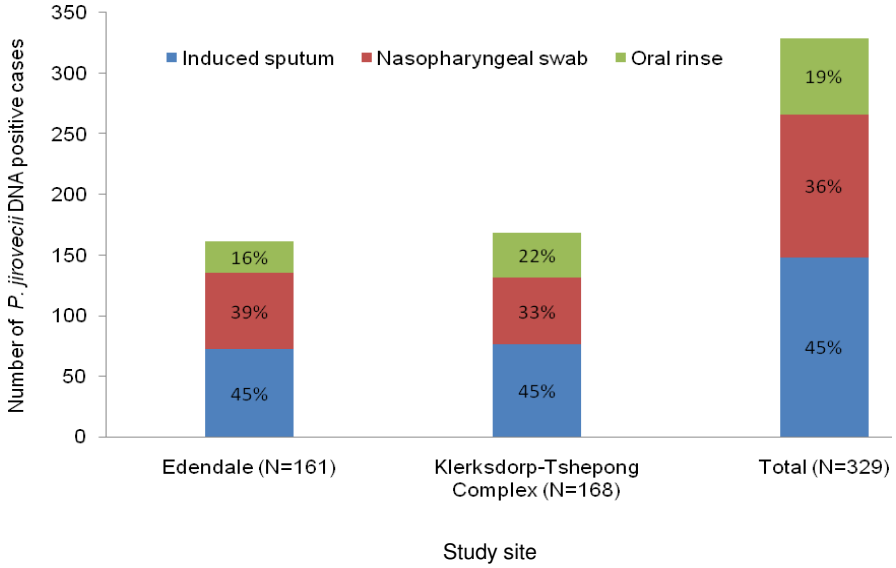


Figure 14: Numbers of *Pneumocystis jirovecii* DNA positive cases by site and specimen type, June 2012 - June 2013. N=329.

Table 3: Characteristics of *Pneumocystis jirovecii* DNA positive cases, June 2012 – June 2013.

Characteristics		**n/N (%)**
Age (years)	<5	112/329 (34)
	5-24	20/329 (6)
	25-44	126/329 (38)
	45-64	63/329 (19)
	≥65	8/329 (2)
Sites	Edendale	161/329 (49)
	Klerksdorp-Tshepong	168/329 (51)
Gender	Female	181/329 (55)
HIV status	HIV infected	199/329 (68)
Outcome	Died	36/302 (12)

Discussion

By expanding the existing respiratory surveillance to include additional pathogens common in South Africa's high HIV prevalence setting and by including surveillance for milder infections, this surveillance programme has enabled descriptions of the prevalence of additional respiratory pathogens in patients with different clinical presentations at the enhanced sites during its first year. These pathogens are not routinely tested for in the public hospital setting due to cost and the difficulty in obtaining appropriate specimens.

The proportion of community-acquired pneumonia (CAP) cases attributed to atypical pneumonia-causing bacteria differs amongst studies based on the populations studied and the methods used for identification. However, it has been reported by Bartlett et al.[6] that atypical pneumonia-causing bacteria are responsible for approximately 10- 20% of CAP worldwide. A study done in Cape Town on specimens collected from hospitalised adults from July 1987 through July 1988, using serology for identification, found that approximately 36% of pneumonia cases were due to atypical bacteria.[7] *Chlamydophila pneumoniae* was identified in 21% of cases, *Legionella pneumophila* in 9% and

M. pneumoniae in 1% of cases. This differs from the findings presented in this study, using real-time PCR for detection, in which *M. pneumoniae* was found in 2% of cases, *Legionella* spp. in 1% and *C. pneumoniae* in 0.2% of cases. The highest detection rate of *M. pneumoniae* was in children aged 1 to 4 years of age. However, it has previously been reported to cause pneumonia in patients 5-25 years of age.[6] *Legionella pneumophila* was common in adults 40-70 years of age. The data presented here indicate that all positive *Legionella* cases were detected in adult patients in the 15-64 years of age category.

During the one year period of this survey, 42 (1%) pertussis cases among patients with SRI and ILI were detected as compared to 60 pertussis cases that were previously reported nationally to the Department of Health over a period of four years (2000-2004).[8] Most clinicians do not consider pertussis in adults and although it is a notifiable condition it is seldom reported. The highest number of pertussis cases was detected in patients with SRI and the majority of cases were detected in samples from the Klerksdorp-Tshepong hospital complex. While disease was identified in all age groups, disease burden was greatest in the 25-64 year age group. Previously, disease was most prevalent in the infant and adolescent age groups, but more recent studies have demonstrated disease burden in the adolescent and adult age groups,[9,10] as indicated in the data presented here. *Bordetella pertussis* was detected in nasopharyngeal and induced sputum specimens although nasopharyngeal specimens gave the highest positive yield (71% vs. 19%).

In this survey the prevalence of TB was 25%. This is lower than that reported in a study conducted in KwaZulu-Natal which was based on the aetiology of CAP. The Kwazulu-Natal study reported *M. tuberculosis* as the commonest isolated pathogen in cases admitted with CAP. *Mycobacterium tuberculosis* was the commonest agent among CAP patients in HIV and non-HIV-infected subjects (40% and 35%, respectively).[11] From the data presented here, nine percent of children less than five years admitted with severe respiratory illness had laboratory confirmed TB. This was similar to the 8% prevalence of culture-confirmed TB reported in other studies in HIV-infected and HIV–uninfected children hospitalised for acute pneumonia.[12,13] The high prevalence of TB in patients admitted with SRI highlights the importance of early detection leading to a reduction of missed opportunities when active testing for TB, particularly in high risk groups, is conducted. Although this survey aimed to screen all SRI patients enrolled in the study for TB, only 59% of patients were tested, and testing varied by age group and site, with a higher proportion of cases tested in the older age groups. Challenges in sample collection have been reported previously, particularly in children.

In this survey the prevalence of *P. jirovecii* in HIV-infected and HIV-uninfected patients of all ages was 15%. The pathogenesis of *P. jirovecii* is not completely understood, which makes the interpretation of whether a positive PCR test is significant or not difficult.[14] Those studies based solely on colonization showed that in immunocompetent children prevalence varied between 16% to 100%, was 20% in immunocompetent adults, and varied from 20% to 43% in HIV-infected adults.[15] Differences in prevalence rates are likely due to differences in study populations, specimen type and the diagnostic tests used. In a previous South African study, the prevalence of *P. jirovecii* pneumonia in children <10 years was 10%[16] but can be as high as 49% in HIV-infected children with severe pneumonia.[17] The use of different specimen types in different subsets of the population as well as clinical factors need to be taken into consideration in order to distinguish between colonisation and true disease when PCR is used for diagnostic purposes.

Further analysis of data gathered from this surveillance programme will allow for the identification of risk groups to be targeted for interventions and to describe how co-infections with these pathogens relate to patient outcome.

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