SURVEILLANCE FOR *BORDETELLA PERTUSSIS*, ATYPICAL BACTERIAL CAUSES OF PNEUMONIA, *HAEMOPHILUS INFLUENZAE* AND *STREPTOCOCCUS PNEUMONIAE* WITHIN THE SEVERE RESPIRATORY ILLNESS SURVEILLANCE PROGRAMME, 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, initially in three of South Africa's provinces:

- Gauteng Province: Chris Hani-Baragwanath Hospital (CHBH) (this site stopped enrolling patients in December 2013)
- KwaZulu-Natal: Edendale Hospital
- Mpumalanga: Matikwana and Mapulaneng Hospitals.

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 severe respiratory illness (SRI) irrespective of symptom duration, as well as to include patients with a clinician admission diagnosis of suspected tuberculosis. 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*, *Chlamydia pneumoniae* and *Legionella* species. Induced sputum specimens were tested for the following pathogens: *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. A sample of individuals without respiratory symptoms was enrolled at ILI sites.

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

- To estimate the prevalence and proportions of patients with *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 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 presents the findings from this surveillance programme for the year 2013 for the pathogens *S. pneumoniae, B. pertussis, H. influenzae* and atypical bacterial causes of pneumonia (*Legionella species, C. pneumoniae* and *M. pneumoniae*). Data from this

surveillance programme from May 2012 to June 2013 have been previously reported.[1] Data are presented from Edendale Hospital and KTHC and the associated ILI sites. Data on individuals without respiratory symptoms are not included in this report.

Methods

Hospitalised patients meeting the clinical case definition for SRI and outpatient cases meeting the case definition for ILI were prospectively enrolled from January to December 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). Induced sputum, blood and oral washes were collected from hospitalised patients only. In those patients where tuberculosis 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 tuberculosis. 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 *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 before being 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 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).[2] 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 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 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.[3] 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.[4]

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

Characteristics of patients with severe respiratory illness (SRI) and influenza-like illness (ILI) enrolled at enhanced surveillance sites

For the period 1 January 2013 to 31 December 2013 a total of 1206 hospitalized individuals with SRI and 1,065 individuals with ILI were enrolled at the two sites.

Influenza-like illness (ILI) patients

Of the patients with ILI, 307/1,065 (29%) were <5 years, and 301/1,065 (28%) were in the age group 25-44 years (table 1). More than half the patients were female (661/1,057, 63%). The HIV prevalence in patients with ILI was 28% (264/934). The highest prevalence was in the age group 25-44 years 160/284 (56%), followed by 43% (40/94) in the age group 45-64 years (figure 1). The majority of patients with ILI were enrolled at the Edendale Gateway clinic (848/1,076, 79%).

Severe respiratory illness (SRI) patients

Most of the patients who were admitted with SRI were in the age group 25-44 years (533/1,206, 44%) and half were female (597/1,206, 50%) (table 1). The HIV prevalence in patients with SRI was 70% (752/1,081). The highest prevalence was in the 25-44 years age group (440/497, 92%) (figure 1). The KTHC site accounted for 820/1,206 (79%) of patients enrolled with SRI.

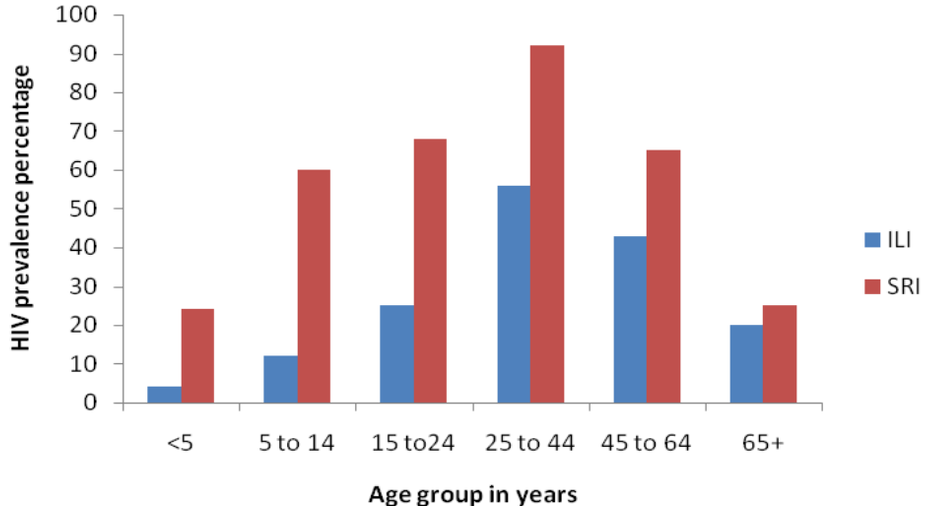

Figure 1: HIV prevalence by age group, influenza-like illness (ILI) and severe respiratory illness (SRI), at enhanced surveillance sites, South Africa, 2013.

Table 1: Characteristics of patients enrolled with severe respiratory illness and influenza-like illness at enhanced surveillance sites, South Africa, 2013.

Characteristic	Influenza-like illness n/N (%)	Severe respiratory illness n/N (%)
Age Group (years)		
0-4	307/1,065 (29)	151/1,206 (13)
5-14	192/1,065 (18)	32/1,206 (3)
15-24	150/1,065 (14)	87/1,206 (7)
25-44	301/1,065 (28)	533/1,206 (44)
45-64	104/1,065 (10)	317/1,206 (26)
≥65	11/1,065 (1)	86/1,206 (7)
Female sex	661/1,057 (63)	597/1,206 (50)
Hospital/Clinic name		
Edendale Hospital	n/a	386/1,206 (32)
KTHC	n/a	820/1,206 (68)
Edendale Gateway clinic	848/1,065 (80)	n/a
Jouberton Gateway clinic	217/1,065 (20)	n/a
HIV prevalence	264/934 (28)	752/1,081 (70)
Underlying illness	45/1,055 (4)	130/1,206 (11)
In-hospital death	n/a	130/1,148 (11)

Bordetella pertussis

ILI patients

Of the enrolled patients 81% (866/1,065) were tested for *B. pertussis*. The prevalence of *B. pertussis* was 1% (5/866). The patients were all aged <25 years (figure 2). Of the five cases, four occurred in the winter months (figure 3) and three were identified at the Jouberton clinic (figure 4). The majority were female (4/5, 80%) and 20% (1/5) tested positive for HIV.

SRI patients

Of the enrolled patients, 91% (1,101/1,206) were tested for *B. pertussis* and 13/1,101 (1%) tested positive for *B. pertussis.* Three of the 13 cases (23%) were aged <5 years, 8/13 (62%) were 25-44 and 2/13 (15%) were 45-64 years (figure 2). The HIV prevalence in *B. pertussis* patients with SRI was 75% (9/12). More than half were female 7/13 (54%) and more than half were enrolled from the Edendale site 7/13 (54%).

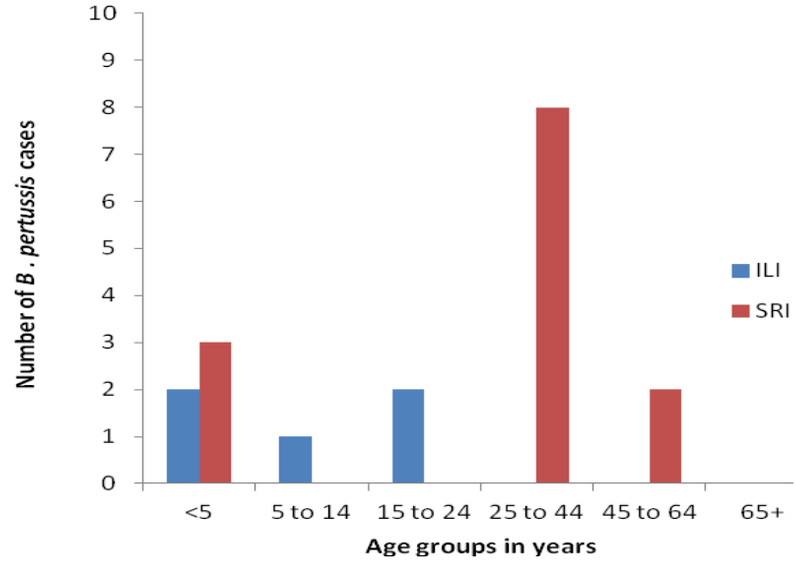

Figure 2: Detection rate of *Bordetella pertussis* among patients with severe respiratory illness (SRI) and influenza-like illness (ILI) by age group at enhanced sites, South Africa, 2013.

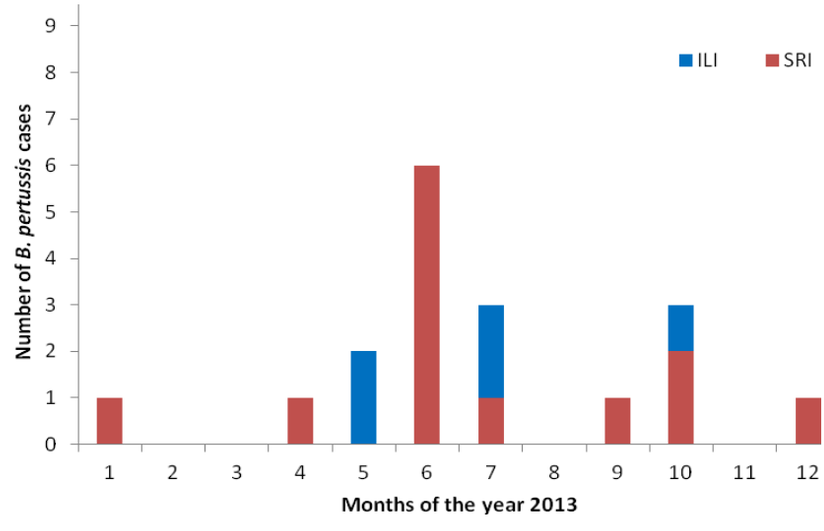

Figure 3: Numbers of cases of *Bordetella pertussis* among patients with severe respiratory illness (SRI) and influenza -like illness (ILI) by month and year at enhanced sites, South Africa, 2013.

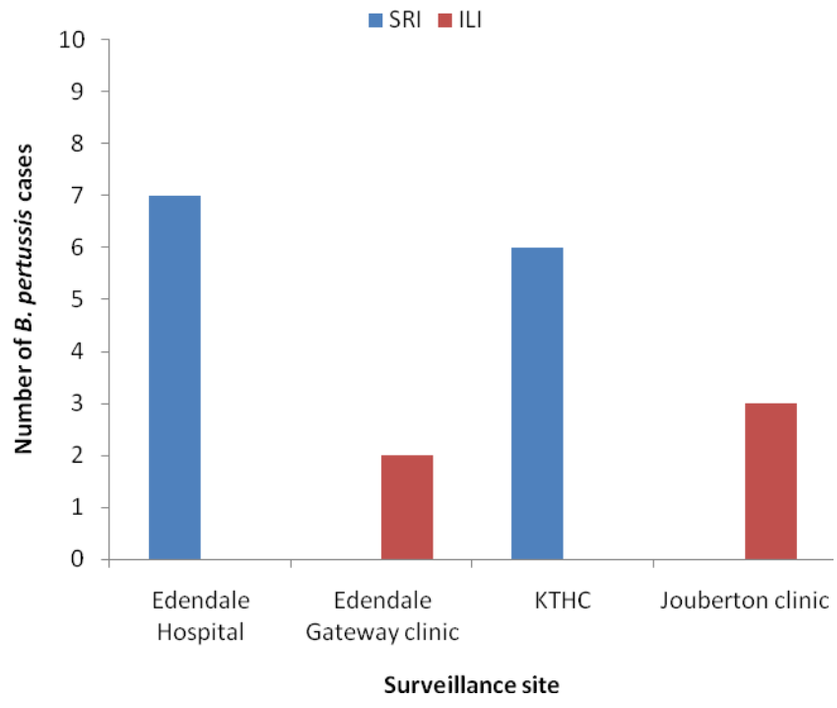

Figure 4: Numbers of cases of *Bordetella pertussis* among patients with severe respiratory illness (SRI) and influenza -like illness (ILI) by study site at enhanced sites, South Africa, 2013.

Atypical pneumonia-causing bacteria

ILI patients

Of the 1,065 patients enrolled, 863 (81%) were tested for *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. Of these, 1% (12/863) tested positive for *M. pneumoniae,* 0.3% (3/863) tested positive for *C. pneumoniae* and none tested positive for *Legionella* spp (figure 6). Cases of *M. pneumoniae* were detected all year round (figure 5), while cases of *C. pneumoniae* were detected between April and September (figure 7). Half of the *M. pneumoniae* cases were under the age of 5 years (6/12, 50%) (figure 8). Only one case was HIV infected. Two of three patients with *C. pneumoniae* were aged 5-14 years and 1 case was under the age of five (figure 10). No *C. pneumoniae* cases were HIV infected.

SRI patients

Of the 1,206 enrolled patients, 91% (1,094) were tested for *M. pneumoniae*, *C. pneumoniae* and *Legionella* spp. Among SRI cases, 21/1,094 (2%) were positive for *M. pneumoniae*, 5/1094 (0.5%) were positive for *Legionella* spp. and 1/1,094 (0.1%) were positive for *C. pneumoniae*. A third of the *M. pneumonia* SRI cases were in the 25-44 age group. The *Legionella* spp cases were all aged >15 years (figures 6 & 9). The majority of *M. pneumoniae cases* were detected at Edendale hospitals (13/21, 62%) (figure 11), whereas all five *Legionella* spp. cases were detected at KTHC hospitals and there were no cases of *Chlamydia* at KTHC (figure 12).

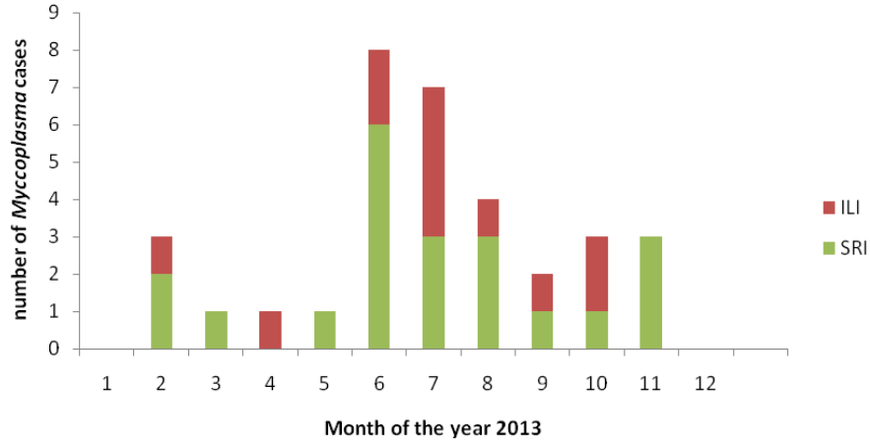

Figure 5: Numbers of cases of *Mycoplasma pneumoniae* among patients with influenza-like illness (ILI) and severe respiratory illness (SRI) by month at enhanced sites, South Africa, 2013.

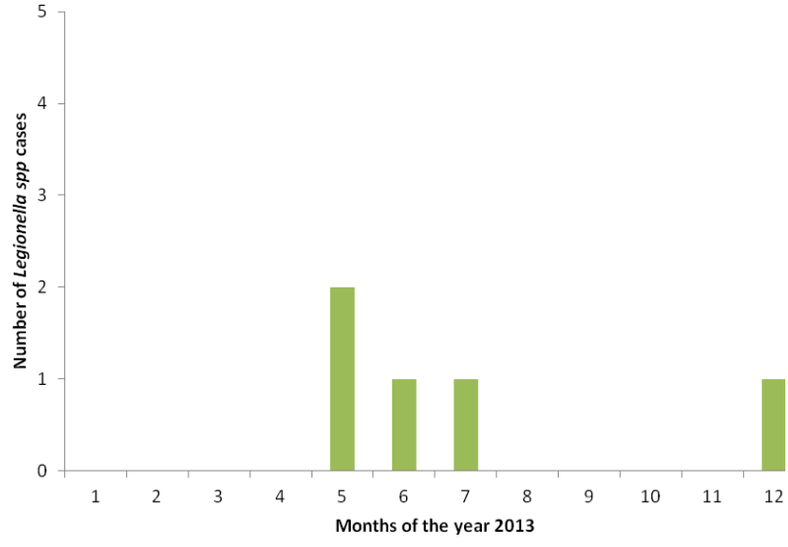

Figure 6: Numbers of cases of *Legionella* spp among patients with severe respiratory illness by month and year at enhanced sites, South Africa, 2013.

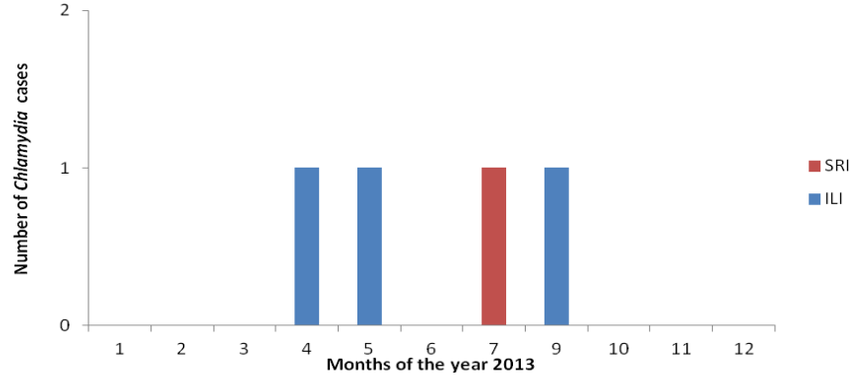

Figure 7: Numbers of cases of *Chlamydia pneumonia* among patients with influenza-like illness (ILI) and severe respiratory illness (SRI) by month and year at enhanced sites, South Africa, 2013.

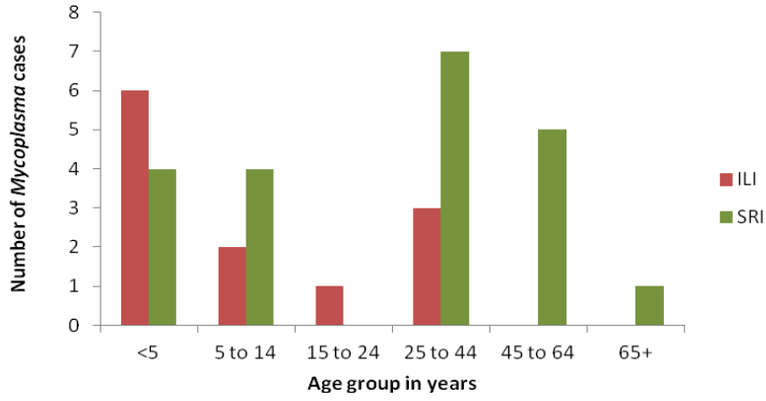

Figure 8: Numbers of cases of *Mycoplasma pneumonia* among patients with influenza-like illness (ILI) and severe respiratory illness (SRI) by age group at enhanced sites, South Africa, 2013.

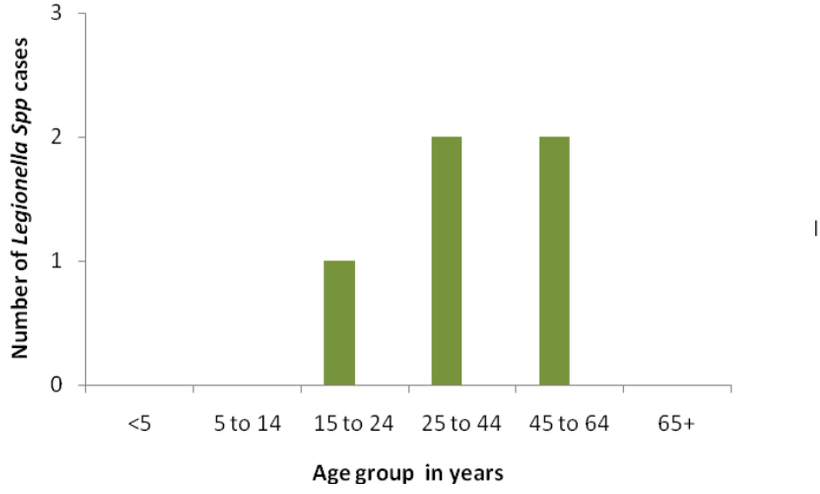

Figure 9: Numbers of cases of *Legionella spp* among patients with severe respiratory illness by age group at enhanced sites, South Africa, 2013.

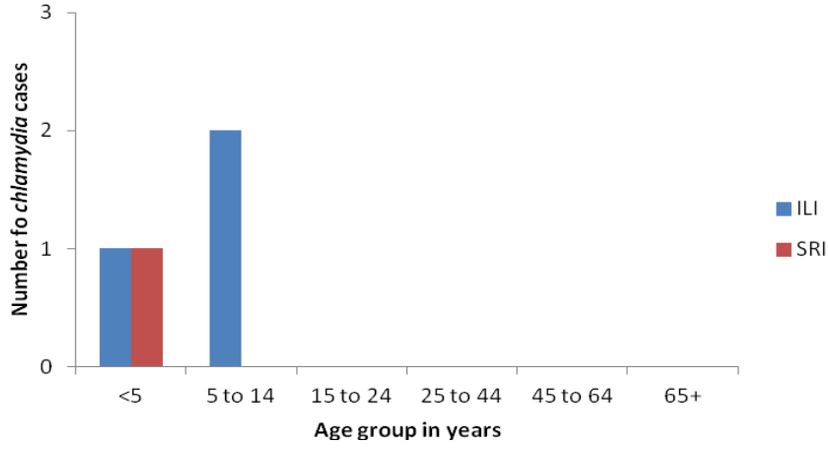

Figure 10: Numbers of cases of *Chlamydia pneumonia*e among patients with influenza-like illness (ILI) and severe respiratory illness (SRI) by age group at enhanced sites, South Africa, 2013.

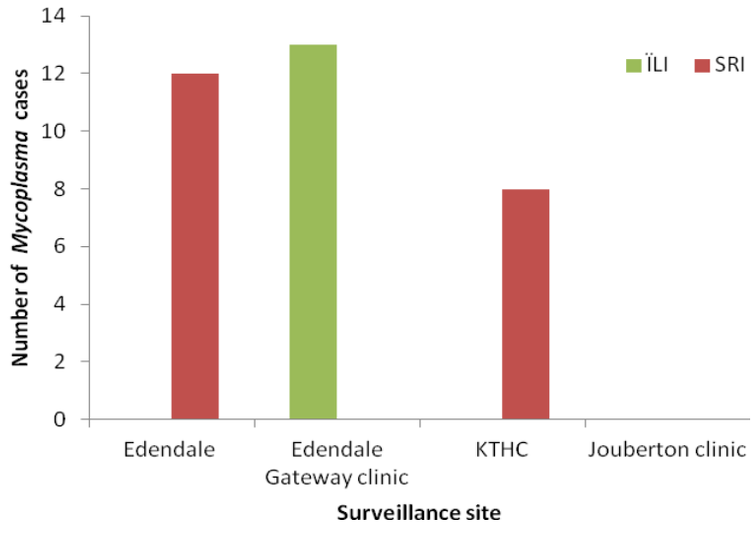

Figure 11: Numbers of cases of *Mycoplasma pneumoniae,* among patients with influenza-like illness (ILI) and severe respiratory illness (SRI) by study site at enhanced sites, South Africa, 2013

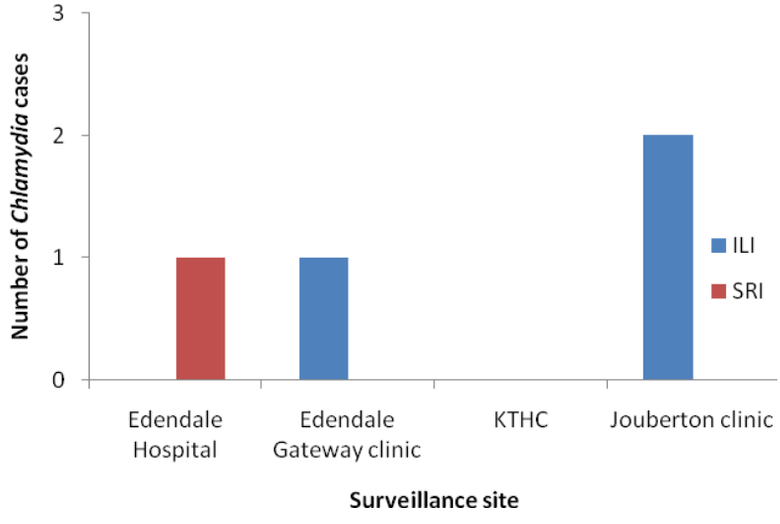

Figure 12: Numbers of cases of *Chlamydia pneumonia* among patients with influenza-like illness (ILI) and severe respiratory illness (SRI) by study site at enhanced sites, South Africa, 2013.

Streptococcus pneumoniae and *Haemophilus influenzae*

Blood specimens were tested for *S. pneumoniae* for 85% (1,637/1,961) of SRI patients enrolled in 2013 and 126 (11%) were positive for *S. pneumoniae*. Pneumococcal infection was detected throughout the year with peaks in the winter and spring months (figure 13). Cases were distributed between the two study sites (figure 14). The detection rate of *S. pneumoniae* ranged from 9% (9/96) in the <5 year age group to 13% in both the 15-24 year (11/84) and the 46-64 year (40/299) age group (figure 15). Of the 1105 SRI cases who had blood specimens tested for *H. influenzae,* 44 (4%) were positive. *Haemophilus influenzae* cases were detected throughout the year (figure 16) and at both hospitals (Edendale 21/44 (48%) and Klerksdorp 23/44 (52%) (figure 17). Of the 44 *H. influenzae* cases, three *H. influenzae* serotype b (Hib) cases were identified and all three were enrolled at the Klerksdorp hospital and were in the age group 25 to 44 years (figure 18).

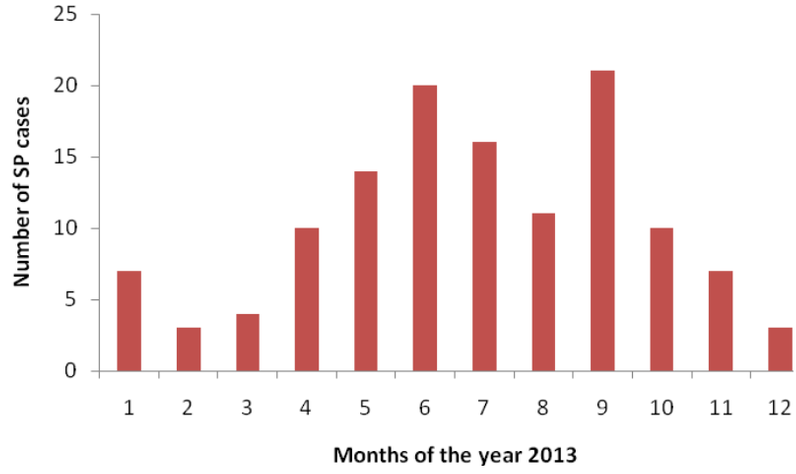

Figure 13: Numbers of cases of *Streptococcus pneumoniae* from patients with severe respiratory illness (SRI) by month and year at enhanced sites, South Africa, 2013.

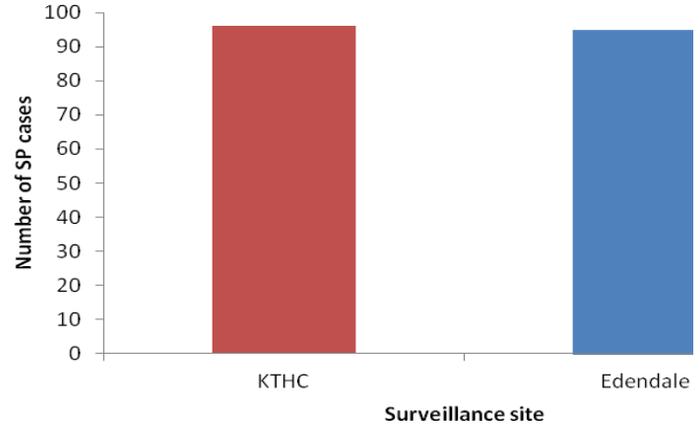

Figure 14: Numbers of cases of *Streptococcus pneumonia* from patients with severe respiratory illness by surveillance site, South Africa, 2013.

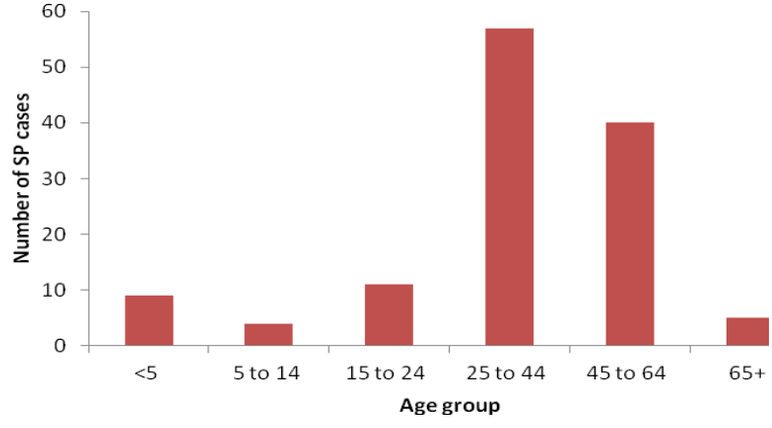

Figure 15: Numbers of cases of *Streptococcus pneumoniae* from patients with severe respiratory illness by age group at enhanced sites, South Africa, 2013.

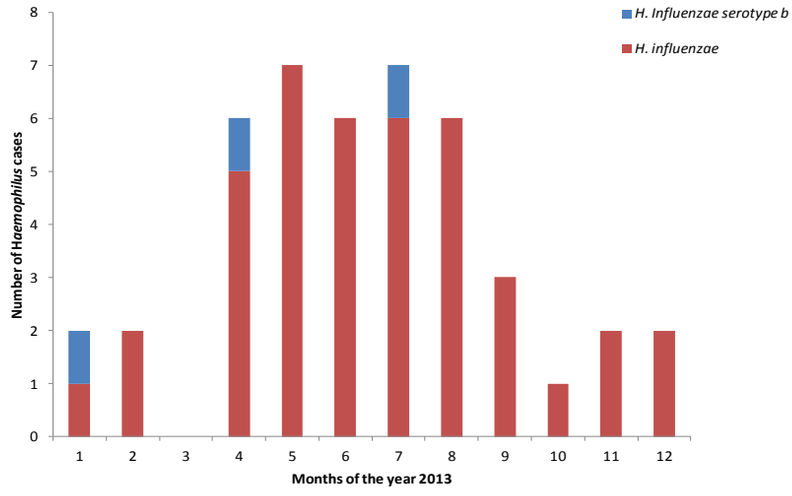

Figure 16: Numbers of cases of *Haemophilus influenzae and Haemophilus influenzae* serotype b from patients with severe respiratory illness by month and year at enhanced sites, South Africa, 2013.

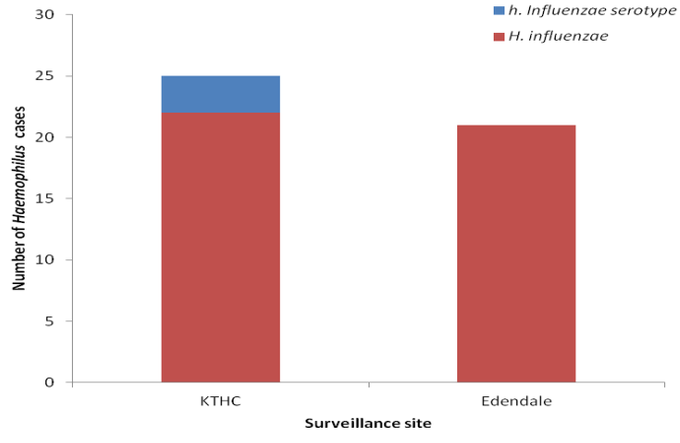

Figure 17: Numbers of cases of *Haemophilus influenzae* and *Haemophilus influenzae* serotype b among patients with severe respiratory infection by study site at enhanced sites, South Africa, 2013.

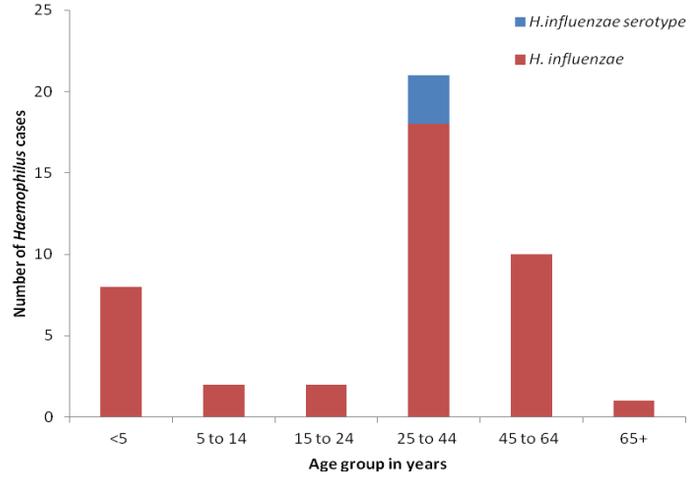

Figure 18: Numbers of cases of *Haemophilus influenzae* and *Haemophilus influenzae* serotype b among patients with severe respiratory infection by age group at enhanced sites, South Africa, 2013.

Discussion

By expanding the existing respiratory surveillance to include additional bacterial 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. These pathogens are not routinely tested for in the public hospital setting due to cost and the difficulty in getting appropriate specimens.

Atypical bacterial pathogens were uncommonly identified: *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 and all positive *Legionella* cases were detected in adult patients in the 15-64 years age category. During the one year period of this survey, 18 (1%) pertussis cases among patients with SRI and ILI were detected. 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 was similar between the two sites. While disease was identified in all age groups, disease burden was greatest in the 25-44 year age group. ILI cases were spread across the following age groups: <5, 5 to 14 and 25 to 44 years. With the change from whole-cell to acellular pertussis vaccine in the routine immunisation schedule from 2009, it is important that monitoring for a possible increase in case numbers continues. 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.

Acknowledgements

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