BMJ Open

Surveillance of influenza and other respiratory viruses in hospitalized patients from distinct regions of Brazil in 2015: Seasonality and implications for immunization schedule. Results from the Global Influenza Hospital Surveillance Network (GIHSN)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017603
Article Type:	Research
Date Submitted by the Author:	10-May-2017
Complete List of Authors:	Raboni, Sonia M; Universidade Federal do Parana, Infectious Diseases Moura, Fernanda; Universidade Federal do Ceara, Departamento de Patologia e Medicina Legal Caetano, Braulia; Fundacao Oswaldo Cruz, Laboratório de Vírus Respiratórios e do Sarampo, IOC Avanzi, Valeria; Universidade Federal do Parana, Laboratório de Virologia Pereira, Luciane; Universidade Federal do Parana, Laboratório de Virologia Nogueira, Meri; Universidade Federal do Parana, Laboratório de Virologia Vidal, Luine; Universidade Federal do Parana, Laboratório de Virologia Tavares, Isabel; Fundacao Oswaldo Cruz, Instituto Nacional de Saúde Pradel, Florence; Mérieux Foundation Picot, Valentina; Fondation Merieux Puig-Barbera, Joan; Centro Superior de Investigacion en Salud publica Siqueira, Marilda; Fundacao Oswaldo Cruz, Laboratório de Vírus Respiratórios e do Sarampo, IOC
Keywords:	influenza, Hospital admission, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

Surveillance of influenza and other respiratory viruses in hospitalized patients from distinct regions of Brazil in 2015: Seasonality and implications for immunization schedule. Results from the Global Influenza Hospital Surveillance Network (GIHSN)

Sonia M Raboni[1], Fernanda E A Moura[2], Braulia C Caetano[3], Valéria M Avanzi[1], Luciane A Pereira[1], Meri B Nogueira[1], Luine R Vidal[1], Isabel Tavares[4], Florence K Pradel[5], Valentina Picot[5], Joan Puig-Barbera[6], Marilda M Siqueira[3].

[1]Laboratório de Virologia, Hospital de Clínicas da Universidade Federal do Paraná, Brazil

[2] Departamento de Patologia e Medicina Legal, Universidade Federal do Ceará, Brazil

[3]Laboratório de Vírus Respiratórios e do Sarampo, IOC, Fiocruz, Brazil

[4]Instituto Nacional de Saúde, Fiocruz, Brazil

[5]Mérieux Foundation, France

[6]FISABIO, Spain

Corresponding author: Sonia Mara Raboni,

Hospital de Clínicas/Universidade Federal do Paraná,

180 General Carneiro Street, 3rd floor, Curitiba, Paraná, Brazil

Zip code: 80060-900

Phone number: +55 41 35256814

Email: sraboni@ufpr.br

Short running head: Seasonality of influenza in Brazil

Word count: Text - 2,999. **Abstract -** 263

ABSTRACT

Objectives To understand the effect of climate on the determinants of influenza seasonality, and to describe epidemiological and clinical features of hospitalized patients with severe acute respiratory infection caused by respiratory viruses.

Design Prospective epidemiological active surveillance study

Setting The study took place in 03 Brazilian hospitals located in cities with different climate conditions: Curitiba (south), Rio de Janeiro (southeast) and Fortaleza (northeast).

Participants This study focused on: (i) all ages in Curitiba, (ii) adults (+18) and elderly (60+) in Rio de Janeiro, and (iiii) children (<18) in Fortaleza. Patients presenting an acute process, whose indication for admission was any of a predefined set of conditions described as potentially associated with a recent influenza infection, were enrolled and epidemiological data were collected.

Interventions There were no interventions.

Primary and secondary outcome measures Influenza infection.

Results A total of 1,666 patients were screened and 595 met the criteria for inclusion. Influenza viruses and ORVs were detected in 6.5% and 59% of patients, respectively. Influenza-positive cases fell in the severe spectrum compared to those with ORV (30% vs. 11%), but without any difference in mortality rates. Epidemiological results revealed variations in the peak time of influenza infections between Northeast (Fortaleza) and South (Curitiba) Brazil, which basically followed the rain period of each region. In the Northeast, in particular, viral circulation was prevalent in the first 4 months of the year, indicating that the vaccination campaign was being carried out in a post-seasonal period, possibly explaining the low effectiveness.

Conclusion The model of active surveillance applied in our study is a valuable tool for investigating the impact of respiratory viruses in hospitalized patients, and monitoring influenza infections enables more adequate preventive measures for the population.

Trial registration Not applicable

Keywords: hospital admissions, influenza, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

Strengths and limitations of this study

- The model of active surveillance applied in our study is a valuable tool for investigating the impact of respiratory viruses, mainly influenza virus, in hospitalized patients.
- Determinants of seasonality and severity of the influenza disease in South America remain mostly unknown.
- This study reports the initial findings of the GIHSN surveillance project regarding the effect of different climatic conditions on the seasonality of influenza infections and the clinical and epidemiological features observed in hospitalized patients from Brazil.
- The low inclusion of patients in Rio de Janeiro does not allow for an assessment of the impact of viral respiratory infections in southeastern Brazil.
- Our data reinforce the growing consensus that vaccinations in Northeast Brazil (tropical climate region) are being carried out in a post-seasonal period, with consequent low effectiveness.

INTRODUCTION

Influenza-like illness (ILI) occurs annually worldwide, but peak timing and severity of the epidemic vary seasonally. [1] Although surveillance for antigenic drift or influenza virus shift is intense, yearly mortality still exceeds 250,000 and results usually from complications such as pneumonia, neurological events, and circulatory failure. [2]

Seasonal influenza epidemics peak during the winter in both the Northern and Southern Hemispheres. In contrast, tropical countries may experience two annual peaks, with shorter and less intense epidemics. [3,4] Besides climate and environmental conditions, host factors such as immune function and body levels of vitamin D have been associated with seasonal variations of this infection, as well as the severity of influenza epidemics. [5,6].

Vaccination has been highlighted as the main public health measure to reduce the frequency of severe influenza cases. [2] In Brazil, influenza vaccination has been carried out annually since 1999. At first, immunization targeted individuals aged 65 years or more; then, in 2000, the age limit was lowered to 60 years. In 2016, the Brazilian Ministry of Health extended the recommendation to children under 5, pregnant women, people with chronic non-communicable diseases, and health professionals. [7-9] Overall, in 2015, national immunization campaigns achieved a vaccination coverage of 82.7% in priority groups. [10]

The Global Influenza Hospital-Based Surveillance Network (GIHSN) is a public–private partnership between research institutes, hospitals, and several laboratories around the world, established to study the epidemiology of severe influenza in consecutive seasons in different countries. In 2015, this network comprised a total of 31 collaborating sites from seven countries, including Brazil. All participants follow the same core investigation protocol developed by the GIHSN scientific committee.

Through broad geographical coverage and data standardization, the GIHSN group seeks to obtain a global picture of the impact of influenza on patients with respiratory pathologies. In addition, this network provides invaluable tools to investigate the seasonality of influenza in regions in which this information is not available, as well as a framework for estimating the effectiveness of seasonal influenza vaccines in preventing severe cases among age and risk groups.

In 2015, the GIHSN group started a study in Rio de Janeiro, Fortaleza, and Curitiba. These three cities are located in distinct regions of Brazil, enabling the evaluation of the effect of different climatic conditions on the seasonality of influenza infections. Here, we report the initial findings of this surveillance project, including seasonality and the clinical and epidemiological features observed.

PATIENTS AND METHODS

Ethical statement

As a multi-centric study, the present project was approved by the Ethical Committee of each institution involved in the research: Instituto Nacional de Infectologia Evandro Chagas (INI/Fiocruz), Rio de Janeiro; Hospital de Clínicas-Universidade Federal do Paraná (HC/UFPR), Curitiba; and Hospital Infantil Albert Sabin (HIAS), Fortaleza. All procedures were performed according to the approved protocols, and consent was obtained from all patients (or guardians).

Study design and site description

The present multi-centric, cross-sectional study was designed to provide information on active surveillance of influenza in hospitalized patients. Data were collected in 2015 in three participating hospitals located in distinct cities and regions of Brazil: Curitiba (South), Fortaleza (Northeast), and Rio de Janeiro (Southeast).

In Curitiba and Rio de Janeiro, patient recruitment and data collection occurred from April to September, coinciding with the period of influenza seasonality in South and Southeast Brazil. In Fortaleza, the study spanned all of 2015, because, in spite of a previous study showing influenza circulation between January and April, [11] the seasonality of influenza in this region is not well established.

In Curitiba, the study was carried out at HC/UFPR, a 310-bed tertiary care academic center, with 47 beds for intensive care. It is also one of the sentinel hospitals for influenza and Severe Acute Respiratory Infection (SARI) surveillance in the region. Curitiba is located at 25° 25′ S, 49° 15′ W, 924 m (3031 ft), and has a mild marine west coast climate, with no dry season and warm summers. Seasonality is moderate, with heavy precipitation during mild winters and a mean temperature of 16.5°C. The city's population numbered approximately 1,879,355 people in 2015.

In Fortaleza, the study was conducted at HIAS, a public health institution of 270 beds. It is a city of 2,500,000 inhabitants at sea level, 4° south of the equator. Its tropical climate is characterized by two distinct seasons: a rainy one between January and May, and a dry one during the rest of the year. There is high relative humidity (79%) and little variation in the average temperature (26.4°C).

In Rio de Janeiro, the study was conducted at Hospital Quinta D'Or, which is a private hospital with 350 beds for general admission and 150 beds in the intensive care unit (ICU). Rio de Janeiro is located at 22° 54′ S, 43° 12′ W and its climate is classified as tropical Atlantic. The year comprises two seasons: a hot and relatively humid one, and another with mild temperatures and less rainfall.

Patient recruitment and inclusion/exclusion criteria

Patients of all ages, who were admitted for any acute condition described as potentially associated to influenza, were considered eligible for enrollment in the study.

To be included, eligible patients were identified in the hospital admission registries and had to comply with all of the following criteria: i) admission within 24 to 72 h prior to recruitment; ii) be a resident in the area of the study (defined for each site) and belong to the base population source; and iii) a referred history of any acute condition associated to influenza with onset within 7 days or less prior to admission to the hospital. The study used the European CDC definition for ILI, which included at least one out of four systemic symptoms (fever, headache, myalgia, or malaise) and at least one out of three respiratory symptoms (cough, sore throat, or shortness of breath).

Patients who did not give consent to participate, institutionalized individuals, and those with a history of hospitalization within 30 days or less prior to recruitment were excluded from the study.

Sample collection and processing

Samples were collected using flocked nylon swabs (Copan, Italy). It was collected one nasopharyngeal and one pharyngeal swab (or nasal swab for patients <14 years). Samples were added to a flask containing 3 mL of universal viral transport medium (UTM™ Medium; Copan, Italy) and stored at -80^{o}C until tested.

Sample testing for the detection of influenza and other respiratory viruses (ORV)

All samples were submitted to nucleic acid extraction using a QIAmp Viral RNA Mini Kit (Qiagen, USA). The presence of influenza A (H1N1pdm and H3N2) and influenza B (B/Yamagata, B/Victoria) was analyzed by reverse transcription-real time PCR (rtRT-PCR) according to the CDC protocol. [12,13]

In a subset of samples (n = 497), the presence of other pathogens was assessed using a commercial multiplex RT-PCR assay (FTD® Respiratory Pathogens 21 Kit, Fast-Track Diagnostics, Luxembourg) designed to detect influenza A (FLUA) (H1N1pdm09); influenza B (FLUB); rhinovirus (RHV); coronavirus (COV) genotypes

NL63, 229E, OC43, HKU1; parainfluenza (PIV) types 1, 2, 3, and 4; human metapneumovirus A/B (HMPV); bocavirus (BOV); respiratory syncytial virus A/B (RSV); adenovirus (ADV); enterovirus (ENV); parechovirus (PRV); and the atypical bacterium *Mycoplasma pneumonia*, or using the Seeplex® RV15 ACE detection kit (Seegene Inc., Korea), a multiplex PCR-based assay allowing for the simultaneous detection of multiple viruses such as ADV; HMPV; PIV types 1, 2, 3, and 4; FLUA; FLUB; RSV types A and B; rhinovirus (HRV) types A, B, and C; ENV; BOV; and COV types 229E/NL63 and OC43/HKU1. Both tests were performed following the manufacturer's protocol.

Statistical analysis

Data were compiled using JMP software version 5.2.1 (SAS Institute Inc., USA) and analyzed using GraphPad Prism version 5.03 (GraphPad Software Inc., USA). Baseline demographic and clinical characteristics with normal and non-normal distributions are presented as means ± standard deviation and medians with interquartile ranges (IQR), respectively. We compared the parameters associated with influenza and ORV infections. Fisher's exact test, chi-squared test, or Wilcoxon-Mann-Whitney test were performed where appropriate. A multivariate logistic regression model was fitted to evaluate clinical or epidemiological characteristics of virus-positive cases to be associated with disease severity. Variables included age, presence of comorbidities, and fatal outcome. All statistical tests were two-sided and considered as significant at $p < 0.05$.

We defined severe disease as that requiring mechanical ventilation, admission to an ICU, or with a fatal outcome.

RESULTS

Study population: Demographics and epidemiological characteristics

During the study period, 1,666 patients from three participating hospitals were screened (161 in Curitiba, 1,362 in Fortaleza, and 143 in Rio de Janeiro). A total of 595 patients met the criteria for inclusion, as shown in Figure 1.

The demographic and clinical characteristics of the population are listed in Table 1. In Curitiba and Fortaleza subsets, males and females were recruited in equal proportions and most individuals were younger than 5 years. In contrast, in Rio de Janeiro, females and adults were predominant, and no child under 2 years was included.

As expected from a predominantly young population, the prevalence of chronic conditions associated to aggravation of influenza was low in Curitiba and Fortaleza subsets. In contrast, the Rio de Janeiro subset was characterized by a significantly higher prevalence of conditions such as cardiovascular disease, COPD, and diabetes.

Other factors that impact the prevalence of severe influenza infections are the use of antivirals and vaccination. Antiviral administration was more frequently reported in Curitiba. Overall, influenza vaccination rates were low, in spite of most age groups included in the study being covered by the Brazilian immunization program: around 31% of patients from Curitiba were vaccinated, against the estimated 14% and 19% in Fortaleza, and Rio de Janeiro, respectively. In general, only 21% of children younger than 5 years who were included in the study were vaccinated. Severe disease was detected in 149/595 cases (24%), but mortality was low: 9/595 (1.5%).

Evaluation of influenza and ORV

All samples were tested for influenza virus, and a total of 39 (6.5%) were positive; 23 (59%) for influenza A H3N2 and 16 (41%) for influenza B - all Yamagata-like lineage. Of these cases, nine (23%) presented co-infection with ORV.

The presence of ORV was tested in 497 patients (83.5%), with 293 (59%) of them being positive and 46 (16%) presenting co-infection. RSV was the most frequently

identified virus and the main source of co-infections (n = 27; 49%), whereas influenza co-infections were found in only 23% (9/39) of cases. The respiratory pathogens detected in this subset of samples are reported in Figure 2.

Severe cases and mortality associated with influenza and ORV

Epidemiological and clinical data comparing patients infected with influenza and those infected with ORV were assessed; only monoinfected cases were included in this analysis. As shown in Table 2, 9/37 influenza-positive cases fell in the severe spectrum, and severe disease was significantly more frequent in influenza-infected patients than in those with ORV (30% vs. 11%). In an adjusted analysis, only age was associated with this outcome (p = 0.01), whereas presence of comorbidities was not (p = 0.12). No difference in mortality was observed between influenza- and ORV-infected patients.

Seasonality of influenza infections in two geographic regions of Brazil

To better understand the seasonality of influenza infections in the study sites, we evaluated the monthly distribution of samples and viral positivity. Then, we plotted this information against the temperature and precipitation (historical means) recorded for each month. Results show variations between Northeast (Fortaleza) and South (Curitiba) Brazil in relation to the peak time of influenza infections, which essentially followed the rain season of each region. Finally, results indicate that immunization against influenza in the Northeast was carried out after the period of virus circulation (Figure 3). Data from Rio de Janeiro were not evaluated owing to the small number of patients included in the study.

DISCUSSION

The GHISN was established with the main goal of better understanding the role of influenza infections in the development of severe respiratory diseases. The information gathered by this network may contribute to the elucidation of influenza

seasonality in some regions, the implementation of more efficient containment measures, as well as the improvement of preventive interventions such as immunization.

In Brazil, the Ministry of Health employs two systems for epidemiological assessment of respiratory diseases: sentinel surveillance of ILI and universal surveillance of SARI. The sentinel surveillance consists of a network of designated health care units (public or private), distributed throughout the country, in which random samples of respiratory cases are periodically collected for detection and genetic characterization of circulating viruses. In the universal SARI surveillance, all severe respiratory cases admitted to ICUs and all deaths related to respiratory disease are investigated in the laboratory. [14] In the present study, only hospitalized patients identified by an active search in the participating health centers were included. In contrast to the Brazilian universal SARI surveillance protocol, which is restricted to ICUs, in this study, the screening included patients admitted to other wards and presenting diseases other than respiratory ones. Individuals admitted with conditions associated with aggravation of influenza infections, such as cardiovascular disease and diabetes, were also screened, thus increasing the number of identified cases.

Overall, the frequency of influenza viruses in the study specimens was 6.5% (39/595), with influenza A comprising 60% of the flu-positive samples. This low prevalence reflected the profile of the 2015 influenza season in Brazil and in most of the Southern Hemisphere and Tropical regions. In that year, viral activity remained low through the entire season, with higher prevalence of H3N2 and lower detection of influenza B and H1N1pdm09. Even in temperate regions of the Southern Hemisphere, activity remained at an inter-seasonal level. In Brazil, a total of 11,945 cases of SARI were reported in 2015, of which 1,089 (9%) were related to influenza infection. Among these cases, 599 (55%) were influenza A/H3N2, 234 (21.5%) were influenza B, 141

(12.9%) were influenza A/H1N1pdm09, and 115 (10.5%) were reported as non-subtyped influenza A. [15]

Since 2014, the Brazilian Ministry of Health included children up to 4 years of age in the group of individuals at risk of severe influenza and, thus, started vaccination of this subpopulation. Data from the National Immunization Program show coverage rates above 83% among young children and pregnant women, 89% in the elderly, and 95% in health workers. [10,14] However, in this study, a much lower coverage was observed, with only 21% of patients reporting or showing proof of influenza vaccination. This was surprising, considering that a significant part of our patients fell in at least one risk category or presented comorbidities. Given a 10.5% mortality rate due to influenza-related SARI cases in 2015, it is essential to assess the reasons for such low immunization frequency. Among influenza deaths, 65% of patients and particularly those aged over 60 presented at least one risk factor likely to cause complications. [10] Our data emphasize the need for a comprehensive identification of the weaknesses of the vaccination campaigns and an intensification of the efforts to bring immunization to these individuals.

We also evaluated the prevalence of ORV in a subset of samples from HC/UFPR, and samples that were selected randomly among patients from Fortaleza and Rio de Janeiro. Overall, different respiratory viruses were found in 59% of the tested samples. This is consistent with previous reports showing high co-circulation of ORV in hospitalized patients during the influenza season, and confirmed that the viruses detected caused very similar ILI respiratory manifestations. [16-18] Moreover, our results reflect the profile of respiratory viruses in children, which was the predominant group included in the study. Interestingly, although circulation of influenza and ORV coincide in time, the frequency of codetection of influenza with ORV in the same

patient was very low, whereas codetection of more than one ORV in a single patient was rather common. This observation is consistent with those in previous reports by our group, and we hypothesize that low codetection of influenza with other ORV is due to the higher pathogenicity of influenza. [17,19]

Next, we compared clinical and epidemiological data from influenza- and ORV-infected patients in an attempt to evaluate the impact of influenza infections. The latter were significantly more abundant in older patients and those with severe diseases, whereas comorbidities were associated with ORV infections. These findings reflect the higher virulence of influenza viruses, but could also be a consequence of a lower adhesion to immunization among older children. No difference in mortality was observed between groups infected with different viruses. This contrasts with a previous study performed in Fortaleza, which showed significant association between RSV infections and infant mortality. [20] We believe that such discrepancy may be due to the low number of positive cases observed in the present study.

Studies on influenza seasonality in Brazil have shown that viral circulation in the Northeast region was more prevalent in the first 4 months of the year, coinciding with a period of higher humidity in that area [11,21], whereas a distinct pattern of viral circulation was observed in the South and Southeast. [22,23] Our data confirm this observation and reinforce the growing consensus that vaccinations in Northeast Brazil are being carried out in a post-seasonal period, with consequent low effectiveness.

The present study presented some limitations: i) the recruitment of patients during the seasonal period of influenza previously defined for two sites (Curitiba and Rio de Janeiro) may have contributed to low influenza B detection, although the frequency observed in our study was similar to that reported by national surveillance programs; ii) there was a predominance of pediatric patients in the study population, so

data on ORV reflect mainly the profile of respiratory viruses in children; iii) the low inclusion of patients in Rio de Janeiro does not allow for an assessment of the impact of viral respiratory infections in that region.

Despite these limitations, the results obtained here indicate that the model of active surveillance applied in our study is a valuable tool for investigating the impact of respiratory viruses in hospitalized patients in distinct regions of Brazil. We also reinforce the importance of monitoring influenza infections, thus enabling more adequate preventive measures for the population.

Authors Contributions

FKP, VP and JPB participated to the conception/design of the study, supervised the analysis, and interpreted the data. MMS and SMR supervised the analysis, interpreted the data and wrote the preliminary manuscript. FEAM and IT supervised the data collection from Fortaleza and Rio de Janeiro, respectively. BCC performed the tests, assembled the data and wrote the preliminary manuscript. VMA, LAP, MBN and LRV performed the molecular tests and collected the data from Curitiba. All authors provided contributions to the paper and approved the final version

Data Sharing Statement

No additional data available.

Competing interests

The authors have no competing interests.

Funding

This work was supported by Sanofi Pasteur through the Global Influenza Hospital Surveillance Network and the Mérieux Foundation.

Role of the funding source

The sponsors of the study had no role in data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for deciding to submit the work for publication.

REFERENCES

1. Bresee J, Hayden FG. Epidemic influenza-responding to the expected but unpredictable. *N Engl J Med* 2013; 368:589–92.
2. World Health Organization. Influenza (Seasonal) FactSheet211. 2014. (http://www.who.int/mediacentre/factsheets/fs211/en/). Accessed 13 September 2016.
3. Bloom-Feshbach K, Alonso WJ, Charu V, *et al*. Latitudinal variations in seasonal activity of influenza and respiratory syncytial virus (RSV): a global comparative review. *PLoS ONE* 2013; 8:e54445.
4. Caini S, Sue Huang Q, Ciblak MA, *et al.* Epidemiological and virological characteristics of influenza B: results of the Global Influenza B Study. *Influenza Other Respir Viruses* 2015; 9:3–12.
5. Shaman J, Kohn M. Absolute humidity modulates influenza survival, transmission, and seasonality. *Proc Nat Acad Sci USA* 2009; 106:3243–48.
6. Lowen AC, Mubareka S, Steel J, *et al*. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* 2007; 3:1470–76.
7. Cunha SS, Camacho LAB, Santos AC, *et al*. Influenza vaccination in Brazil: rationale and caveats. *Rev Sauude Puublica* 2005; 39:129–36.
8. Luna EJ, Gattás VL. Effectiveness of the Brazilian influenza vaccination policy, a systematic review. *Rev Inst Med Trop Sao Paulo* 2010; 52:175–81.
9. Oliveira JF, Boing AF, Waldman EA, *et al*. Ecological study on mortality from influenza and pneumonia before and after influenza vaccination in the Northeast

and South of Brazil. *Cad Saude Publica* 2013; 29:2535–45.

10. Brazilian Health Ministry. Departamento de informática do SUS (DATASUS). (http://sipni.datasus.gov.br/si-pni-web/faces/relatorio/consolidado/coberturaVacinalCampanhaInfluenza.jsf). Accessed 11 December 2016.
11. Moura FE, Perdigão AC, Siqueira MM. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. *Am J Trop Med Hyg* 2009; 81:180–83.
12. World Health Organization. CDC protocol of realtime RTPCR for influenza A (H1N1). 2009. (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/). Accessed 3 October 2015.
13. Biere B, Bauer B, Schweiger B. Differentiation of influenza B virus lineages Yamagata and Victoria by real-time PCR. *J Clin Microbiol* 2010; 48:1425–27.
14. Barros ENC, Cintra O, Rosseto E, *et al*. Patterns of influenza B circulation in Brazil and its relevance to seasonal vaccine composition. *Braz J Infect Dis* 2016; 20:81–90.
15. Brazilian Epidemiological Bulletim. Informe técnico – Situação epidemiológica da influenza (CREMESP). (http://www.cremesp.org.br/pdfs/Informe_tecnico_flu_17mar2016.pdf). Accessed 11 December 2016.
16. Brittain-Long R, Nord S, Olofsson S, *et al*. Multiplex real-time PCR for detection of respiratory tract infections. *J Clin Virol* 2008; 41:53–6.
17. Raboni SM, Stella V, Cruz CR, *et al*. Laboratory diagnosis, epidemiology, and clinical outcomes of pandemic influenza A and community respiratory viral infections in southern Brazil. *J Clin Microbiol* 2011; 49:1287–93.

18. Giamberardino HI, Homsani S, Bricks LF, *et al*. Clinical and epidemiological features of respiratory virus infections in preschool children over two consecutive influenza seasons in southern Brazil. *J Med Virol* 2016; 88:1325–33.
19. **T**rombetta H, Faggion HZ, Leotte J, *et al*. Human coronavirus and severe acute respiratory infection in Southern Brazil. *Pathog Glob Health* 2016; 110:113–18.
20. Alonso WJ, Laranjeira BJ, Pereira SA, *et al*. Comparative dynamics, morbidity and mortality burden of pediatric viral respiratory infections in an equatorial city. *Ped Infect Dis J* 2012; 31:e09–e14.
21. Moura FE. Influenza in the tropics. *Curr Opin Infect Dis* 2010; 23:415–20.
22. Tsuchiya LR, Costa LM, Raboni SM, *et al*. Viral respiratory infection in Curitiba, Southern Brazil. *J Infect* 2005; 51:401–07.
23. Belley N, Carraro E, Perosa A, *et al*. Patterns of influenza infections among different risk groups in Brazil. *Braz J Infect Dis* 2007; 11:399–402.

Table 1. Epidemiological and clinical characteristics of the patients included in the study.

Characteristics	Curitiba n = 136 (%)	Fortaleza n = 427 (%)	RJ n = 32 (%)
Sex			
Male	61 (45)	243 (57)	12 (37)
Female	75 (55)	184 (43)	20 (63)
Age (Median, years/IQR)	1.4 (0.5–87.9)	1.3 (0–16.6)	1.4 (3.3–89.3)
Age group (years)			
< 2	86 (63)	254 (59)	0
2–4	23 (17)	100 (24)	3 (9)
5–17	11 (8)	73 (17)	4 (12)
18–64	12 (9)	0	9 (28)
> 65	4 (3)	0	16 (51)
Time to onset of symptoms (Median, days/IQR)	2 (0–7)	2 (0–7)	2 (0–6)
Exposure to smoking			
Yes	22 (16)	109 (25)	1 (3)
No	90 (66)	277 (65)	21 (66)
Ex-smoker	24 (18)	41 (10)	10 (31)
Chronic diseases*			
None	76 (56)	341 (78)	9 (28)

Cardiovascular	16 (12)	26 (6)	12 (38)
Pulmonary	32 (23)	8 (2)	7 (22)
Asthma	13 (10)	37 (9)	9 (28)
Diabetes	1 (1)	3 (1)	6 (19)
Immunosuppressed	2 (1.5)	9 (2)	1 (3)
Renal	0	7 (1.5)	2 (6)
Rheumatic disease	0	8 (2)	0
Cirrhosis	0	2 (0.5)	2 (6)
Neuromuscular	11 (8)	22 (5)	0
Neoplasm	1 (1)	1 (0.2)	5 (16)
Autoimmune	1 (1)	8 (2)	1 (3)
Length of stay in hospital (Median days/IQR)	2 (1–53)	1 (0–14)	2 (1–53)
Polypnea			
Yes	108 (80)	51 (12)	17 (53)
No	7 (5)	303 (71)	3 (9)
NI	21 (15)	73 (17)	12 (38)
Use of antiviral drugs			
Yes	13 (10)	10 (2)	0
Days of use (Median/IQR)	5 (2–5)	15 (1–35)	-
Vaccination in 2014			
Yes	42 (31)	59 (14)	6 (19)
No	94 (69)	344 (81)	26 (81)

NI	0	24 (5)	0
ICU admission			
Yes	37 (27)	10 (2)	26 (81)
No	99 (73)	417 (98)	6 (19)
Mechanical ventilation			
Yes	29 (21.3)	13 (3.0)	0
No	107 (78.7)	414 (97.0)	0
Death			
Yes	4 (3)	4 (1)	1 (3)
No	132 (97)	423 (99)	31 (97)
Severe disease	99 (73)	16 (4)	26 (81)

*Some patients presented more than one comorbidity. ICU, intensive care unit; IQR, interquartile range; NI, no information; RJ, Rio de Janeiro.

Table 2. Epidemiological and clinical characteristics of patients with positive diagnosis for respiratory viruses treated in reference hospitals in Curitiba, Fortaleza, and Rio de Janeiro, 2015.

Characteristics	Positive cases		
	Influenza virus n = 30 (%)	ORV n = 247 (%)	*p* value
Sex			
Male	13 (43)	121 (49)	0.5694
Female	17 (57)	126 (51)	-
Age (Median, years/IQR)	2.6 (1.3–5.9)	0.8 (0.2–2.0)	< 0.0001
Age group (years)			
< 2	9 (37)	180 (73)	0.0002
2–4	10 (33)	43 (17)	-
5–17	4 (13)	15 (6)	-
> 18	5 (17)	9 (4)	-
Time to onset of symptoms (Median, days/IQR)	1.5 (0–4)	2 (1–4)	0.1882
Chronic diseases*			
No	16 (53)	192 (78)	**0.0064** (OR 3.0 95% CI 1.4–6.6)
Pulmonary	6 (20)	40 (16)	-
Cardiovascular	7 (23)	16 (6)	-

Other	3 (10)	27 (11)	-
Length of stay in hospital (Median days/IQR)	2 (1–6)	1.5 (1–9.7)	0.8726
Polypnea			
Yes	11 (37)	88 (36)	0.8325
No	15 (50)	139 (56)	-
NI	4 (13)	20 (8)	-
Use of antiviral drugs			
Yes	0	9 (4)	NA
Days of use (Median/ IQR)	-	05 (5–35)	-
Vaccination in 2014			
Yes	4 (13)	28 (11)	0.7634
No	26 (87)	216 (87)	-
NI	0	3 (2)	-
ICU admission			
Yes	8 (27)	22 (9)	**0.0081** (OR 3.7 95% CI 1.4–9.3)
No	22 (73)	225 (91)	-
Mechanical ventilation			
Yes	6 (20)	15 (6)	**0.0164** (OR 3.8, 95% CI 1.3–10.9)
No	24 (80)	232 (94)	-

Death			
Yes	1 (3)	6 (2)	0.5006
No	29 (97)	241 (98)	
Severe disease			
Yes	9 (30)	26 (11)	**0.0061** (OR 3.6, 95% CI 1.5–8.7)
No	21 (70)	221 (89)	-

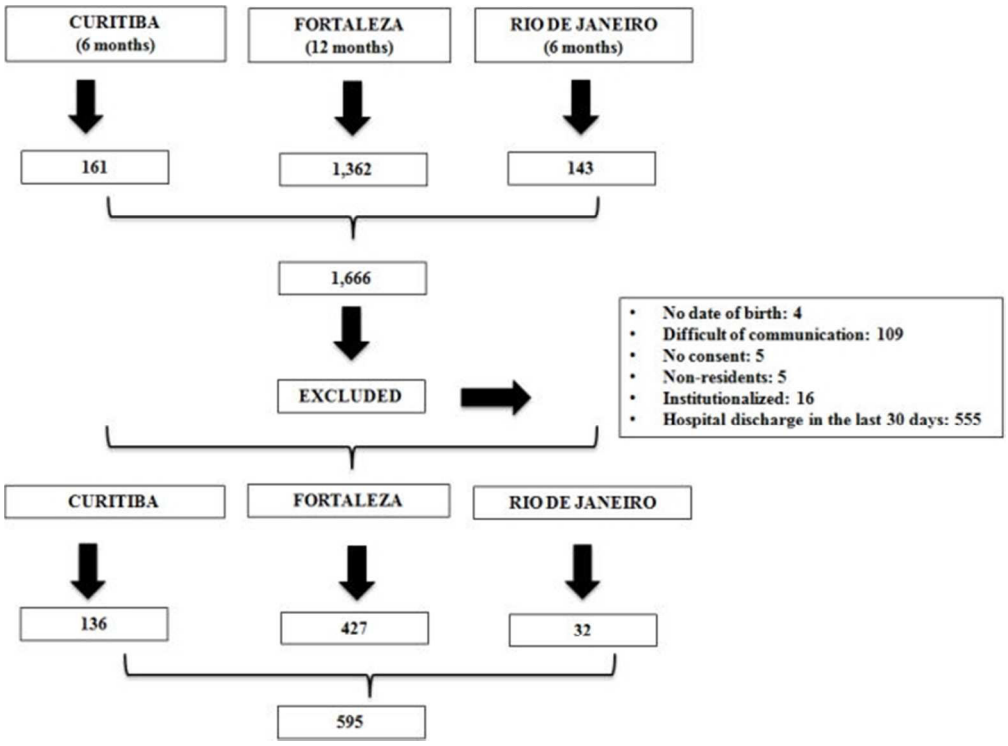
*Some patients presented more than one comorbidity. CI, confidence interval; ICU, intensive care unit; IQR, interquartile range. NI, no information; OR, odds ratio. In bold, statistically significant values.

Figure Legends

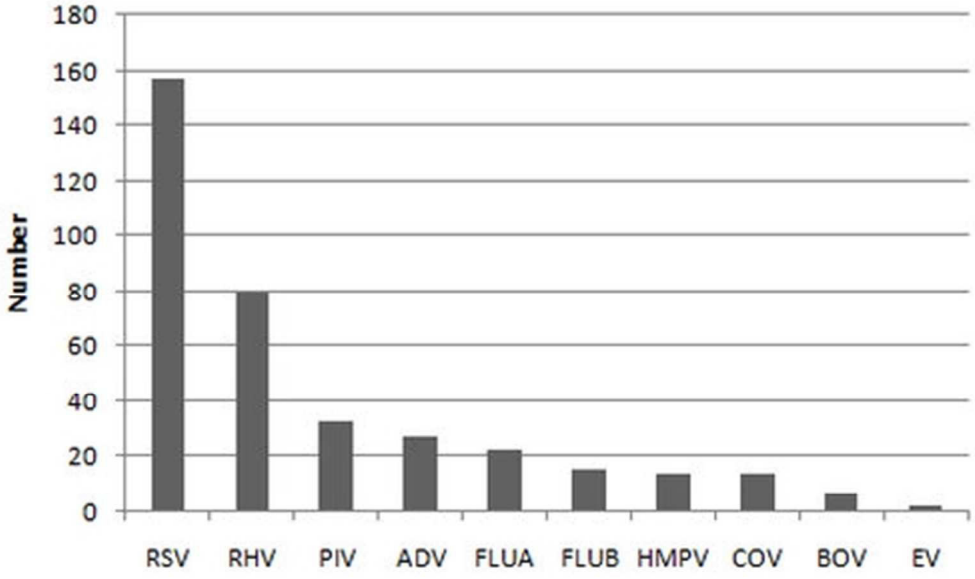
Figure 1. Flowchart summarizing the process of patient inclusion in the study, performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

Figure 2. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

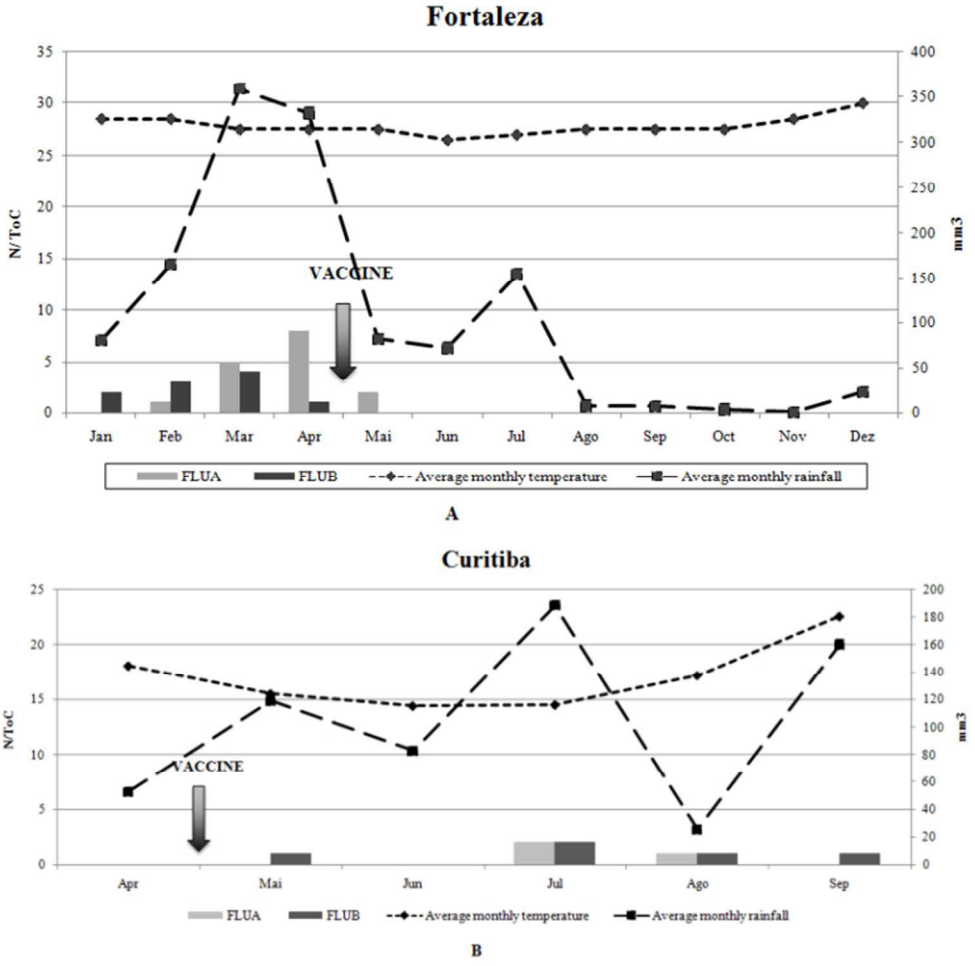
Figure 3. Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow.



169x124mm (96 x 96 DPI)



132x78mm (96 x 96 DPI)



211x207mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	01
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	02-03
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	04-05
Objectives	3	State specific objectives, including any prespecified hypotheses	05
Methods			
Study design	4	Present key elements of study design early in the paper	05
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	05-07
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls ***Cross-sectional study***—Give the eligibility criteria, and the sources and methods of selection of participants	07
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Not applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	07
Bias	9	Describe any efforts to address potential sources of bias	08
Study size	10	Explain how the study size was arrived at	09 Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	08
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	08
		(*b*) Describe any methods used to examine subgroups and interactions	Not applicable
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	08
		(*e*) Describe any sensitivity analyses	Not applicable

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	09
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	09
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	*09-10*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	09-10 Table 1
		(*b*) Report category boundaries when continuous variables were categorized	Table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14-15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Global Influenza Hospital-based Surveillance Network (GIHSN) – Results of surveillance of influenza and other respiratory viruses in hospitalized patients in 2015, Brazil

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017603.R1
Article Type:	Research
Date Submitted by the Author:	07-Jul-2017
Complete List of Authors:	Raboni, Sonia M; Universidade Federal do Parana, Infectious Diseases Moura, Fernanda; Universidade Federal do Ceara, Departamento de Patologia e Medicina Legal Caetano, Braulia; Fundacao Oswaldo Cruz, Laboratório de Vírus Respiratórios e do Sarampo, IOC Avanzi, Valeria; Universidade Federal do Parana, Laboratório de Virologia Pereira, Luciane; Universidade Federal do Parana, Laboratório de Virologia Nogueira, Meri; Universidade Federal do Parana, Laboratório de Virologia Vidal, Luine; Universidade Federal do Parana, Laboratório de Virologia Tavares, Isabel; Fundacao Oswaldo Cruz, Instituto Nacional de Saúde Pradel, Florence; Mérieux Foundation Picot, Valentina; Fondation Merieux Puig-Barbera, Joan; Centro Superior de Investigacion en Salud publica Siqueira, Marilda; Fundacao Oswaldo Cruz, Laboratório de Vírus Respiratórios e do Sarampo, IOC
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Public health, Epidemiology
Keywords:	influenza, Hospital admission, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

Global Influenza Hospital-based Surveillance Network (GIHSN) – Results of surveillance of influenza and other respiratory viruses in hospitalized patients in 2015, Brazil

Sonia M Raboni[1], Fernanda E A Moura[2], Braulia C Caetano[3], Valéria M Avanzi[1], Luciane A Pereira[1], Meri B Nogueira[1], Luine R Vidal[1], Isabel Tavares[4], Florence K Pradel[5], Valentina Picot[5], Joan Puig-Barbera[6], Marilda M Siqueira[3].

[1]Laboratório de Virologia, Hospital de Clínicas da Universidade Federal do Paraná, Brazil

[2] Departamento de Patologia e Medicina Legal, Universidade Federal do Ceará, Brazil

[3]Laboratório de Vírus Respiratórios e do Sarampo, IOC, Fiocruz, Brazil

[4]Instituto Nacional de Saúde, Fiocruz, Brazil

[5]Mérieux Foundation, France

[6]FISABIO, Spain

Corresponding author: Sonia Mara Raboni,

Hospital de Clínicas/Universidade Federal do Paraná,

180 General Carneiro Street, 3rd floor, Curitiba, Paraná, Brazil

Zip code: 80060-900

Phone number: +55 41 35256814

Email: sraboni@ufpr.br

Short running head: Seasonality of influenza in Brazil

Word count: Text - 2,999. **Abstract -** 263

ABSTRACT

Objectives To describe epidemiological and clinical features of hospitalized patients with severe acute respiratory infection caused by influenza and other respiratory viruses, to report the effect of climate on the determinants of influenza seasonality, and. to correlate the findings of influenza circulation and time of immunization in Brazil.

Design Prospective epidemiological active surveillance study

Setting The study took place in 3 Brazilian hospitals located in cities with different climate conditions: Curitiba (south), Rio de Janeiro (southeast) and Fortaleza (northeast).

Participants This study focused on: (i) all ages in Curitiba, (ii) adults (+18) and elderly (60+) in Rio de Janeiro, and (iiii) children (<18) in Fortaleza. Patients presenting an acute process, whose indication for admission was any of a predefined set of conditions described as potentially associated with a recent influenza infection, were enrolled and epidemiological data were collected.

Interventions There were no interventions.

Primary and secondary outcome measures Influenza infection.

Results A total of 1,666 patients were screened and 595 met the criteria for inclusion. Influenza viruses and other respiratory viruses (ORVs) were detected in 6.5% and 59% of patients, respectively. Influenza-positive cases fell in the severe spectrum compared to those with ORV (30% vs. 11%), but without any difference in mortality rates. Epidemiological results revealed variations in the peak time of influenza infections between Northeast (Fortaleza) and South (Curitiba) Brazil, which basically followed the rain period of each region. In the Northeast, in particular, viral circulation was prevalent in the first 4 months of the year, indicating that the vaccination campaign was being carried out in a post-seasonal period, possibly explaining the low effectiveness.

Conclusion The model of active surveillance applied in our study is a valuable tool for investigating the impact of respiratory viruses in hospitalized patients, and monitoring influenza infection enables the implementation of adequate preventive measures.

Trial registration Not applicable

Keywords: hospital admissions, influenza, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

Strengths and limitations of this study

- The strengths of this study are the use of the same model of active surveillance applied in other outside centers allowing subsequent comparisons on the impact of respiratory viruses, mainly influenza virus, in hospitalized patients worldwide; Second, to add more information on seasonality and severity of the influenza disease in South America, whose data are limited. An finally, our data reinforce the growing consensus that vaccinations in Northeast Brazil (tropical climate region) are being carried out in a post-seasonal period, with consequent low effectiveness.
- The main weakness of the data is the low inclusion of patients in Rio de Janeiro that does not allow for an assessment of the impact of viral respiratory infections in southeastern Brazil.

INTRODUCTION

Influenza-like illness (ILI) occurs annually worldwide, but peak timing and severity of the epidemic vary seasonally. [1] Although surveillance for antigenic drift or influenza virus shift is intense, yearly mortality still exceeds 250,000 and results usually from complications such as pneumonia, neurological events, and circulatory failure. [2]

Seasonal influenza epidemics peak during the winter in temperate latitudes of both the Northern and Southern Hemispheres. In contrast, tropical countries may experience two annual peaks, with shorter and less intense epidemics. [3,4] Moreover, previous studies showed that influenza activity has been frequently out of phase with the hemispheric winter in many tropical regions, and even temperate ones, with the consequence that optimal timing for routine influenza vaccination recommendations does not necessarily correspond to the one expected for their hemisphere. [5]. In Brazil, which presents temperate and tropical regions, it has been showed an important regional heterogeneity in influenza peak. [6,7] Besides climate and environmental conditions, which have shown an association between seasonal influenza epidemics with "cold-dry" and "humid-rainy". [8] host factors such as age, preexisting immunity, genetic polymorphisms, and presence of comorbidities have been associated with seasonal variations of this infection, vaccine responsiveness, as well as the severity of influenza epidemics. [9].

Vaccination has been highlighted as the main public health measure to reduce the frequency of severe influenza cases. [2] In Brazil, influenza vaccination has been carried out annually since 1999. At first, immunization targeted individuals aged 65 years or more; then, in 2000, the age limit was lowered to 60 years. In 2016, the Brazilian Ministry of Health extended the recommendation to children under 5, pregnant women, people with chronic non-communicable diseases, and health

professionals. [10-12] Overall, in 2015, national immunization campaigns achieved a vaccination coverage of 82.7% in priority groups. [13]

The Global Influenza Hospital-Based Surveillance Network (GIHSN) is a public–private partnership between research institutes, hospitals, and several laboratories around the world, established to study the epidemiology of severe influenza in consecutive seasons in different countries. In 2015, this network comprised a total of 31 collaborating sites from seven countries, including Brazil. All participants follow the same core investigation protocol developed by the GIHSN scientific committee. Through broad geographical coverage and data standardization, the GIHSN group seeks to obtain a global picture of the impact of influenza on patients with respiratory pathologies. In addition, this network provides invaluable tools to investigate the seasonality of influenza in regions in which this information is not available, as well as a framework for estimating the effectiveness of seasonal influenza vaccines in preventing severe cases among age and risk groups.

In 2015, the GIHSN group started a study in Rio de Janeiro, Fortaleza, and Curitiba. These three cities are located in distinct regions of Brazil, enabling the evaluation of the effect of different climatic conditions on the seasonality of influenza infections. Here, we report the initial findings of this surveillance project, including seasonality and the clinical and epidemiological features observed.

PATIENTS AND METHODS

Ethical statement

As a multi-centric study, the present project was approved by the Ethical Committee of each institution involved in the research: Instituto Nacional de Infectologia Evandro Chagas (INI/Fiocruz), Rio de Janeiro; Hospital de Clínicas-

Universidade Federal do Paraná (HC/UFPR), Curitiba; and Hospital Infantil Albert Sabin (HIAS), Fortaleza. All procedures were performed according to the approved protocols, and consent was obtained from all patients (or guardians).

Study design and site description

The present multi-centric, cross-sectional study was designed to provide information on active surveillance of influenza in hospitalized patients. Data were collected in 2015 in three participating hospitals located in distinct cities and regions of Brazil: Curitiba (South), Fortaleza (Northeast), and Rio de Janeiro (Southeast) (Figure 1).

In Curitiba and Rio de Janeiro, patient recruitment and data collection occurred from April to September, coinciding with the period of influenza seasonality in South and Southeast Brazil. In Fortaleza, the study spanned all of 2015, because, in spite of a previous study showing influenza circulation between January and April, [14] the seasonality of influenza in this region is not well established.

In Curitiba, the study was carried out at HC/UFPR, a 310-bed tertiary care academic center, with 47 beds for intensive care. It is also one of the sentinel hospitals for influenza and Severe Acute Respiratory Infection (SARI) surveillance in the region. Curitiba is located at 25° 25′ S, 49° 15′ W, 924 m (3031 ft), and has a mild marine west coast climate, with no dry season and warm summers. Seasonality is moderate, with heavy precipitation during mild winters and a mean temperature of 16.5°C. The city's population numbered approximately 1,879,355 people in 2015.

In Fortaleza, the study was conducted at HIAS, a public health institution of 270 beds. It is a city of 2,500,000 inhabitants at sea level, 4° south of the equator. Its tropical climate is characterized by two distinct seasons: a rainy one between January and May,

and a dry one during the rest of the year. There is high relative humidity (79%) and little variation in the average temperature (26.4°C).

In Rio de Janeiro, the study was conducted at Hospital Quinta D'Or, which is a private hospital with 350 beds for general admission and 150 beds in the intensive care unit (ICU). Rio de Janeiro is located at 22° 54′ S, 43° 12′ W and its climate is classified as tropical Atlantic. The year comprises two seasons: a hot and relatively humid one, and another with mild temperatures and less rainfall.

Patient recruitment and inclusion/exclusion criteria

Patients of all ages, who were admitted for any acute condition described as potentially associated to influenza, were considered eligible for enrollment in the study. To be included, eligible patients were identified in the hospital admission registries and had to comply with all of the following criteria: i) admission within 24 to 72 h prior to recruitment; ii) be a resident in the area of the study (defined for each site) and belong to the base population source; and iii) a referred history of any acute condition associated to influenza with onset within 7 days or less prior to admission to the hospital. The study used the European CDC definition for ILI, which included at least one out of four systemic symptoms (fever, headache, myalgia, or malaise) and at least one out of three respiratory symptoms (cough, sore throat, or shortness of breath).

Patients who did not give consent to participate, institutionalized individuals, and those with a history of hospitalization within 30 days or less prior to recruitment were excluded from the study.

Sample collection and processing

Samples were collected using flocked nylon swabs (Copan, Italy). It was collected one nasopharyngeal and one pharyngeal swab (or nasal swab for patients <14 years). Samples were added to a flask containing 3 mL of universal viral transport

medium (UTM™ Medium; Copan, Italy) and stored at -80°C until tested.

Sample testing for the detection of influenza and other respiratory viruses (ORV)

All samples were submitted to nucleic acid extraction using a QIAmp Viral RNA Mini Kit (Qiagen, USA). The presence of influenza A (H1N1pdm and H3N2) and influenza B (B/Yamagata, B/Victoria) was analyzed by reverse transcription-real time PCR (rtRT-PCR) according to the CDC protocol. [15,16]

In a subset of samples (n = 497), the presence of other pathogens was assessed using a commercial multiplex RT-PCR assay (FTD® Respiratory Pathogens 21 Kit, Fast-Track Diagnostics, Luxembourg) designed to detect influenza A (FLUA) (H1N1pdm09); influenza B (FLUB); rhinovirus (RHV); coronavirus (COV) genotypes NL63, 229E, OC43, HKU1; parainfluenza (PIV) types 1, 2, 3, and 4; human metapneumovirus A/B (HMPV); bocavirus (BOV); respiratory syncytial virus A/B (RSV); adenovirus (ADV); enterovirus (ENV); parechovirus (PRV); and the atypical bacterium *Mycoplasma pneumonia*, or using the Seeplex® RV15 ACE detection kit (Seegene Inc., Korea), a multiplex PCR-based assay allowing for the simultaneous detection of multiple viruses such as ADV; HMPV; PIV types 1, 2, 3, and 4; FLUA; FLUB; RSV types A and B; rhinovirus (HRV) types A, B, and C; ENV; BOV; and COV types 229E/NL63 and OC43/HKU1. Both tests were performed following the manufacturer's protocol.

Data analysis

Data were compiled using JMP software version 5.2.1 (SAS Institute Inc., USA) and analyzed using GraphPad Prism version 5.03 (GraphPad Software Inc., USA). Baseline demographic and clinical characteristics with normal and non-normal distributions are presented as means ± standard deviation and medians with interquartile ranges (IQR), respectively. Epidemiological and clinical data comparing patients

infected with influenza and those infected with ORV were assessed; only monoinfected cases were included in this analysis. Fisher's exact test, chi-squared test, or Wilcoxon-Mann-Whitney test were performed where appropriate. A multivariate logistic regression model was fitted to evaluate clinical or epidemiological characteristics of virus-positive cases to be associated with disease severity. Variables included age, presence of comorbidities, and fatal outcome. All statistical tests were two-sided and considered as significant at $p < 0.05$.

We defined severe disease as that requiring mechanical ventilation, admission to an ICU, or with a fatal outcome.

To better understand the seasonality of influenza infections in the study sites, we evaluated the monthly distribution of samples and viral positivity. Then, we plotted this information against the temperature and precipitation (historical means) recorded for each month.

RESULTS

Study population: Demographics and epidemiological characteristics

During the study period, 1,666 patients from three participating hospitals were screened (161 in Curitiba, 1,362 in Fortaleza, and 143 in Rio de Janeiro). A total of 595 patients met the criteria for inclusion, as shown in Figure 2.

The demographic and clinical characteristics of the population are listed in Table 1. In Curitiba and Fortaleza subsets, males and females were recruited in equal proportions and most individuals were younger than 5 years. In contrast, in Rio de Janeiro, females and adults were predominant, and no child under 2 years was included.

As expected from a predominantly young population, the prevalence of chronic conditions associated to aggravation of influenza was low in Curitiba and Fortaleza

subsets. In contrast, the Rio de Janeiro subset was characterized by a significantly higher prevalence of conditions such as cardiovascular disease, COPD, and diabetes.

Other factors that impact the prevalence of severe influenza infections are the use of antivirals and vaccination. Antiviral administration was more frequently reported in Curitiba. Overall, influenza vaccination rates were low, in spite of most age groups included in the study being covered by the Brazilian immunization program: around 31% of patients from Curitiba were vaccinated, against the estimated 14% and 19% in Fortaleza, and Rio de Janeiro, respectively. In general, only 21% of children younger than 5 years who were included in the study were vaccinated. Severe disease was detected in 149/595 cases (24%), but mortality was low: 9/595 (1.5%).

Evaluation of influenza and ORV

All samples were tested for influenza virus, and a total of 39 (6.5%) were positive; 23 (59%) for influenza A H3N2 and 16 (41%) for influenza B - all Yamagata-like lineage. Of these cases, nine (23%) presented co-infection with ORV.

The presence of ORV was tested in 497 patients (83.5%), with 293 (59%) of them being positive and 46 (16%) presenting co-infection. RSV was the most frequently identified virus and the main source of co-infections (n = 27; 49%), whereas influenza co-infections were found in only 23% (9/39) of cases. The respiratory pathogens detected in this subset of samples are reported in Figure 3.

Severe cases and mortality associated with influenza and ORV

As shown in Table 2, 9/37 influenza-positive cases fell in the severe spectrum, and severe disease was significantly more frequent in influenza-infected patients than in those with ORV (30% vs. 11%). In an adjusted analysis, only age was associated with this outcome (p = 0.01), whereas presence of comorbidities was not (p = 0.12). No difference in mortality was observed between influenza- and ORV-infected patients.

Seasonality of influenza infections in two geographic regions of Brazil

Regarding seasonality of influenza infections and temperature and precipitation (historical means) recorded for each month. Results show variations between Northeast (Fortaleza) and South (Curitiba) Brazil in relation to the peak time of influenza infections, which essentially coincided with the peak of the rainy season of each region. Finally, results indicate that immunization against influenza in the Northeast was carried out after the period of virus circulation (Figure 4). Data from Rio de Janeiro were not evaluated owing to the small number of patients included in the study.

DISCUSSION

The GHISN was established with the main goal of better understanding the role of influenza infections in the development of severe respiratory diseases. The information gathered by this network may contribute to the elucidation of influenza seasonality in some regions, the implementation of more efficient containment measures, as well as the improvement of preventive interventions such as immunization.

In Brazil, the Ministry of Health employs two systems for epidemiological assessment of respiratory diseases: sentinel surveillance of ILI and universal surveillance of SARI. The sentinel surveillance consists of a network of designated health care units (public or private), distributed throughout the country, in which random samples of respiratory cases are periodically collected for detection and genetic characterization of circulating viruses. In the universal SARI surveillance, all severe respiratory cases admitted to ICUs and all deaths related to respiratory disease are investigated in the laboratory. [17] In the present study, only hospitalized patients identified by an active search in the participating health centers were included. In contrast to the Brazilian universal SARI surveillance protocol, which is restricted to ICUs, in this study, the screening included patients admitted to other wards and

presenting diseases other than respiratory ones. Individuals admitted with conditions associated with aggravation of influenza infections, such as cardiovascular disease and diabetes, were also screened, thus increasing the number of identified cases.

Overall, the frequency of influenza viruses in the study specimens was 6.5% (39/595), with influenza A comprising 60% of the flu-positive samples. This low prevalence reflected the profile of the 2015 influenza season in Brazil and in most of the Southern Hemisphere and Tropical regions. In that year, viral activity remained low through the entire season, with higher prevalence of H3N2 and lower detection of influenza B and H1N1pdm09. Even in temperate regions of the Southern Hemisphere, activity remained at an inter-seasonal level. In Brazil, a total of 11,945 cases of SARI were reported in 2015, of which 1,089 (9%) were related to influenza infection. Among these cases, 599 (55%) were influenza A/H3N2, 234 (21.5%) were influenza B, 141 (12.9%) were influenza A/H1N1pdm09, and 115 (10.5%) were reported as non-subtyped influenza A. [18]

Since 2014, the Brazilian Ministry of Health included children up to 4 years of age in the group of individuals at risk of severe influenza and, thus, started vaccination of this subpopulation. Data from the National Immunization Program show coverage rates above 83% among young children and pregnant women, 89% in the elderly, and 95% in health workers. [13,17]. In the present study, it was observed that proportionally there were a higher percentage of patients from Curitiba vaccinated among the 3 sites. However, overall a lower coverage was observed, with only 21% of patients reporting or showing proof of influenza vaccination. This was surprising, considering that a significant part of our patients fell in at least one risk category or presented comorbidities. Given a 10.5% mortality rate due to influenza-related SARI cases in 2015, it is essential to assess the reasons for such low immunization frequency. Among

influenza deaths, 65% of patients and particularly those aged over 60 presented at least one risk factor likely to cause complications. [13] Our data emphasize the need for a comprehensive identification of the weaknesses of the vaccination campaigns and an intensification of the efforts to bring immunization to these individuals.

We also evaluated the prevalence of ORV in a subset of samples from HC/UFPR, and samples that were selected randomly among patients from Fortaleza and Rio de Janeiro. Overall, different respiratory viruses were found in 59% of the tested samples. This is consistent with previous reports showing high co-circulation of ORV in hospitalized patients during the influenza season, and confirmed that the viruses detected caused very similar ILI respiratory manifestations. [19-21] Moreover, our results reflect the profile of respiratory viruses in children, which was the predominant group included in the study. Interestingly, although circulation of influenza and ORV coincide in time, the frequency of codetection of influenza with ORV in the same patient was very low, whereas codetection of more than one ORV in a single patient was rather common. This observation is consistent with those in previous reports by our group, and we hypothesize that low codetection of influenza with other ORV is due to the higher pathogenicity of influenza. [20,22]

Next, we compared clinical and epidemiological data from influenza- and ORV-infected patients in an attempt to evaluate the impact of influenza infections. The latter were significantly more abundant in older patients and those with severe diseases, whereas comorbidities were associated with ORV infections. These findings reflect the higher virulence of influenza viruses, but could also be a consequence of a lower adhesion to immunization among older children. No difference in mortality was observed between groups infected with different viruses. This contrasts with a previous study performed in Fortaleza, which showed significant association between RSV

infections and infant mortality. [23] We believe that such discrepancy may be due to the low number of positive cases observed in the present study.

Studies on influenza seasonality in Brazil have shown that viral circulation in the Northeast region was more prevalent in the first 4 months of the year, coinciding with a period of higher humidity in that area [14,24], whereas a distinct pattern of viral circulation was observed in the South and Southeast. [25,26] Our data confirm this observation and reinforce the growing consensus that vaccinations in Northeast Brazil are being carried out in a post-seasonal period, with consequent low effectiveness.

The factors associated with the diversity of seasonal patterns displayed during influenza epidemics in tropics are not completely understood. Though previous study identified a seasonal southward traveling wave across Brazil (from equatorial to temperate regions), suggesting that environmental issues play more important role than population factors in driving the influenza epidemics in the country. [6]

To know the dynamics of dispersion of influenza viruses in tropical regions have been considered of crucial importance, since several reports have suggested that in these regions the influenza activity is more temporally diffuse and may contain the foci for the emergence of new variants. [6,7,27] In addition to the need for a different influenza immunization schedule in Brazil, the composition of this vaccine should also be evaluated, since its antigenic composition for this region may be different from that recommended for the temperate region. [7] However, information on strains circulating in tropics is still scarce, since most of the countries in this region have low income and the health surveillance is not completely employed. Besides that, the logistical challenges to implementing a national immunization program with different calendars and distinct antigenic compositions in Brazil meet the need for investments that provide self-sufficiency in its production [7].

The present study presented some limitations: i) the recruitment of patients during the seasonal period of influenza previously defined for two sites (Curitiba and Rio de Janeiro) may have contributed to low influenza B detection, although the frequency observed in our study was similar to that reported by national surveillance programs; ii) there was a predominance of pediatric patients in the study population, so data on ORV reflect mainly the profile of respiratory viruses in children; iii) the low inclusion of patients in Rio de Janeiro does not allow for an assessment of the impact of viral respiratory infections in that region; and iv) the monitoring of weather conditions is performed by organs linked to the states of each region of the country, so we decided to limit the description of the data from similar information that were made available by these institutes.

Despite these limitations, the results obtained here indicate that the model of active surveillance applied in our study is a valuable tool for investigating the impact of respiratory viruses in hospitalized patients in distinct regions of Brazil. We also reinforce the importance of monitoring influenza infections, thus enabling more adequate preventive measures for the population.

Authors Contributions

FKP, VP and JPB participated to the conception/design of the study, supervised the analysis, and interpreted the data. MMS and SMR supervised the analysis, interpreted the data and wrote the preliminary manuscript. FEAM and IT supervised the data collection from Fortaleza and Rio de Janeiro, respectively. BCC performed the tests, assembled the data and wrote the preliminary manuscript. VMA, LAP, MBN and LRV performed the molecular tests and collected the data from Curitiba. All authors provided contributions to the paper and approved the final version

Data Sharing Statement

No additional data available.

Competing interests

The authors have no competing interests.

Funding

This work was supported by Sanofi Pasteur through the Global Influenza Hospital Surveillance Network and the Mérieux Foundation.

Role of the funding source

The sponsors of the study had no role in data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for deciding to submit the work for publication.

REFERENCES

1. Bresee J, Hayden FG. Epidemic influenza-responding to the expected but unpredictable. *N Engl J Med* 2013; 368:589–92.
2. World Health Organization. Influenza (Seasonal) FactSheet211. 2014. (http://www.who.int/mediacentre/factsheets/fs211/en/). Accessed 13 September 2016.
3. Bloom-Feshbach K, Alonso WJ, Charu V, *et al*. Latitudinal variations in seasonal activity of influenza and respiratory syncytial virus (RSV): a global comparative review. *PLoS ONE* 2013; 8:e54445.
4. Caini S, Sue Huang Q, Ciblak MA, *et al.* Epidemiological and virological characteristics of influenza B: results of the Global Influenza B Study. *Influenza Other Respir Viruses* 2015; 9:3–12.
5. Alonso WJ, Yu C, Viboud C, *et al.* A global map of hemispheric influenza vaccine recommendations based on local patterns of viral circulation. *Sci Rep* 2015; 5:17214.

6. Alonso WJ, Viboud C, Simonsen L, et al. Seasonality of influenza in Brazil: A travelling wave from the Amazon to the subtropics. *Am J Epidemiol* 2007; 165:1434-1442.
7. Mello WA, de Paiva TM, Ishida MA, *et al*. The dilemma of influenza vaccine recommendations when applied to the tropics: The Brazilian case examined under alternative scenarios. *PLoS ONE* 2009; 8:e5095.
8. Tamerius JD, Shaman J, Alonso WJ, et al. Environmental Predictors of Seasonal Influenza Epidemics across Temperate and Tropical Climates. PLoS Pathog. 2013; 9(3):e1003194.
9. Castrucci MR. Factors affecting immune responses to the influenza vaccine. Hum *Vaccin Immunother* 2017 Jun 15:0. doi: 10.1080/21645515.2017.1338547
10. Cunha SS, Camacho LAB, Santos AC, *et al*. Influenza vaccination in Brazil: rationale and caveats. *Rev Sauude Puublica* 2005; 39:129–36.
11. Luna EJ, Gattás VL. Effectiveness of the Brazilian influenza vaccination policy, a systematic review. *Rev Inst Med Trop Sao Paulo* 2010; 52:175–81.
12. Oliveira JF, Boing AF, Waldman EA, *et al*. Ecological study on mortality from influenza and pneumonia before and after influenza vaccination in the Northeast and South of Brazil. *Cad Saude Publica* 2013; 29:2535–45.
13. Brazilian Health Ministry. Departamento de informática do SUS (DATASUS). (http://sipni.datasus.gov.br/si-pni-web/faces/relatorio/consolidado/coberturaVacinalCampanhaInfluenza.jsf). Accessed 11 December 2016.
14. Moura FE, Perdigão AC, Siqueira MM. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. *Am J Trop Med Hyg* 2009; 81:180–83.
15. World Health Organization. CDC protocol of realtime RTPCR for influenza A

(H1N1). 2009. (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/). Accessed 3 October 2015.

16. Biere B, Bauer B, Schweiger B. Differentiation of influenza B virus lineages Yamagata and Victoria by real-time PCR. *J Clin Microbiol* 2010; 48:1425–27.
17. Barros ENC, Cintra O, Rosseto E, *et al*. Patterns of influenza B circulation in Brazil and its relevance to seasonal vaccine composition. *Braz J Infect Dis* 2016; 20:81–90.
18. Brazilian Epidemiological Bulletim. Informe técnico – Situação epidemiológica da influenza (CREMESP). (http://www.cremesp.org.br/pdfs/Informe_tecnico_flu_17mar2016.pdf). Accessed 11 December 2016.
19. Brittain-Long R, Nord S, Olofsson S, *et al*. Multiplex real-time PCR for detection of respiratory tract infections. *J Clin Virol* 2008; 41:53–6.
20. Raboni SM, Stella V, Cruz CR, *et al*. Laboratory diagnosis, epidemiology, and clinical outcomes of pandemic influenza A and community respiratory viral infections in southern Brazil. *J Clin Microbiol* 2011; 49:1287–93.
21. Giamberardino HI, Homsani S, Bricks LF, *et al*. Clinical and epidemiological features of respiratory virus infections in preschool children over two consecutive influenza seasons in southern Brazil. *J Med Virol* 2016; 88:1325–33.
22. **T**rombetta H, Faggion HZ, Leotte J, *et al*. Human coronavirus and severe acute respiratory infection in Southern Brazil. *Pathog Glob Health* 2016; 110:113–18.
23. Alonso WJ, Laranjeira BJ, Pereira SA, *et al*. Comparative dynamics, morbidity and mortality burden of pediatric viral respiratory infections in an equatorial city. *Ped Infect Dis J* 2012; 31:e09–e14.

24. Moura FE. Influenza in the tropics. *Curr Opin Infect Dis* 2010; 23:415–20.

25. Tsuchiya LR, Costa LM, Raboni SM, *et al*. Viral respiratory infection in Curitiba, Southern Brazil. *J Infect* 2005; 51:401–07.

26. Belley N, Carraro E, Perosa A, *et al*. Patterns of influenza infections among different risk groups in Brazil. *Braz J Infect Dis* 2007; 11:399–402.

27. Rambaut A, Pybus OG, Nelson MI, *et al*. The genomic and epidemiological dynamics of human influenza A virus. *Nature* 2008; 453:615-619

Table 1. Epidemiological and clinical characteristics of the patients included in the study.

Characteristics	Curitiba n = 136 (%)	Fortaleza n = 427 (%)	RJ n = 32 (%)
Sex			
Male	61 (45)	243 (57)	12 (37)
Female	75 (55)	184 (43)	20 (63)
Age (Median, years/IQR)	1.4 (0.5–87.9)	1.3 (0–16.6)	1.4 (3.3–89.3)
Age group (years)			
< 2	86 (63)	254 (59)	0
2–4	23 (17)	100 (24)	3 (9)
5–17	11 (8)	73 (17)	4 (12)
18–64	12 (9)	0	9 (28)
> 65	4 (3)	0	16 (51)
Time to onset of symptoms (Median, days/IQR)	2 (0–7)	2 (0–7)	2 (0–6)
Exposure to smoking			
Yes	22 (16)	109 (25)	1 (3)
No	90 (66)	277 (65)	21 (66)
Ex-smoker	24 (18)	41 (10)	10 (31)
Chronic diseases*			
None	76 (56)	341 (78)	9 (28)

Cardiovascular	16 (12)	26 (6)	12 (38)
Pulmonary	32 (23)	8 (2)	7 (22)
Asthma	13 (10)	37 (9)	9 (28)
Diabetes	1 (1)	3 (1)	6 (19)
Immunosuppressed	2 (1.5)	9 (2)	1 (3)
Renal	0	7 (1.5)	2 (6)
Rheumatic disease	0	8 (2)	0
Cirrhosis	0	2 (0.5)	2 (6)
Neuromuscular	11 (8)	22 (5)	0
Neoplasm	1 (1)	1 (0.2)	5 (16)
Autoimmune	1 (1)	8 (2)	1 (3)
Length of stay in hospital (Median days/IQR)	2 (1–53)	1 (0–14)	2 (1–53)
Polypnea			
Yes	108 (80)	51 (12)	17 (53)
No	7 (5)	303 (71)	3 (9)
NI	21 (15)	73 (17)	12 (38)
Use of antiviral drugs			
Yes	13 (10)	10 (2)	0
Days of use (Median/IQR)	5 (2–5)	15 (1–35)	-
Vaccination in 2014			
Yes	42 (31)	59 (14)	6 (19)
No	94 (69)	344 (81)	26 (81)

NI	0	24 (5)	0
ICU admission			
Yes	37 (27)	10 (2)	26 (81)
No	99 (73)	417 (98)	6 (19)
Mechanical ventilation			
Yes	29 (21.3)	13 (3.0)	0
No	107 (78.7)	414 (97.0)	0
Death			
Yes	4 (3)	4 (1)	1 (3)
No	132 (97)	423 (99)	31 (97)
Severe disease	99 (73)	16 (4)	26 (81)

*Some patients presented more than one comorbidity. ICU, intensive care unit; IQR, interquartile range; NI, no information; RJ, Rio de Janeiro.

Table 2. Epidemiological and clinical characteristics of patients with positive diagnosis for respiratory viruses treated in reference hospitals in Curitiba, Fortaleza, and Rio de Janeiro, 2015.

Characteristics	Positive cases		
	Influenza virus n = 30 (%)	ORV n = 247 (%)	*p* value
Sex			
Male	13 (43)	121 (49)	0.5694
Female	17 (57)	126 (51)	-
Age (Median, years/IQR)	2.6 (1.3–5.9)	0.8 (0.2–2.0)	< 0.0001
Age group (years)			
< 2	9 (37)	180 (73)	0.0002
2–4	10 (33)	43 (17)	-
5–17	4 (13)	15 (6)	-
> 18	5 (17)	9 (4)	-
Time to onset of symptoms (Median, days/IQR)	1.5 (0–4)	2 (1–4)	0.1882
Chronic diseases*			
No	16 (53)	192 (78)	**0.0064** (OR 3.0 95% CI 1.4–6.6)
Pulmonary	6 (20)	40 (16)	-
Cardiovascular	7 (23)	16 (6)	-

Other	3 (10)	27 (11)	-
Length of stay in hospital (Median days/IQR)	2 (1–6)	1.5 (1–9.7)	0.8726
Polypnea			
Yes	11 (37)	88 (36)	0.8325
No	15 (50)	139 (56)	-
NI	4 (13)	20 (8)	-
Use of antiviral drugs			
Yes	1 (3)	9 (4)	NA
Days of use (Median/ IQR)	5	5 (5–35)	-
Vaccination in 2014			
Yes	4 (13)	28 (11)	0.7634
No	26 (87)	216 (87)	-
NI	0	3 (2)	-
ICU admission			
Yes	8 (27)	22 (9)	**0.0081** (OR 3.7 95% CI 1.4–9.3)
No	22 (73)	225 (91)	-
Mechanical ventilation			
Yes	6 (20)	15 (6)	**0.0164** (OR 3.8, 95% CI 1.3–10.9)
No	24 (80)	232 (94)	-

Death			
Yes	1 (3)	6 (2)	0.5006
No	29 (97)	241 (98)	
Severe disease			
Yes	9 (30)	26 (11)	**0.0061** (OR 3.6, 95% CI 1.5–8.7)
No	21 (70)	221 (89)	-

*Some patients presented more than one comorbidity. CI, confidence interval; ICU, intensive care unit; IQR, interquartile range. NI, no information; OR, odds ratio. In bold, statistically significant values.

Figure Legends

Figure 1. Map of Brazil, showing the location of Curitiba, Rio de Janeiro and Fortaleza, and the total of screened (Blue) and included (Red) patients in each site.

Figure 2. Flowchart summarizing the process of patient inclusion in the study, performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

Figure 3. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

Figure 4. Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow (green and red lines represent mean temperatures and precipitations, respectively).

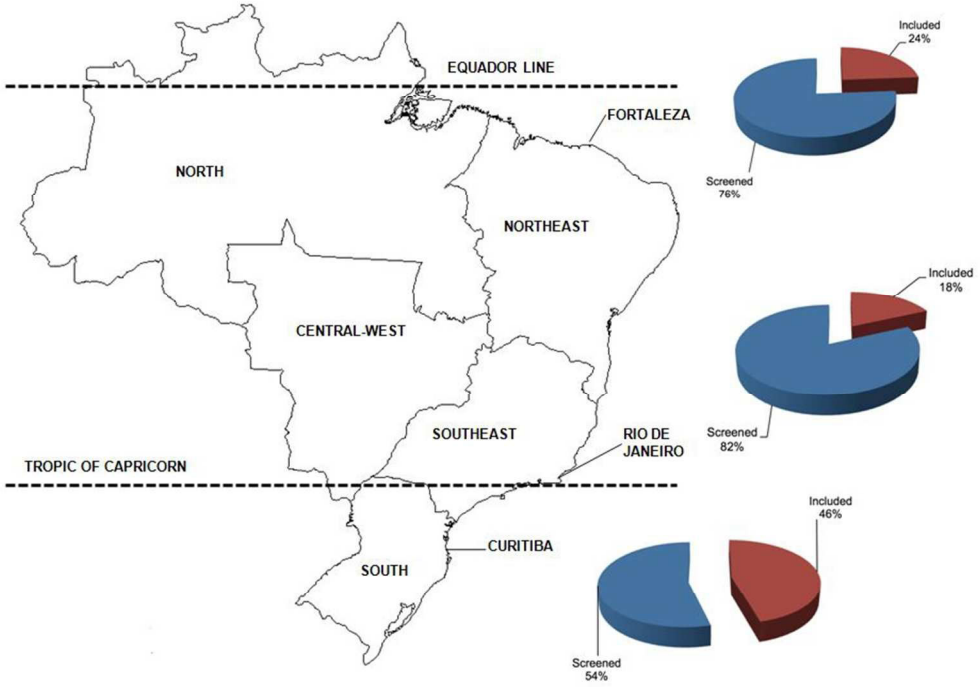


Figure 1. Map of Brazil, showing the location of Curitiba, Rio de Janeiro and Fortaleza, and the total of screened (Blue) and included (Red) patients in each site.

79x55mm (300 x 300 DPI)

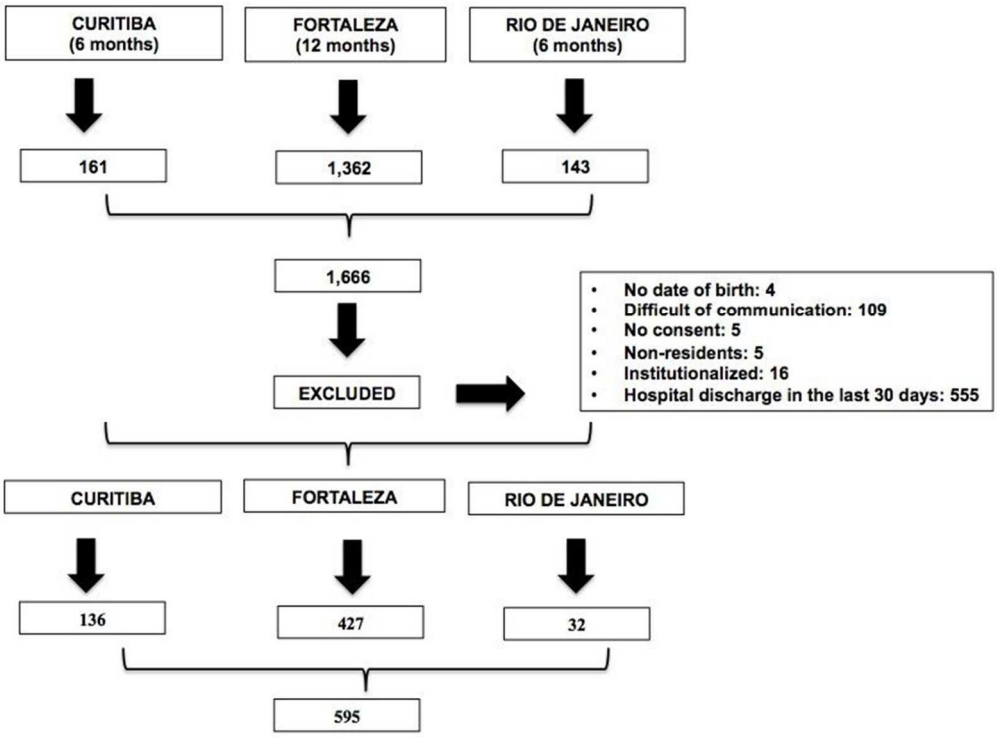


Figure 2. Flowchart summarizing the process of patient inclusion in the study, performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

66x49mm (300 x 300 DPI)

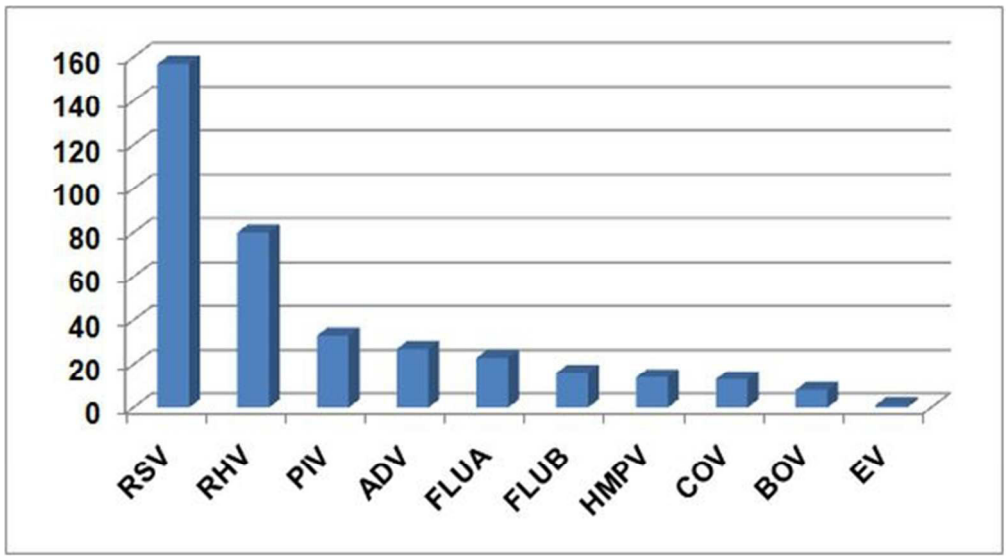


Figure 3. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

54x29mm (300 x 300 DPI)

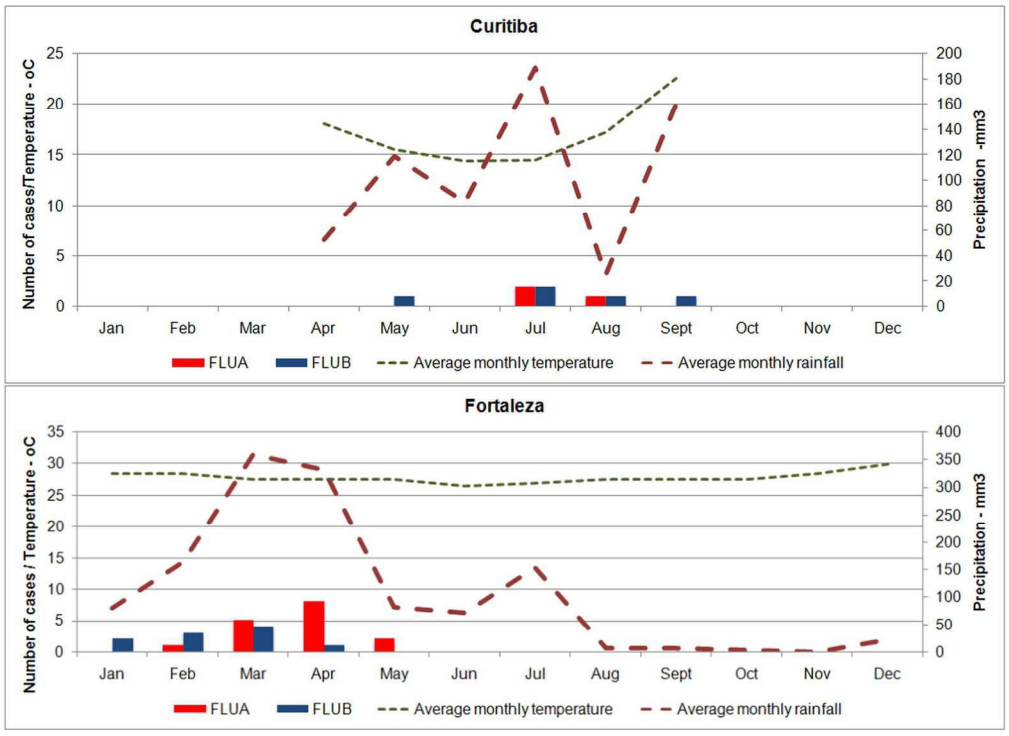


Figure 4. Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow (green and red lines represent mean temperatures and precipitations, respectively).

185x134mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	01
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	02-03
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	04-05
Objectives	3	State specific objectives, including any prespecified hypotheses	05
Methods			
Study design	4	Present key elements of study design early in the paper	05
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	05-07
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls ***Cross-sectional study***—Give the eligibility criteria, and the sources and methods of selection of participants	07
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Not applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	07
Bias	9	Describe any efforts to address potential sources of bias	08
Study size	10	Explain how the study size was arrived at	09 Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	08
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	08
		(*b*) Describe any methods used to examine subgroups and interactions	Not applicable
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	08
		(*e*) Describe any sensitivity analyses	Not applicable

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	09
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	09
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	*09-10*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	09-10 Table 1
		(*b*) Report category boundaries when continuous variables were categorized	Table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14-15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Global Influenza Hospital-based Surveillance Network (GIHSN): results of surveillance of influenza and other respiratory viruses in hospitalized patients in Brazil, 2015

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017603.R2
Article Type:	Research
Date Submitted by the Author:	22-Sep-2017
Complete List of Authors:	Raboni, Sonia M; Universidade Federal do Parana, Infectious Diseases Moura, Fernanda; Universidade Federal do Ceara, Departamento de Patologia e Medicina Legal Caetano, Braulia; Fundacao Oswaldo Cruz, Laboratório de Vírus Respiratórios e do Sarampo Avanzi, Valeria; Universidade Federal do Parana, Laboratório de Virologia Pereira, Luciane; Universidade Federal do Parana, Laboratório de Virologia Nogueira, Meri; Universidade Federal do Parana, Laboratório de Virologia Vidal, Luine; Universidade Federal do Parana, Laboratório de Virologia Tavares, Isabel; Fundacao Oswaldo Cruz, Instituto Nacional de Saúde Pradel, Florence; Fondation Merieux Picot, Valentina; Fondation Merieux Puig-Barbera, Joan; FISABIO-Public Health, Vaccines Research Siqueira, Marilda; Fundacao Oswaldo Cruz, Laboratório de Vírus Respiratórios e do Sarampo
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Public health, Epidemiology
Keywords:	influenza, Hospital admission, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

Global Influenza Hospital-based Surveillance Network (GIHSN): results of surveillance of influenza and other respiratory viruses in hospitalized patients in Brazil, 2015

Sonia M. Raboni[1,*], Fernanda E. A. Moura[2], Braulia C. Caetano[3], Valéria M. Avanzi[1], Luciane A. Pereira[1], Meri B. Nogueira[1], Luine R. Vidal[1], Isabel Tavares[4], Florence K. Pradel[5], Valentina Picot[5], Joan Puig-Barbera[6], Marilda M. Siqueira[3]

[1]Infectious Diseases Department, Universidade Federal do Paraná, Curitiba, Brazil

[2] Departamento de Patologia e Medicina Legal, Universidade Federal do Ceará, Fortaleza, Brazil

[3]Laboratório de Vírus Respiratórios e do Sarampo, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

[4]Instituto Nacional de Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

[5] Fondation Mérieux, Lyon, France

[6] Vaccines Research, FISABIO-Health Public, Valencia, Spain

* Corresponding author.

Hospital de Clínicas/Universidade Federal do Paraná, 180 General Carneiro Street, 3rd floor, Curitiba, Paraná, Brazil 80060-900.

Phone: +55 41 35256814

E-mail: sraboni@ufpr.br

Running head: Seasonality of influenza in Brazil

SUMMARY

Background: Influenza-like illness occurs annually worldwide, with peak timing and severity varying seasonally and resulting in significant annual mortality.

Objectives: There were three objectives: 1) describe the epidemiological and clinical features of hospitalized patients with severe acute respiratory infection caused by influenza and other respiratory viruses; 2) report the influenza seasonality in the region, and 3) correlate findings of influenza circulation and immunization time in Brazil.

Patients/Methods: This study took place in three Brazilian hospitals located in cities with different climatic conditions [Curitiba (south), Rio de Janeiro (southeast), and Fortaleza (northeast)]. Patients presenting an acute process with indication for admission consisting of a predefined set of conditions potentially associated with recent influenza infection were enrolled.

Results: We screened 1666 patients, with 595 meeting the inclusion criteria. Influenza viruses and other respiratory viruses (ORVs) were detected in 6.5% and 59% of patients, respectively. Influenza-positive cases fell into the severe spectrum as compared with those with ORV (30% vs. 11%), but without any difference in mortality rates. Epidemiological results revealed variations in the peak time of influenza infections between northeast (Fortaleza) and south (Curitiba) Brazil, basically following the rain period of each region. In northeast Brazil, viral circulation was prevalent in the first 4 months of the year, indicating that the vaccination campaign occurred in a post-seasonal period, possibly explaining the low effectiveness.

Conclusions: The active-surveillance model is a valuable tool for investigating respiratory virus impact on hospitalized patients, with influenza-infection monitoring enabling implementation of adequate preventive measures.

Keywords: hospital admissions, influenza, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection.

Strengths and limitations of this study

- The strengths of this study are the use of the same model of active surveillance applied in other outside centers allowing subsequent comparisons on the impact of respiratory viruses, mainly influenza virus, in hospitalized patients worldwide. Second, to add more information on seasonality and severity of the influenza disease in South America, whose data are limited. Finally, our data reinforce the growing consensus that vaccinations in Northeast Brazil (tropical climate region) are being carried out in a post-seasonal period, with consequent low effectiveness.
- The main weakness of the data is the low inclusion of patients in Rio de Janeiro that does not allow for an assessment of the impact of viral respiratory infections in southeastern Brazil. The study was carried out with data collected in one year, which limits predictions on the impact of influenza longitudinally in these locales.

1. INTRODUCTION

Influenza-like illness (ILI) occurs annually worldwide, but peak timing and severity of the epidemic vary seasonally.[1] Although surveillance for antigenic drift or influenza-virus shift is intense, annual mortality still exceeds 250,000 and results usually from complications, such as pneumonia, neurological events, and circulatory failure.[2]

Seasonal influenza epidemics peak during the winter in temperate latitudes of both the Northern and Southern Hemispheres. By contrast, tropical countries might experience two annual peaks, with shorter and less intense epidemics.[3,4] Moreover, previous studies showed that influenza activity is frequently out of phase with the hemispheric winter in many tropical regions and some temperate ones, with the consequence that optimal timing for routine influenza-vaccination recommendations does not necessarily correspond to the activity expected for that hemisphere.[5]

Brazil, which includes temperate and tropical regions, exhibits an important regional heterogeneity in influenza peak.[6,7] Seasonal influenza epidemics are usually associated with "cold-dry" and "humid-rainy" climates[8]; however, in addition to climate and environmental conditions, host factors, such as age, preexisting immunity, genetic polymorphisms, and presence of comorbidities, are associated with seasonal variations of this infection, vaccine responsiveness, as well as the severity of influenza epidemics.[9]

Vaccination is the main public health measure used to reduce the frequency of severe influenza cases.[2] In Brazil, influenza vaccination has been performed annually since 1999. Initially, immunization targeted individuals aged ≥65 years; however, in 2000, the age limit was lowered to 60 years. In 2016, the Brazilian Ministry of Health extended the recommendation to children <5 years, pregnant women, people with

chronic non-communicable diseases, and health professionals.[10–12] Overall, in 2015, national immunization campaigns achieved a vaccination coverage of 82.7% in priority groups.[13]

The Global Influenza Hospital-Based Surveillance Network (GIHSN) is a public–private partnership between research institutes, hospitals, and several laboratories around the world, and was established to study the epidemiology of severe influenza in consecutive seasons in different countries. (http://www.gihsn.org/?page=map). In 2015, this network comprised a total of 31 collaborating sites from seven countries, including Brazil. All participants follow the same core investigation protocol developed by the GIHSN scientific committee. Through broad geographical coverage and data standardization, the GIHSN group seeks to obtain a global picture of the impact of influenza on patients with respiratory pathologies. Additionally, this network provides invaluable tools to investigate the seasonality of influenza in regions where this information is not available, as well as a framework for estimating the effectiveness of seasonal influenza vaccines in preventing severe cases among age and risk groups.

In 2015, the GIHSN group began a study in Rio de Janeiro, Fortaleza, and Curitiba, three cities located in distinct regions of Brazil and enabling the evaluation of effects of different climatic conditions on the seasonality of influenza infections. Here, we report the initial findings of this surveillance project, including seasonality activity and the clinical and epidemiological features observed.

2. PATIENTS AND METHODS

2.1. Ethical statement

As a multicenter study, this project was approved by the Ethical Committee of each institution involved in the research: Instituto Nacional de Infectologia Evandro Chagas (INI/Fiocruz), Rio de Janeiro; Hospital de Clínicas-Universidade Federal do Paraná (HC/UFPR), Curitiba; and Hospital Infantil Albert Sabin (HIAS), Fortaleza. All procedures were performed according to approved protocols, and consent was obtained from all patients (or guardians).

2.2. Study design and site description

This multicenter, cross-sectional study was designed to provide information on active surveillance of influenza in hospitalized patients. Data were collected in 2015 from three participating hospitals located in distinct cities and regions of Brazil: Curitiba (south), Fortaleza (northeast), and Rio de Janeiro (southeast) (Figure 1).

In Curitiba and Rio de Janeiro, patient recruitment and data collection occurred from April to September, coinciding with the period of influenza seasonality in south and southeast Brazil. In Fortaleza, the study spanned all of 2015, because, despite a previous study showing influenza circulation between January and April,[14] the seasonality of influenza in this region is not well established.

In Curitiba, the study was performed at HC/UFPR, a 310-bed, tertiary care, academic center, with 47 beds in the intensive care unit (ICU). It is also among the sentinel hospitals for influenza and severe acute respiratory infection (SARI) surveillance in the region. Curitiba is located at 25° 25′ S, 49° 15′ W, 924 m (3031 ft) and has a mild marine west coast climate, with no dry season and warm summers. Seasonality is moderate, with heavy precipitation during mild winters and a mean temperature of 16.5°C. The city population numbered ~1,879,355 people in 2015.

In Fortaleza, this study was conducted at HIAS, a public health institution of 270 beds. Fortaleza is a city of 2,500,000 inhabitants and is located at sea level, 4° south of

the equator. Its tropical climate is characterized by two distinct seasons: a rainy one occurring between January and May, and a dry one occurring during the rest of the year. There is high relative humidity (79%) and little variation in the average temperature (26.4°C).

In Rio de Janeiro, this study was conducted at Hospital Quinta D'Or, which is a private hospital with 350 beds for general admission and 150 beds in the ICU. Rio de Janeiro is located at 22° 54′S, 43° 12′W, and its climate is classified as tropical Atlantic. The year comprises two seasons: a hot and relatively humid one and another with mild temperatures and less rainfall.

2.3. Patient recruitment and inclusion/exclusion criteria

Patients of all ages who were admitted for any acute condition described as potentially associated with influenza were considered eligible for enrollment in the study. To be included, eligible patients were identified in the hospital admission registries and had to comply with all of the following criteria: 1) admission within 24 h to 72 h prior to recruitment; 2) a resident of the area of the study (defined for each site) and belonging to the base population source; and 3) a referral history for any acute condition associated with influenza and exhibiting onset within ≤7 days prior to hospital admission. This study used the European Center for Disease Control definition for ILI, which included at least one of four systemic symptoms (fever, headache, myalgia, or malaise) and at least one of three respiratory symptoms (cough, sore throat, or shortness of breath). Patients who did not give consent to participate, institutionalized individuals, and those with a history of hospitalization ≤30 days prior to recruitment were excluded from the study.

2.4. Sample collection and processing

Samples were collected using nylon FLOQswabs (Copan, Brescia, Italy) and

included one nasopharyngeal and one pharyngeal swab (or nasal swab for patients <14 years). Samples were added to a flask containing 3 mL of universal viral-transport medium (UTM medium; Copan) and stored at −80°C until testing.

2.4.1. Sample testing to detect influenza and other respiratory viruses (ORVs)

All samples were submitted for nucleic acid extraction using a QIAmp viral RNA mini kit (Qiagen, Hilden, Germany). The presence of influenza A (H1N1pdm and H3N2) and influenza B (B/Yamagata or B/Victoria) was analyzed by reverse transcription real-time polymerase chain reaction (RT-PCR) according to Center for Disease Control (CDC) protocol.[15,16]

In a subset of samples (n = 497), the presence of other pathogens was assessed using a commercial multiplex RT-PCR assay (FTD respiratory pathogens 21 kit; Fast-Track Diagnostics, Esch-sur-Alzette, Luxembourg) designed to detect the following: influenza A (FLUA) (H1N1pdm09); influenza B (FLUB); rhinovirus (RHV); coronavirus (COV) genotypes NL63, 229E, OC43, and HKU1; parainfluenza (PIV) types 1, 2, 3, and 4; human metapneumovirus A/B (HMPV); bocavirus (BOV); respiratory syncytial virus A/B (RSV); adenovirus (ADV); enterovirus (ENV); parechovirus (PRV); and the atypical bacterium *Mycoplasma pneumonia*.

Alternatively, we used the Seeplex RV15 ACE detection kit (Seegene Inc., Seoul, Korea), a multiplex PCR-based assay allowing for the simultaneous detection of the following viruses: ADV; HMPV; PIV types 1, 2, 3, and 4; FLUA; FLUB; RSV types A and B; rhinovirus (HRV) types A, B, and C; ENV; BOV; and COV types 229E/NL63 and OC43/HKU1. Both tests were performed according to manufacturer protocol.

2.5 Data analysis

Data were compiled using JMP software version 5.2.1 (SAS Institute Inc., Cary, NC, USA) and analyzed using GraphPad Prism version 5.03 (GraphPad Software, Inc., La Jolla, CA, USA). Baseline demographic and clinical characteristics with normal and non-normal distributions were presented as the mean ± standard deviation and medians with interquartile ranges (IQRs), respectively. Epidemiological and clinical data comparing patients infected with influenza and those infected with ORV were assessed; however, only monoinfected cases were included in this analysis. Fisher's exact test, chi-squared test, or the Wilcoxon-Mann-Whitney test was performed as appropriate. A multivariate logistic regression model was fitted to evaluate clinical or epidemiological characteristics of virus-positive cases to determine association with disease severity. Variables included age, presence of comorbidities, and fatal outcome. All statistical tests were two-sided and considered significant at $p < 0.05$.

We defined severe disease as that requiring mechanical ventilation, admission to an ICU, or resulting in a fatal outcome. To improve our understanding of the seasonality of influenza infections at the study sites, we evaluated the monthly distribution of samples and the viruses detected by RT-PCR. This information was then plotted against the temperature and precipitation (historical means) recorded for each month.

3. RESULTS

3.1 Demographics and epidemiological characteristics of the study population

During the study period, 1666 patients from three participating hospitals were screened (161 in Curitiba, 1362 in Fortaleza, and 143 in Rio de Janeiro), with 595 patients meeting the inclusion criteria (Figure 2).

The demographic and clinical characteristics of the population are listed in Table 1. In the Curitiba and Fortaleza subsets, males and females were recruited in equal

proportions, with most individuals <5 years of age. By contrast, in Rio de Janeiro, females and adults were predominant, as the hospital did not evaluate children.

As expected from a predominantly young population, the prevalence of chronic conditions associated with aggravation of influenza was low in the Curitiba and Fortaleza subsets, whereas the Rio de Janeiro subset was characterized by a significantly higher prevalence of conditions, such as cardiovascular disease, chronic obstructive pulmonary disease, and diabetes.

Other factors that impact the prevalence of severe influenza infections are the use of antivirals and vaccination. Antiviral administration was more frequently reported in Curitiba. Overall, influenza vaccination rates were low, despite most age groups included in the study being covered by the Brazilian immunization program: ~31% of patients from Curitiba were vaccinated, and an estimated 14% and 19% were vaccinated in Fortaleza and Rio de Janeiro, respectively. Only 21% of children <5 years who were included in the study were vaccinated. Severe disease was detected in 149/595 cases (24%), but mortality was low: 9/595 (1.5%).

3.2. Influenza and ORV evaluation

All samples were tested for influenza virus, with 39 (6.5%) positive: 23 (59%) influenza A H3N2 and 16 (41%) for influenza B (all Yamagata-like lineages). Of these cases, nine (23%) presented ORV co-infection.

The presence of ORV was tested in 497 patients (83.5%), with 293 (59%) positive and 46 (16%) presenting co-infection. RSV was the most frequently identified virus and the main source of co-infections (n = 27; 49%), whereas influenza co-infections were found in only 23% (9/39) of cases. The respiratory pathogens detected in this subset of samples are reported in Figure 3.

3.3. Severe cases and mortality associated with influenza and ORV

As shown in Table 2, 9/37 influenza-positive cases fell into the severe spectrum, and severe disease was significantly more frequent in influenza-infected patients than in those with ORV (30% vs. 11%). In an adjusted analysis, only age was associated with this outcome ($p = 0.01$), whereas the presence of comorbidities was not ($p = 0.12$). No difference in mortality was observed between influenza- and ORV-infected patients.

3.4. Seasonality of influenza infections in two geographic regions of Brazil

Comparison of the seasonality of influenza infections with temperature and precipitation (historical means) recorded for each month revealed variations between the northeast (Fortaleza) and south (Curitiba) regions of Brazil in relation to the peak period of influenza infections; however, in both regions, influenza coincided with the peak of the rainy season. Our results indicated that immunization against influenza in the northeast was performed after the period of virus circulation (Figure 4), whereas data from Rio de Janeiro were not evaluated due to the small number of patients included in the study.

4. DISCUSSION

The GHISN was established with the main goal of improving the understanding of roles of influenza infections in the development of severe respiratory diseases. The information gathered by this network can potentially contribute to the elucidation of influenza seasonality in some regions, implementation of more efficient containment measures, and the improvement of preventive interventions, such as immunization.

In Brazil, the Ministry of Health employs two systems for epidemiological assessment of respiratory diseases: sentinel surveillance of ILI and universal surveillance of SARI. Sentinel surveillance consists of a network of designated health care units (public or private) distributed throughout the country, in which random

samples of respiratory cases are periodically collected for detection and genetic characterization of circulating viruses. In universal SARI surveillance, all severe respiratory cases admitted to ICUs and all deaths related to respiratory disease are investigated in the laboratory.[17] In the present study, only hospitalized patients identified by an active search of the participating health centers were included. In contrast to the Brazilian universal SARI surveillance protocol, which is restricted to ICUs, screening for our study included patients admitted to other wards and presenting diseases other than respiratory related. Individuals admitted with conditions associated with aggravation of influenza infections, such as cardiovascular disease and diabetes, were also screened, thereby increasing the number of identified cases.

Overall, the frequency of influenza viruses in the study specimens was 6.5% (39/595), with influenza A comprising 60% of the flu-positive samples. This low prevalence reflected the profile of the 2015 influenza season in Brazil and in most of the Southern Hemisphere and tropical regions. During that year, viral activity remained low through the entire season, with higher prevalence of H3N2 and lower detection of influenza B and H1N1pdm09. Even in temperate regions of the Southern Hemisphere, activity remained at an interseasonal level. In Brazil, a total of 11,945 cases of SARI were reported in 2015, of which 1089 (9%) were related to influenza infection. Among these cases, 599 (55%) were influenza A/H3N2, 234 (21.5%) were influenza B, 141 (12.9%) were influenza A/H1N1pdm09, and 115 (10.5%) were reported as non-subtyped influenza A.[18]

Since 2014, the Brazilian Ministry of Health has included children ≤4 years of age in the group of individuals at risk of severe influenza and subsequently began vaccinating this subpopulation. Data from the National Immunization Program show coverage rates >83% among young children and pregnant women, 89% in the elderly,

and 95% in health workers.[13,17] In the present study, we observed that there was a higher percentage of patients from Curitiba vaccinated among the three sites; however, an overall lower coverage was observed, with only 21% of patients reporting or showing proof of influenza vaccination. This was surprising, considering that a significant number of our patients fell in at least one risk category or presented comorbidities. Given a 10.5% mortality rate due to influenza-related SARI cases in 2015, it is essential to assess the reasons for such low immunization frequency. Among influenza deaths, 65% of patients and particularly those aged over 60 presented at least one risk factor likely to cause complications.[13] Our data emphasized the need for comprehensive identification of the weaknesses in the vaccination campaigns and an intensification of the efforts to bring immunization to these individuals.

We also evaluated the prevalence of ORV in a subset of samples from HC/UFPR and in samples selected randomly from among patients from Fortaleza and Rio de Janeiro. Overall, different respiratory viruses were found in 59% of the tested samples. This was consistent with previous reports showing high co-circulation of ORV in hospitalized patients during the influenza season, and confirmed that the viruses detected caused very similar ILI respiratory manifestations.[19–21] Moreover, our results reflected the profile of respiratory viruses in children, which was the predominant group included in the study. Interestingly, although circulation of influenza and ORV coincided over time, the frequency of co-detection of influenza with ORV was very low, whereas co-detection of more than one ORV in a single patient was rather common along with RSV. This observation was consistent with those in our previous reports, and we hypothesize that low codetection of influenza with other ORVs in these hospitalized patients was likely due to the higher pathogenicity of influenza.[20,22]

We then compared clinical and epidemiological data from influenza- and ORV-infected patients to evaluate the impact of these infections. Influenza was significantly more frequent in older patients and those with severe diseases, whereas comorbidities were associated with ORV infections. These findings reflected a lower adhesion to immunization among older children, with the presence of severe disease a possible consequence of the higher virulence of influenza viruses, although no difference in mortality was observed between both groups. This contrasted with results from a previous study performed in Fortaleza, showing significant association between RSV infections and infant mortality.[23] We believe that such a discrepancy might be due to the low number of positive cases observed in the present study.

Studies of influenza seasonality in Brazil show that viral circulation in the northeast region is more prevalent in the first 4 months of the year, coinciding with a period of higher humidity in that area,[14,24] whereas viral circulation in the south and southeast appears associated with lower temperature and unlikely to be linked to humidity data.[25,26] Our data confirmed this observation and reinforced the growing consensus that vaccinations in northeast Brazil are being carried out in a post-seasonal period, with consequent low effectiveness.

The factors associated with the diversity of seasonal patterns displayed during influenza epidemics in the tropics are not completely understood. A previous study identified a seasonal southward-traveling wave across Brazil (from equatorial to temperate regions), suggesting that environmental issues play more important roles than population factors, such as immune function and levels of vitamin D, in driving influenza epidemics in the country.[6]

Understanding the dispersion dynamics of influenza viruses in tropical regions is considered of crucial importance, given that several reports suggested that in these

regions, influenza activity is more temporally diffuse and might contain foci for the emergence of new variants.[6,7,27] In addition to the need for a different influenza-immunization schedule in Brazil, the composition of this vaccine should also be evaluated, because the antigenic composition for this region might differ from that recommended for the temperate region.[7] However, information on strains circulating in tropical regions remains scarce, because most countries in these regions have low income, and health surveillance is not completely employed. Additionally, policies and resources need to be established to overcome the logistical challenges to implementing national immunization programs using different calendars and distinct antigenic compositions in Brazil.[7]

This study contained some limitations. Patient recruitment during the seasonal period of influenza previously defined for two sites (Curitiba and Rio de Janeiro) might have contributed to low influenza B detection, although the frequency observed here was similar to that reported by national surveillance programs. Additionally, the study was performed using data collected over the course of 1 year, which limited predictions of the impact of influenza longitudinally in these locales. There was a predominance of pediatric patients in the study population; therefore, data concerning ORV reflect mainly the profile of respiratory viruses in children. The low number of patients from Rio de Janeiro did not allow for an assessment of the impact and seasonality of viral respiratory infections in that region. Finally, the monitoring of weather conditions was performed by organizations linked to the states of each region of the country; therefore limited the description of data to that from similar information made available by these institutions.

Despite these limitations, our results indicated that the model of active surveillance applied in our study represents a valuable tool for investigating the impact

of respiratory viruses in hospitalized patients in distinct regions of Brazil. We also reinforced the importance of monitoring influenza infections to enable more adequate preventive measures for the population.

Acknowledgments

This work was supported by Sanofi Pasteur through the Global Influenza Hospital Surveillance Network and the Mérieux Foundation.

Author contributions

FKP, VP, and JPB participated to the conception/design of the study, supervised the analysis, and interpreted the data. MMS and SMR supervised the analysis, interpreted the data, and wrote the preliminary manuscript. FEAM and IT supervised the data collection from Fortaleza and Rio de Janeiro, respectively. BCC performed the tests, assembled the data, and wrote the preliminary manuscript. VMA, LAP, MBN, and LRV performed the molecular tests and collected the data from Curitiba. All authors contributed to the writing and review of the manuscript and approved the final version.

Competing interests

The authors have no competing interests.

REFERENCES

1. Bresee J, Hayden FG. Epidemic influenza—responding to the expected but unpredictable. N Engl J Med 2013;368:589-592.
2. World Health Organization. Influenza (Seasonal) Fact Sheet 211. 2014.

(http://www.who.int/mediacentre/factsheets/fs211/en/). Accessed 13 September 2016.

3. Bloom-Feshbach K, Alonso WJ, Charu V, et al. Latitudinal variations in seasonal activity of influenza and respiratory syncytial virus (RSV): a global comparative review. PLoS ONE 2013;8:e54445.
4. Caini S, Sue Huang Q, Ciblak MA, et al. Epidemiological and virological characteristics of influenza B: results of the Global Influenza B Study. Influenza Other Respir Viruses 2015;9:3-12.
5. Alonso WJ, Yu C, Viboud C, et al. A global map of hemispheric influenza vaccine recommendations based on local patterns of viral circulation. Sci Rep 2015;5:17214.
6. Alonso WJ, Viboud C, Simonsen L, et al. Seasonality of influenza in Brazil: a travelling wave from the Amazon to the subtropics. Am J Epidemiol 2007;165:1434-1442.
7. Mello WA, de Paiva TM, Ishida MA, et al. The dilemma of influenza vaccine recommendations when applied to the tropics: the Brazilian case examined under alternative scenarios. PLoS ONE 2009;8:e5095.
8. Tamerius JD, Shaman J, Alonso WJ, et al. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. PLoS Pathog 2013;9:e1003194.
9. Castrucci MR. Factors affecting immune responses to the influenza vaccine. Hum Vaccin Immunother 2017. doi: 10.1080/21645515.2017.1338547.
10. Cunha SS, Camacho LAB, Santos AC, et al. Influenza vaccination in Brazil: rationale and caveats. Rev Sauude Puublica 2005;39:129-136.
11. Luna EJ, Gattás VL. Effectiveness of the Brazilian influenza vaccination policy, a

systematic review. Rev Inst Med Trop Sao Paulo 2010;52:175-181.

12. Oliveira JF, Boing AF, Waldman EA, et al. Ecological study on mortality from influenza and pneumonia before and after influenza vaccination in the northeast and south of Brazil. Cad Saude Publica 2013;29:2535-2545.
13. Brazilian Health Ministry. Departamento de informática do SUS (DATASUS). (http://sipni.datasus.gov.br/si-pni-web/faces/relatorio/consolidado/coberturaVacinalCampanhaInfluenza.jsf). Accessed 11 December 2016.
14. Moura FE, Perdigão AC, Siqueira MM. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. Am J Trop Med Hyg 2009;81:180-183.
15. World Health Organization. CDC protocol of real-time RT-PCR for influenza A (H1N1). 2009. (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/). Accessed 3 October 2015.
16. Biere B, Bauer B, Schweiger B. Differentiation of influenza B virus lineages Yamagata and Victoria by real-time PCR. J Clin Microbiol 2010;48:1425-1427.
17. Barros ENC, Cintra O, Rosseto E, et al. Patterns of influenza B circulation in Brazil and its relevance to seasonal vaccine composition. Braz J Infect Dis 2016;20:81-90.
18. Brazilian Epidemiological Bulletim. Informe técnico – Situação epidemiológica da influenza (CREMESP). (http://www.cremesp.org.br/pdfs/Informe_tecnico_flu_17mar2016.pdf). Accessed 11 December 2016.
19. Brittain-Long R, Nord S, Olofsson S, et al. Multiplex real-time PCR for detection of respiratory tract infections. J Clin Virol 2008;41:53-56.
20. Raboni SM, Stella V, Cruz CR, et al. Laboratory diagnosis, epidemiology, and

clinical outcomes of pandemic influenza A and community respiratory viral infections in southern Brazil. J Clin Microbiol 2011;49:1287-1293.

21. Giamberardino HI, Homsani S, Bricks LF, et al. Clinical and epidemiological features of respiratory virus infections in preschool children over two consecutive influenza seasons in southern Brazil. J Med Virol 2016;88:1325-1333.
22. Trombetta H, Faggion HZ, Leotte J, et al. Human coronavirus and severe acute respiratory infection in Southern Brazil. Pathog Glob Health 2016;110:113-118.
23. Alonso WJ, Laranjeira BJ, Pereira SA, et al. Comparative dynamics, morbidity and mortality burden of pediatric viral respiratory infections in an equatorial city. Ped Infect Dis J 2012;31:e09-e14.
24. Moura FE. Influenza in the tropics. Curr Opin Infect Dis 2010;23:415-420.
25. Tsuchiya LR, Costa LM, Raboni SM, et al. Viral respiratory infection in Curitiba, southern Brazil. J Infect 2005;51:401-407.
26. Belley N, Carraro E, Perosa A, et al. Patterns of influenza infections among different risk groups in Brazil. Braz J Infect Dis 2007;11:399-402.
27. Rambaut A, Pybus OG, Nelson MI, et al. The genomic and epidemiological dynamics of human influenza A virus. Nature 2008;453:615-619.

Table 1. Epidemiological and clinical characteristics of the patients included in this study.

Characteristics	Curitiba (*n* = 136) (%)	Fortaleza (*n* = 427) (%)	RJ (*n* = 32) (%)
Sex			
Male	61 (45)	243 (57)	12 (37)
Female	75 (55)	184 (43)	20 (63)
Age (median, y/IQR)	1.4 (0.5–87.9)	1.3 (0–16.6)	1.4 (3.3–89.3)
Age group (y)			
<2	86 (63)	254 (59)	0
2–4	23 (17)	100 (24)	3 (9)
5–17	11 (8)	73 (17)	4 (12)
18–64	12 (9)	0	9 (28)
> 65	4 (3)	0	16 (51)
Time to onset of symptoms (Median, days/IQR)	2 (0–7)	2 (0–7)	2 (0–6)
Exposure to smoking			
Yes	22 (16)	109 (25)	1 (3)
No	90 (66)	277 (65)	21 (66)
Ex-smoker	24 (18)	41 (10)	10 (31)
Chronic diseases*			
None	76 (56)	341 (78)	9 (28)

Cardiovascular	16 (12)	26 (6)	12 (38)
Pulmonary	32 (23)	8 (2)	7 (22)
Asthma	13 (10)	37 (9)	9 (28)
Diabetes	1 (1)	3 (1)	6 (19)
Immunosuppressed	2 (1.5)	9 (2)	1 (3)
Renal	0	7 (1.5)	2 (6)
Rheumatic disease	0	8 (2)	0
Cirrhosis	0	2 (0.5)	2 (6)
Neuromuscular	11 (8)	22 (5)	0
Neoplasm	1 (1)	1 (0.2)	5 (16)
Autoimmune	1 (1)	8 (2)	1 (3)
Length of stay in hospital (Median days/IQR)	2 (1–53)	1 (0–14)	2 (1–53)
Polypnea			
Yes	108 (80)	51 (12)	17 (53)
No	7 (5)	303 (71)	3 (9)
NI	21 (15)	73 (17)	12 (38)
Use of antiviral drugs			
Yes	13 (10)	10 (2)	0
Days of use (Median/IQR)	5 (2–5)	15 (1–35)	—
Vaccination in 2014			
Yes	42 (31)	59 (14)	6 (19)
No	94 (69)	344 (81)	26 (81)

NI	0	24 (5)	0
ICU admission			
Yes	37 (27)	10 (2)	26 (81)
No	99 (73)	417 (98)	6 (19)
Mechanical ventilation			
Yes	29 (21.3)	13 (3.0)	0
No	107 (78.7)	414 (97.0)	0
Death			
Yes	4 (3)	4 (1)	1 (3)
No	132 (97)	423 (99)	31 (97)
Severe disease	99 (73)	16 (4)	26 (81)

*Some patients presented more than one comorbidity. ICU, intensive care unit; IQR, interquartile range; NI, no information; RJ, Rio de Janeiro.

Table 2. Epidemiological and clinical characteristics of patients with positive diagnosis for respiratory viruses treated in reference hospitals in Curitiba, Fortaleza, and Rio de Janeiro, 2015.

Characteristics	Positive cases		
	Influenza virus *n* = 30 (%)	ORV *n* = 247 (%)	*p*
Sex			
Male	13 (43)	121 (49)	0.5694
Female	17 (57)	126 (51)	—
Age (median, y/IQR)	2.6 (1.3–5.9)	0.8 (0.2–2.0)	<0.0001
Age group (y)			
<2	9 (37)	180 (73)	0.0002
2–4	10 (33)	43 (17)	—
5–17	4 (13)	15 (6)	—
> 18	5 (17)	9 (4)	—
Time to onset of symptoms (median, days/IQR)	1.5 (0–4)	2 (1–4)	0.1882
Chronic diseases*			
No	16 (53)	192 (78)	**0.0064** (OR 3.0 95% CI 1.4–6.6)
Pulmonary	6 (20)	40 (16)	—
Cardiovascular	7 (23)	16 (6)	—

Other	3 (10)	27 (11)	—
Length of stay in hospital (median days/IQR)	2 (1–6)	1.5 (1–9.7)	0.8726
Polypnea			
Yes	11 (37)	88 (36)	0.8325
No	15 (50)	139 (56)	—
NI	4 (13)	20 (8)	—
Use of antiviral drugs			
Yes	1 (3)	9 (4)	NA
Days of use (median/IQR)	5	5 (5–35)	—
Vaccination in 2014			
Yes	4 (13)	28 (11)	0.7634
No	26 (87)	216 (87)	—
NI	0	3 (2)	—
ICU admission			
Yes	8 (27)	22 (9)	**0.0081** (OR 3.7 95% CI 1.4–9.3)
No	22 (73)	225 (91)	—
Mechanical ventilation			
Yes	6 (20)	15 (6)	**0.0164** (OR 3.8, 95% CI 1.3–10.9)
No	24 (80)	232 (94)	—

Death			
Yes	1 (3)	6 (2)	0.5006
No	29 (97)	241 (98)	—
Severe disease			
Yes	9 (30)	26 (11)	**0.0061** (OR 3.6, 95% CI 1.5–8.7)
No	21 (70)	221 (89)	—

Bold numerals indicated statistically significant values. *Some patients presented more than one comorbidity. CI, confidence interval; ICU, intensive care unit; IQR, interquartile range. NI, no information; OR, odds ratio.

Figure legends

Figure 1. Map of Brazil showing the location of Curitiba, Rio de Janeiro, and Fortaleza and the total of screened (Blue) and included (Red) patients at each site.

Figure 2. Flowchart summarizing the process of patient inclusion in the study performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

Figure 3. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

Figure 4. Number of detected cases of influenza A and B associated with meteorological data in Fortaleza (northeast) and Curitiba (south) in 2015. The time of the influenza-vaccination campaign is indicated by an arrow (green and red lines represent mean temperatures and precipitation, respectively).

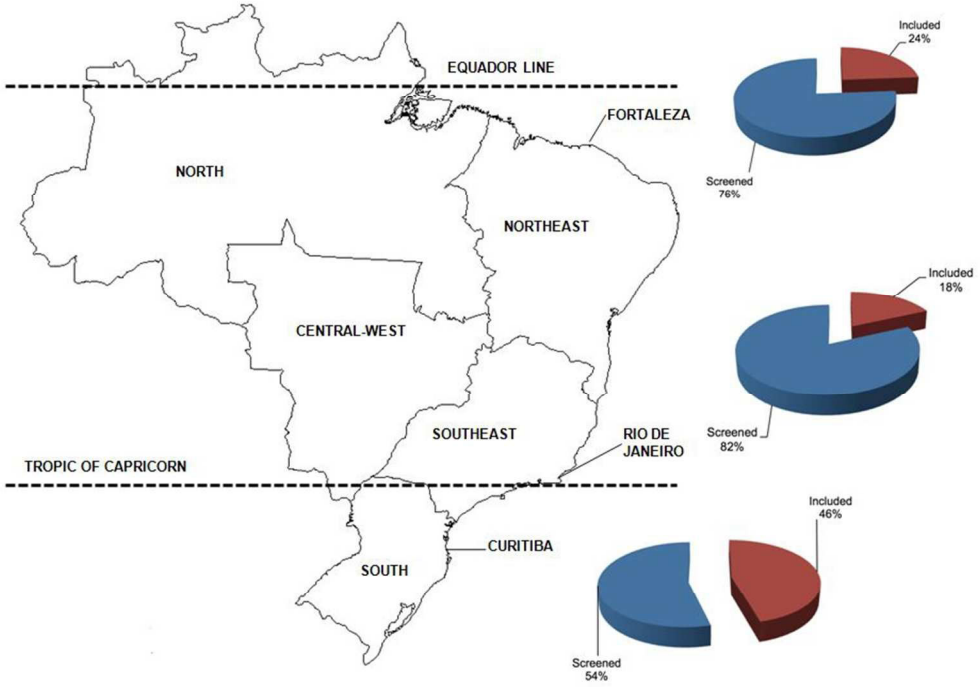


Figure 1. Map of Brazil, showing the location of Curitiba, Rio de Janeiro and Fortaleza, and the total of screened (Blue) and included (Red) patients in each site.

79x55mm (300 x 300 DPI)

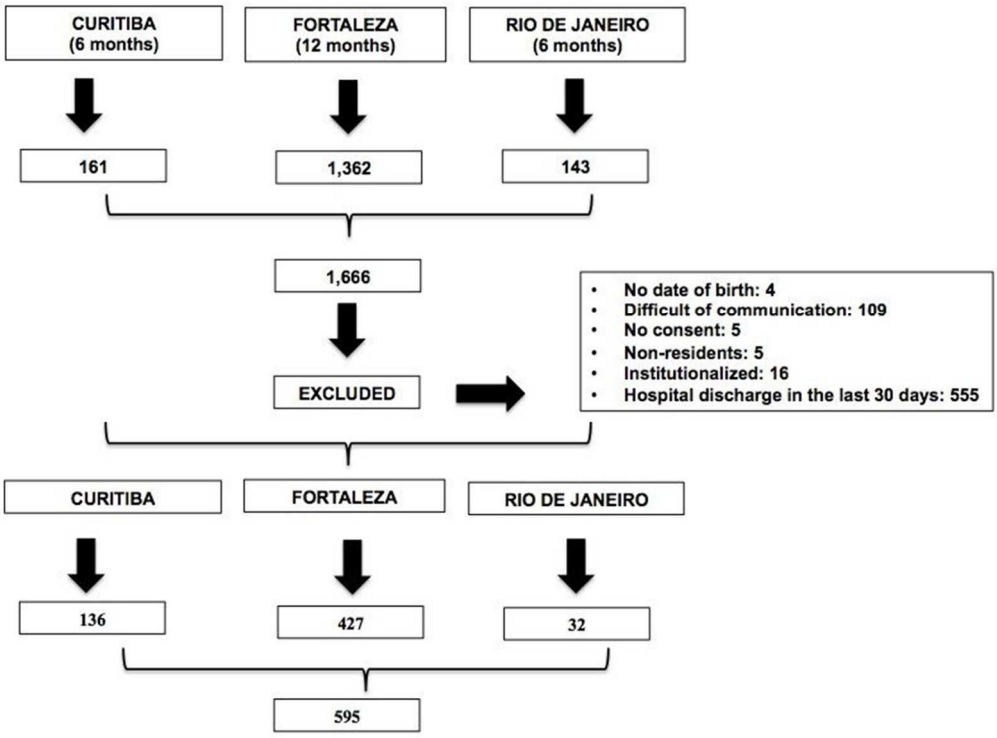


Figure 2. Flowchart summarizing the process of patient inclusion in the study, performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

66x49mm (300 x 300 DPI)

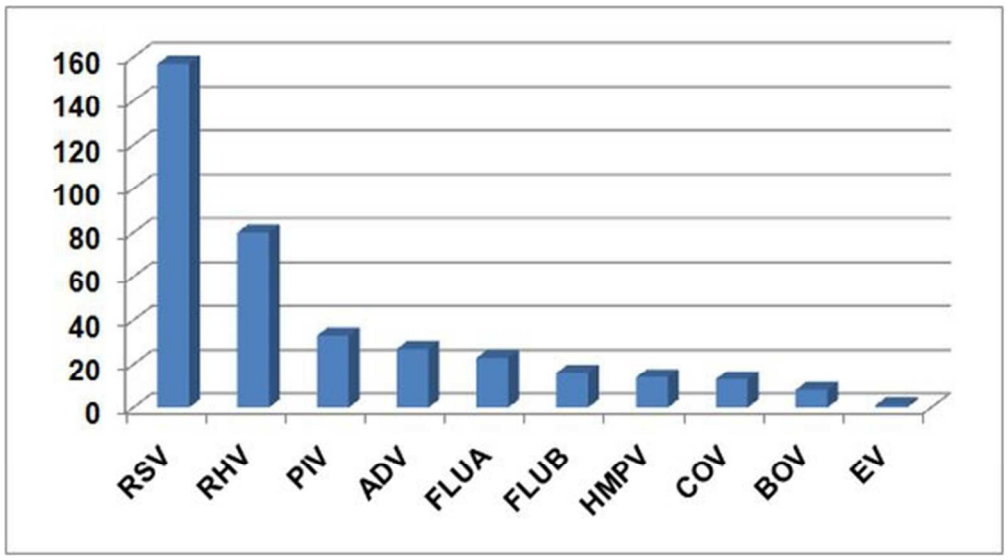


Figure 3. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

54x29mm (300 x 300 DPI)

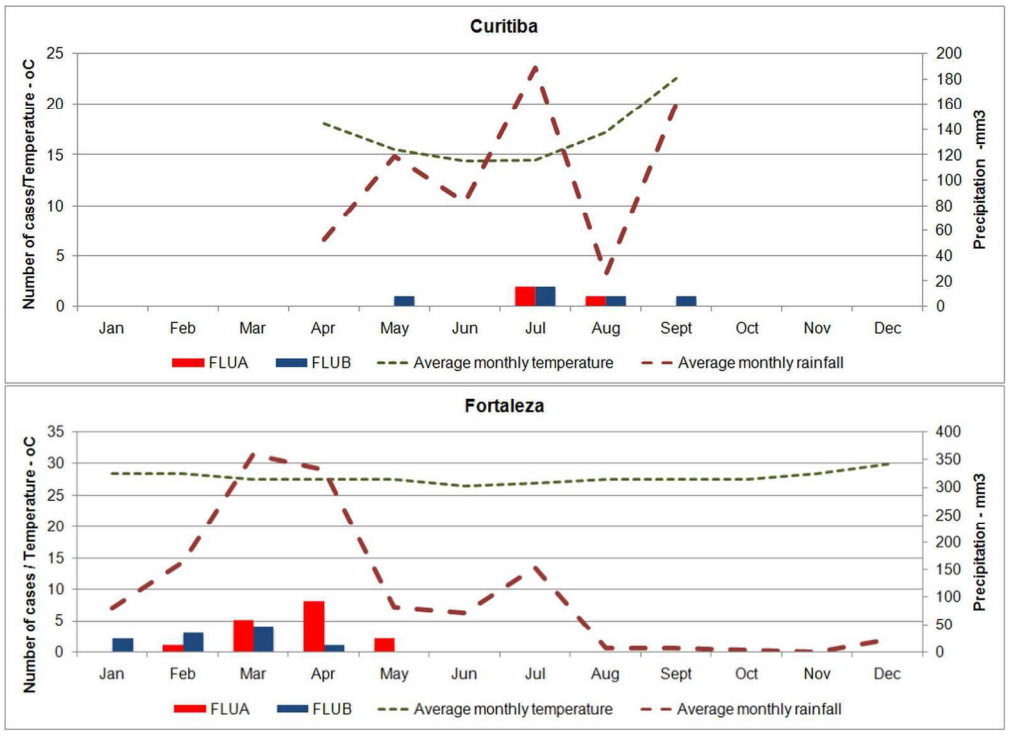


Figure 4. Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow (green and red lines represent mean temperatures and precipitations, respectively).

185x134mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	01
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	02-03
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	04-05
Objectives	3	State specific objectives, including any prespecified hypotheses	05
Methods			
Study design	4	Present key elements of study design early in the paper	05
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	05-07
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls ***Cross-sectional study***—Give the eligibility criteria, and the sources and methods of selection of participants	07
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Not applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	07
Bias	9	Describe any efforts to address potential sources of bias	08
Study size	10	Explain how the study size was arrived at	09 Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	08
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	08
		(*b*) Describe any methods used to examine subgroups and interactions	Not applicable
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	08
		(*e*) Describe any sensitivity analyses	Not applicable

Continued on next page

Results			**Page**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	09
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	09
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	*09-10*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	09-10 Table 1
		(*b*) Report category boundaries when continuous variables were categorized	Table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14-15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.