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## 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)

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Keywords:	influenza, Hospital admission, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

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Manuscripts

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

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49 **Short running head:** Seasonality of influenza in Brazil

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## 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 (iii) 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.

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3 **Conclusion** The model of active surveillance applied in our study is a valuable tool for  
4 investigating the impact of respiratory viruses in hospitalized patients, and monitoring  
5 influenza infections enables more adequate preventive measures for the population.  
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9 **Trial registration** Not applicable  
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13 **Keywords:** hospital admissions, influenza, seasonality, severe acute respiratory  
14 infection, vaccination, viral respiratory infection  
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### 17 18 19 **Strengths and limitations of this study** 20

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

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3 Through broad geographical coverage and data standardization, the GIHSN group seeks  
4 to obtain a global picture of the impact of influenza on patients with respiratory  
5 pathologies. In addition, this network provides invaluable tools to investigate the  
6 seasonality of influenza in regions in which this information is not available, as well as  
7 a framework for estimating the effectiveness of seasonal influenza vaccines in  
8 preventing severe cases among age and risk groups.  
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15 In 2015, the GIHSN group started a study in Rio de Janeiro, Fortaleza, and  
16 Curitiba. These three cities are located in distinct regions of Brazil, enabling the  
17 evaluation of the effect of different climatic conditions on the seasonality of influenza  
18 infections. Here, we report the initial findings of this surveillance project, including  
19 seasonality and the clinical and epidemiological features observed.  
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## 28 **PATIENTS AND METHODS**

### 29 **Ethical statement**

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

47  
48 The present multi-centric, cross-sectional study was designed to provide  
49 information on active surveillance of influenza in hospitalized patients. Data were  
50 collected in 2015 in three participating hospitals located in distinct cities and regions of  
51 Brazil: Curitiba (South), Fortaleza (Northeast), and Rio de Janeiro (Southeast).  
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3 In Curitiba and Rio de Janeiro, patient recruitment and data collection occurred  
4 from April to September, coinciding with the period of influenza seasonality in South  
5 and Southeast Brazil. In Fortaleza, the study spanned all of 2015, because, in spite of a  
6 previous study showing influenza circulation between January and April, [11] the  
7 seasonality of influenza in this region is not well established.  
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13 In Curitiba, the study was carried out at HC/UFPR, a 310-bed tertiary care  
14 academic center, with 47 beds for intensive care. It is also one of the sentinel hospitals  
15 for influenza and Severe Acute Respiratory Infection (SARI) surveillance in the region.  
16 Curitiba is located at 25° 25' S, 49° 15' W, 924 m (3031 ft), and has a mild marine west  
17 coast climate, with no dry season and warm summers. Seasonality is moderate, with  
18 heavy precipitation during mild winters and a mean temperature of 16.5°C. The city's  
19 population numbered approximately 1,879,355 people in 2015.  
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29 In Fortaleza, the study was conducted at HIAS, a public health institution of 270  
30 beds. It is a city of 2,500,000 inhabitants at sea level, 4° south of the equator. Its tropical  
31 climate is characterized by two distinct seasons: a rainy one between January and May,  
32 and a dry one during the rest of the year. There is high relative humidity (79%) and little  
33 variation in the average temperature (26.4°C).  
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39 In Rio de Janeiro, the study was conducted at Hospital Quinta D'Or, which is a  
40 private hospital with 350 beds for general admission and 150 beds in the intensive care  
41 unit (ICU). Rio de Janeiro is located at 22° 54' S, 43° 12' W and its climate is  
42 classified as tropical Atlantic. The year comprises two seasons: a hot and relatively  
43 humid one, and another with mild temperatures and less rainfall.  
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#### 50 **Patient recruitment and inclusion/exclusion criteria**

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52 Patients of all ages, who were admitted for any acute condition described as  
53 potentially associated to influenza, were considered eligible for enrollment in the study.  
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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. [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<sup>®</sup> 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  $\pm$  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

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3 During the study period, 1,666 patients from three participating hospitals were  
4 screened (161 in Curitiba, 1,362 in Fortaleza, and 143 in Rio de Janeiro). A total of 595  
5 patients met the criteria for inclusion, as shown in Figure 1.  
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9 The demographic and clinical characteristics of the population are listed in Table  
10 1. In Curitiba and Fortaleza subsets, males and females were recruited in equal  
11 proportions and most individuals were younger than 5 years. In contrast, in Rio de  
12 Janeiro, females and adults were predominant, and no child under 2 years was included.  
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16 As expected from a predominantly young population, the prevalence of chronic  
17 conditions associated to aggravation of influenza was low in Curitiba and Fortaleza  
18 subsets. In contrast, the Rio de Janeiro subset was characterized by a significantly  
19 higher prevalence of conditions such as cardiovascular disease, COPD, and diabetes.  
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26 Other factors that impact the prevalence of severe influenza infections are the  
27 use of antivirals and vaccination. Antiviral administration was more frequently reported  
28 in Curitiba. Overall, influenza vaccination rates were low, in spite of most age groups  
29 included in the study being covered by the Brazilian immunization program: around  
30 31% of patients from Curitiba were vaccinated, against the estimated 14% and 19% in  
31 Fortaleza, and Rio de Janeiro, respectively. In general, only 21% of children younger  
32 than 5 years who were included in the study were vaccinated. Severe disease was  
33 detected in 149/595 cases (24%), but mortality was low: 9/595 (1.5%).  
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#### 44 **Evaluation of influenza and ORV**

45 All samples were tested for influenza virus, and a total of 39 (6.5%) were  
46 positive; 23 (59%) for influenza A H3N2 and 16 (41%) for influenza B - all Yamagata-  
47 like lineage. Of these cases, nine (23%) presented co-infection with ORV.  
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52 The presence of ORV was tested in 497 patients (83.5%), with 293 (59%) of  
53 them being positive and 46 (16%) presenting co-infection. RSV was the most frequently  
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3 identified virus and the main source of co-infections ( $n = 27$ ; 49%), whereas influenza  
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5 co-infections were found in only 23% (9/39) of cases. The respiratory pathogens  
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7 detected in this subset of samples are reported in Figure 2.

### 8 9 **Severe cases and mortality associated with influenza and ORV**

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

### 24 25 26 **Seasonality of influenza infections in two geographic regions of Brazil**

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28 To better understand the seasonality of influenza infections in the study sites, we  
29  
30 evaluated the monthly distribution of samples and viral positivity. Then, we plotted this  
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32 information against the temperature and precipitation (historical means) recorded for  
33  
34 each month. Results show variations between Northeast (Fortaleza) and South  
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36 (Curitiba) Brazil in relation to the peak time of influenza infections, which essentially  
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38 followed the rain season of each region. Finally, results indicate that immunization  
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40 against influenza in the Northeast was carried out after the period of virus circulation  
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42 (Figure 3). Data from Rio de Janeiro were not evaluated owing to the small number of  
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44 patients included in the study.

## 45 46 47 **DISCUSSION**

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49 The GHISN was established with the main goal of better understanding the role  
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51 of influenza infections in the development of severe respiratory diseases. The  
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53 information gathered by this network may contribute to the elucidation of influenza  
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3 seasonality in some regions, the implementation of more efficient containment  
4 measures, as well as the improvement of preventive interventions such as immunization.  
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7 In Brazil, the Ministry of Health employs two systems for epidemiological  
8 assessment of respiratory diseases: sentinel surveillance of ILI and universal  
9 surveillance of SARI. The sentinel surveillance consists of a network of designated  
10 health care units (public or private), distributed throughout the country, in which  
11 random samples of respiratory cases are periodically collected for detection and genetic  
12 characterization of circulating viruses. In the universal SARI surveillance, all severe  
13 respiratory cases admitted to ICUs and all deaths related to respiratory disease are  
14 investigated in the laboratory. [14] In the present study, only hospitalized patients  
15 identified by an active search in the participating health centers were included. In  
16 contrast to the Brazilian universal SARI surveillance protocol, which is restricted to  
17 ICUs, in this study, the screening included patients admitted to other wards and  
18 presenting diseases other than respiratory ones. Individuals admitted with conditions  
19 associated with aggravation of influenza infections, such as cardiovascular disease and  
20 diabetes, were also screened, thus increasing the number of identified cases.  
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37 Overall, the frequency of influenza viruses in the study specimens was 6.5%  
38 (39/595), with influenza A comprising 60% of the flu-positive samples. This low  
39 prevalence reflected the profile of the 2015 influenza season in Brazil and in most of the  
40 Southern Hemisphere and Tropical regions. In that year, viral activity remained low  
41 through the entire season, with higher prevalence of H3N2 and lower detection of  
42 influenza B and H1N1pdm09. Even in temperate regions of the Southern Hemisphere,  
43 activity remained at an inter-seasonal level. In Brazil, a total of 11,945 cases of SARI  
44 were reported in 2015, of which 1,089 (9%) were related to influenza infection. Among  
45 these cases, 599 (55%) were influenza A/H3N2, 234 (21.5%) were influenza B, 141  
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3 (12.9%) were influenza A/H1N1pdm09, and 115 (10.5%) were reported as non-  
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5 subtyped influenza A. [15]

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7 Since 2014, the Brazilian Ministry of Health included children up to 4 years of  
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9 age in the group of individuals at risk of severe influenza and, thus, started vaccination  
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11 of this subpopulation. Data from the National Immunization Program show coverage  
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13 rates above 83% among young children and pregnant women, 89% in the elderly, and  
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15 95% in health workers. [10,14] However, in this study, a much lower coverage was  
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17 observed, with only 21% of patients reporting or showing proof of influenza  
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19 vaccination. This was surprising, considering that a significant part of our patients fell  
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21 in at least one risk category or presented comorbidities. Given a 10.5% mortality rate  
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23 due to influenza-related SARI cases in 2015, it is essential to assess the reasons for such  
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25 low immunization frequency. Among influenza deaths, 65% of patients and particularly  
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27 those aged over 60 presented at least one risk factor likely to cause complications. [10]  
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29 Our data emphasize the need for a comprehensive identification of the weaknesses of  
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31 the vaccination campaigns and an intensification of the efforts to bring immunization to  
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33 these individuals.  
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37 We also evaluated the prevalence of ORV in a subset of samples from  
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39 HC/UFPR, and samples that were selected randomly among patients from Fortaleza and  
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41 Rio de Janeiro. Overall, different respiratory viruses were found in 59% of the tested  
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43 samples. This is consistent with previous reports showing high co-circulation of ORV in  
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45 hospitalized patients during the influenza season, and confirmed that the viruses  
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47 detected caused very similar ILI respiratory manifestations. [16-18] Moreover, our  
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49 results reflect the profile of respiratory viruses in children, which was the predominant  
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51 group included in the study. Interestingly, although circulation of influenza and ORV  
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53 coincide in time, the frequency of codetection of influenza with ORV in the same  
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3 patient was very low, whereas codetection of more than one ORV in a single patient  
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5 was rather common. This observation is consistent with those in previous reports by our  
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7 group, and we hypothesize that low codetection of influenza with other ORV is due to  
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9 the higher pathogenicity of influenza. [17,19]  
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12 Next, we compared clinical and epidemiological data from influenza- and ORV-  
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14 infected patients in an attempt to evaluate the impact of influenza infections. The latter  
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16 were significantly more abundant in older patients and those with severe diseases,  
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18 whereas comorbidities were associated with ORV infections. These findings reflect the  
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20 higher virulence of influenza viruses, but could also be a consequence of a lower  
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22 adhesion to immunization among older children. No difference in mortality was  
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24 observed between groups infected with different viruses. This contrasts with a previous  
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26 study performed in Fortaleza, which showed significant association between RSV  
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28 infections and infant mortality. [20] We believe that such discrepancy may be due to the  
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30 low number of positive cases observed in the present study.  
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34 Studies on influenza seasonality in Brazil have shown that viral circulation in the  
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36 Northeast region was more prevalent in the first 4 months of the year, coinciding with a  
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38 period of higher humidity in that area [11,21], whereas a distinct pattern of viral  
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40 circulation was observed in the South and Southeast. [22,23] Our data confirm this  
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42 observation and reinforce the growing consensus that vaccinations in Northeast Brazil  
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44 are being carried out in a post-seasonal period, with consequent low effectiveness.  
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47 The present study presented some limitations: i) the recruitment of patients  
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49 during the seasonal period of influenza previously defined for two sites (Curitiba and  
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51 Rio de Janeiro) may have contributed to low influenza B detection, although the  
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53 frequency observed in our study was similar to that reported by national surveillance  
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55 programs; ii) there was a predominance of pediatric patients in the study population, so  
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3 data on ORV reflect mainly the profile of respiratory viruses in children; iii) the low  
4 inclusion of patients in Rio de Janeiro does not allow for an assessment of the impact of  
5 viral respiratory infections in that region.  
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9 Despite these limitations, the results obtained here indicate that the model of  
10 active surveillance applied in our study is a valuable tool for investigating the impact of  
11 respiratory viruses in hospitalized patients in distinct regions of Brazil. We also  
12 reinforce the importance of monitoring influenza infections, thus enabling more  
13 adequate preventive measures for the population.  
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### 19 **Authors Contributions**

20  
21 FKP, VP and JPB participated to the conception/design of the study, supervised the  
22 analysis, and interpreted the data. MMS and SMR supervised the analysis, interpreted  
23 the data and wrote the preliminary manuscript. FEAM and IT supervised the data  
24 collection from Fortaleza and Rio de Janeiro, respectively. BCC performed the tests,  
25 assembled the data and wrote the preliminary manuscript. VMA, LAP, MBN and LRV  
26 performed the molecular tests and collected the data from Curitiba. All authors provided  
27 contributions to the paper and approved the final version  
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### 39 **Data Sharing Statement**

40 No additional data available.  
41  
42

### 43 **Competing interests**

44 The authors have no competing interests.  
45  
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47

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3 The sponsors of the study had no role in data analysis, data interpretation, or writing of  
4 the report. The corresponding author had full access to all the data in the study and had  
5 final responsibility for deciding to submit the work for publication.  
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**Table 1.** Epidemiological and clinical characteristics of the patients included in the study.

<b>Characteristics</b>	<b>Curitiba n = 136 (%)</b>	<b>Fortaleza n = 427 (%)</b>	<b>RJ n = 32 (%)</b>
<b>Sex</b>			
Male	61 (45)	243 (57)	12 (37)
Female	75 (55)	184 (43)	20 (63)
<b>Age (Median, years/IQR)</b>			
	1.4 (0.5–87.9)	1.3 (0–16.6)	1.4 (3.3–89.3)
<b>Age group (years)</b>			
< 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)
<b>Time to onset of symptoms (Median, days/IQR)</b>			
	2 (0–7)	2 (0–7)	2 (0–6)
<b>Exposure to smoking</b>			
Yes	22 (16)	109 (25)	1 (3)
No	90 (66)	277 (65)	21 (66)
Ex-smoker	24 (18)	41 (10)	10 (31)
<b>Chronic diseases*</b>			
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)
<b>Length of stay in hospital (Median days/IQR)</b>	<b>2 (1–53)</b>	<b>1 (0–14)</b>	<b>2 (1–53)</b>
<b>Polypnea</b>			
Yes	108 (80)	51 (12)	17 (53)
No	7 (5)	303 (71)	3 (9)
NI	21 (15)	73 (17)	12 (38)
<b>Use of antiviral drugs</b>			
Yes	13 (10)	10 (2)	0
Days of use (Median/IQR)	5 (2–5)	15 (1–35)	-
<b>Vaccination in 2014</b>			
Yes	42 (31)	59 (14)	6 (19)
No	94 (69)	344 (81)	26 (81)

NI	0	24 (5)	0
<b>ICU admission</b>			
Yes	37 (27)	10 (2)	26 (81)
No	99 (73)	417 (98)	6 (19)
<b>Mechanical ventilation</b>			
Yes	29 (21.3)	13 (3.0)	0
No	107 (78.7)	414 (97.0)	0
<b>Death</b>			
Yes	4 (3)	4 (1)	1 (3)
No	132 (97)	423 (99)	31 (97)
<b>Severe disease</b>	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	ORV	<i>p</i> value
	n = 30 (%)	n = 247 (%)	
<b>Sex</b>			
Male	13 (43)	121 (49)	0.5694
Female	17 (57)	126 (51)	-
<b>Age (Median, years/IQR)</b>	2.6 (1.3–5.9)	0.8 (0.2–2.0)	< 0.0001
<b>Age group (years)</b>			
< 2	9 (37)	180 (73)	0.0002
2–4	10 (33)	43 (17)	-
5–17	4 (13)	15 (6)	-
> 18	5 (17)	9 (4)	-
<b>Time to onset of symptoms (Median, days/IQR)</b>	1.5 (0–4)	2 (1–4)	0.1882
<b>Chronic diseases*</b>			
No	16 (53)	192 (78)	<b>0.0064</b> (OR 3.0 95% CI 1.4– 6.6)
Pulmonary	6 (20)	40 (16)	-
Cardiovascular	7 (23)	16 (6)	-

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



<b>Death</b>			
Yes	1 (3)	6 (2)	0.5006
No	29 (97)	241 (98)	
<b>Severe disease</b>			
Yes	9 (30)	26 (11)	<b>0.0061</b> (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.

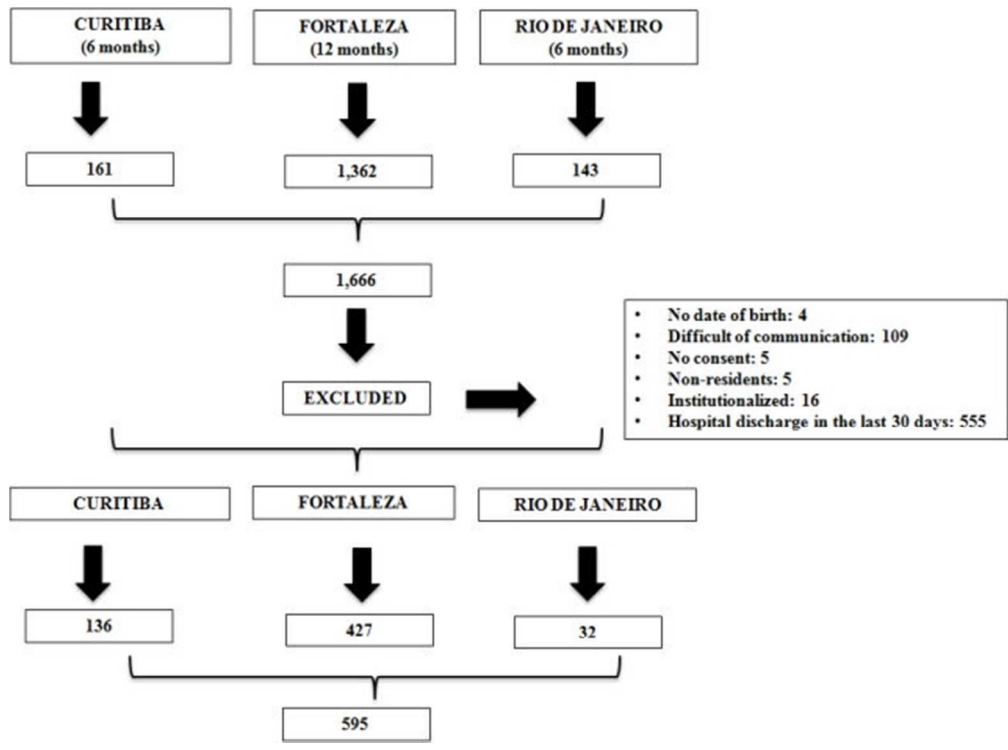
### Figure Legends

**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.

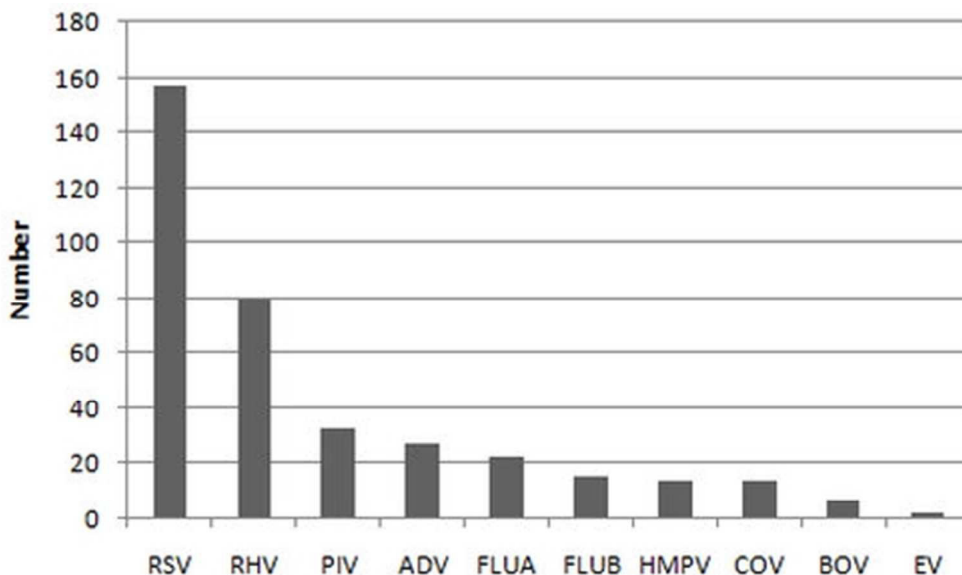
**Figure 3.** Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow.

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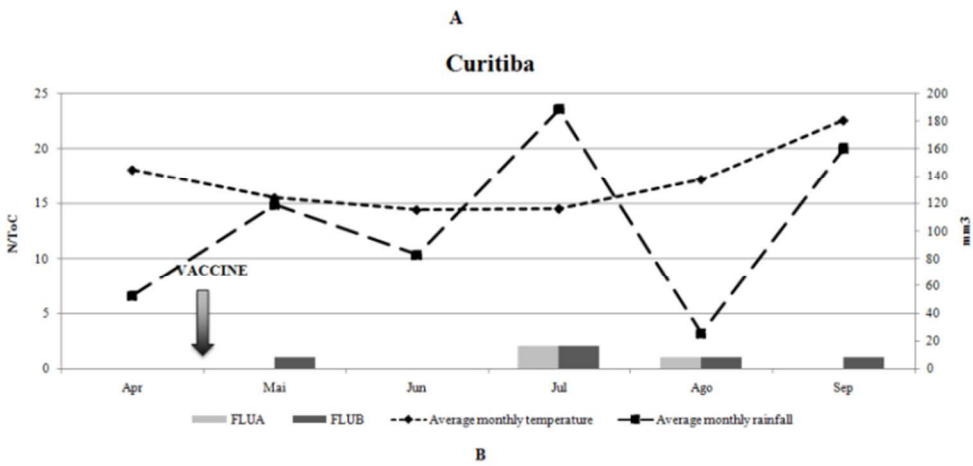
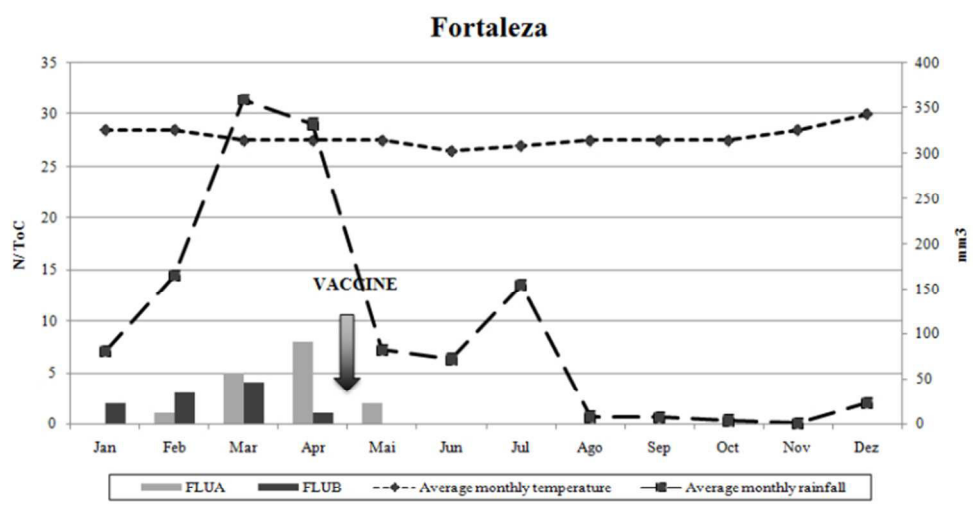


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## STROBE Statement—checklist of items that should be included in reports of observational studies

	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
<b>Introduction</b>			
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
<b>Methods</b>			
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	07
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	

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		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	08
		(g) Describe any sensitivity analyses	Not applicable

Continued on next page

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<b>Results</b>			<b>Page</b>
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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://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

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<b>Primary Subject Heading</b>:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Public health, Epidemiology
Keywords:	influenza, Hospital admission, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

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Manuscripts

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3 **Global Influenza Hospital-based Surveillance Network (GIHSN) – Results of**  
4 **surveillance of influenza and other respiratory viruses in hospitalized patients in**  
5 **2015, Brazil**  
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52 **Short running head:** Seasonality of influenza in Brazil  
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## 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 (iii) 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.

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3 **Conclusion** The model of active surveillance applied in our study is a valuable tool for  
4 investigating the impact of respiratory viruses in hospitalized patients, and monitoring  
5 influenza infection enables the implementation of adequate preventive measures.  
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9 **Trial registration** Not applicable  
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13 **Keywords:** hospital admissions, influenza, seasonality, severe acute respiratory  
14 infection, vaccination, viral respiratory infection  
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### 17 18 19 **Strengths and limitations of this study** 20

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

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3 professionals. [10-12] Overall, in 2015, national immunization campaigns achieved a  
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5 vaccination coverage of 82.7% in priority groups. [13]  
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7 The Global Influenza Hospital-Based Surveillance Network (GIHSN) is a  
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9 public-private partnership between research institutes, hospitals, and several  
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11 laboratories around the world, established to study the epidemiology of severe influenza  
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13 in consecutive seasons in different countries. In 2015, this network comprised a total of  
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15 31 collaborating sites from seven countries, including Brazil. All participants follow the  
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17 same core investigation protocol developed by the GIHSN scientific committee.  
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19 Through broad geographical coverage and data standardization, the GIHSN group seeks  
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21 to obtain a global picture of the impact of influenza on patients with respiratory  
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23 pathologies. In addition, this network provides invaluable tools to investigate the  
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25 seasonality of influenza in regions in which this information is not available, as well as  
26  
27 a framework for estimating the effectiveness of seasonal influenza vaccines in  
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29 preventing severe cases among age and risk groups.  
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33 In 2015, the GIHSN group started a study in Rio de Janeiro, Fortaleza, and  
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35 Curitiba. These three cities are located in distinct regions of Brazil, enabling the  
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37 evaluation of the effect of different climatic conditions on the seasonality of influenza  
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39 infections. Here, we report the initial findings of this surveillance project, including  
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41 seasonality and the clinical and epidemiological features observed.  
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## 46 **PATIENTS AND METHODS**

### 47 **Ethical statement**

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49 As a multi-centric study, the present project was approved by the Ethical  
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51 Committee of each institution involved in the research: Instituto Nacional de  
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53 Infectologia Evandro Chagas (INI/Fiocruz), Rio de Janeiro; Hospital de Clínicas-  
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3 Universidade Federal do Paraná (HC/UFPR), Curitiba; and Hospital Infantil Albert  
4 Sabin (HIAS), Fortaleza. All procedures were performed according to the approved  
5 protocols, and consent was obtained from all patients (or guardians).  
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### 8 9 **Study design and site description**

10  
11 The present multi-centric, cross-sectional study was designed to provide  
12 information on active surveillance of influenza in hospitalized patients. Data were  
13 collected in 2015 in three participating hospitals located in distinct cities and regions of  
14 Brazil: Curitiba (South), Fortaleza (Northeast), and Rio de Janeiro (Southeast) (Figure  
15 1).  
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19 In Curitiba and Rio de Janeiro, patient recruitment and data collection occurred  
20 from April to September, coinciding with the period of influenza seasonality in South  
21 and Southeast Brazil. In Fortaleza, the study spanned all of 2015, because, in spite of a  
22 previous study showing influenza circulation between January and April, [14] the  
23 seasonality of influenza in this region is not well established.  
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27 In Curitiba, the study was carried out at HC/UFPR, a 310-bed tertiary care  
28 academic center, with 47 beds for intensive care. It is also one of the sentinel hospitals  
29 for influenza and Severe Acute Respiratory Infection (SARI) surveillance in the region.  
30 Curitiba is located at 25° 25' S, 49° 15' W, 924 m (3031 ft), and has a mild marine west  
31 coast climate, with no dry season and warm summers. Seasonality is moderate, with  
32 heavy precipitation during mild winters and a mean temperature of 16.5°C. The city's  
33 population numbered approximately 1,879,355 people in 2015.  
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37 In Fortaleza, the study was conducted at HIAS, a public health institution of 270  
38 beds. It is a city of 2,500,000 inhabitants at sea level, 4° south of the equator. Its tropical  
39 climate is characterized by two distinct seasons: a rainy one between January and May,  
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3 and a dry one during the rest of the year. There is high relative humidity (79%) and little  
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5 variation in the average temperature (26.4°C).  
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7 In Rio de Janeiro, the study was conducted at Hospital Quinta D'Or, which is a  
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9 private hospital with 350 beds for general admission and 150 beds in the intensive care  
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11 unit (ICU). Rio de Janeiro is located at 22° 54' S, 43° 12' W and its climate is  
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13 classified as tropical Atlantic. The year comprises two seasons: a hot and relatively  
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15 humid one, and another with mild temperatures and less rainfall.  
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### 18 **Patient recruitment and inclusion/exclusion criteria**

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20 Patients of all ages, who were admitted for any acute condition described as  
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22 potentially associated to influenza, were considered eligible for enrollment in the study.  
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24 To be included, eligible patients were identified in the hospital admission registries and  
25  
26 had to comply with all of the following criteria: i) admission within 24 to 72 h prior to  
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28 recruitment; ii) be a resident in the area of the study (defined for each site) and belong  
29  
30 to the base population source; and iii) a referred history of any acute condition  
31  
32 associated to influenza with onset within 7 days or less prior to admission to the  
33  
34 hospital. The study used the European CDC definition for ILI, which included at least  
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36 one out of four systemic symptoms (fever, headache, myalgia, or malaise) and at least  
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38 one out of three respiratory symptoms (cough, sore throat, or shortness of breath).  
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42 Patients who did not give consent to participate, institutionalized individuals,  
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44 and those with a history of hospitalization within 30 days or less prior to recruitment  
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46 were excluded from the study.  
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### 48 **Sample collection and processing**

49  
50 Samples were collected using flocked nylon swabs (Copan, Italy). It was  
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52 collected one nasopharyngeal and one pharyngeal swab (or nasal swab for patients <14  
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54 years). Samples were added to a flask containing 3 mL of universal viral transport  
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3 medium (UTM™ Medium; Copan, Italy) and stored at -80°C until tested.

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

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

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

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43 **Data analysis**

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46 Data were compiled using JMP software version 5.2.1 (SAS Institute Inc., USA)  
47 and analyzed using GraphPad Prism version 5.03 (GraphPad Software Inc., USA).  
48 Baseline demographic and clinical characteristics with normal and non-normal  
49 distributions are presented as means ± standard deviation and medians with interquartile  
50 ranges (IQR), respectively. Epidemiological and clinical data comparing patients  
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3 infected with influenza and those infected with ORV were assessed; only monoinfected  
4 cases were included in this analysis. Fisher's exact test, chi-squared test, or Wilcoxon-  
5 Mann-Whitney test were performed where appropriate. A multivariate logistic  
6 regression model was fitted to evaluate clinical or epidemiological characteristics of  
7 virus-positive cases to be associated with disease severity. Variables included age,  
8 presence of comorbidities, and fatal outcome. All statistical tests were two-sided and  
9 considered as significant at  $p < 0.05$ .

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

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

## 20 21 22 **RESULTS**

### 23 24 25 **Study population: Demographics and epidemiological characteristics**

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

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32  
33 The demographic and clinical characteristics of the population are listed in Table  
34 1. In Curitiba and Fortaleza subsets, males and females were recruited in equal  
35 proportions and most individuals were younger than 5 years. In contrast, in Rio de  
36 Janeiro, females and adults were predominant, and no child under 2 years was included.

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

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3 subsets. In contrast, the Rio de Janeiro subset was characterized by a significantly  
4  
5 higher prevalence of conditions such as cardiovascular disease, COPD, and diabetes.  
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7 Other factors that impact the prevalence of severe influenza infections are the  
8  
9 use of antivirals and vaccination. Antiviral administration was more frequently reported  
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11 in Curitiba. Overall, influenza vaccination rates were low, in spite of most age groups  
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13 included in the study being covered by the Brazilian immunization program: around  
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15 31% of patients from Curitiba were vaccinated, against the estimated 14% and 19% in  
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17 Fortaleza, and Rio de Janeiro, respectively. In general, only 21% of children younger  
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19 than 5 years who were included in the study were vaccinated. Severe disease was  
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21 detected in 149/595 cases (24%), but mortality was low: 9/595 (1.5%).  
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#### 24 **Evaluation of influenza and ORV**

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26 All samples were tested for influenza virus, and a total of 39 (6.5%) were  
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28 positive; 23 (59%) for influenza A H3N2 and 16 (41%) for influenza B - all Yamagata-  
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30 like lineage. Of these cases, nine (23%) presented co-infection with ORV.  
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33 The presence of ORV was tested in 497 patients (83.5%), with 293 (59%) of  
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35 them being positive and 46 (16%) presenting co-infection. RSV was the most frequently  
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37 identified virus and the main source of co-infections ( $n = 27$ ; 49%), whereas influenza  
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39 co-infections were found in only 23% (9/39) of cases. The respiratory pathogens  
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41 detected in this subset of samples are reported in Figure 3.  
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#### 44 **Severe cases and mortality associated with influenza and ORV**

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

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3 presenting diseases other than respiratory ones. Individuals admitted with conditions  
4 associated with aggravation of influenza infections, such as cardiovascular disease and  
5 diabetes, were also screened, thus increasing the number of identified cases.  
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9 Overall, the frequency of influenza viruses in the study specimens was 6.5%  
10 (39/595), with influenza A comprising 60% of the flu-positive samples. This low  
11 prevalence reflected the profile of the 2015 influenza season in Brazil and in most of the  
12 Southern Hemisphere and Tropical regions. In that year, viral activity remained low  
13 through the entire season, with higher prevalence of H3N2 and lower detection of  
14 influenza B and H1N1pdm09. Even in temperate regions of the Southern Hemisphere,  
15 activity remained at an inter-seasonal level. In Brazil, a total of 11,945 cases of SARI  
16 were reported in 2015, of which 1,089 (9%) were related to influenza infection. Among  
17 these cases, 599 (55%) were influenza A/H3N2, 234 (21.5%) were influenza B, 141  
18 (12.9%) were influenza A/H1N1pdm09, and 115 (10.5%) were reported as non-  
19 subtyped influenza A. [18]  
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33 Since 2014, the Brazilian Ministry of Health included children up to 4 years of  
34 age in the group of individuals at risk of severe influenza and, thus, started vaccination  
35 of this subpopulation. Data from the National Immunization Program show coverage  
36 rates above 83% among young children and pregnant women, 89% in the elderly, and  
37 95% in health workers. [13,17]. In the present study, it was observed that proportionally  
38 there were a higher percentage of patients from Curitiba vaccinated among the 3 sites.  
39 However, overall a lower coverage was observed, with only 21% of patients reporting  
40 or showing proof of influenza vaccination. This was surprising, considering that a  
41 significant part of our patients fell in at least one risk category or presented  
42 comorbidities. Given a 10.5% mortality rate due to influenza-related SARI cases in  
43 2015, it is essential to assess the reasons for such low immunization frequency. Among  
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3 influenza deaths, 65% of patients and particularly those aged over 60 presented at least  
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5 one risk factor likely to cause complications. [13] Our data emphasize the need for a  
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7 comprehensive identification of the weaknesses of the vaccination campaigns and an  
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9 intensification of the efforts to bring immunization to these individuals.  
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11 We also evaluated the prevalence of ORV in a subset of samples from  
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13 HC/UFPR, and samples that were selected randomly among patients from Fortaleza and  
14  
15 Rio de Janeiro. Overall, different respiratory viruses were found in 59% of the tested  
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17 samples. This is consistent with previous reports showing high co-circulation of ORV in  
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19 hospitalized patients during the influenza season, and confirmed that the viruses  
20  
21 detected caused very similar ILI respiratory manifestations. [19-21] Moreover, our  
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23 results reflect the profile of respiratory viruses in children, which was the predominant  
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25 group included in the study. Interestingly, although circulation of influenza and ORV  
26  
27 coincide in time, the frequency of codetection of influenza with ORV in the same  
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29 patient was very low, whereas codetection of more than one ORV in a single patient  
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31 was rather common. This observation is consistent with those in previous reports by our  
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33 group, and we hypothesize that low codetection of influenza with other ORV is due to  
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35 the higher pathogenicity of influenza. [20,22]  
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39 Next, we compared clinical and epidemiological data from influenza- and ORV-  
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41 infected patients in an attempt to evaluate the impact of influenza infections. The latter  
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43 were significantly more abundant in older patients and those with severe diseases,  
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45 whereas comorbidities were associated with ORV infections. These findings reflect the  
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47 higher virulence of influenza viruses, but could also be a consequence of a lower  
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49 adhesion to immunization among older children. No difference in mortality was  
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51 observed between groups infected with different viruses. This contrasts with a previous  
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53 study performed in Fortaleza, which showed significant association between RSV  
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3 infections and infant mortality. [23] We believe that such discrepancy may be due to the  
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5 low number of positive cases observed in the present study.  
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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].

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3 The present study presented some limitations: i) the recruitment of patients  
4 during the seasonal period of influenza previously defined for two sites (Curitiba and  
5 Rio de Janeiro) may have contributed to low influenza B detection, although the  
6 frequency observed in our study was similar to that reported by national surveillance  
7 programs; ii) there was a predominance of pediatric patients in the study population, so  
8 data on ORV reflect mainly the profile of respiratory viruses in children; iii) the low  
9 inclusion of patients in Rio de Janeiro does not allow for an assessment of the impact of  
10 viral respiratory infections in that region; and iv) the monitoring of weather conditions  
11 is performed by organs linked to the states of each region of the country, so we decided  
12 to limit the description of the data from similar information that were made available by  
13 these institutes.  
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26 Despite these limitations, the results obtained here indicate that the model of  
27 active surveillance applied in our study is a valuable tool for investigating the impact of  
28 respiratory viruses in hospitalized patients in distinct regions of Brazil. We also  
29 reinforce the importance of monitoring influenza infections, thus enabling more  
30 adequate preventive measures for the population.  
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### 37 **Authors Contributions**

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40 FKP, VP and JPB participated to the conception/design of the study, supervised the  
41 analysis, and interpreted the data. MMS and SMR supervised the analysis, interpreted  
42 the data and wrote the preliminary manuscript. FEAM and IT supervised the data  
43 collection from Fortaleza and Rio de Janeiro, respectively. BCC performed the tests,  
44 assembled the data and wrote the preliminary manuscript. VMA, LAP, MBN and LRV  
45 performed the molecular tests and collected the data from Curitiba. All authors provided  
46 contributions to the paper and approved the final version  
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### 56 **Data Sharing Statement**



No additional data available.

### **Competing interests**

The authors have no competing interests.

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### **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.

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

	<b>Curitiba</b>	<b>Fortaleza</b>	<b>RJ</b>
<b>Characteristics</b>	<b>n = 136 (%)</b>	<b>n = 427 (%)</b>	<b>n = 32 (%)</b>
<b>Sex</b>			
Male	61 (45)	243 (57)	12 (37)
Female	75 (55)	184 (43)	20 (63)
<b>Age (Median, years/IQR)</b>			
	1.4 (0.5–87.9)	1.3 (0–16.6)	1.4 (3.3–89.3)
<b>Age group (years)</b>			
< 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)
<b>Time to onset of symptoms (Median, days/IQR)</b>			
	2 (0–7)	2 (0–7)	2 (0–6)
<b>Exposure to smoking</b>			
Yes	22 (16)	109 (25)	1 (3)
No	90 (66)	277 (65)	21 (66)
Ex-smoker	24 (18)	41 (10)	10 (31)
<b>Chronic diseases*</b>			
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)
<b>Length of stay in hospital (Median days/IQR)</b>	<b>2 (1–53)</b>	<b>1 (0–14)</b>	<b>2 (1–53)</b>
<b>Polypnea</b>			
Yes	108 (80)	51 (12)	17 (53)
No	7 (5)	303 (71)	3 (9)
NI	21 (15)	73 (17)	12 (38)
<b>Use of antiviral drugs</b>			
Yes	13 (10)	10 (2)	0
Days of use (Median/IQR)	5 (2–5)	15 (1–35)	-
<b>Vaccination in 2014</b>			
Yes	42 (31)	59 (14)	6 (19)
No	94 (69)	344 (81)	26 (81)

NI	0	24 (5)	0
<b>ICU admission</b>			
Yes	37 (27)	10 (2)	26 (81)
No	99 (73)	417 (98)	6 (19)
<b>Mechanical ventilation</b>			
Yes	29 (21.3)	13 (3.0)	0
No	107 (78.7)	414 (97.0)	0
<b>Death</b>			
Yes	4 (3)	4 (1)	1 (3)
No	132 (97)	423 (99)	31 (97)
<b>Severe disease</b>	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	ORV	<i>p</i> value
	n = 30 (%)	n = 247 (%)	
<b>Sex</b>			
Male	13 (43)	121 (49)	0.5694
Female	17 (57)	126 (51)	-
<b>Age (Median, years/IQR)</b>	2.6 (1.3–5.9)	0.8 (0.2–2.0)	< 0.0001
<b>Age group (years)</b>			
< 2	9 (37)	180 (73)	0.0002
2–4	10 (33)	43 (17)	-
5–17	4 (13)	15 (6)	-
> 18	5 (17)	9 (4)	-
<b>Time to onset of symptoms (Median, days/IQR)</b>	1.5 (0–4)	2 (1–4)	0.1882
<b>Chronic diseases*</b>			
No	16 (53)	192 (78)	<b>0.0064</b> (OR 3.0 95% CI 1.4– 6.6)
Pulmonary	6 (20)	40 (16)	-
Cardiovascular	7 (23)	16 (6)	-



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

<b>Death</b>			
Yes	1 (3)	6 (2)	0.5006
No	29 (97)	241 (98)	
<b>Severe disease</b>			
Yes	9 (30)	26 (11)	<b>0.0061</b> (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).

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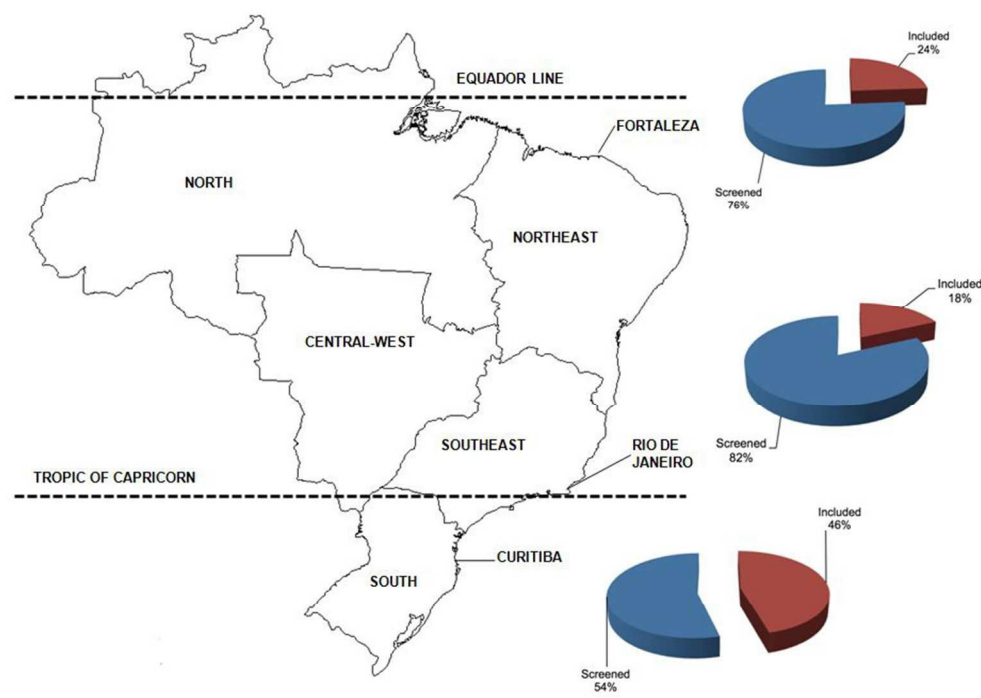


Figure 1. Map of Brazil, showing the location of Curitiba, Rio de Janeiro and Fortaleza, and the total of screened (Blue) and included (Red) patients in each site.

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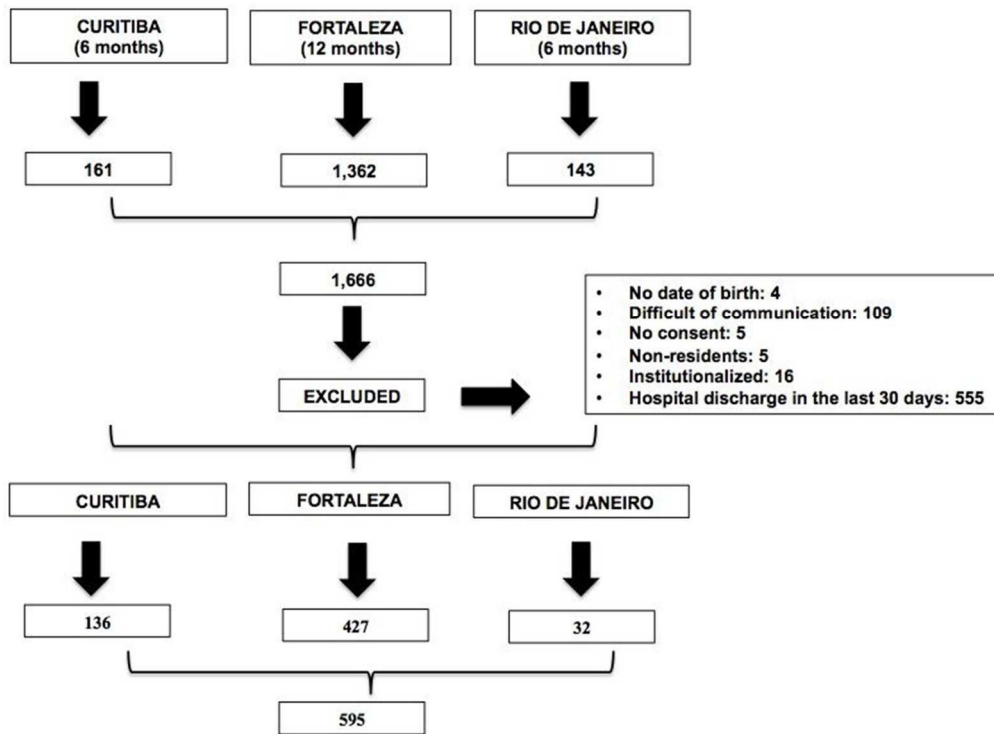


Figure 2. Flowchart summarizing the process of patient inclusion in the study, performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

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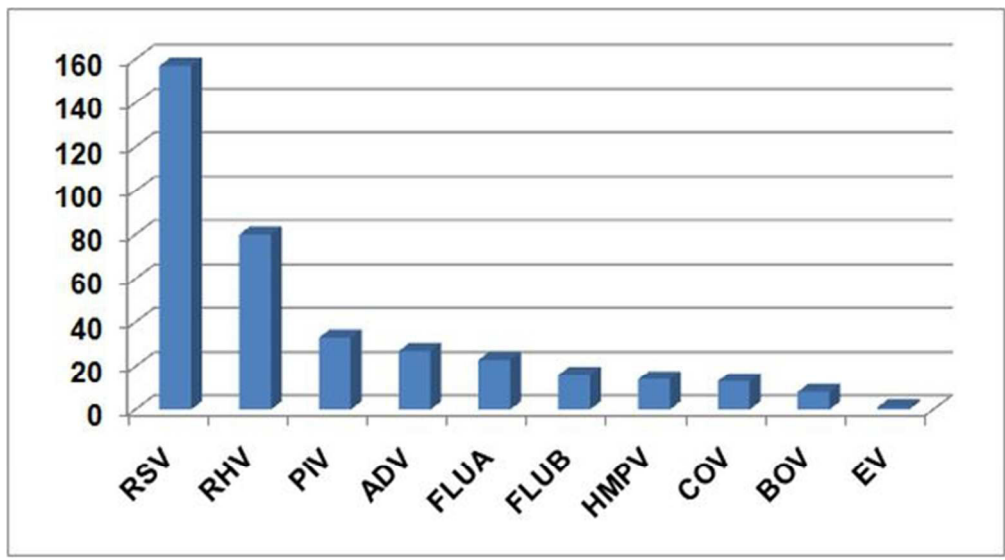


Figure 3. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

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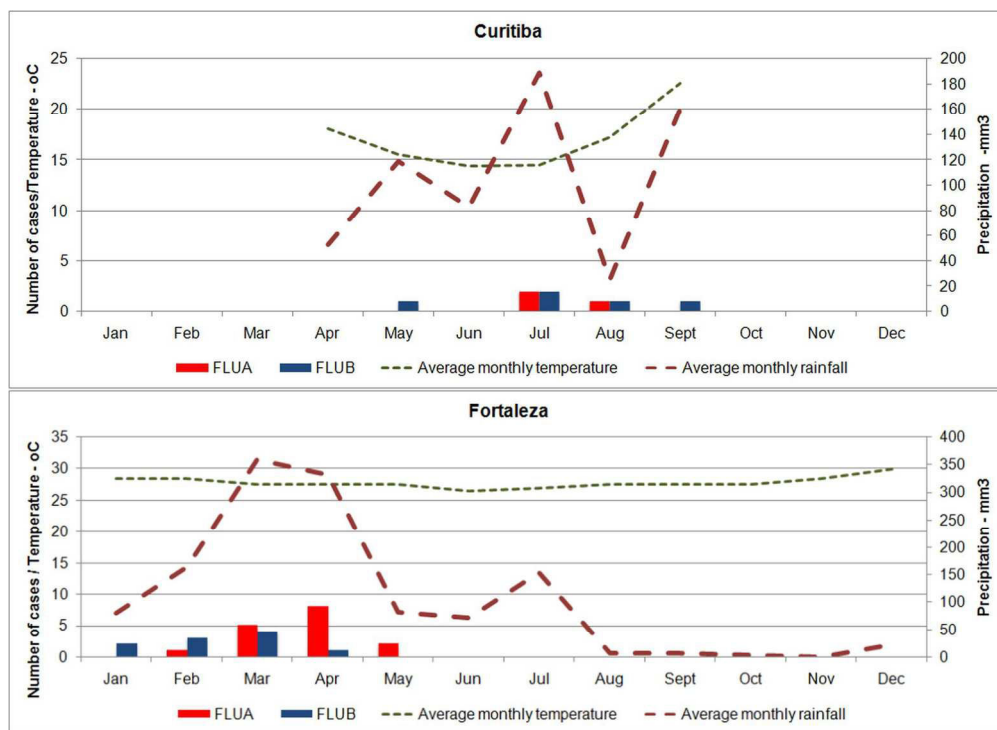


Figure 4. Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow (green and red lines represent mean temperatures and precipitations, respectively).

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
<b>Introduction</b>			
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
<b>Methods</b>			
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	07
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	



	<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	08
	(g) Describe any sensitivity analyses	Not applicable

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<b>Results</b>			<b>Page</b>
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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://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

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<b>Primary Subject Heading</b>:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Public health, Epidemiology
Keywords:	influenza, Hospital admission, seasonality, severe acute respiratory infection, vaccination, viral respiratory infection

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3 **Global Influenza Hospital-based Surveillance Network (GIHSN): results of**  
4 **surveillance of influenza and other respiratory viruses in hospitalized patients in**  
5 **Brazil, 2015**  
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50 **Running head:** Seasonality of influenza in Brazil  
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## 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.

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3 **Keywords:** hospital admissions, influenza, seasonality, severe acute respiratory  
4 infection, vaccination, viral respiratory infection.  
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9 **Strengths and limitations of this study**  
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- 11 • The strengths of this study are the use of the same model of active surveillance  
12 applied in other outside centers allowing subsequent comparisons on the impact  
13 of respiratory viruses, mainly influenza virus, in hospitalized patients  
14 worldwide. Second, to add more information on seasonality and severity of the  
15 influenza disease in South America, whose data are limited. Finally, our data  
16 reinforce the growing consensus that vaccinations in Northeast Brazil (tropical  
17 climate region) are being carried out in a post-seasonal period, with consequent  
18 low effectiveness.  
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- 21 • The main weakness of the data is the low inclusion of patients in Rio de Janeiro  
22 that does not allow for an assessment of the impact of viral respiratory infections  
23 in southeastern Brazil. The study was carried out with data collected in one year,  
24 which limits predictions on the impact of influenza longitudinally in these  
25 locales.  
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## 1. INTRODUCTION

Influenza-like illness (ILI) occurs annually worldwide, but peak timing and severity of the epidemic vary seasonally.<sup>1</sup> 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.<sup>2</sup>

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.<sup>3,4</sup> 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.<sup>5</sup>

Brazil, which includes temperate and tropical regions, exhibits an important regional heterogeneity in influenza peak.<sup>6,7</sup> Seasonal influenza epidemics are usually associated with "cold-dry" and "humid-rainy" climates<sup>8</sup>; 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.<sup>9</sup>

Vaccination is the main public health measure used to reduce the frequency of severe influenza cases.<sup>2</sup> In Brazil, influenza vaccination has been performed annually since 1999. Initially, immunization targeted individuals aged  $\geq 65$  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

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3 chronic non-communicable diseases, and health professionals.<sup>10-12</sup> Overall, in 2015,  
4 national immunization campaigns achieved a vaccination coverage of 82.7% in priority  
5 groups.<sup>13</sup>  
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9 The Global Influenza Hospital-Based Surveillance Network (GIHSN) is a  
10 public-private partnership between research institutes, hospitals, and several  
11 laboratories around the world, and was established to study the epidemiology of severe  
12 influenza in consecutive seasons in different countries.  
13 (http://www.gihsn.org/?page=map). In 2015, this network comprised a total of 31  
14 collaborating sites from seven countries, including Brazil. All participants follow the  
15 same core investigation protocol developed by the GIHSN scientific committee.  
16 Through broad geographical coverage and data standardization, the GIHSN group seeks  
17 to obtain a global picture of the impact of influenza on patients with respiratory  
18 pathologies. Additionally, this network provides invaluable tools to investigate the  
19 seasonality of influenza in regions where this information is not available, as well as a  
20 framework for estimating the effectiveness of seasonal influenza vaccines in preventing  
21 severe cases among age and risk groups.  
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37 In 2015, the GIHSN group began a study in Rio de Janeiro, Fortaleza, and  
38 Curitiba, three cities located in distinct regions of Brazil and enabling the evaluation of  
39 effects of different climatic conditions on the seasonality of influenza infections. Here,  
40 we report the initial findings of this surveillance project, including seasonality activity  
41 and the clinical and epidemiological features observed.  
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## 50 **2. PATIENTS AND METHODS**

### 51 **2.1. Ethical statement**

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3 As a multicenter study, this project was approved by the Ethical Committee of  
4 each institution involved in the research: Instituto Nacional de Infectologia Evandro  
5 Chagas (INI/Fiocruz), Rio de Janeiro; Hospital de Clínicas-Universidade Federal do  
6 Paraná (HC/UFPR), Curitiba; and Hospital Infantil Albert Sabin (HIAS), Fortaleza. All  
7 procedures were performed according to approved protocols, and consent was obtained  
8 from all patients (or guardians).  
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## 15 **2.2. Study design and site description**

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18 This multicenter, cross-sectional study was designed to provide information on  
19 active surveillance of influenza in hospitalized patients. Data were collected in 2015  
20 from three participating hospitals located in distinct cities and regions of Brazil:  
21 Curitiba (south), Fortaleza (northeast), and Rio de Janeiro (southeast) (Figure 1).  
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26 In Curitiba and Rio de Janeiro, patient recruitment and data collection occurred  
27 from April to September, coinciding with the period of influenza seasonality in south  
28 and southeast Brazil. In Fortaleza, the study spanned all of 2015, because, despite a  
29 previous study showing influenza circulation between January and April,<sup>14</sup> the  
30 seasonality of influenza in this region is not well established.  
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37 In Curitiba, the study was performed at HC/UFPR, a 310-bed, tertiary care,  
38 academic center, with 47 beds in the intensive care unit (ICU). It is also among the  
39 sentinel hospitals for influenza and severe acute respiratory infection (SARI)  
40 surveillance in the region. Curitiba is located at 25° 25' S, 49° 15' W, 924 m (3031 ft)  
41 and has a mild marine west coast climate, with no dry season and warm summers.  
42 Seasonality is moderate, with heavy precipitation during mild winters and a mean  
43 temperature of 16.5°C. The city population numbered ~1,879,355 people in 2015.  
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52 In Fortaleza, this study was conducted at HIAS, a public health institution of 270  
53 beds. Fortaleza is a city of 2,500,000 inhabitants and is located at sea level, 4° south of  
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3 the equator. Its tropical climate is characterized by two distinct seasons: a rainy one  
4 occurring between January and May, and a dry one occurring during the rest of the year.  
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6 There is high relative humidity (79%) and little variation in the average temperature  
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8 (26.4°C).  
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11 In Rio de Janeiro, this study was conducted at Hospital Quinta D'Or, which is a private  
12 hospital with 350 beds for general admission and 150 beds in the ICU. Rio de Janeiro is  
13 located at 22° 54'S, 43° 12'W, and its climate is classified as tropical Atlantic. The year  
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15 comprises two seasons: a hot and relatively humid one and another with mild  
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17 temperatures and less rainfall.  
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### 20 21 22 **2.3. Patient recruitment and inclusion/exclusion criteria**

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24 Patients of all ages who were admitted for any acute condition described as  
25 potentially associated with influenza were considered eligible for enrollment in the  
26 study. To be included, eligible patients were identified in the hospital admission  
27 registries and had to comply with all of the following criteria: 1) admission within 24 h  
28 to 72 h prior to recruitment; 2) a resident of the area of the study (defined for each site)  
29 and belonging to the base population source; and 3) a referral history for any acute  
30 condition associated with influenza and exhibiting onset within  $\leq 7$  days prior to hospital  
31 admission. This study used the European Center for Disease Control definition for ILI,  
32 which included at least one of four systemic symptoms (fever, headache, myalgia, or  
33 malaise) and at least one of three respiratory symptoms (cough, sore throat, or shortness  
34 of breath). Patients who did not give consent to participate, institutionalized individuals,  
35 and those with a history of hospitalization  $\leq 30$  days prior to recruitment were excluded  
36 from the study.  
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### 52 **2.4. Sample collection and processing**

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54 Samples were collected using nylon FLOQswabs (Copan, Brescia, Italy) and  
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3 included one nasopharyngeal and one pharyngeal swab (or nasal swab for patients <14  
4 years). Samples were added to a flask containing 3 mL of universal viral-transport  
5 medium (UTM medium; Copan) and stored at  $-80^{\circ}\text{C}$  until testing.  
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#### 8 9 **2.4.1. Sample testing to detect influenza and other respiratory viruses (ORVs)**

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11 All samples were submitted for nucleic acid extraction using a QIAmp viral  
12 RNA mini kit (Qiagen, Hilden, Germany). The presence of influenza A (H1N1pdm and  
13 H3N2) and influenza B (B/Yamagata or B/Victoria) was analyzed by reverse  
14 transcription real-time polymerase chain reaction (RT-PCR) according to Center for  
15 Disease Control (CDC) protocol.<sup>15,16</sup>  
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19 In a subset of samples ( $n = 497$ ), the presence of other pathogens was assessed  
20 using a commercial multiplex RT-PCR assay (FTD respiratory pathogens 21 kit; Fast-  
21 Track Diagnostics, Esch-sur-Alzette, Luxembourg) designed to detect the following:  
22 influenza A (FLUA) (H1N1pdm09); influenza B (FLUB); rhinovirus (RHV);  
23 coronavirus (COV) genotypes NL63, 229E, OC43, and HKU1; parainfluenza (PIV)  
24 types 1, 2, 3, and 4; human metapneumovirus A/B (HMPV); bocavirus (BOV);  
25 respiratory syncytial virus A/B (RSV); adenovirus (ADV); enterovirus (ENV);  
26 parechovirus (PRV); and the atypical bacterium *Mycoplasma pneumonia*.  
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40 Alternatively, we used the Seplex RV15 ACE detection kit (Seegene Inc.,  
41 Seoul, Korea), a multiplex PCR-based assay allowing for the simultaneous detection of  
42 the following viruses: ADV; HMPV; PIV types 1, 2, 3, and 4; FLUA; FLUB; RSV  
43 types A and B; rhinovirus (HRV) types A, B, and C; ENV; BOV; and COV types  
44 229E/NL63 and OC43/HKU1. Both tests were performed according to manufacturer  
45 protocol.  
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#### 52 **2.5 Data analysis**

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

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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

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2 proportions, with most individuals <5 years of age. By contrast, in Rio de Janeiro,  
3 females and adults were predominant, as the hospital did not evaluate children.  
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7 As expected from a predominantly young population, the prevalence of chronic  
8 conditions associated with aggravation of influenza was low in the Curitiba and  
9 Fortaleza subsets, whereas the Rio de Janeiro subset was characterized by a  
10 significantly higher prevalence of conditions, such as cardiovascular disease, chronic  
11 obstructive pulmonary disease, and diabetes.  
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15 Other factors that impact the prevalence of severe influenza infections are the  
16 use of antivirals and vaccination. Antiviral administration was more frequently reported  
17 in Curitiba. Overall, influenza vaccination rates were low, despite most age groups  
18 included in the study being covered by the Brazilian immunization program: ~31% of  
19 patients from Curitiba were vaccinated, and an estimated 14% and 19% were vaccinated  
20 in Fortaleza and Rio de Janeiro, respectively. Only 21% of children <5 years who were  
21 included in the study were vaccinated. Severe disease was detected in 149/595 cases  
22 (24%), but mortality was low: 9/595 (1.5%).  
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### 3.2. Influenza and ORV evaluation

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35 All samples were tested for influenza virus, with 39 (6.5%) positive: 23 (59%)  
36 influenza A H3N2 and 16 (41%) for influenza B (all Yamagata-like lineages). Of these  
37 cases, nine (23%) presented ORV co-infection.  
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44 The presence of ORV was tested in 497 patients (83.5%), with 293 (59%)  
45 positive and 46 (16%) presenting co-infection. RSV was the most frequently identified  
46 virus and the main source of co-infections ( $n = 27$ ; 49%), whereas influenza co-  
47 infections were found in only 23% (9/39) of cases. The respiratory pathogens detected  
48 in this subset of samples are reported in Figure 3.  
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### 3.3. Severe cases and mortality associated with influenza and ORV

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3 As shown in Table 2, 9/37 influenza-positive cases fell into the severe spectrum,  
4 and severe disease was significantly more frequent in influenza-infected patients than in  
5 those with ORV (30% vs. 11%). In an adjusted analysis, only age was associated with  
6 this outcome ( $p = 0.01$ ), whereas the presence of comorbidities was not ( $p = 0.12$ ). No  
7 difference in mortality was observed between influenza- and ORV-infected patients.  
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### 10 11 12 13 **3.4. Seasonality of influenza infections in two geographic regions of Brazil**

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15 Comparison of the seasonality of influenza infections with temperature and  
16 precipitation (historical means) recorded for each month revealed variations between the  
17 northeast (Fortaleza) and south (Curitiba) regions of Brazil in relation to the peak period  
18 of influenza infections; however, in both regions, influenza coincided with the peak of  
19 the rainy season. Our results indicated that immunization against influenza in the  
20 northeast was performed after the period of virus circulation (Figure 4), whereas data  
21 from Rio de Janeiro were not evaluated due to the small number of patients included in  
22 the study.  
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## 35 36 **4. DISCUSSION**

37 The GHISN was established with the main goal of improving the understanding  
38 of roles of influenza infections in the development of severe respiratory diseases. The  
39 information gathered by this network can potentially contribute to the elucidation of  
40 influenza seasonality in some regions, implementation of more efficient containment  
41 measures, and the improvement of preventive interventions, such as immunization.  
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48 In Brazil, the Ministry of Health employs two systems for epidemiological  
49 assessment of respiratory diseases: sentinel surveillance of ILI and universal  
50 surveillance of SARI. Sentinel surveillance consists of a network of designated health  
51 care units (public or private) distributed throughout the country, in which random  
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3 samples of respiratory cases are periodically collected for detection and genetic  
4 characterization of circulating viruses. In universal SARI surveillance, all severe  
5 respiratory cases admitted to ICUs and all deaths related to respiratory disease are  
6 investigated in the laboratory.<sup>17</sup> In the present study, only hospitalized patients  
7 identified by an active search of the participating health centers were included. In  
8 contrast to the Brazilian universal SARI surveillance protocol, which is restricted to  
9 ICUs, screening for our study included patients admitted to other wards and presenting  
10 diseases other than respiratory related. Individuals admitted with conditions associated  
11 with aggravation of influenza infections, such as cardiovascular disease and diabetes,  
12 were also screened, thereby increasing the number of identified cases.  
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24 Overall, the frequency of influenza viruses in the study specimens was 6.5%  
25 (39/595), with influenza A comprising 60% of the flu-positive samples. This low  
26 prevalence reflected the profile of the 2015 influenza season in Brazil and in most of the  
27 Southern Hemisphere and tropical regions. During that year, viral activity remained low  
28 through the entire season, with higher prevalence of H3N2 and lower detection of  
29 influenza B and H1N1pdm09. Even in temperate regions of the Southern Hemisphere,  
30 activity remained at an interseasonal level. In Brazil, a total of 11,945 cases of SARI  
31 were reported in 2015, of which 1089 (9%) were related to influenza infection. Among  
32 these cases, 599 (55%) were influenza A/H3N2, 234 (21.5%) were influenza B, 141  
33 (12.9%) were influenza A/H1N1pdm09, and 115 (10.5%) were reported as non-  
34 subtyped influenza A.<sup>18</sup>  
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48 Since 2014, the Brazilian Ministry of Health has included children  $\leq 4$  years of  
49 age in the group of individuals at risk of severe influenza and subsequently began  
50 vaccinating this subpopulation. Data from the National Immunization Program show  
51 coverage rates  $>83\%$  among young children and pregnant women, 89% in the elderly,  
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3 and 95% in health workers.<sup>13,17</sup> In the present study, we observed that there was a higher  
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5 percentage of patients from Curitiba vaccinated among the three sites; however, an  
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7 overall lower coverage was observed, with only 21% of patients reporting or showing  
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9 proof of influenza vaccination. This was surprising, considering that a significant  
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11 number of our patients fell in at least one risk category or presented comorbidities.  
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13 Given a 10.5% mortality rate due to influenza-related SARI cases in 2015, it is essential  
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15 to assess the reasons for such low immunization frequency. Among influenza deaths,  
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17 65% of patients and particularly those aged over 60 presented at least one risk factor  
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19 likely to cause complications.<sup>13</sup> Our data emphasized the need for comprehensive  
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21 identification of the weaknesses in the vaccination campaigns and an intensification of  
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23 the efforts to bring immunization to these individuals.  
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27 We also evaluated the prevalence of ORV in a subset of samples from HC/UFPR  
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29 and in samples selected randomly from among patients from Fortaleza and Rio de  
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31 Janeiro. Overall, different respiratory viruses were found in 59% of the tested samples.  
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33 This was consistent with previous reports showing high co-circulation of ORV in  
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35 hospitalized patients during the influenza season, and confirmed that the viruses  
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37 detected caused very similar ILI respiratory manifestations.<sup>19-21</sup> Moreover, our results  
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39 reflected the profile of respiratory viruses in children, which was the predominant group  
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41 included in the study. Interestingly, although circulation of influenza and ORV  
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43 coincided over time, the frequency of co-detection of influenza with ORV was very  
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45 low, whereas co-detection of more than one ORV in a single patient was rather common  
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47 along with RSV. This observation was consistent with those in our previous reports, and  
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49 we hypothesize that low codetection of influenza with other ORVs in these hospitalized  
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51 patients was likely due to the higher pathogenicity of influenza.<sup>20,22</sup>  
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3 We then compared clinical and epidemiological data from influenza- and ORV-  
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5 infected patients to evaluate the impact of these infections. Influenza was significantly  
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7 more frequent in older patients and those with severe diseases, whereas comorbidities  
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9 were associated with ORV infections. These findings reflected a lower adherence to  
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11 immunization among older children, with the presence of severe disease a possible  
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13 consequence of the higher virulence of influenza viruses, although no difference in  
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15 mortality was observed between both groups. This contrasted with results from a  
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17 previous study performed in Fortaleza, showing significant association between RSV  
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19 infections and infant mortality.<sup>23</sup> We believe that such a discrepancy might be due to the  
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21 low number of positive cases observed in the present study.  
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25 Studies of influenza seasonality in Brazil show that viral circulation in the  
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27 northeast region is more prevalent in the first 4 months of the year, coinciding with a  
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29 period of higher humidity in that area,<sup>14,24</sup> whereas viral circulation in the south and  
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31 southeast appears associated with lower temperature and unlikely to be linked to  
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33 humidity data.<sup>25,26</sup> Our data confirmed this observation and reinforced the growing  
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35 consensus that vaccinations in northeast Brazil are being carried out in a post-seasonal  
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37 period, with consequent low effectiveness.  
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40 The factors associated with the diversity of seasonal patterns displayed during  
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42 influenza epidemics in the tropics are not completely understood. A previous study  
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44 identified a seasonal southward-traveling wave across Brazil (from equatorial to  
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46 temperate regions), suggesting that environmental issues play more important roles than  
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48 population factors, such as immune function and levels of vitamin D, in driving  
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50 influenza epidemics in the country.<sup>6</sup>  
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53 Understanding the dispersion dynamics of influenza viruses in tropical regions is  
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55 considered of crucial importance, given that several reports suggested that in these  
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3 regions, influenza activity is more temporally diffuse and might contain foci for the  
4 emergence of new variants.<sup>6,7,27</sup> In addition to the need for a different influenza-  
5 immunization schedule in Brazil, the composition of this vaccine should also be  
6 evaluated, because the antigenic composition for this region might differ from that  
7 recommended for the temperate region.<sup>7</sup> However, information on strains circulating in  
8 tropical regions remains scarce, because most countries in these regions have low  
9 income, and health surveillance is not completely employed. Additionally, policies and  
10 resources need to be established to overcome the logistical challenges to implementing  
11 national immunization programs using different calendars and distinct antigenic  
12 compositions in Brazil.<sup>7</sup>

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

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

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3 of respiratory viruses in hospitalized patients in distinct regions of Brazil. We also  
4 reinforced the importance of monitoring influenza infections to enable more adequate  
5 preventive measures for the population.  
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### 10 11 **Acknowledgments**

12  
13 This work was supported by Sanofi Pasteur through the Global Influenza Hospital  
14 Surveillance Network and the Mérieux Foundation.  
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### 20 21 **Author contributions**

22 FKP, VP, and JPB participated to the conception/design of the study, supervised the  
23 analysis, and interpreted the data. MMS and SMR supervised the analysis, interpreted  
24 the data, and wrote the preliminary manuscript. FEAM and IT supervised the data  
25 collection from Fortaleza and Rio de Janeiro, respectively. BCC performed the tests,  
26 assembled the data, and wrote the preliminary manuscript. VMA, LAP, MBN, and LRV  
27 performed the molecular tests and collected the data from Curitiba. All authors  
28 contributed to the writing and review of the manuscript and approved the final version.  
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### 48 49 **Competing interests**

50 The authors have no competing interests.  
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**Table 1.** Epidemiological and clinical characteristics of the patients included in this study.

	<b>Curitiba</b>	<b>Fortaleza</b>	<b>RJ</b>
<b>Characteristics</b>	<b>(n = 136) (%)</b>	<b>(n = 427) (%)</b>	<b>(n = 32) (%)</b>
<b>Sex</b>			
Male	61 (45)	243 (57)	12 (37)
Female	75 (55)	184 (43)	20 (63)
<b>Age (median, y/IQR)</b>			
	1.4 (0.5–87.9)	1.3 (0–16.6)	1.4 (3.3–89.3)
<b>Age group (y)</b>			
<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)
<b>Time to onset of symptoms (Median, days/IQR)</b>			
	2 (0–7)	2 (0–7)	2 (0–6)
<b>Exposure to smoking</b>			
Yes	22 (16)	109 (25)	1 (3)
No	90 (66)	277 (65)	21 (66)
Ex-smoker	24 (18)	41 (10)	10 (31)
<b>Chronic diseases*</b>			
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)
<b>Length of stay in hospital (Median days/IQR)</b>	<b>2 (1–53)</b>	<b>1 (0–14)</b>	<b>2 (1–53)</b>
<b>Polypnea</b>			
Yes	108 (80)	51 (12)	17 (53)
No	7 (5)	303 (71)	3 (9)
NI	21 (15)	73 (17)	12 (38)
<b>Use of antiviral drugs</b>			
Yes	13 (10)	10 (2)	0
Days of use (Median/IQR)	5 (2–5)	15 (1–35)	—
<b>Vaccination in 2014</b>			
Yes	42 (31)	59 (14)	6 (19)
No	94 (69)	344 (81)	26 (81)



NI	0	24 (5)	0
<b>ICU admission</b>			
Yes	37 (27)	10 (2)	26 (81)
No	99 (73)	417 (98)	6 (19)
<b>Mechanical ventilation</b>			
Yes	29 (21.3)	13 (3.0)	0
No	107 (78.7)	414 (97.0)	0
<b>Death</b>			
Yes	4 (3)	4 (1)	1 (3)
No	132 (97)	423 (99)	31 (97)
<b>Severe disease</b>	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	ORV	<i>p</i>
	<i>n</i> = 30 (%)	<i>n</i> = 247 (%)	
<b>Sex</b>			
Male	13 (43)	121 (49)	0.5694
Female	17 (57)	126 (51)	—
<b>Age (median, y/IQR)</b>	2.6 (1.3–5.9)	0.8 (0.2–2.0)	<0.0001
<b>Age group (y)</b>			
<2	9 (37)	180 (73)	0.0002
2–4	10 (33)	43 (17)	—
5–17	4 (13)	15 (6)	—
> 18	5 (17)	9 (4)	—
<b>Time to onset of symptoms (median, days/IQR)</b>	1.5 (0–4)	2 (1–4)	0.1882
<b>Chronic diseases*</b>			
No	16 (53)	192 (78)	<b>0.0064</b> (OR 3.0 95% CI 1.4– 6.6)
Pulmonary	6 (20)	40 (16)	—
Cardiovascular	7 (23)	16 (6)	—

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

<b>Death</b>			
Yes	1 (3)	6 (2)	0.5006
No	29 (97)	241 (98)	—
<b>Severe disease</b>			
Yes	9 (30)	26 (11)	<b>0.0061</b> (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).

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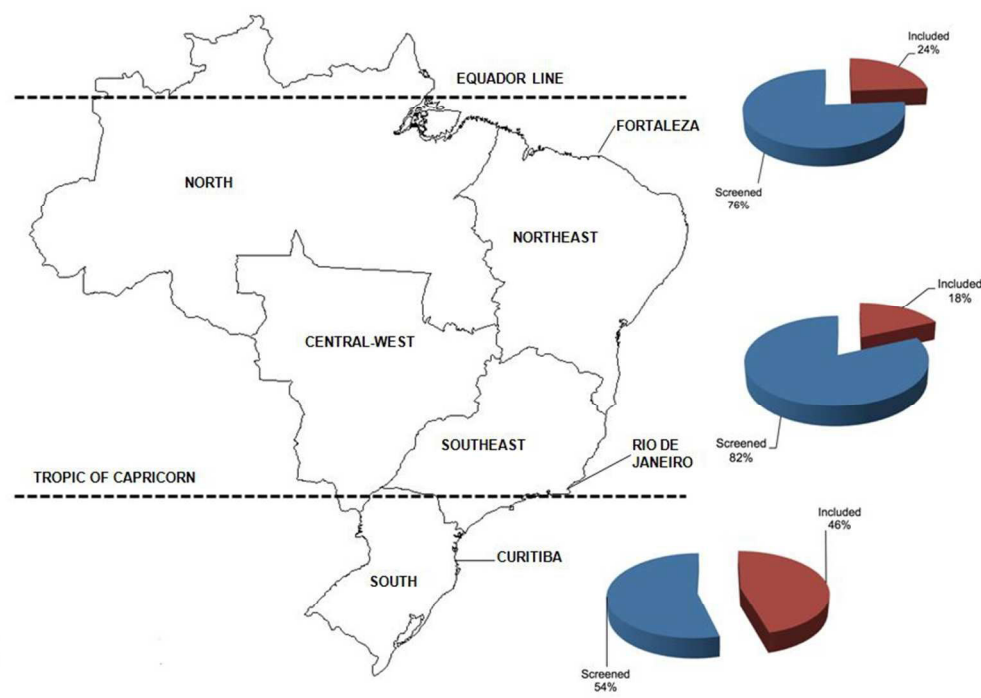


Figure 1. Map of Brazil, showing the location of Curitiba, Rio de Janeiro and Fortaleza, and the total of screened (Blue) and included (Red) patients in each site.

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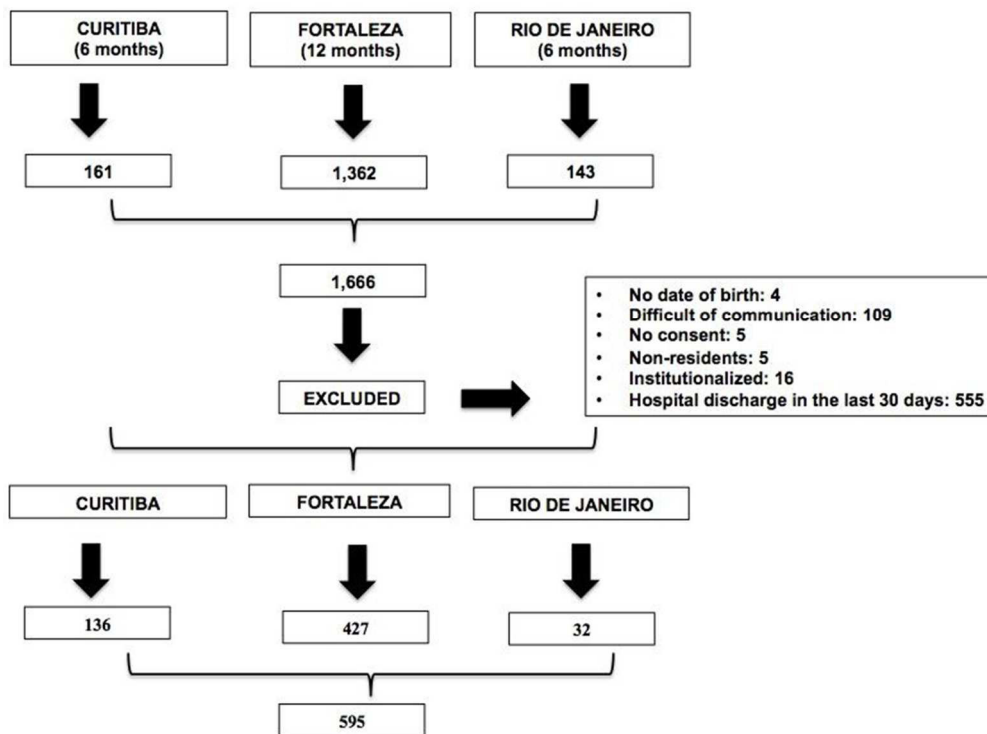


Figure 2. Flowchart summarizing the process of patient inclusion in the study, performed in GIHSN hospitals in Curitiba, Fortaleza, and Rio de Janeiro over 6, 12, and 6 months of collection, respectively.

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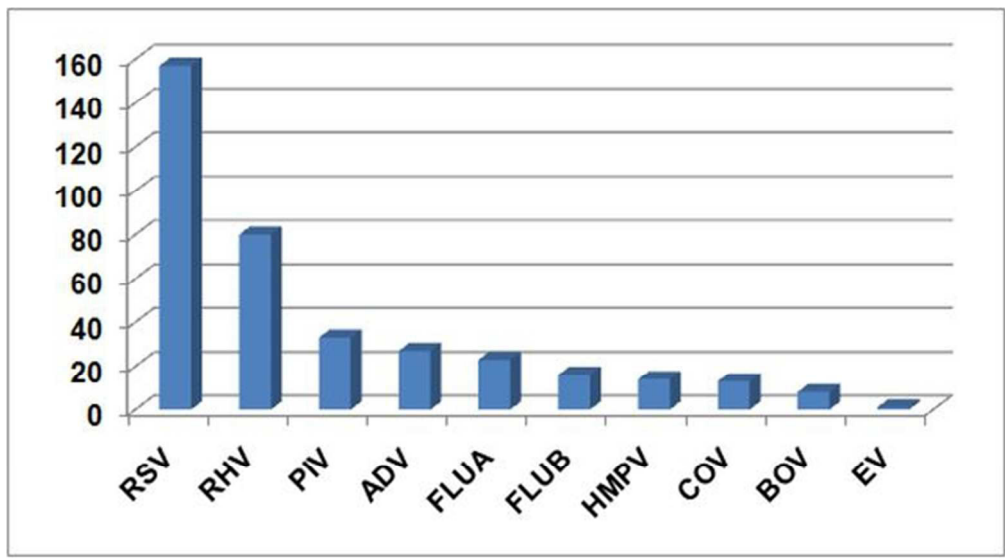


Figure 3. Respiratory viruses detected in a subset of samples (n = 497) from patients included in the study.

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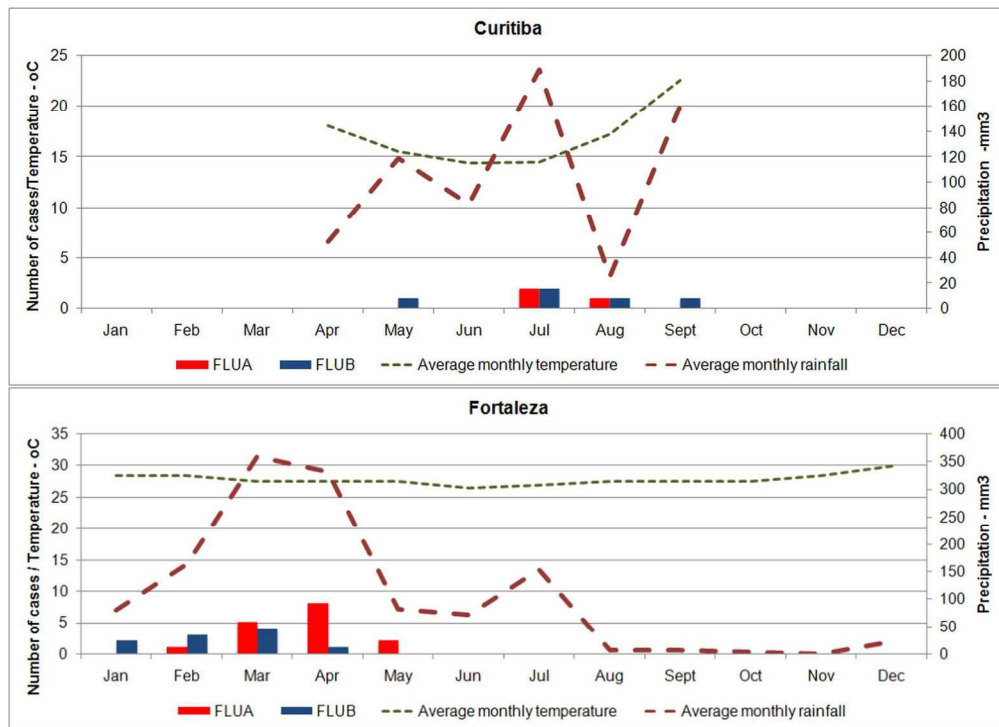


Figure 4. Number of detected cases of influenza A and B, associated with meteorological data in Fortaleza (Northeast) and Curitiba (South), in 2015. The time of the influenza vaccination campaign is indicated by an arrow (green and red lines represent mean temperatures and precipitations, respectively).

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
<b>Introduction</b>			
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
<b>Methods</b>			
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	07
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	

	<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	08
	(g) Describe any sensitivity analyses	Not applicable

Continued on next page

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<b>Results</b>			<b>Page</b>
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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).