TITLE PAGE

Full-length title:

Epidemiological surveillance of respiratory viral infections at IHU Méditerranée Infection and its application to SARS-CoV-2

Short title (for the running head): Respiratory viral infections at IHU Méditerranée Infection

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ABSTRACT

Epidemiological surveillance of infections at IHU Méditerranée Infection is based on in-house systems that use data from our microbiology-virology laboratory and continuously expand and evolve. Until 2020, respiratory samples were the third most frequent clinical samples sent to our laboratory. In 2019 we received ≈18,000 respiratory samples to search for bacteria and fungi and 17,600 to search for viruses. Over the 2015-2019 5-year period, we diagnosed >26,000 infections with respiratory viruses. The onset of the SARS-CoV-2 pandemic has dramatically boosted the number of tests and diagnoses of viral respiratory infections. On December 31st, over 339 days of daily surveillance, 427,787 SARS-CoV-2 tests had been performed for 306,363 patients. The mean number of daily tests was 1.262±930 (range, 8-3,596) and that of new patients tested was 904±688 (7-2,835). A total of 26,327 patients were diagnosed positive, the mean daily number being 78±94 (0-416), corresponding to a rate of new positive patients of 8.6% (mean: 6.1±5.4% (0-25.9%)). We first diagnosed SARS-CoV-2 on February 27th. The number of cases then peaked on March 26th (n= 362), was on average 2.5 between May 9th and July 5th, and increased and peaked again on October 26th (n= 416). Our surveillance strategy allowed observing SARS-CoV-2 temporal and age distributions and coinfections with other respiratory viruses. Data accumulated using and improving our existing tools show that comprehensive real-time surveillance of emerging infections is essential. Indeed it allows observing their epidemiological characteristics that cannot be predicted or extrapolated from other infections as some are new and unexpected and whose timely knowledge is valuable for optimal biological and clinical managements.

TEXT

Principle of the surveillance of infections at IHU Méditerranée Infection

Surveillance of infections has been implemented in our microbiology and virology laboratory since 2003 [1, 2]. It follows the recommendations made in a report on bio-terrorism and infectious diseases by one of us (DR) [3]. This report recommended in particular to implement a surveillance of abnormal events, without *a priori*, including syndromic surveillance, and of mortality. Our laboratory is the only one carrying out microbiology-virology diagnoses for all public and university hospitals (Assistance Publique des Hôpitaux de Marseille (AP-HM)) of Marseille, the second largest city in France with around 860,000 inhabitants (https://www.insee.fr/fr/statistiques/1405599?geo=COM-13055). It performs the diagnoses of all infections including those related to bacterial, fungal, parasitic and viral pathogens. Our syndromic surveillance strategy consists in counting on a weekly basis the number of samples received, classified by their nature, as well as the number of tests carried out, these two elements being situated upstream of the positive diagnosis of infections [4]. This surveillance is supplemented with a “traditional” surveillance corresponding to the follow-up of positive diagnoses for all the microbial and viral pathogens. Since 2003, we have thus followed a “roadmap” leading to monitoring abnormal events related to infections, and this monitoring has adapted from a technical point of view, and to our environment which has been modified over time. In 2012, the creation of the IHU Méditerranée Infection (IHU-MI) made it possible to professionalize surveillance tools with the establishment of a dedicated IT platform (MIDAS), and we were joined on this occasion by a team of epidemiologists of the military health service [2]. In addition, the principle of surveillance based on data from the microbiology-virology laboratory has been extended to the southeastern region of France (Provence-Alpes-Côte d'Azur region, or South region) which includes ≈7% of the population of metropolitan France. A collaborative network called PACASurvE has been in place since 2013 and the majority of hospitals (n= 17) and around half of private medical biology analysis laboratories (n= 285) participate [5, 6] and up to 386 when considering specialized medical biology analyses [7]. Data from our surveillance systems are examined weekly. Alarms are triggered automatically in the event of an abnormal increase in the number of samples, tests, or positive diagnoses. These events may lead to additional investigations, studies and reports [2]. In addition, since 2014, weekly monitoring of deaths at AP-HM has been integrated into the monitoring [2, 8, 9]. It can detect the infections most frequently associated with death.

Respiratory samples and diagnosis of respiratory infections

Until 2020, respiratory samples were the third most frequent type of sample among those sent to our laboratory, after urine samples and blood cultures. In 2019 we received ≈18,000 respiratory samples to search for bacteria and fungi and 17,600 to search for viruses. Regarding the search for bacteria, during a 63-month period from February 1st 2014 to April 25, 2019, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most frequently isolated bacteria from respiratory samples in 6,189, 3,190, and 973 cases, respectively (≈1,180, 610 and 185/year, respectively). There were ≈11,700 searches for mycobateria and 160 positive diagnoses/year (in 2019). Regarding fungi, during a 40-month period from June 1st 2017 to October 31th 2020, 15,976 respiratory samples (≈4,800 /year) from 12,032 patients were analyzed, and ≥1 fungus was isolated from 1,636 (10%) of them (≈490/year). The most frequent species were *Candida albicans* (54%), followed by *C. glabrata* (6%), *C. tropicalis* (6%), and *Aspergillus fumigatus* (6%). Overall, regarding microbiology, 8 of the 15 agents appearing in the top 15 of the most frequently diagnosed microbial agents are strictly or possibly respiratory pathogens: *S. aureus*, *C. albicans*, *K. pneumoniae*, *P. aeruginosa*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *C. glabrata*.

Viral respiratory infections

Respiratory viruses are an important part of infectious agents in our clinical microbiology-virology laboratory, and the most frequently diagnosed agents of respiratory tract infections. Over the 2015-2019 5-year period, we diagnosed 7,412 influenza A viruses; 2,882 influenza B viruses; 6,754 rhinoviruses; 4,851 respiratory syncytial viruses (RSV); 1,617 metapneumoviruses; 1,239 adenoviruses; 763 infections with human parainfluenza viruses 1 to 4; 480 enteroviruses; and 469 infections with the four seasonal human coronaviruses (HCoV) (229E, NL63, OC43 and HKU1) (**Figure 1**). Since 2010, viral respiratory infection diagnoses have been carried out mainly by real-time PCR (qPCR), based on in-house or commercial simplex or multiplex tests. The numbers of direct diagnoses of respiratory viruses were only exceeded or competed by *Escherichia coli* (5,800 in 2019) and *K. pneumoniae* (1,400) in urines (49,000), and by coagulase negative staphylococci (1,200) in blood cultures (49,000).

An important point in the surveillance of respiratory infections (as for that of other infections) is their unpredictability [10]. This can be observed including for viruses for which we have numerous data such as influenza viruses. Thus, if we consider the PCR diagnoses of influenza infections in our clinical microbiology-virology laboratory during the winters from 2010-2011 to 2019-2020, we observe important variations from year to year of the time of the emergence of the winter epidemic, of its duration, of the level of incidence reached at the epidemic peak, of the period during which this peak is reached, and of the viral types (A and B) and subtypes (H3N2, H1N1) predominant throughout the epidemic period (**Figure 1**). This unpredictability makes surveillance of considerable interest. The unpredictability of respiratory viral infections also applies to the 4 HPIV types circulating in humans as it turns out that these do not have the same seasonality. Thus, HPIV-3 circulates mainly during spring while HPIV-4 shows peaks of incidence from September to November and between February and March [11, 12]. The epidemic curves of the 4 seasonal HCoV are also not fully superimposed [13-18]. This shows the value of monitoring these viruses separately, which we have performed more comprehensively since 2019. Another point regarding respiratory viral infections is the need for an accurate diagnosis. Attributing cases of respiratory infections to a given virus without documentation by a diagnosis can lead to a very imperfect knowledge of the causes and the epidemiology of these infections [19-21]. In addition, we were able to observe among our diagnoses associations between microbial and viral pathogens in respiratory samples, and their interactions are being investigated in our institute [22, 23]. Finally, the monitoring of the weekly numbers of respiratory samples is a very useful element in addition to that of the diagnoses, since it can lead to earlier alerts triggered by increases in respiratory samples compared to alerts based on positive diagnoses [1].

Another important component of viral respiratory disease surveillance is mortality surveillance (https://www.mediterranee-infection.com/le-global-burden-of-infections-des-hopital-publics-de-marseille-and-the-region-provence-alpes-cote-dazur/). It allows us to observe among the most frequently diagnosed respiratory agents those most frequently associated with death. We do not speculate on the imputability of these infectious agents in the death, but we observe associations between these agents and deaths. A preliminary study carried out between February 1st, 2014 and April 25th, 2019, covering 63 months, measured that among 347,877 patients hospitalized at the AP-HM, 15,235 had died and for 62% of them, i.e. 9,480 patients, ≥1 clinical sample had been sent to our microbiology-virology laboratory. This corresponds to an average of 35 deaths/week for which we analyzed ≥1 clinical sample. We found as agents most frequently associated with death pathogens frequently involved in respiratory infections such as *S. aureus*, *K. pneumoniae*, *P. aeruginosa* as well as *Candida* spp.. However, there were also per year, associated with deaths, around 25 diagnoses of influenza A virus, 32 of influenza B virus, 18 of rhinovirus, and 9 of RSV. In another study that analyzed mortality between weeks 47 and 14 during the winters of 2018, 2019 and 2019-2020, we found that 0.4% of diagnoses of influenza viruses (10 for 2,815 cases), 1.0% of those of rhinoviruses (15 for 1,565 cases), and 1.5% of those of a seasonal HCoV (9 for 615 cases) were in patients who had died [9]. The diagnoses of respiratory infections among travelers are also monitored in our institute [24, 25], in particular those made on return from the Hajj pilgrimage for which for instance the acquisition rates of rhinovirus/enterovirus, HCoV-229E and influenza A virus were determined to be 39%, 20% and 2%, respectively [26]. Our infection surveillance also covers specific populations such as homeless people [27].

Surveillance of SARS-CoV-2 infections

Surveillance of SARS-CoV-2 qPCR tests and positive diagnoses

Our surveillance of respiratory viral infections has been completed and adapted following the emergence of SARS-CoV-2 that has introduced a scale change regarding the number of tests and diagnoses of viral respiratory infections. In fact, while we performed a maximum of between ≈400 and 1,000 tests/week during 2010-2011 and 2018-2019 winters, we received up to ≈20,000 respiratory samples/week during year 2020 (**Figures 2, 3**). Our surveillance went from a weekly rhythm to a daily rhythm. We have set up molecular tests to diagnose infections with this virus as quickly as possible. So three days after the release of the first viral genome (on January 10th, 2020) we had designed and ordered in-house real-time reverse transcription PCR (qPCR) systems. We subsequently used an internationally-validated qPCR system from the virology laboratory of Charité Hospital in Berlin [28]. We tested our SARS-CoV-2 detection tests on January 25th and performed the first test for a patient whose sample was referred to our institute on January 29th. At the beginning of February, we carried out 674

SARS-CoV-2 qPCR tests for 337 people repatriated from China to France [29], and retrospectively tested 137 patients who had died with a respiratory infection between 2018 and 2019, 135 medical students returning in 2018-2019 from Asia, and 144 people in whom a respiratory sample had been collected in Senegal between March 2019 and February 2020; all these tests being negative. The first 280 patients tested between January 29th and March 1st (210 and 60 on return from Italy and Asia, respectively) were negative for SARS-CoV-2, but other respiratory viruses were identified in 49% of the cases (n= 137) [21]. The most frequent viruses detected were influenza A virus (12%); rhinovirus/enterovirus (12%); common HCoV (229E, OC43, NL63 and HKU1 in 1%, 1%, 4% and 7%, respectively); influenza B virus (8%); metapneumovirus (7%); RSV (2%); and adenovirus (1%). In addition, 12 patients (4%) were coinfected with different respiratory viruses, most often with rhinovirus/enterovirus and metapneumovirus.

The first positive SARS-CoV-2 qPCR result was obtained on February 27 th for a patient hospitalized at Nice University Hospital located in the Provence Alpes Côte d'Azur region (PACA; Southern France), since we were at this time the only center in this French region to perform SARS-CoV-2 testing. The first SARS-CoV-2-positive patient hospitalized in Marseille in our institute was detected on March 2nd, after we had routinely performed 4,149 SARS-CoV-2 qPCR tests for 3,417 symptomatic or asymptomatic patients. The surveillance of SARS-CoV-2 infections has been accompanied by daily reports on the IHU Méditerranée Infection website since March 26th in the form of a “Southern France Morning Post” posted every day (https: //www.mediterranee-infection.com/covid-19/). This information available to everyone includes the total number of samples received at the laboratory and of tests performed, the number of positive tests and the percentage of positives. This also includes the numbers of tests and positives for the newly-tested patients, for symptomatic and asymptomatic patients, for patients sampled at IHU MI or at AP-HM, and still more precisely for patients residing in our department of Bouches-du-Rhône, and in the city of Marseille. We have indeed received a large number of samples from other hospitals and laboratories in the Provence Alpes Côte d'Azur region and also carried out tests for patients domiciled in other French regions (who may have traveled to Marseille to be tested), mainly Auvergne Rhône-Alpes region and Ile-de-France region, particularly Paris (as of July 7th: n= 470, 547 and 235, respectively). As already available for other infectious agents on our intranet platform for the epidemiological surveillance of infections (MIDAS), surveillance charts for SARS-CoV-2 infections were added specifically for SARS-CoV-2, and separately for each French department and each arrondissement of the city of Marseille.

The surveillance of SARS-CoV-2 infections carried out in our institute has been optimized by the testing strategy that has been implemented there. The tests were thus carried out, from the beginning and until now, for all patients regardless of whether they were symptomatic or not, contact-cases or not, and with a medical prescription or not. All these tests were performed by qPCR on nasopharyngeal swabs, the only diagnostic approach that has been used in our institute. In fact, our evaluation of a recommended antigen test on nasopharyngeal swabs from 204 qPCR-positive patients (including 182 (89%) symptomatic) showed a high false-negative rate (21% in symptomatic patients, and 45% in asymptomatic) and positive and negative predictive values of 96% and 72%, respectively [30]. We used various qPCR assays, including in-house techniques in microplates [21, 29] and later during the year a commercial reagent for microplate assays as well as commercial simplex (n= 3) or multiplex (2) tests, including some evaluated in our laboratory [31]. In addition, we tested 7 alternative qPCR systems as backup tests in case of genetic evolution of the viruses that would generate mismatches of primers and/or PCR probes possibly. As of December 31st, 2020, over a period of 339 days, 427,787 tests had been performed for 306,363 patients. The mean number of daily tests was 1.262 (standard deviation, 930; range: 8-3,596; median= 979) and that of new patients tested was 904±688 (7-2,35; median= 693). A total of 26,327 patients were diagnosed positive, the mean daily number being 78±94 (0-416; median= 35), corresponding to a rate of new positive patients of 8.6% (mean: 6.1±5.4% (0-25.9; median= 5.6%). We observed different phases between February and December. Indeed, the daily number of SARS-CoV-2 diagnoses peaked on March 26th (n= 362), dramatically decreased in May with a mean of 2.5/day for 58 days between May 9th and July 5th (https://www.mediterranee-infection.com/covid-19/). Then, incidence re-increased from early July and peaked again on October 26th (n= 416) before a new drop (**Figure 2**).

Epidemiological features of SARS-CoV-2 infections and associated respiratory viruses

Over the year 2020 and from the first days of the emergence of the SARS-CoV-2 epidemic in our region, we have carried out studies relating to the surveillance of infections by this virus and relying on the observation of our laboratory data. First, we compared the temporal distribution of infections by this emerging coronavirus with that of the 4 other seasonal human coronaviruses [32]. At the end of May, for each of these five coronaviruses, we observed a bell-shaped curve with a lag of a few weeks, SARS-CoV-2 having occurred later than the seasonal HCoVs. These data suggested that the epidemic curve of SARS-CoV-2 may be very similar to that of common HCoV and to that of some other respiratory viruses (**Figure 4**). A second element observed was the age distribution of SARS-CoV-2 infections. A study carried out early until March 14th demonstrated a low proportion of cases in children (0 between 0-1 year, 3 (1%) between 1-5 years and 7 (4%) between 5-10 years), significantly lower than in adults [32]. These results were verified in a larger study carried out on the first 302 pediatric cases (<18 years of age) diagnosed at Marseille university hospital on April 15th, which showed that they corresponded to 5% of the positive patients (n= 5,861) and included 107 (2% of all positive patients) and 70 (1%) children under 10 and 6 years of age, respectively [33]. All these infected children clinically recovered. If we compare these data to those for seasonal HCoVs, we observe that children are spared only by SARS-CoV-2 while they are the age group mainly affected by the four season-endemic coronaviruses [32]. These data showed early in the pandemic that the epidemiology of SARS-CoV-2 could not be predicted based on prior knowledge of other coronavirus infections, nor on that of other respiratory infections such as influenza virus infections. Indeed, other respiratory viral infections affect children extensively, especially the youngest of them [33].

Our ability to diagnose all infectious pathogens in the same laboratory, including bacterial, fungal, parasitic and viral pathogens, allows us to analyze possible coinfections. This is another element of respiratory infection surveillance. The multiplex PCR diagnostic approach has been developed since 10 years ago in our laboratory through our POC laboratories [34, 35] but also our core laboratory. It has recently expanded with technical progress and the increasing availability of commercial multiplex tests with rapid results [19, 21, 36]. Regarding respiratory infections it is tricky to clinically narrow down a differential diagnosis to a single one due to the significant overlap in the clinical presentations. Multiplex PCR diagnosis allows a more exhaustive coverage of respiratory viruses, as we have shown for example in the context of the first research of SARS-CoV-2 infections in our laboratory [21], and for the diagnosis of respiratory viral coinfections [19]. We thus studied coinfections with SARS-CoV-2 and other respiratory viruses among 4,222 patients during March and April 2020 [19]. A total of 643 patients (15%) were diagnosed with SARS-CoV-2, 1,095 (26%) were diagnosed with ≥1 non-SARS-CoV-2 respiratory viruses, and 27 (4% of those SARS-CoV-2-positive) were coinfected with SARS-CoV-2 and another respiratory virus, including a rhinovirus (n= 11), an endemic coronavirus (HCoV-OC43 (2), HCoV-HKU1 (2), HCoV-229E (1)), influenza viruses A (2) or B (2), HPIV 4 (2) and 2 (1), bocavirus (2), and adenovirus (1). The number of coinfections with SARS-CoV-2 and other respiratory viruses decreased by 3.5 times between March and April while the number of infections with non-SARS-CoV-2 respiratory viruses decreased by 18 times between these 2 months, indicating that the frequency of such coinfections largely depends on the rate of coincidence of these viruses. The surveillance of these coinfections for the more recent period between August and November thus revealed a frequency of coinfections of 0.3% with 46 cases involving overwhelmingly, in 37 cases, rhinoviruses that circulate along the whole year. Interestingly, over the recent period from November to December 2020, rhinoviruses (426 diagnoses (13% of tests)) and adenoviruses (140 (3%)) have been detected, but the incidence rates of infections with other respiratory viruses (apart from SARS-CoV-2) were unexpectedly very low, and lower than those observed during this 2 month-period during the 10 previous years. Thus only two diagnoses of RSV infection, one diagnosis of metapneumovirus infection, and no influenza virus infection were detected in 2020 vs. on average 433±131, 67±44, and 113±134 diagnoses, respectively, during the years 2010 to 2019. The daily surveillance of the number of samples and diagnoses of SARS-CoV-2 infections has been accompanied by other surveillance needs. It included the implementation of the genomic epidemiological surveillance that can reveal some aspects of the SARS-CoV-2 infection that cannot be deduced from the mere observation of the numbers of cases. This surveillance showed that several epidemics have occurred since July that involved different SARS-CoV-2 variants [37-39]. Finally, our daily monitoring of positive diagnoses allowed us to detect SARS-CoV-2 reinfections. We observed that among the 6,799 patients diagnosed positive between February and May, 837 had been retested since June and 15 patients had been found positive again for SARS-CoV-2 >2 months after viral clearance following the first infection. Viral genome sequencing made it possible to demonstrate reinfection with a virus of a different genotype compared to that of the first episode [40].

Conclusion

In total, in the context of the SARS-CoV-2 pandemic, we have completed and adapted our epidemiological surveillance of respiratory viral infections by relying on the strategies and versatile tools that pre-existed in IHU MI. Data accumulated in 2020 show that it is essential to perform a real-time surveillance of emerging infections to be able to observe all their epidemiological characteristics, whose timely knowledge is useful for an optimal biological and clinical management of the cases. These characteristics cannot be predicted or extrapolated from other infections with similar agents since some of them are new and unexpected. Our surveillance strategy, combined with the strategy of massive SARS-CoV-2 screening conducted from February at IHU-MI, allowed us to be the first to observe and communicate on several features of the infections with this emerging virus.

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This manuscript has been edited by a native English speaker.

Author contributions

Conceived and designed the experiments: PC, HC, DR. Contributed materials/analysis tools: all authors. Analyzed the data: all authors. Wrote the paper: PC and DR.

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Conflicts of interest

The authors have no conflicts of interest to declare. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Ethics

All the data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille university hospitals), and this study results from routine standard clinical management. This study has been approved by the ethics committee of our institution (N°2020-029). Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019-73.

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FIGURE LEGENDS

Figure 1. Number of diagnoses by qPCR of respiratory viruses during the period from 2010 to 2019

Figure 2. Weekly number of diagnoses by qPCR of respiratory viruses in 2020

Figure 3. Weekly number of respiratory samples sent to our laboratory to test for viruses by qPCR during the period from 2010 to 2020

Figure 4. Weekly numbers of diagnoses by qPCR of respiratory viruses

HCoV, human common coronavirus; HPIV, human parainfluenza virus; RSV, respiratory syncytial virus