TITLE PAGE

Full-length title:

Consequences of the COVID-19 outbreak on the diversity of non-viral infectious agents and the evolution of antibiotic resistance by using a syndromic surveillance based on laboratory data at the IHU Méditerranée Infection, Marseille, France.

Running title : Consequences of the COVID-19 outbreak on the diversity of non-viral infectious agents and the evolution of antibiotic resistance

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Word count: abstract: 274; text: 2,268

Figures: 4; **Tables:** 5; **References:** 19

Supplementary Data: Figures: 0; Table: 0; References: 0

Key words (6): Syndromic surveillance; clinical microbiology laboratory; epidemiology; lockdown, COVID-19; diversity; wild

Abstract

For controlling the rapid spread of this SARS-CoV-2 virus, French Government implemented various measures including a lockdown. The objective of this paper is to describe the surveillance system MIDaS and to show how this system has been used for evaluating the consequences of the French lockdown on the bacterial mix of the AP-HM, as well as the evolution of antibiotic resistance. We were particulary interested by bacterial agents identified in respiratory, blood and urine samples during the lockdown period, that is from week 12 (mi-march) to week 35 (end of august) 2020, with the years 2017 to 2019 as control period and we used the percentage of non-resistant (wild) isolates for each species monitored by the surveillance system without differentiating the resistance phenotypes. The follow-up of the laboratory bacterial identifcation activity showed a drop in the moving average of the number of patients during the lockdown. The relative abundance in respiratory samples for the whole studied period was higher in 2020 while it decreased for urine samples and was constant for blood samples. *S. epidermidis* and *C. albicans* species increased during the lockdown, restoration and post-lockdown periods, conversely *E. coli* significaly decreased. A significant increase in the percentage of wild phenotypes during 2020 compared to 2017-2019 is observed for *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa*. The use of the MIDaS syndromic collection and surveillance system at IHU-MI has thus made it possible to detect aberrations in the epidemic signal, to observe and analyze unexpected increases or decreased in observed cases and to implement actions to stop the spread of a pathogen but also to understand the underlying mechanisms of its transmission.

TEXT

Introduction

Hospital University Institute Méditerranée Infection (IHU-MI) hosts the clinical microbiology and virology laboratory for all four public university hospitals of Marseille (AP-HM) that performs the diagnosis of all infectious agents including bacteria, microscopic fungi, parasites and viruses. Since 2013, it has improved or implemented five syndromic epidemiological surveillance sub-systems that uses the results of this laboratory, unified in a single collection and analysis system named MIDaS (for Mediterranée Infection Data Warehousing and Surveillance). Besides traditional surveillance based on patients' clinical diagnoses of notifiable infectious diseases, syndromic surveillance uses data about specimen received at the laboratory and about tests performed based on prescriptions of clinicians, as well as other laboratory markers through innovative approaches.

In December 2019, Wuhan in Hubei province became the epicenter of the spread of a new emerging pathogen called SARS-CoV-2. It spread rapidly to other continents, the pandemic being declared in March 2020. For controlling the rapid spread of this virus, French Government implemented various measures: closure of schools, culture centers and socialization places such as bars and restaurants [1,2] before announcing a total lockdown one week later, from 16 March 2020 [3] until 11 May 2020 (week 12 to week 19). Several European, American and Asian countries have made the same choice with a more or less strict lockdown [4]. Several studies have already assessed the effectiveness and impact of the diverse responses on various fields: medical, economic or sociological [5-8], but, to our knowledge, its microbiological impact has not been yet studied.

The objective of this paper is to describe the surveillance system and to show how this system has been used for evaluating the consequences of the French lockdown on the bacterial mix of the AP-HM, as well as the evolution of antibiotic resistance.

Materials and methods

MIDaS, an epidemiological hub

The MIDaS system can be considered as a surveillance activity hub. Data collection coming from the hospital information system is mainly collected and processed for surveillance purposes in the search of statistical aberrations [9] of activity indicator time series, i.e a higher than expected number of observed cases. These statistical events are discussed each week during a staff meeting that is attended by biologists, clinicians, and epidemiologists, and during which in-silico investigations, notably comparisons and cross-referencing of data, can be used. Further epidemiological investigations and measures can be initiated if the investigation confirms the alarm and, if required, an epidemiological alert may be simultaneously transmitted to the health institutions concerned, including the Regional Health Agency (Agence Régionale de Santé, ARS) or the Infection Control Committee (Comité de Lutte contre les Infections nosocomiales, CLIN). The surveillance results are also weekly disseminated through the IHU Méditerranée Infection website, and. As an surveillance activity hub, this system also suggests the weekly enrichment of our microbial strain collection (CSUR) [10], and supports the quality control of laboratory activities, in the search of deviations in laboratory processes. The overall structure of MIDaS is presented in Figure 1.

MIDaS data collection

The main role of MIDaS is to collect from the hospital information system by weekly extraction-transform-load processes diverse surveillance-related data. The AP-HM consists in four public university hospitals: Timone (1,307 beds), Conception (767 beds), North Hospital (793 beds) and South Hospital (421 beds) and has approximately 125,000 admissions and 1 million consultations per year. The clinical microbiology and virology laboratory performs approximately 8 million tests per year. Tests results as well as patients and specimen information are collected from the laboratory information system. Other data from hospital information systems as the hospital medico-economic data (Programme de Médicalisation des Systèmes d'Information, PMSI) are weekly collected, especially information about patients' death in order to study death-associated infections. Finally, data from other automated systems are also collected, such as spectra files generated by the Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) mass spectrometry instruments used for bacterial and fungal routine identification. MIDaS therefore populate a data warehouse that groups together microbiological analysis results (sample number, requesting unit, sample date, type of analysis, antibiotic susceptibility test results, antibiotic resistance phenotype) and patient information (anonymized patient identifier, age, gender, postal code of residence, anonymized identifier of hospital stay, date of hospitalization, length of stay, death). Six million of microbiological results are stored in this data warehouse, representing 240,000 antibiotic susceptibility tests, 2,300,000 samples, 850,000 patients and nearly 1 million MALDI-TOF clinical spectra (more than 3 million for spectra being produced for research purposes).

MIDaS domain-specific monitoring systems

Five domain-specific monitoring sub-systems are connected to the data warehouse for producing fully automated dashboards. Historically, EPIMIC (for EPIdemiological surveillance and alert based on MICrobiological data) is the first monitoring system that was implemented, in 2002 in our laboratory to allow monitoring the weekly counts of clinical specimens sent by clinicians, of diagnosis tests performed and of diagnosis results obtained [11]. It has been later updated in 2013 for its integration in MIDaS. Since 2013, bacteria have been more comprehensively monitored by BALYSES (Bacterial real-time Laboratory-based Surveillance System) while SFY (Surveillance of Fungi and Yeasts) has focused on microscopic fungi and yeasts and MARSS (Marseille Antibiotic Resistance Surveillance System) has monitored antibiotic resistance patterns [12]. In addition, MALDI-TOF spectra have been used as an additional tool to support surveillance and are analyzed by the SpectraSurv system (for MALDI-TOF based surveillance) [13].

Data for the lockdown analysis

In this study, we were particulary interested by bacterial agents identified in respiratory, blood and urine samples during the lockdown period, that is from week 12 (mi-march) to week 35 (end of august) 2020, with the years 2017 to 2019 as control period.

Hospital activities

The analysis based on BALYSES allowed us to define three periods according to the evolution of the laboratory activities during 2020: a lockdown phase (weeks 12-19), a restoration phase (weeks 20-24) and a post-lockdown phase (weeks 25-35) (figure 2).

Bacterial and fungal community

The bacterial community was studied in terms of species richness and abundance for the 3 most frequent samples: urine, respiratory and blood samples. The specific richness represents

the total number of species present in a sample and relative abundance (or relative frequency) indicates the frequency of a species.

Evolution of antibiotic resistance

We used the percentage of non-resistant (wild) isolates for each species monitored by the surveillance system without differentiating the resistance phenotypes, taking in account the nosocomial or community origin of the isolate.

Statistical analysis

The log-linear model, and secondly the Fisher and Chi2 tests for point comparisons, were used to evaluate the evolution of diversity and antimicrobial resistance, with a statistical significance threshold of 0.05 [14].

Results

Hospital activities

The follow-up of the laboratory bacterial identifcation activity (Figure 2) showed a drop in the moving average of the number of patients during the lockdown period from 641.5 patients on March 11 to 412.5 patients on May 13, 2020. After the end of the lockdown, the activity level gradually returned to the normal, from 412.5 patients on May 13 to 512.5 patients on June 17 then to 611.25 patients on July 15, 2020.

Bacterial and fungal community

From the weeks 12 to 35 in 2017-2020, a total of 349 bacterial and fungal species were identified from 30,918 identifications including 24,946 from urine samples (186 distinct species), 4,555 from respiratory samples (230 distinct species) and 1,417 from blood samples (111 distinct species). The top 20 species alone represent 87.4% (27,037/30,918) of the total

number of identifications. The relative abundance in respiratory samples for the whole studied period was higher in 2020 while it decreased for urine samples and was constant for blood samples (Figure 3A). However, the species richness was constant over time in respiratory and urine samples but decreased in blood samples (Figure 3B).

When comparing diversities between 2020 and 2017-2019 for the pooled three kinds of samples (urine, respiratory and blood samples), we found a significant variation in the relative frequency of 9 species out of the top-20 (45%) during the lockdown period, of 4 species during the restoration and post-lockdown periods, although not for the same species (Table 1, Figure 4). Species that significantly decreased during the lockdown are *Escherichia coli* (39.3% to 28.6%, p-value < 2.2e-16), *Klebsiella oxytoca* (1.5% to 0.8%, p-value = 0.02) and *Haemophilus influenzae* (1.2% to 0.7%, p-value = 0.02). There is a significant increased for *Candida albicans*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Staphylococcus haemolyticus*, *Enterobacter aerogenes* and *Candida glabrata* (Table 1). *S. epidermidis* and *C. albicans* species increased during all three time periods, conversely *E. coli* significaly decreased. *Citrobacter koseri* experienced a significant decrease only during the restoration period and *Staphylococcus aureus* experienced significant growth during the post-lockdown (Table 1).

Diversity in respiratory samples

During the lockdown and restoration periods, 5 species out of the top 20 recorded a significant variation in their relative frequency, and 1 during the post-lockdown (Table 2A). *E. coli*, *S. pneumoniae* and H. *influenzae* significatly decreased during the lockdown and remained stable during the next two phases. *C. albicans* is the only species that increased during the three periods. *K. pneumoniae* decreased during the restoration period whereas species including *E. cloacae* and *S. agalactia* increased.

Diversity in blood samples

A significant increase in relative frequency is observed for *E. faecalis* and *S. haemolyticus* for blood samples during the lockdown, which is maintained during the post-lockdown only for *S. haemolyticus* (Table 2B). No other variation in relative frequency is observed during the restoration period.

Diversity in urine samples

E. coli significaly decreased from 46.5% to 38.4% during the lockdown in contrast to *C. albicans* (3.0% to 5.1%), *E. cloacae* (2.3% to 3.5%) and *C. glabrata* (0.6% to 1.1%) (Table 2C) that significaly increased (Table 2C). During the restoration period, only *C. albicans* (2.9% to 4.9%) increased and *C. koseri* (1.6% to 0.8%) decreased. No significant variation is observed for the post-lockdown period.

Evolution of antibiotic resistance

Whathever the origin of the infection, the analysis of the evolution of bacterial antibiotic resistance showed a significant increase in the percentage of wild phenotypes during 2020 compared to 2017-2019 for *E. coli* (45.4% to 48.5%), *K. pneumoniae* (59.6% to 67.7%), *P. mirabilis* (56.4% to 64.1%) and *P. aeruginosa* (56.0% to 64.9%) (Table 3). Other species out of the top-20 did not record any significant change. The wild percentage for community infection significantly increased for *E. coli* and *P. aeruginosa* whereas it decreased for *K. pneumoniae* and *P. mirabilis*. However, for nosocomial infection, this percentage significantly decreased for only *P. aeruginosa* and increased for *K. pneumoniae*.

However, in regards of the origin of infection (nosocomial or community), the percentage of wild phenotypes significantly decreased when the origin of infection is nosocomial and significantly increased when the origin of infection is community for *E. aerogenes*, *E. faecium*, *K. oxytoca* and *M. morganii* (Table 3). *E. faecalis* presented a decreased percentage for nosocomial infection and *E. cloacae* an increased percentage for

community infection.

Discussion

The consequences of COVID-19 on the diversity of non-viral infectious agents and on the evolution of antibiotic resistance is notable. Infectious agents may be separated into 2 groups according to their behaviour during this first lockdown.

The first group is characterised by a decrease of the species relative frequency during the whole study period, but with a decrease confirmed only during the lockdown period when the statistical analysis takes the kind of the sample in account. We have here an example of Simpson's paradox [15]. *Escherichia coli* is characteristic of this first group.

Species of the second group show a significant increase of their relative frequency whatever the type of sample and the period studied. *Candida albicans* and *glabrata* belong to this group, particularly found in intensive care units, which were heavily impacted during the SARS-CoV-2 epidemic, possibly explaining their significant increases during the lockdown [16,17].

The wild phenotype population has also increased in comparison with the previous 3 years for the most identified species in our institute. *E. coli* and *P. aeuruginosa* present more frequently a wild phenotype than usually in the context of community-acquired infection. However, the susceptibility of *E. coli* to most antibiotics involved in community-acquired urinary tract infections tended to decrease before the COVID-19 pandemic [18].

In France, government responses taken to limit the spread of the virus, such as lockdown, probably played a role in the evolution of identification of bacteria and fungi [2,3]. Indeed, it was recommended that individuals should stay at home and contact the emergency call centre (number 15) only in the event of respiratory distress, to avoid clogging up hospitals and the spread of the disease [19]. In addition, in order to manage patients with COVID-19,

many hospital departments have been transformed to accommodate these SARS-CoV-2 positive patients, which explains this increase in the number of hospitalisations. Non-emergency hospital activities were suspended. Thus, the number of patients and ordinary hospitalisation outside of COVID-19 decreased considerably during the first containment, partly explaining this decrease in some pathogens and the increase in others.

The use of the MIDaS syndromic collection and surveillance system at IHU-MI has thus made it possible to detect aberrations in the epidemic signal, to observe and analyze unexpected increases in observed cases and to implement actions to stop the spread of a pathogen but also to understand the underlying mechanisms of its transmission. Syndromic surveillance is central in the surveillance of infectious agents and particularly adapted to the unpredictability of their epidemiological trends and to new and re-emerging pathogens.

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Acknowlegments

This manuscript has been edited by a native English speaker.

Figures

Figure 1 – Structure of Méditerranée Infection Data warehousing and Surveillance (MIDaS)

Figure 2 – Follow-up of patients with at least one bacterial identification at IHU Méditerranée Infection from January to September, 2020.

Figure 3 – Evolution of the relative abundance (A) and the specific richness (B) from weeks 12 to 35, 2017 to 2020 at Assistance Publique – Hôpitaux de Marseille, Marseille, France.

Figure 4 - Weekly incidence of the 5 most identified species in our institute and SARS-CoV-2 from 2018 to 2020, Marseille, France.