Title: Integrated environmental and clinical-syndromic surveillance for the prevention of acute respiratory infections (ARIs) in indoor environments and vulnerable communities (Stell-ARI)

Abstract

Background and Objectives:

The epidemiological relevance of viral acute respiratory infections (VARIs) has been dramatically highlighted by COVID19. However, other viruses cannot be neglected, such as the influenza virus, respiratory syncytial virus, rhinovirus, other coronavirus, adenovirus, metapneumovirus. The transmission of these viruses occurs mainly in closed spaces and is influenced both by human and environmental factors. Besides schools, closed communities, such as nursing homes and prisons are the most vulnerable settings, where the real extent of VARIs is often difficult to evaluate, due to the natural progression of the diseases and challenges in identifying cases. During the COVID19 pandemic, Wastewater-Based surveillance has demonstrated its great potential for monitoring the diffusion and evolution of the virus in the environment. Our aim is to pilot an integrated surveillance system for closed communities, employing syndromic surveillance, environmental monitoring (air, surfaces, and wastewater), and the collection of data on environmental and behavioral risk factors. Through this comprehensive approach, we aim to gain a better understanding of the prevalence and dynamics of VARIs within closed communities, enabling more effective prevention and control strategies to safeguard the health of vulnerable populations.

Methods:

The Project funded by the National Recovery and Resilience Plan NRRP - Tuscany Health Ecosystem will consist of designing and validating tools for epidemiological and environmental surveillance, as well as analytical methods for environmental matrices, including viral sensors for detecting viruses. The integration of all this information into risk assessment models will provide a useful tool for early warning and risk management.

Results:

In the first phase of the project, we will design and validate tools, such as clinical, epidemiological and environmental-based questionnaires, for each setting, coupled with analytical methods for detecting viruses. The integrated surveillance system for closed communities will be carried-out through a syndromic surveillance approach, environmental monitoring (air, surfaces, and wastewater) and the collection of environmental risk factors.

Conclusion:

Integrating different surveillance systems for VARIs appears crucial to inform early warning and risk assessment tools available at the local, regional and national levels, but methods and strategies need to be set up.

i. Abbreviations

RSA= LTCF

CUP= Unique Project Code

THE = Tuscany Health Ecosystem

ARI = Acute Respiratory Infections

NRP = National Recovery and Resilience Plan

RSV = Respiratory Syncytial Virus

WBE= Wastewater Based Epidemiology

QA= Environmental Questionnaire

QCB=basal clinical questionnaire

QCA=acute clinical questionnaire

Inmates=People Who Live in Prison.

RSA Workers, correctional facility, School = Workers

People Living in RSA = Guests Of RSA

CO2 = Carbon Dioxide

PM2.5 = Particulate matter (atmospheric particles - 2.5 µm)

VOCs = Volatile organic compounds (volatile organic compounds)

1. Summary

Viral Acute Respiratory Infections (ARI) are a broad spectrum of illnesses caused by different viruses that can infect both the upper and lower respiratory tracts. Symptoms can vary from mild to more severe clinical pictures, with the onset of fever, cough, sore throat, breathlessness, headache and fatigue. These diseases are highly contagious and can spread through various mechanisms, such as direct contact, fomites and droplets of saliva or mucus expelled by infected individuals during sneezing or coughing [1]. Surveillance of acute

respiratory infections is therefore crucial and requires the implementation of specific procedures to monitor and collect data on the incidence, prevalence and spread of these diseases in the community. Several sources of information are required for this analysis, such as clinical data, epidemiological indicators, mortality and morbidity data, and data from environmental surveillance activities, such as water, air and surface analysis.

Specifically, Wastewater Based Epidemiology (WBE) represents respiratory viruses as a complementary resource to clinical surveillance in identifying and monitoring the course of an epidemic. Infected individuals, whether symptomatic or asymptomatic, can shade the virus through faeces, allowing WBE to measure the spread of the pathogen throughout the community without relying solely on clinical tests [2]. Air and surfaces play a crucial role in virus transmission: environmental matrices allow viruses to spread through the air and make contact with humans, potentially leading to infections. Additionally, viruses can contaminate surfaces, facilitating a faster spread of infections. Given this significance, it is essential to conduct an in-depth study to provide valuable and complementary information, to the existing data, helping us better understand and manage viral infections in various settings.

Therefore, our project aims to provide important insights into how WBE, implemented by the data obtained from the other environmental matrices, can provide predictions of possible viral circulation and clinical cases in closed environments and vulnerable communities, such as schools, correctional facilities and nursing homes.

The experimental field study will last 18 months: it will be preceded by a phase of method and instrument development, followed by data analysis and result presentation. The experimental study will be conducted in three closed communities, including an assisted living residence (RSA), a correctional facility and a primary school. Participants involved will consist of RSA guests, people living in the correctional facility, students, and all workers of the facilities. They can join the study voluntarily after signing the consent form and can withdraw at any time.

The project is funded by the European Union within the framework of the National Plan for Recovery and Resilience (PNRR), in which the Tuscany Health Ecosystem (THE), coordinated by the University of Florence, is part, to strengthen the life sciences sector in the Region of Tuscany. The following departments of the University of Pisa are participating in the study: Biology; Chemistry and Industrial Chemistry; Clinical and Experimental Medicine; Surgical, Medical, Molecular and Critical Area Pathology; Translational Research and New Technologies in Medicine and Surgery.

Clinical data will be collected through pseudonymised questionnaires, which will be administered to study participants either in self-administered form or through interviews. These questionnaires will be conducted at the beginning of the study and in case of any symptoms of acute respiratory infections (ARI) are suspected. They will aim to investigate the

presence of symptoms and risk factors related to ARI development. The questionnaires will be distributed as paper forms in the prison, on tablets in the RSA and online via the Influweb platform in schools. A confirmatory nasopharyngeal swab will be administered to subjects showing symptoms compatible with ARI. For students in schools, additional data on close contacts will be collected using proximity sensors (tags) that will allow contact tracing revealing the identity of the participants.

Data on the environmental characteristics of the facilities will be obtained through questionnaires administered to the facility managers. Wastewater from the facilities will be sampled weekly and analysed for the presence of adenovirus, influenza virus, SARS-CoV-2, and respiratory syncytial virus (RSV). Furthermore, air and surface monitoring will take place in communal areas, assessing the temperature, humidity, CO2 level, PM2.5 particles, VOCs(volatile organic compounds), total microbial load, and the presence of Staphylococcus aureus, adenovirus, influenza virus, SARS-CoV-2 and respiratory syncytial virus (RSV).

2. Background

The emergence of new human viral diseases affecting the respiratory tract continues to threaten global public health security [3]. COVID-19 disease, which results in severe acute respiratory syndrome due to SARS-CoV-2, has caused millions of infections and deaths worldwide since its emergence in December 2019, along with social and economic disruption [4]. SARS-CoV-2 spread rapidly, within months, despite global public health strategies to curb transmission by testing symptomatic patients and encouraging social distancing.

Moreover, the global incidence of acute respiratory tract infections caused by influenza virus and respiratory syncytial virus should not be overlooked. It is estimated that influenza virus causes 39.1 million episodes of acute lower respiratory tract infection (95% uncertainty interval 30.5-48.4) and 58,200 deaths (44,000-74,200) per year and respiratory syncytial virus causes 24.8 million episodes (19.7-31.4) and 76,600 deaths (55,100-103,500) per year [5].

In this context, robust epidemiological and clinical surveillance systems are needed to efficiently target prevention and control measures for areas and populations at risk. However, to date, clinical surveillance has some limitations and concerns, including the availability and cost of clinical trials as well as objective difficulties in intercepting asymptomatic individuals [6]. Current public health strategies that rely solely on 'symptom onset' for the identification of infections need urgent reassessment due to an underestimation of the number of infections due to the presence of asymptomatic patients, who are not promptly reported to the competent authorities. This scenario allows only the tip of the iceberg of viral diseases (hospitalised patients or laboratory-diagnosed cases) to be captured.

In the COVID-19 pandemic scenario, SARS-CoV-2 surveillance has been suggested as a tool to determine the extent of COVID-19 in cities and act as an early warning for SARS-CoV- 2 re-

emergence and circulation in communities [7]. Most people infected with SARS-CoV-2 develop mild or no symptoms of respiratory illness, but excrete large numbers of SARS-CoV-2, particularly in nasal fluids, faeces and, less frequently, urine. Therefore, detecting the genome of SARS-CoV-2 in urban wastewater has been useful in capturing the full extent of the disease at a community level.

Although, scientific research has focused, especially in the aftermath of the pandemic, on the possible application of wastewater surveillance as an environmental surveillance tool, the analysis of additional matrices such as air and surfaces should not be overlooked. It is well known, in fact, that most respiratory viruses, e.g. SARS-CoV-2, are transported via aerosols and fomites and can, moreover, remain viable in the air and on surfaces [8,9]. Furthermore, individual risk factors and underlying chronic diseases are often associated with frail conditions, which can facilitate infections. Environmental characteristics, in particular air quality and living/working conditions, are important determinants of disease, deserving of targeted surveillance activities. Transmission may be particularly high in enclosed or semi-enclosed crowded places with limited recirculation of ambient air, such as schools, patients in nursing homes or people living in residential homes [10]. In addition, frequent contacts, especially typical of school age, are a further element in the spread of infectious diseases. Disease surveillance, therefore, is a key element of effective early warning systems and preparedness infrastructure for current and future threats.

The National Recovery and Resilience Plan (NRP) 'Italy Tomorrow' is a vast programme of reforms, including public administration, justice, simplification of legislation, competition, taxation accompanied by adequate investments.

The NRP is part of the Next Generation EU (NGEU) programme, also often referred to as the 'Recovery fund', the EUR 750 billion package, roughly half of which consists of subsidies, agreed by the European Union in response to the pandemic crisis. The Plan is built around three strategic axes shared at European level: digitalisation and innovation, ecological transition, and social inclusion. It is an intervention intended to remedy the economic and social damage caused by the SARS-CoV-2 pandemic crisis, as well as to help resolve certain structural weaknesses of the Italian economy and accompany the country on a path of ecological and environmental transition.

The PNRR is divided into 6 missions, corresponding to the 6 areas of intervention envisaged in Next Generation EU. The missions are in turn divided into 16 components. Component 2 of mission 4 'from Research to Enterprise' is divided into 11 investments grouped into 3 lines of intervention, with a total allocation of EUR 11.44 billion. The support measures planned under the three lines of intervention in which the University of Pisa is currently involved: Ecosystems of Innovation (EI) - investment line 1.5 envisages the creation and/or

strengthening of Ecosystems of Innovation 'territorial leaders of R&D' on the national territory. These are diffuse networks of universities, public research bodies, local institutions and highly qualified public and private entities which, organised with a hub and spoke governance structure, through joint activities of other training, research and innovation, intervene in areas of technological specialisation consistent with the industrial and research vocations of the reference territory, promoting and strengthening collaboration between the research system, the production system and territorial institutions. The projects presented, do not have predetermined themes, but an approach oriented towards major challenges, favouring the creation of impactful innovation and entrepreneurship (Duration 2022-2026).

The THE - Tuscany Health Ecosystem, with the University of Florence as proposer and with a priority objective falling within the 'Life Sciences' area of the National Research Plan (NRP), is part of this framework. The main objective is to stimulate and support the growth and consolidation of Tuscany's life sciences ecosystem, an important scientific and economic sector in the region, thanks to the involvement of public and private players that will provide the critical mass, skills, infrastructures, connections and integration necessary to meet the innovation needs of stakeholders, such as companies and the regional health system. Entrepreneurial training programmes, dedicated not only to spin-offs but also to researchers and students, have been organised as joint initiatives by the partners to stimulate the development of business ideas as additional support activities for academic spin-offs. In detail, the ecosystem will address the topics of advanced radiotherapies and diagnostics in oncology, preventive and predictive medicine, advanced technologies, methods and materials for human health and wellbeing, nanotechnologies for diagnosis and therapy, implementation and innovation for health and wellbeing, precision medicine and personalised healthcare, innovation in translational medicine, biotechnology and imaging in neuroscience, robotics and automation for health, and population health.

The THE includes the study 'Integrated Environmental and Clinical Surveillance for the Prevention of Acute Respiratory Infections (ARI) in Enclosed Environments and Vulnerable Communities', in which the following Departments of the University of Pisa participate:

- Biology (lead)

- Chemistry and Industrial Chemistry

- Clinical and Experimental Medicine

- Surgical, medical, molecular and critical area pathology

- Translational Research and New Technologies in Medicine and Surgery

As reported earlier, acute respiratory infections (ARIs) cause a considerable burden of disease especially in community settings and in closed environments where physical vulnerability is very high. Therefore, it is appropriate to investigate and design surveillance activities in closed communities based on the environmental characteristics that determine the spread of acute respiratory infections, particularly SARS-CoV-2, Influenza Virus, Respiratory Syncytial Virus, Adenovirus.

The present work aims to design and validate tools for an integration of clinical-syndromic surveillance and the detection of environmental parameters, either through the use of questionnaires or actual analytical monitoring.

Closed communities could benefit from such tools in two ways:

Early warning: although known for some 80 years now, wastewater-based epidemiology has recently attracted great interest because of the possibility of early detection of SARS-CoV-2 circulation both in large settings (cities) and in more restricted settings such as communities (university campuses and residences for the elderly primarily). This type of surveillance can take place before individuals in a facility are directly involved, prompting preventive interventions (such as mask use, spacing and vaccination).

Risk assessment: based on knowledge of the environmental context in which people are located and by monitoring contamination, it is possible to predict the risk of transmission of respiratory infections and the effects of preventive measures to be activated before infection spreads.

By combining early warning and risk assessment it would therefore be possible to enhance the preventive capacities of such settings, but the development and validation of such a system requires the integration of clinical-epidemiological and environmental data, which can only be achieved by conducting these surveys in parallel and in a coordinated manner.

Among the closed communities that could make use of such an integrated surveillance system, prisons are undoubtedly a priority, both because of the presence of particularly vulnerable groups (e.g. drug addicts and foreigners with a poor health culture), and because of environmental characteristics such as overcrowding and structural deficiencies (e.g. ventilation systems) that may favour the spread of infectious diseases.

3. Study objectives

General objective: to create an integrated clinical and environmental surveillance system of ARI and its determinants in community settings in order to plan prevention strategies and interventions to minimise the spread of infection.

Specific objectives:

1. Establishment of sentinel clinical-syndromic surveillance and risk factors for ARI in selected closed environments for the detection of:

(a) individual and social behaviour

b) early symptoms and signs, diagnostic criteria for specific respiratory diseases and their evolution, biological samples for diagnosis of the causative pathogen;

(c) individual risk factors, including comorbidities and contact tracing.

2. Establishment of sentinel environmental surveillance in closed environments selected for:

(a) environmental parameters (e.g. space, crowding, microclimate conditions, ventilation);

b) indoor air quality monitoring (air pollutants such as volatile organic compounds, microbiological indicators, index pathogens);

(c) microbiological monitoring of wastewater.

3. Implementation of electrochemical sensors for monitoring viruses and bacteria

4. Integration of clinical, behavioural and environmental data to develop early warning and risk prediction models that can be transferred to the regional/national level.

4. Study design

This is an open, multi-setting, epidemiological cohort study that plans to include subjects from among RSA guests, people living in the prison, students and operators.

Duration and phases:

The project has a duration of three years: it started on 1 December 2022, will end on 30 November 2025 and will be divided into successive phases, with very strict timelines for the purpose of funding disbursement:

Phase 1: Duration 1 year (1 December 2022 - 30 November 2023). Enrolment of facilities and development of survey instruments (questionnaires and analytical methods). Authorisation by the ethics committee. Information on the project at facilities to operators and health education programme. Pilot study;

Phase 2: Duration 1 year (1 December 2023 - 30 November 2024). Field study for data collection;

Phase 3: Duration 1 year (1 December 2024 - 30 November 2025). Data processing and model building. Production of information material and dissemination.

The following measurements will be carried out for each recruitment site area:

Clinical-syndromic surveillance: semi-structured pseudonymised questionnaires administered in total respect for privacy to samples of participating subjects and facility staff, for the detection of individual symptoms and risk factors. In the case of suspected ARI, nasopharyngeal swabs will be taken for the identification of potentially responsible viruses by means of molecular PCR tests. In addition, for students in schools, data will be collected during school hours regarding close contact using proximity sensors (tags) based on RFID (Radio Frequency Identification) technology (devices not mass-produced by the company Bitmanufaktur, Cambridge UK). RFID tags will enable the accurate, automated and pseudonymised detection of close encounters between individuals within the school environment. This technology offers a promising approach for monitoring and managing social interactions, especially in settings such as schools, where controlling the spread of disease is a priority.

Environmental surveillance: semi-structured questionnaires will be administered to facility managers to detect the characteristics of the spaces and their attendance. In addition, periodic monitoring of microclimate parameters and the chemical-microbiological quality of the air will be carried out. Existing or ad hoc developed electrochemical sensors will be used for this purpose.

At the same time, virological monitoring of the facilities' wastewater will be performed, both with traditional methods (biomolecular) and with the aforementioned sensors.

Finally, data processing and model development will take place. The data collected from a clinical perspective will be integrated with behavioural and environmental data in order to develop and validate models for ARI alerts and risk assessment in closed communities.

5. Study setting

5.1 RSA

The RSA 'Le Sorgenti' is a socio-medical institution located in San Giuliano Terme, Via Di Giacomo 1, which provides care for elderly persons over 65 years of age who are unable to take care of themselves physically and psychologically as well as being unable to live independently at home. However, in special cases, it can also accommodate persons under the age of 65 who present a condition of senile decline and cannot find another care facility. The RSA offers services of medium complexity and care intensity, both temporary and

permanent, and has 71 beds divided into four units: three with 20 beds each and one with 11 beds. The facility is part of the 'Centro Polifunzionale di Cura e Assistenza' (Multifunctional Care and Assistance Centre), a complex that includes various social-health units, both residential and outpatient, and is authorised by the Municipality of San Giuliano Terme to receive residents with hospitality fees paid in full by the guest. The RSA is located in a modern, 50,000 square metre building with a total surface area of approximately 7,400 square metres, on three levels (basement, ground floor and first floor), consisting of two rectangular bodies and a connecting element. The ground floor houses the administrative offices, reception, a gymnasium, medical and nursing surgeries, a room for the podiatrist and hairdresser/beauty therapist, a multipurpose room and a bar and refreshment room. The first floor comprises three nuclei: two with 20 beds and one with 11 beds, all with exclusive services. The basement is intended for support services for the residence, such as storage, technical rooms and changing rooms. The rooms, 31 in total, are equipped with private bathrooms and are divided into 32 double rooms and 7 single rooms. All rooms have centralised oxygen systems, bathrooms equipped for the disabled, ergonomic beds and provision for TV installation. The Residence has an outdoor car park and a garden set up for the use of residents and their families.

5.2 Correctional facility:

The Casa Circondariale di Lucca, located on the eastern outskirts of the Tuscan city, was opened in the early 19th century. The prison facility, although very old, is equipped with new treatment spaces in good condition. At the moment, only two of the four ordinary prison sections are in operation and are divided into two levels. In addition, there is a section for disabled prisoners with special sanitary equipment. The sleeping rooms are mostly double and without an internal shower, with the exception of one section which has triple rooms with a shower. The cells are equipped with working heating and a separate toilet, and all windows are fitted with screens.

As far as common areas are concerned, there are no common areas in the detention sections. However, the eighth section, which is entirely dedicated to the treatment of prisoners, was recently inaugurated. It is spread over two floors and is equipped with numerous rooms, including interview rooms, two gyms, two libraries, a large multi-purpose space, a room dedicated to digitalisation, spaces dedicated to schooling, table football and other empty rooms used according to the needs of the moment. In addition, there are two libraries within the treatment section, accessible to guests as a reading room without time restrictions. There are spaces specifically dedicated to education and training, but no areas for work or worship for non-Catholic inmates.

The Casa Circondariale di Lucca currently houses 76 inmates, with the presence of 68 prison police officers, 9 administrative staff and 1 educator.

5.3 School:

The Istituto Comprensivo 'V. Galilei', located in Via di Padule, comprises five pre-schools, four primary schools and one secondary school, together with kindergartens and public schools. Of the four primary schools of interest, the following descriptions can be given:

- The primary school 'Sante De Sanctis', located in Via Cisanello, 6, Pisa, consists of several buildings offering a wide range of services to students. In the main building there are seven main classrooms, each accommodating a class with an average of 20 pupils. On the ground floor of the main building is a large refectory, where students can take their meals during lunchtime. On the first floor of the main building is a classroom used for alternative activities and workshops. Next to the main building is a separate facility housing the gymnasium, equipped with toilets and changing rooms. In addition, the school has a large garden that provides an outdoor space for recreational activities and leisure time for the students.

- The 'Giovanni Parmini' primary school, located at Via di Parigi, 3, Pisa, is a state school in a spacious building that accommodates a total of 170 pupils, divided into eight classes. In addition to the main classrooms, the school has a computer lab and a plexus library. With regard to sports activities, there is a gymnasium with a multi-purpose field, which allows for physical activities and participation in sports events within the school. In addition, the school has a large garden with an area dedicated to a vegetable garden.

- The 'Guglielmo Oberdan' primary school, located in Via San Michele degli Scalzi, Pisa, is a state school that accommodates 104 pupils divided into five classes. The school building has two floors, ground floor and first floor. In addition to the classrooms for the five classes, the school has a library, a music room, a classroom with high counters for special work, a free classroom used for drama and a gymnasium. With regard to sanitary facilities, there are two toilets on the ground floor and two on the first floor. In addition, the school is equipped with a modern lift.

- The primary school 'Livia Gereschi' is a state school located at Via Viale 16, Pisa and accommodates 154 pupils divided into eight classes. The school building has two floors, and on the first floor there is a space dedicated to physical activity and the development of motor skills. In addition, there is a multimedia room for interactive activities. At the back of the school is a multi-purpose field that provides the pupils with an outdoor space for motor activities and leisure time.

6. Study populations

6.1. RSA:

Guests over the age of 65 and all carers will be included in the RSA in Pisa, according to the admission criteria established for the RSA and with different health conditions, including those with chronic diseases, degenerative diseases, physical disabilities, and other known or diagnosed medical conditions. Subjects will have different levels of functional autonomy, including those who require assistance with daily activities such as mobility, personal hygiene, mealtimes and other routine activities. Individuals included may have different socio-economic statuses, including those with different financial circumstances or levels of education.

6.2 Correctional facility:

The subjects under study are all persons living in the prison and all operators of the Lucca prison, aged over 18, according to the national law in force for the age of majority.All subjects with different lengths of detention and various clinical conditions will be included, including those with chronic diseases, infectious diseases and other known or diagnosed medical conditions.In addition, subjects with known or reported risk behaviour, such as drug use, violent behaviour as well as violations of prison regulations will be included. The subjects included will have different educational and socio-economic levels.

6.3 School:

Subjects included are students between the ages of 5 and 11 years and primary school operators in Pisa.All subjects will be included regardless of health status, language spoken or ethnic origin.Only students for whom written informed consent has been obtained from parents or legal guardians, in accordance with local or national laws on child protection and research ethics, will be considered.

7. Inclusion criteria

7.1 RSA

- Gender of subjects: All persons, of all gender identities, will be allowed to participate in this study without any restrictions.

- Age of subjects: All guests and operators of the RSA over the age of 18.

- Origin: All persons, guests and operators, of all origins will be authorised to participate in this study without any restrictions.

- Acknowledgement: those who have signed the informed consent.

7.2 Prison

- Gender of subjects: All persons, of all gender identities, will be allowed to participate in this study without any restriction.

- Age of subjects: All persons living in the prison and all workers over the age of 18.

- Origin: All persons living in the prison and all operators of any origin will be allowed to participate in this study without any restriction.

- Acknowledgement: those who have signed the informed consent.

7.3 School

- Gender of subjects: All students and all primary school staff of all gender identities will be allowed to participate in this study without any restrictions.

- Age of subjects: All primary school students between the ages of 5 and 11 years, and all operators over the age of 18 years.

- Origin: All students and operators of any origin will be allowed to participate in this study without any restrictions.

- Acknowledgement: For all students whose parents/guardians have signed the informed consent and for all operators who have signed the informed consent.

8. Exclusion criteria

8.1 RSA:

- Subjects under 18 years of age;

- Subjects who do not provide consent;

- Subjects incapable of understanding instructions or answering questions.

8.2 Prison:

- Subjects under the age of 18;

- Subjects who do not provide consent;

- Subjects incapable of understanding instructions or answering questions.

8.3 School:

- Non-primary school students between 5 and 11 years of age, and all staff under the age of 18;

- Subjects/families who do not provide consent;

- Subjects unable to understand instructions or answer questions.

9. Study exit criteria

- Withdrawal of consent

- Death or transfer to another facility

10. Outcomes of the Study

The expected outcomes corresponding to each specific objective are detailed below:

1.1 Data collection tools for clinical-syndromic surveillance and risk factors tested and validated in different indoor settings

1.2 Sentinel clinical-syndromic surveillance system for acute respiratory diseases tested in selected closed environments in the region of Tuscany, including RSAs, prisons and schools

2.1 Data collection instruments for the evaluation of environmental parameters

2.2 Indoor air quality surveillance conducted in selected closed environments in the region of Tuscany, including RSAs, prisons and schools

2.2 Microbiological surveillance of environmental wastewater conducted in selected closed environments in the region of Tuscany, including RSAs, prisons and schools

3. Preliminary feasibility study for monitoring using electrochemical sensors

4.1 Integrated environmental and clinical-syndromic surveillance system established and tested in at least one relevant environment

4.2 Recommendations for expansion of the acute respiratory disease sentinel surveillance system for early warning and pandemic preparedness

4.3 Assessment of the association of respiratory disease incidence with environmental and individual factors. A predictive model using a nomogram will be developed, validated and visualised.

The socio-economic impact of ARI is greatest in fragile and disadvantaged populations [11]. Our project aims to bring improvement in terms of public health: reducing the incidence of ARIs, their economic consequences on the NHS, reducing inequalities such as those due to age or marginalisation.

11. Variables

In brief, information will be collected on:

- the environmental characteristics of the settings: general characteristics of the facility, daily activities performed, hygiene rules followed in the facility

- the individual characteristics of the participants: general characteristics of the individual, general pathology of the subject including respiratory and allergic pathology, COVID-19 disease, vaccination status, exposure to risk factors, socio-economic factors, acute pathology occurring during the study period, contact with other individuals and consent for a possible nose-pharyngeal swab.

12. Data collection

12.1 Clinical samples

Biological material (surface cells, mucus and secretions from the nasopharynx and tonsils) from the respiratory tract of the study subjects will be collected by nasopharyngeal swab. The nasopharyngeal swab is a minimally invasive, easy to perform and painless diagnostic test used to detect pathogenic micro-organisms responsible for infections of the upper respiratory tract. Sampling is carried out using a thin, sterile, single-use wadded stick. This procedure, subject to the consent of the subject in the study or his or her guardian, will be carried out by the facility operators (nursing staff in the RSA and prison settings) and by parents and the operators themselves in the school setting. For the purposes of correct execution and non-contamination of the sample, after washing hands and wearing personal protective equipment, the person taking the sample will explain the procedure to the subject, inviting him/her to tilt his/her head back and keep his/her torso erect. The swab will

then be gently inserted through one nostril and continued along the floor of the nasal coana until it reaches the back of the nasopharynx. Once in place, the swab will be gently rotated and then held in situ for a few seconds to collect abundant nasal secretion. Taking care not to contaminate it, with the same swab, the manoeuvre will be repeated for the contralateral nostril. Once extracted, the applicator will be placed in a sterile tube containing the transport medium. After washing his hands, the sample collector will place the pseudonymised code on the tube and send it to the laboratory by a dedicated transport service.

12.2 Microbiological samples

For air and surfaces, the parameters monitored will be indicators of general/human microbial contamination: total bacterial load at 22°C and 37°C, Staphylococcus aureus. Respiratory viruses: adenovirus (index pathogen), influenza virus, SARS-CoV-2, respiratory syncytial virus (RSV). Chemical-physical parameters: microclimate parameters (temperature, humidity), CO2, PM2.5, VOCs.

Air samples will be collected by automatic samplers positioned in the common areas. Sampling of the most exposed surfaces will also be carried out in the same areas, by means of special swabs. Sampling will generally be carried out on a monthly basis; occasionally, weekly sampling may be arranged, depending on need and clinical trends. Once collected, they will be transported to the laboratory for analysis.

For wastewater, the parameters monitored will be respiratory viruses sought in air and clinical surveillance: adenovirus (index pathogen), influenza virus, SARS-CoV-2, RSV. Sampling will be performed at the exit of each facility by appropriately trained personnel via a sewer drain outside the building. All necessary safety measures (e.g. use of Personal Protective Equipment - PPE) must be applied. Raw sewage samples will be collected on a weekly basis, in bottles with safety caps, appropriately labelled indicating the date and place of collection. They will then be transported to the laboratory, maintaining a temperature of 4°C, and pre-treated in a thermal bath for 30 minutes at 56°C for viral particle inactivation. This will be followed by a concentration step by precipitation using PEG/NaCl, and a nucleic acid extraction step using a commercial kit (BioMérieux Nuclisens System).

Finally, a quantitative analysis by PCR will be carried out for each sample of each matrix, through which information will be obtained on the presence and eventual quantity of each viral agent.

12.3 Chemical Samples

The chemical characterisation of the ambient air samples of the three structures under examination (RSA, district house and school) will be carried out monthly by suitably trained personnel using an analysis protocol based on thermal desorption coupled with gas chromatography and mass spectrometry (TD-GC-MS). Specifically, the method involves the

quantitative transfer by means of a suction pump of an aliquot of gaseous sample into stainless steel absorbent systems packed with a suitable stationary phase capable of quantitatively retaining a wide range (C3-C30) of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs). Prior to sampling, each absorbent tube will be systematically spiked with a known amount of labelled internal standards (ISs) ensuring accurate quantification of the analytes of interest as well as quality control of the analytical data. In order to reduce any drift in instrumental response over time, environmental samples will be analysed in batches of 20-30 tubes. Between the sampling and the instrumental analysis, the absorbent tubes containing the samples from the three structures will be hermetically sealed with Swagelock caps, so as to avoid any external contamination, and stored at 4 °C at the Department of Chemistry and Industrial Chemistry of the University of Pisa for the necessary time. The result of the chemical analysis will contain information on the qualitative chemical composition of the air samples as well as the concentration levels of each contaminant found.

12.4 Questionnaires

12.4.1 Environmental Questionnaire

The environmental questionnaire (QA) is a survey aimed exclusively at the managers of the various facilities under review: RSA, Prison and School, and is based on the assessment of environmental conditions, individual and group practices and behaviour within the facility in order to prevent the spread of respiratory viruses. The purpose of the questionnaire is also to understand the hygiene policies and practices currently in use in the facilities and to identify any aspects that may increase the risk of respiratory virus infections.

The questionnaire will cover several aspects, including personal and environmental hygiene practices, cleaning and disinfection of common areas, ventilation, air quality and daily activities. It will consist of a series of multiple-choice and open-ended questions to cover different environmental factors in order to obtain specific information on residents' behaviour, their movements and contacts within the facility. In addition, the questionnaire will assess the effectiveness of existing policies on preventing the spread of respiratory viruses, as well as the facility's ability to adopt any policy changes in a timely and effective manner. The questionnaires will be administered once at the beginning of the survey to the facility managers involved, and will be administered again later if there are substantial changes in the facility. The questionnaire will take approximately 8 minutes to complete.

12.4.2 Baseline Clinical Questionnaire

The baseline clinical assessment questionnaire (QCB) aims to identify patients presenting symptoms or risk factors associated with respiratory virus infections and to provide an initial assessment of the subject. The QCB is intended for all participants and consists of a series of multiple-choice and open-ended questions covering different aspects of the participant's

medical history, such as the presence of previous illnesses, risk factors for respiratory infections, previous vaccinations, respiratory and allergic symptoms, socio-economic factors and exposure to risk factors such as smoking and alcohol consumption.The information collected by the QCB will allow a baseline assessment of the subject's condition and evaluate a possible correlation with respiratory viruses. The questionnaires will only be administered once at the beginning of the survey, or when a new participant is added (e.g. transfer to the facility).The questionnaire will take approximately 12 minutes to complete.

The objective of the questionnaire is to provide a complete and accurate picture of the participant's clinical situation, identifying any risk factors and identifying individuals at risk of developing complications from respiratory infections.

12.4.3 Acute Clinical Questionnaire

The Acute Clinical Assessment Questionnaire (AQCA) is an essential tool for the early identification of any symptoms associated with ARIs and for monitoring the evolution of the subject's condition over time. The QCA is designed to identify all participants presenting with respiratory symptoms, such as cough, sore throat, difficulty breathing or fever, and is intended to provide a detailed and timely clinical assessment of participants.The questionnaire consists of a series of specific questions regarding the subject's current symptoms, the duration of symptoms, any current therapies and contact with other people who have experienced similar symptoms.The questionnaires will be administered weekly for the duration of the project.The completion of each questionnaire will take approximately 6 minutes.The objective of the QCA is to collect information that will allow us to monitor the evolution of the subject's condition over time, identify possible clusters of respiratory infections within the community or an enclosed environment, in order to take the necessary prevention and control measures to prevent the spread of infection.

12.4.4 Methods of questionnaire administration

The Stell-ARI project, in order to identify individual symptoms and risk factors, will use two main methods to collect information and data from participants:

- Self-administered questionnaires, where participants will read the questions and answer them themselves. The questionnaires will be simply formulated, easy to understand and fill out, and accompanied by short, clear instructions. They will be distributed with a brief explanation of the objectives of the survey, motivating the participants and asking for their cooperation. The completed questionnaires will be collected in a special urn to guarantee the anonymity of the participant and transported to the Biology Department of the University of Pisa, Via San Zeno 35/39, Pisa. In the case of administration via tablet or the "Influweb" portal (https://influweb.org/welcome), a letter of introduction and detailed instructions for completion on the platform will be provided.

- Questionnaires will be administered by an interviewer who will ask the questions and record the answers during an interview with the participants. Training will be provided for the interviewers to ensure the quality of the answers. The questionnaires may be administered either during a face-to-face interview or by means of a telephone interview with the facility manager(s).

Some dissemination strategies, depending on the setting and the type of subjects (operators, guests, students, people living in the prison), will be implemented to invite them to participate. Some possible strategies are: organisation of presentation events of the study on site or online, posters, distribution of flyers.

12.4.4.1. RSA:

Type of questionnaires	Method of administration	Referred to	Frequency
Environmental Assessment Questionnaires	Self-administered paper	Heads of structure	1 time
Baseline clinical evaluation questionnaires	Interviewed by operators via tablet	Residents of the facility	1 time
	Self-administered by tablet	Facility workers	1 time
Acute Clinical Assessment Questionnaires	Interviewed by operators via tablet	Residents of the facility	Weekly
	Self-administered by tablet	Facility workers	Weekly

12.4.4.2. Correctional facility:

Type of questionnaires	Method of administration	Referred to	Frequency
Environmental Assessment Questionnaires	Self-administered paper	Heads of structure	1 time

Baseline clinical evaluation questionnaires	Interviewed by operators in paper form	People living in the correctional facility	1 time
	Self-administered paper	Facility workers	1 time
Acute Clinical Assessment Questionnaires	Interviewed by operators in paper form	People living in the correctional facility	Weekly
	Self-administered paper	Facility workers	Weekly

12.4.4.3. School:

Type of questionnaires	**Method of administration**	**Referred to**	**Frequency**
Environmental Assessment Questionnaires	Self-administered paper	Heads of structure	1 time
Baseline clinical evaluation questionnaires	Self-administered via the 'Influweb' platform	Parents of students	1 time
	Self-administered via the 'Influweb' platform	Facility workers	1 time
Acute Clinical Assessment Questionnaires	Self-administered via the 'Influweb' platform	Parents of students	Weekly
	Self-administered via the 'Influweb' platform	Facility workers	Weekly

12.5 Proximity sensors (students only)

Proximity sensors collect information that will be stored internally in the devices (tags) and extracted at the end of data collection, via a physical connection between the sensors and

the operators' computers. The data collected by each sensor represent the time-resolved proximity relationships between that sensor and the other sensors in the system, along with information on the sensor's state of motion obtained through an on-board accelerometer in the device.

In addition to representing a minimally invasive method of data collection, the tags require only minimal participation from those involved, who will be asked to wear a pin on their apron once they enter the classroom. Proximity sensors will enable accurate, automated and pseudonymised detection of close encounters between students within the school environment. This technology offers a promising approach for monitoring and managing social interactions, especially in contexts such as schools, where controlling the spread of disease and maintaining safety are priorities, allowing for both frequency and duration of contact.

All students who have given their consent will be required to wear the proximity devices every day in class for a period of 10 to 15 days.

13. Sample size

The sample size is a parameter that can provide the number of persons to be included in the study in order to intercept, taking into account a certain margin of error, any differences on the basis of the prevalence of the disease under study. As this project aims to study a large set of respiratory diseases, it is not possible to calculate the sample size on the basis of a specific prevalence. The maximum sample size is reached when the prevalence of the disease in the study population is 50%; therefore, applying the precautionary principle, we calculated the sample size right at its maximum point; therefore, assuming a disease prevalence of 50% and with a margin of error of 5%, we obtain a sample size of 400 subjects in total in order to reach the objective. In order to obtain data from this number of subjects, all students, residents, guests and operators of the facilities concerned who meet the criteria listed above will be invited to participate in this initiative.

14. Follow-up procedure

Follow-up will be performed exclusively for the clinical-syndromic evaluation of the study subjects by means of QCA. The latter will be administered weekly for the entire duration of the project to study subjects or to those who join as new participants, after administration of the QCB.

15. Data management and storage

The collected data will be converted from a paper form to a local computerised archive for processing. Data collected via the 'Influweb' platform and 'tag' devices will be managed

directly by the platform operator, who will extract the data by means of a pseudonymised code provided to the user with informed consent to provide it to the principal investigator. All data collected will be pseudonymised and no sensitive data will be requested from recruited subjects.

Electronic databases will be constructed and maintained to allow for adequate security and reliability, ensuring no loss, alteration or corruption of data and documents. Paper questionnaires will be kept archived for a period of at least 10 years, at the Department of Biology of the University of Pisa in via san Zeno 35/39, Pisa, under the responsibility of the principal investigator.

The data will be processed in accordance with the provisions of the European Regulation for the processing of personal data no. 2016/679 and Legislative Decree of 30 June 2003 no. 196 ss.ii. as amended by Legislative Decree of 10 August 2018, no. 101 "Provisions for the adaptation of national legislation to the provisions of the GDPR" as well as the Deliberation of the Privacy Guarantor no. 52 of 24 July 2008 and subsequent updates.

The data, processed by electronic means, will be disseminated only in aggregate and anonymous form, for example through scientific publications, reports and scientific conferences.

16. Statistical analysis

Subject characteristics will be described by means of medians and interquartile ranges for numerical variables and absolute frequencies and percentages for categorical variables. The time course of the environmental variables will be represented by means of graphical displays.

The association between infections detected by means of nasopharyngeal and salivary swabs (where necessary) or questionnaires and the exposure factors detected in the QCB will be assessed by means of logistic regression models taking possible confounders into account.

Statistical methods and analysis algorithms will be used to examine the collected data and identify contact patterns, interaction frequencies and other relevant metrics. In short, the patterns of close encounters between people and the interaction patterns between different groups of people will be analysed using different indicators. Thanks to the high spatial and temporal resolution infrastructure, we will be able to monitor the number of contacts each person has made with other people, record the time spent in each encounter, the sum of contact times between two individuals, and the frequency of encounters between any pair of people. For each pair of individuals 'i' and 'j', different weights will be defined corresponding to different measures based on the data collected: the occurrence of contact, which takes the value 1 if at least one contact was established between 'i' and 'j' and 0 otherwise; the frequency of contact, which indicates how many times contact was observed

between 'i' and 'j' during the study; the time spent in each encounter; and the cumulative duration of contact, which indicates the sum of the durations of all contacts established between 'i' and 'j' observed during the study. In addition to the quantities defined above, which are weights defined for the pairs of individuals 'i' and 'j', it will be possible to define corresponding quantities for each individual 'i', aggregating all individuals 'j' who had contact with 'i'. In relation to the weights defined above, the following quantities will be obtained: the number of distinct contacts, which indicates the number of distinct individuals with whom 'i' has established at least one contact (i.e. a contact between 'i' and 'j' that occurs will be counted only once); the total number of contacts, which indicates the total number of contacts established by individual 'i', counting repeated contacts with the same individual 'j' as distinct events; the cumulative contact time, corresponding to the total sum of the duration of all contacts involving individual 'i'.

Environmental data will be treated as continuous variables. Regression models with splines will be used to estimate the time course of the measured parameters.

The relationship between ARI incidence and environmental data will be investigated by means of time series regression models.

In the case of missing data, imputation models will be used; the impact of any outliers on the results will be assessed by sensitivity analyses.

All analyses will be conducted for all data or stratifying by setting. All statistical tests will be two-sided with a significance level set at $\alpha = 0.05$.

Data will be analysed using the statistical software R [12].

17. Funding of the study

In accordance with the PNRR, the period of eligibility of the expenditure of the Research and Innovation Programme "THE - Tuscany Health Ecosystem", lasting 36 months, starts on 01/12/2022 and can be extended no later than 28/02/2026. Investment Line 1.5 (1.300 M€) provides for the creation and/or strengthening of 12 Innovation Ecosystems (THE) "territorial R&D leaders" on the national territory with the proposing entity University of Florence - UNIFI, HUB: "Tuscany Health Ecosystem Società Consortile a Responsabilità Limitata", the SPOKE with UNIPI involvement (budget: 17,662,130.40 €), affiliated Spoke 2 (Preventive and Predictive Medicine), scientific heads Prof. Bellina and Prof. Carducci, 1,308,657.86 €.

18. Ethical considerations and privacy

The project with the planned interventions and the administration of questionnaires at various times during the course of the study will not have an impact on the health status of the participants.

Respect for persons and human dignity, fair distribution of the benefits and burdens of research will be ensured, while protecting the values, rights and interests of study participants. The project will also consider the social, cultural and historical experiences of the patients involved. The research process will not perpetrate or endorse, directly or indirectly, any aspect of discrimination, stigma or exclusion. During the activities, the project will uphold the Avoiding Harm Principle: risks will be assessed with respect to their physical, social and psychological effects on patients.

In addition, the investigators ensure that the trial will be conducted in full compliance with the European legislation on clinical trials [Regulation (EU) No 536/2014] and its national transposition [DM 15 July 1997; D.Lvo 211/2003; D.L.vo 200/2007 and D.L.vo. 52/2019] and the principles of the Declaration of Helsinki in order to ensure maximum protection of the subjects involved. The principal investigator agrees that the trial will be conducted in accordance with this protocol and Good Clinical Practice (GCP). The principal investigator will provide the relevant Ethics Committee and Competent Authorities with the study protocol and any related documents provided to the patient (Information Notice and Informed Consent Form). Approval of the Ethics Committee and the Competent Authority should be obtained prior to the start of any trial-related procedures and should be documented by official communication to the investigator. If changes to the protocol become necessary during the course of the trial, the sponsor will submit an appropriate protocol amendment request to the relevant Ethics Committee, the approval of which will follow the procedures established by the Ethics Committee's own rules.

Participants will be invited to take part in the project in the manner described under 'Method of administration of questionnaires', once they have agreed to participate they may actually take part in the project after signing the informed consent form.

It will be the responsibility of the investigators, or their designees, to obtain informed consent from patients after they have been adequately informed about the aims, methods, expected benefits and foreseeable risks of the study. The investigators or designees shall also inform the participants that non-participation or discontinuation will not cause harm or damage to them.

Participation will be free and voluntary, and the signed informed consent forms will be kept by the individual facilities (RSA, School and District Home) for the duration of the study.

19. Communication and dissemination

The project will be illustrated in the enrolled facilities, intermediate and final results will be presented in publications and scientific conferences. Information material concerning the use of the results will be produced for the parties involved, including the client (Ministry of

University and Research with reference to mission 4 (Education and Research) of the National Resilience Plan (NRP)).

20. Conflict of interest

The investigators declare that they have received no funding and that there is no conflict of interest in relation to this study.

21. Responsibility and publication policies

The results of this research will be published in peer reviewed journals, scientific reports, presentations at conferences, seminars and symposia and will be used to inform public agencies and authorities about evidence-based strategies to link wastewater, air and surface matrices with respiratory health in closed communities, the data will be presented in an aggregated and anonymous manner and no information that could identify participants will be reported in any way.

However, the views and opinions expressed are solely those of the authors and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

22. Role of the Promoter and the Investigators

The role of the promoter and the investigators in the study is established in the technical annex of the Stell-ARI project, on the basis of which the financial resources corresponding to the planned commitment have been allocated. In detail, the promoter is responsible for developing the study design, the data collection tools and the analysis plan. The investigators are involved in the definition of the study design and are responsible for enrolment. Data collection will be performed by the investigators, commissioned facilities and, limited to schools, by the ISI Foundation via the Influweb platform. The promoter, with input from all investigators, is responsible for writing the scientific and dissemination reports. All investigators will be included in the authorship of the products resulting from this study.

23. Ownership of data processing

The University of Pisa with headquarters in Pisa, Lungarno Pacinotti 43, in the person of the Rector pro tempore and, limited to the school setting, shared with the ISI Foundation with headquarters in Turin, via Chisola 5, in the person of the President.

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