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EPIDEMIOLOGY
OF INFECTIOUS DISEASES

SCIENTIFIC SEMINAR ON INFECTIOUS DISEASES

Brussels, 16 May 2024

.be

Sciensano
Epidemiology and public health
Epidemiology of infectious diseases
Rue Juliette Wytsmanstraat 14 | 1050 Brussels | Belgium

Scientific seminar on infectious diseases | 16 May 2024 |
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TABLE OF CONTENTS

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Under the auspices of the Belgian Association of Public Health

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PROGRAMME

08:30	Registration with walking breakfast / Visit of stands
	SESSION 1 **Erika Vlieghe (UAntwerp) & Jorgen Stassiins (Sciensano)**
09:00	Welcome address
09:15	New vaccines in the market - S. Callens (UZGent)
09:45	Unveiling infectious diseases: the power of next generation sequencing – J. W. A Rossens (University of Groningen)
10:10	Cluster of cysticercosis in a school – A. Devos (Departement Zorg)
10:35	Respi-radar: a tool to monitor respiratory infections – G. De Muylder, (Sciensano)
11:00	Coffee break / Visit of stands
	SESSION 2 **Denis Piérard (UZ Brussels) & Adrae Taame (Vivalis)**
11:30	Indications of molecular diagnosis for respiratory pathogens: update on the current situation – R. Naesens (Ziekenhuis Netwerk Antwerpen)
11:55	Diphteria: clinical picture and management of cases – C. Martin (CHU Saint Pierre Brussels)
12:20	Diphteria among asylum seekers: outbreak management inside a reception center – J.F. Léonard (AVIQ)
12:45	Lunch and poster session
	SESSION 3 **Steven Van Gucht (Sciensano) & Rémy Demeester (CHU Charleroi)**
14:00	Estimated numbers of covid-19 deaths averted by vaccination in Belgium – V. Stouten (Sciensano)
14:10	Clinical impact of a direct rapid antimicrobial susceptibility testing (dRAST) – C. Fontaine (CHBA Seraing)
14:35	West Nile virus and Dengue in South Europe: epidemiology and public health responce – H. Devalk (Santé Publique France)
15:05	Increase of invasive group A Streptococcus infection – P. Smeesters (ULBruxelles)
15:35	Increase in gonorrhea : overvieuw of the situation - A. Lecompte (Sciensano)
15:45	Closing address and end of seminar

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POSTER SESSION SCHEDULE DURING LUNCH BREAK

Title abstract	time oral presentation
Individual level analysis of digital proximity tracing for COVID-19 in Belgium highlights major bottlenecks	**13:15**
Rapid increase of vaccine serotype 4 invasive pneumococcal disease in young adults since 2020 in Belgium	**13:15**
Identification of the new HSV-2 variant (HSV-2v) in three patients suffering from recurrent infections unresponsive to antiviral therapy	**13:15**
Epidemiological situation of tick-borne encephalitis in Belgium, an overview	**13:15**
Genomic surveillance of *Staphylococcus aureus* in Belgian hospitals, 2022-2023	**13:15**
Wastewater-based epidemiology in Belgium – a complementary surveillance system for early warning of outbreaks for infectious diseases	**13:15**
Description of the measles clusters in Belgium in 2023-2024	**13:15**
Assessment of COVID-19 contact tracing network accuracy via phylogenetic analysis of community-level SARS-CoV-2 genomic data	**13:20**
Indirect effect on adult IPD of changes in childhood immunization programme from PCV13 to PCV10 and again to PCV13 in Belgium (2014-2023)	**13:20**
Facilitated interpretation of metagenomics data for detection of bacterial pathogens and their antimicrobial resistance genes	**13:20**
Emergence and spread of a mupirocin-resistant variant of the European epidemic fusidic acid-resistant impetigo clone of *Staphylococcus aureus* in Belgium, 2013 to 2023	**13:20**
Advancing Wastewater Surveillance: Development and Validation of AMR Detection Methods	**13:20**
Climpathic: advancing genomic surveillance of climate-linked pathogens in wastewater	**13:20**
Rapid On-Site Metagenomic Sequencing for One Health Pathogen and Antimicrobial Resistance Surveillance	**13:20**
Doubling of triazole resistance rates in invasive aspergilloses in Belgium over a period of 10 years	**13:25**
Surveillance of non-invasive *streptococcus pneumoniae* infections in Belgium to evaluate national vaccination strategy (2020-2023)	**13:25**
Emergence of invasive Haemophilus influenzae isolates with ftsI mutations associated with high beta-lactam resistance in Belgium: identification and genomic characterization	**13:25**
Epidemiology of invasive *Haemophilus influenzae* infections in Belgium: 2018 - 2022	**13:25**
National reference centre for Shiga toxin-producing *Escherichia coli* (NRC STEC): annual report 2023	**13:25**
Prolonged VIM-producing *Pseudomonas aeruginosa* outbreak at a Belgian intensive care department, an outbreak investigation report 2019-2023	**13:25**
Healthcare-Associated Infections and Antimicrobial Use in Belgian Acute Care Hospitals: Results of the 2022 ECDC Point Prevalence Survey	**13:25**
Citizen science as an effective tool to detect the tiger mosquito (Aedes albopictus) in Belgium	**13:25**
Immunogenicity of V116 (21-valent PCV) in pneumococcal-naïve adults 50-64, 65-74, and ≥75 years of age: subgroup analysis of a randomized phase 3 trial (Stride-3)	**13:25**
Epidemiology of *Haemophilus influenzae* isolates from otitis media in Belgium	**13:30**

Uncovering gaps in knowledge: A survey of Belgian general practitioners' awareness of Legionnaires' disease diagnostic testing.	**13:30**
Rapid molecular typing of Shiga toxin-producing *Escherichia coli* O157 by IS629-printing for cluster detection at the Belgian National Reference Centre	**13:30**
Comparison of Belgian COVID-19 mortality between epidemiological surveillance and death certificates for the years 2020 and 2021	**13:30**
Monitoring of the immune response to SARS-CoV-2 in vulnerable populations: the COVICO study (2023-2026)	**13:30**
A phase 3 clinical study to evaluate the safety, tolerability, and immunogenicity of V116 in pneumococcal vaccine-experienced adults 50 years of age or older (Stride-6)	**13:30**
Third dose of COVID-19 mRNA vaccine closes the gap in immune response between naïve nursing home residents and healthy adults	**13:30**
Epidemiology of *Tropheryma whipplei* in Belgium: Insights from the National Reference Laboratory	**13:35**
Is equitable priority vaccination of the vulnerable feasible in a real-world context? The case of Belgium	**13:35**
Detection of measles virus genotype D8 in wastewater of Brussels capital region, Belgium, March 2024	**13:35**
Closing the gap: Oxford Nanopore Technologies R10 sequencing allows comparable results to Illumina sequencing for SNP-based outbreak investigation of bacterial pathogens	**13:35**
Molecular characterization of serogroup B *Neisseria meningitidis* clinical isolates collected in Belgium (2016-2022) and assessment of their predicted Bexsero vaccine coverage	**13:35**
Retrospective study on *Campylobacter* spp. bacteremia in Belgium: 2014-2023.	**13:35**
A diagnostic framework for *Tropheryma whipplei* proposed by the Belgian National Reference Laboratory	**13:40**
The prevalence of pathogens in ticks collected from humans in Belgium, 2021 versus 2017	**13:40**
Genotyping and antifungal susceptibility testing of *candida auris* isolates detected in Belgium from 2016 to 2023	**13:40**
Comprehensive epidemiological and genomic analysis of enteric *Campylobacter jejuni* strains in Brussels: Insights into antimicrobial resistance and virulence profiles.	**13:40**
Q-Net-Assess project: improved molecular surveillance and assessment of host adaption and virulence of *Coxiella burnetii* in Europe	**13:40**
Bartonella quintana endocarditis complicated with cerebral stroke: a case report	**13:40**
Influenza like-illness surveillance using a Belgian sentinel network of nursing homes: results of season 2022-23	**13:40**
Shotgun metagenomics on air: Longitudinal surveillance of viral pathogens in a daycare center	**13:40**
Assessing the prevalence and dynamics of emerging Campylobacterales in human stool samples in Brussels by filtration culture	**13:45**
Have we forgotten how to RIME? A case report on a rare infection-triggered mucocutaneous complication	**13:45**
Epidemiological evolution of scabies in Belgium, 2000-2022	**13:45**
Emergence of emm3.93 *Streptococcus pyogenes* clone in iGAS infections: Epidemiological insights from Belgium (2022 - February 2024)	**13:45**
Rotavirus epidemiology shifts due to pandemic: 14-Year Experience of Belgian National Reference Center in the COVID-19 Context	**13:45**

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ABSTRACTS OF PRESENTATIONS

STEVEN CALLENS

UZ GENT

BIOGRAPHY

Prof. Dr. Steven Callens, an internist and infectious diseases specialist at UZ Gent, leads the Department of General Internal Medicine & Infectious Diseases. His field of interest in internal medicine include fever and inflammation of unknown origin and includes diagnosing and managing opportunistic infections.

Prof. Callens also presides over the Belgian Society of Internal Medicine and the Belgian National Immunization Technical Advisory Group of the Superior Helath Council.

Prof. Callens has a profound commitment to global health, evidenced by his early career in Kenya and research on pediatric HIV treatment in the Democratic Republic of Congo. He holds several advisory roles in agencies, particularly in assisting managing epidemics like H1N1 and COVID-19.

He continues to shape future medical professionals and the field of internal medicine and infectious diseases, serving as the supervisor of residents and contributing to medical curriculum.

NEW VACCINES IN THE MARKET

The development and implementation of new vaccines are pivotal in combating infectious diseases globally. This lecture will explore the advancements and challenges in vaccine research and policy, focusing on vaccines against Respiratory Syncytial Virus (RSV), pneumococcal disease, dengue fever, chikungunya, MPox (formerly known as Monkeypox), Influenza, and development in malaria prevention... Each vaccine's development, efficacy, challenges, and impact on current public health policies as these are shaped in the Superior Health Council.

JOHN W. A ROSSEN

UNIVERSITY OF GRONINGEN

BIOGRAPHY

Prof. Dr. John W.A. Rossen (born 1966) has over 30 years of experience in molecular biology, virology, and microbiology, having published more than 225 peer-reviewed research articles. In 1996, he completed his Ph.D. at Utrecht University's Faculty of Veterinary Medicine, focusing on coronavirus-host cell interactions. Prof. Rossen is a Professor in the Department of Medical Microbiology and Infection Control at the University of Groningen and the Laboratory for Medical Microbiology and Infectious Diseases at Isala Hospital (Zwolle, the Netherlands). He was an early adopter of next-generation sequencing (NGS) for clinical microbiology and infection prevention, implementing the technology for its routine use in these areas. Prof. Rossen's research emphasizes the application of translational molecular microbiology, metagenomics, and metatranscriptomics in clinical microbiology and public health. His "Personalized Molecular Microbiology" research group investigates samples from patients, animals, foods, and environmental sources using a One Health approach. From 2020-2021, Prof. Rossen held a sabbatical position at IDbyDNA, a U.S.-based startup, where he further advanced metagenomics methods for elucidating microorganisms' roles in human disease. Since March 2020, he has also served as Adjunct Professor at the University of Utah School of Medicine.

UNVEILING INFECTIOUS DISEASES: THE POWER OF NEXT GENERATION SEQUENCING

This presentation explores the power of advanced technologies and machine learning in the diagnosis, prevention, and combat of infectious diseases. I will explain how these techniques contribute to infection prevention, for instance, by accurate monitoring of pathogens. I will also discuss how (personalized) molecular diagnostics, which detect the DNA/RNA of pathogens, can lead to faster and more accurate diagnoses.

Next, I will delve into the impact of (meta)genomics and metatranscriptomics. Metagenomics allows us to analyze the complete genetic material in a sample for diagnosis. Metatranscriptomics focuses on the genes that are being expressed at a particular time.

I will conclude with an overview of how artificial intelligence and machine learning can analyze the large amounts of data from these techniques. These tools enhance our understanding of the interaction between pathogen and host and the host response, which leads to better predictions and insights. The presentation emphasizes the promising possibilities of this cutting-edge approach in the fight against infectious diseases.

AARON DEVOS

DEPARTMENT ZORG

BIOGRAPHY

After 3,5 years working as a general practitioner I started work as a doctor infectious disease control for the department of care since June 2023 and I got involved in this outbreak in December 2023.

CLUSTER OF CYSTICERCOSIS IN A SCHOOL

Neurocysticercosis is a disease caused by taenia solium, pork tapeworms when a human is infected by the eggs of the tapeworm and functions as an intermediate host instead of pigs. The larvae of the tapeworm form cysts, usually in the central nervous system that can cause severe neurological symptoms.
In this presentation I will provide background on this disease and describe an outbreak of neurocysticercosis in a school in Flanders. We focus on the decision making process and the management of this outbreak due to its unique character: an outbreak in a non-endemic region is rare and there are no clear guidelines on its management.

GÉRALDINE DE MUYLDER

SCIENSANO

BIOGRAPHY

Géraldine De Muylder is a scientist in the service of Epidemiology of Infectious Diseases at Sciensano. She started working in the service in the summer of 2020 and was actively involved in reporting of the COVID-19 epidemiological situation and risk assessment. She holds a PhD from the ULB.

RESPI-RADAR: A TOOL TO MONITOR RESPIRATORY INFECTIONS

During the COVID-19 crisis, the Risk Assessment Group (RAG) was asked to set up a system for a standardized interpretation of the epidemiological situation of COVID-19 which would assist decision-making. Hence, several "management" tools were successively developed and used between September 2020 and August 2023, which proved useful in evaluating and communicating the epidemiological situation to authorities.
The Respi-Radar was developed in the summer 2023 with the aim of assessing the epidemiological situation and informing public health preparedness and response for respiratory infections in general, so not only focusing on SARS-CoV-2.

The Respi-Radar was divided into four levels, based on combinations of indicators: Level Yellow when the epidemic threshold was reached but the situation remained under control and the impact on the healthcare system (first and second line) remained limited ; level Orange when there was a moderate viral circulation with pressure on the healthcare system; in this case public health measures are necessary to reverse the trend ; level Red when there was an important or very important viral circulation with a high risk of overwhelming of the healthcare system ; in this case measures to mitigate the epidemic might be needed. The Green level reflected the baseline situation, when the tool is not needed.

The Respi-Radar was based on six main indicators, from the Influenza-Like Illness and Severe Acute Respiratory Infection sentinel surveillances (in nursing homes, primary and secondary care), as well as from the wastewater surveillance. Additional information fed the assessment of the situation, such as data from the national reference laboratories which provided pathogen-specific information.

Based on the Respi-Radar tool, the RAG regularly evaluated the epidemiological situation of respiratory infections between September 2023 and March 2024. The situation was assessed as being in the yellow level from week 46 2023 to week 3 2024; in the orange level from week 4 2024 to week 7 2024, back to yellow on week 8 2024 and green since week 11 2024. When the orange level was reached, measures were proposed to the healthcare

sector by the Risk Management Group (RMG). The evaluation of the Respi-Radar (indicators used, thresholds, levels) is currently ongoing in order to determine the best approach for the assessment and management of the epidemiological situation during the next respiratory season.

Authors (team involved in the project) : Géraldine De Muylder; Simon Couvreur, Giulietta Stefani, Nathalie Bossuyt, Jorgen Stassijns.

REFERENCES

https://www.sciensano.be/fr/biblio/20230823ragrespi-radartool-monitor-respiratory-viruses

REINOUT NAESENS

ZIEKENHUIS NETWERK ANTWERPEN

BIOGRAPHY

Reinout Naesens has been a clinical microbiologist at ZiekenhuisNetwerk Antwerpen (ZNA) since 2013 and currently also serves as the head of the Infection Prevention & Control department. He actively participates in the Hospital Outbreak Support Team (HOST) project and is a member of the antibiotic therapy steering committee of the GemeenschapsZusters Antwerpen (GZA) and ZNA network. Additionally, he holds the position of chairman of the Microbiology Working Group within the Commission of Clinical Biology, overseeing and guiding nomenclature changes in the microbiology field. He was involved in the Belgian SARS-CoV-2 sequencing consortium and is a member of the Belgian Risk Assessment Group (RAG) and the Severe Acute Respiratory Infection (SARI) surveillance group. Reinout Naesens has authored or co-authored more than 20 papers in peer-reviewed journals.

INDICATIONS OF MOLECULAR DIAGNOSIS FOR RESPIRATORY PATHOGENS: UPDATE ON THE CURRENT SITUATION

Molecular techniques have become a cornerstone in the diagnosis of respiratory infections. Although possibilities in this domain have been expanding and continue to do so, medical analyses always come with a cost. Difficulties in result interpretation are also always just around the corner. If implemented thoughtfully and interpreted carefully, however, these tests have the potential to improve clinical decision-making.

Initially, the presentation will focus on the benefits and limitations of molecular testing for individual patient care, thereby seeking the underlying rationale and supporting evidence.

Subsequently, it will provide insights into the reasons for testing from an epidemiological and public health perspective, including systematic testing for Infection Prevention and Control (IPC) in settings with vulnerable populations, such as hospitals. Results from some own projects will be presented for both SARS-CoV-2 and Influenza A.

Lastly, the presentation will briefly mention the prospects of changes in the reimbursement of these tests.

CHARLOTTE MARTIN

CHU SAINT PIERRE BRUSSELS

BIOGRAPHY

Charlotte Martin is a specialist in Internal Medicine and Infectious Diseases.

She is Head of Department since March 2022.

She is Head of the MIA Center, which includes the HIV Reference Center, the HIV Testing Center and the STI Clinic.

She is also Head of the Travel & Vaccine Clinic and of the ImmunoStart consultation, created on her initiative.

She teaches the Medical Virology course at the Université Libre de Bruxelles, and she is a training supervisor for specialist doctors in training in infectiology.

She wrote her doctoral thesis on the impact of HIV infection on vaccine response to yellow fever.

She is expert at Conseil Supérieur de la Santé, Vaccines group.

DIPHTERIA: CLINICAL PICTURE AND MANAGEMENT OF CASES

JEAN-FRANCOIS LÉONARD

AVIQ

BIOGRAPHY

Jean François Léonard has a nursing background (with experience in intensive care) and a master in Public Health.
He currently works for the AVIQ (Agence pour une Vie de Qualité) as a Health Manager (Infectious Disease Surveillance Department) / and as a Manager for BelRAI Projects.
He is also a Scientific Collaborator at the Health and Society Research Institute of UCLouvain (BelRAI Integrated Screener Research - KULeuven).

DIPHTERIA AMONG ASYLUM SEEKERS: OUTBREAK MANAGEMENT INSIDE A RECEPTION CENTER

Authors: Leonard, JF; Purnelle, N; Tchatchie, E; Boulouffe, C.
Direction de la Surveillance des Maladies Infectieuses, AVIQ

Diphtheria, historically feared as one of the most devastating childhood diseases, has seen a significant decline in Belgium due to vaccination. However, sporadic cases still occur, posing a threat to unvaccinated or incompletely vaccinated individuals. The disease, caused primarily by Corynebacterium diphtheriae, is still endemic in several regions of the world.
In June 2023, a cluster of diphtheria cases occurred in a refugee center in Namur. Three confirmed cases and two suspected cases among high-risk contacts have been identified. The first case, a 16-year-old Pakistani girl, presented with severe symptoms, leading to her death despite timely hospitalization. Her two siblings also showed signs of the disease. Prompt treatment, including antibiotic therapy and vaccination, was crucial in managing these cases.
The management strategy extended beyond individual cases to include the refugee center and surrounding community. High-risk contacts (HRC) were identified, screened, and provided with prophylactic treatment. Mass vaccination and hygiene measures were implemented within the center to prevent further transmission. Additionally, healthcare workers and other individuals exposed to the cases were monitored and treated as necessary.
Challenges arose in managing HRC in school settings and in the communication with different healthcare providers. Coordination between public health agencies, schools, and healthcare providers was essential to ensure comprehensive follow-up and response measures.
At a broader level, the outbreak called into question an update of vaccination strategies for asylum seekers, particularly children. Issues such as incomplete vaccination records and logistical barriers to vaccine supply were addressed through collaborative efforts between government agencies and healthcare providers.
In conclusion, the management of the diphtheria outbreak in the Namur refugee center exemplifies the importance of a coordinated public health response. Timely identification and treatment associated with prevention measures, and effective communication between stakeholders, are essential in controlling such outbreaks and safeguarding public health.

VEERLE STOUTEN

SCIENSANO

BIOGRAPHY

Veerle obtained a BSc and MSc in Biological Sciences from the Catholic University of Leuven. From 2011 to 2015 she worked as a study coordinator at the Rheumatology department of UZLeuven. In this position she coordinated several clinical studies of pharmaceutical companies regarding innovative medicines for rheumatological diseases. In 2016, she started her doctoral research in Biomedical Sciences in the same department. During this PhD, which she obtained in 2020, she conducted research into various intensive treatment strategies for rheumatoid arthritis, the results of which contributed to the adjustment of the European guidelines on the treatment of rheumatoid arthritis.
Since March 2021, Veerle has been working for Sciensano at the Epidemiology of Infectious Diseases department. On one hand, she is involved in monitoring the COVID-19 vaccination rate and in assessing the effectiveness of COVID-19 vaccination. She is also involved in renewing the existing data collection within Epilabo, a network of sentinel microbiology laboratories to collect test results at the national level.

ESTIMATED NUMBER OF COVID-19 DEATHS AVERTED BY VACCINATION IN BELGIUM

Background/Aims
Vaccination campaigns have been rolled out globally in order to limit the impact of COVID-19 on severe health outcomes, including mortality. We aimed to estimate the number of averted deaths in the Belgian population aged ≥65 years by COVID-19 vaccination between January 2021 and January 2023.

Methods
Nationwide data on COVID-19 infections, vaccinations, all-cause mortality and demographic characteristics were collected and linked at the individual level. We estimated Vaccine Effectiveness against COVID mortality (VE) in persons having received a vaccine dose in the last 6 months using a Cox proportional hazards model adjusted for age, sex, time since vaccination, previous infection, comorbidities province and income. COVID-19 death was defined as a death within 1-7 weeks of a positive SARS-CoV-2 laboratory-test. Based on these VE estimates, COVID-19 vaccine coverage data and COVID-19 mortality data from the national surveillance, we estimated the number of averted deaths (expected number of deaths without vaccinating minus the reported number of deaths) by age group, and by variant.

Results
By January 31st 2023, 11,033 COVID-19 deaths have been reported through the Belgian surveillance. The average vaccine coverage was 48.2%

during alpha, 84.7% during delta and 62.8% during omicron dominance. VE against COVID- 19 mortality was estimated at the timepoint of 0-59 days after vaccination, for 65-79 year and ≥80 year-olds respectively, at 64.6% (58.4%-69.8%) and 34.9% (28.0%-41.1%) during Alpha, at 84.3% (81.8%-86.5%) and 79.1% (76.8%-81.2%) during Delta and at 82.6% (80.3%-84.7%) and 70.8% (68.4%-73.0%) during Omicron dominance. We estimated 10,042 deaths averted (range: 8,917-11,188) among the Belgian population aged ≥65 years, representing a 47.7% reduction (range 44.8%-50.4%) in the expected COVID-19 deaths. Out of 10,042 averted deaths, there were slightly more persons ≥80 year (53.6%; 5,382) than between 65-79 years (46.4%; 4,660), and the majority was averted during Delta (46.5%; 4,670) or Omicron dominance (43.5%; 4,366), compared to during Alpha dominance (10.0%; 1,006)

Conclusion

The multiple COVID-19 vaccinations in Belgium led to an important reduction in COVID-19-related mortality among the Belgian population ≥65 years, in particular during Delta and Omicron dominance, underscoring effectiveness of vaccines against COVID-19 mortality.

CORENTIN FONTAINE

CHBA SERAING

BIOGRAPHY

I graduated in pharmaceutical sciences in 2016 at the University of Liège. I directly started an intership in Clinical Biology. As soon as my third year, I specialised in Clinical Microbiology (internships at the University Hospital of Liège and Hospital Bois-de-l'Abbaye (CHBA) in Seraing). I completed various certificates in the field: Antimicrobial stewardship, infectious diseases and clinical microbiology, IPC (Infection prevention and control) in Belgium; and therapeutic and preventive strategies in infectious diseases in France (Sorbonne University). Since November 2021, I evolve in the CHBA Laboratory as a clinical microbiologist. In addition to my position in the lab, I also work as a IPC specialist and AMS delegate.

CLINICAL IMPACT OF A DIRECT RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING (DRAST)

Background: A critical step to reduce mortality during sepsis is the prompt administration of an adequate antibiotic treatment. In order to adjust empirical therapy, clinicians need identification of the microorganism and antimicrobial susceptibility testing (AST). The rapid AST from QuantaMatrix (dRAST™) provides AST directly from positive blood culture (PBC) within 6 hours. We evaluated the contribution of dRAST™ in the management of patients with bloodstream infection (BSI).

Methods: We reviewed, retrospectively in 150 patients and prospectively in 15 patients, antimicrobial therapies and classified them into 3 categories (optimal, suboptimal, ineffective) according to timing of microbiological results. We also compared dRAST™ and classic AST (VitekÒ2) in terms of « time-to-result » (TTR, time between sampling and availability of results) in the retrospective study.

Results: In the retrospective study, adaptation to optimal therapy (OT) according to AST occurred in 46/100 of Gram-negative PBC and in 4/50 of Gram-positive PBC. For these patients, TTR was significantly lower with dRAST™ compared with classic AST (29:35 (± 08:48) hours versus 50:55 (± 12:45) hours, $p<0.001$). In the prospective study, adaptation in OT according to dRAST™ occurred in 27% (3/11) of Gram-negative PBC and in 0% (0/4) of Gram-positive PBC.

Conclusion: For Gram-positive PBC, dRAST™ seems to have limited utility. On the contrary, for Gram-negative PBC, a higher proportion of treatments were adjusted using AST. These adaptations were significantly faster with dRAST™. Therefore, for patients with BSI requiring adaptation of empirical

antimicrobial therapy thanks to AST, dRAST™ allows a faster administration of optimal therapy and may thus contribute to a better clinical outcome.

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HENRIETTE DEVALK

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BIOGRAPHY

Henriette de Valk is a medical epidemiologist at the French National Public Health Agency (Santé Publique France). As the head of the Foodborne, Vector-borne and Zoonotic Infections Unit she is actively involved in European networking activities for surveillance, as national focal point for France and as chairperson of the coordination group of the Emerging and Vector-borne Diseases network of the European Centre for Disease Prevention and Control (ECDC) and member of the Vectornet Scientific Coordination Committee. She recently worked on guidelines for surveillance of emerging arboviruses, in France as well as at European level with ECDC. Henriette de Valk graduated from the University of Leyden in the Netherlands, the London school of Hygiene and Tropical Medicine in the United Kingdom and the Institut for Tropical Medicine in Antwerp, Belgium and is an alumnus of The European Programme for Intervention Epidemiology Training. Prior to coming to Santé Publique France she worked for the NGO Médecins Sans Frontières in Sudan, Uganda and Mali, for the German Technical Cooperation (GTZ) in Cameroon and for the World Health Organization in Indonesia.

WEST NILE VIRUS AND DENGUE IN SOUTH EUROPE: EPIDEMIOLOGY AND PUBLIC HEALTH RESPONSE.

PIERRE SMEETERS

ULBRUXELLES

BIOGRAPHY

Pierre Smeesters is a paediatrician working as the head of the paediatric department from the Academic Children's Hospital Queen Fabiola at the Free University of Brussels (ULB) in Belgium. He is also leading a research group in Microbiology and Infectious Disease at the Free University of Brussels. Pierre has had a broad range of clinical and research experiences in a number of diverse settings including Peru, Brazil, Australia and Belgium. Pierre now shares his time between the Children's Hospital, teaching of paediatrics and microbiology at University and scientific research. His research interests include translational research in Strep A, vaccination, new diagnostic tests and societal issues related to childhood. Pierre's team has set up and validated a new typing tool for Strep A which has been endorsed by the American CDC and is now broadly used for molecular typing and vaccine development. His team has also developed new diagnostic tools for pharyngitis and pneumonia.

INCREASE OF INVASIVE GROUP A STREPTOCOCCUS INFECTIONS

Group A Streptococcus (GAS or Strep A) is a leading bacterial pathogen responsible for significant disease burden in Belgium and elsewhere. While the incidence of invasive Strep A infection shas been slowly - but constantly - increasing over time, a more significant upsurge has been observed after covid- 19 outbreak. This recent upsurge is likely to be explained by a combination of various factors including new strain emergence (M1UK especially), lack of exposure during Covid-19 outbreaks and epidemiological variations in viral infections such as RSV, Flu or Varicela. A recent systematic review highlighted the importance of human social determinants as an epidemiological marker of Strep A genetic diversity. These social factors should therefore be taken more seriously into account when designing future epidemioloigcal studies. Finaly, an efficient global vaccine appears to be the most efficient way forward to decrease Strep A disease burden.

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AMARYL LECOMPTE

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BIOGRAPHY

Amaryl Lecompte graduated from KU Leuven as a general practitioner and obtained a master's degree in epidemiology at the University of Antwerp in 2023. Since 2020 , she joined Sciensano as scientific collaborator where she works at the Department of Epidemiology of Infectious Diseases within the team that monitors HIV, hepatitis B and C and sexually transmitted infections (STIs) in Belgium. She is responsible for the surveillance of STIs.

INCREASE IN GONORRHEA : OVERVIEW OF THE SITUATION

Sexually transmitted infections are on the rise in Belgium as in other European countries. In particular, the rise in gonorrhea has been striking in the past few years . Gonorrhea primarily affects young people, and the increase is seen in both women and men. This indicates that there is also heterosexual transmission in addition to transmission among men who have sex with men. In Europe, the majority of cases of gonorrhea were reported in men who have sex with men.

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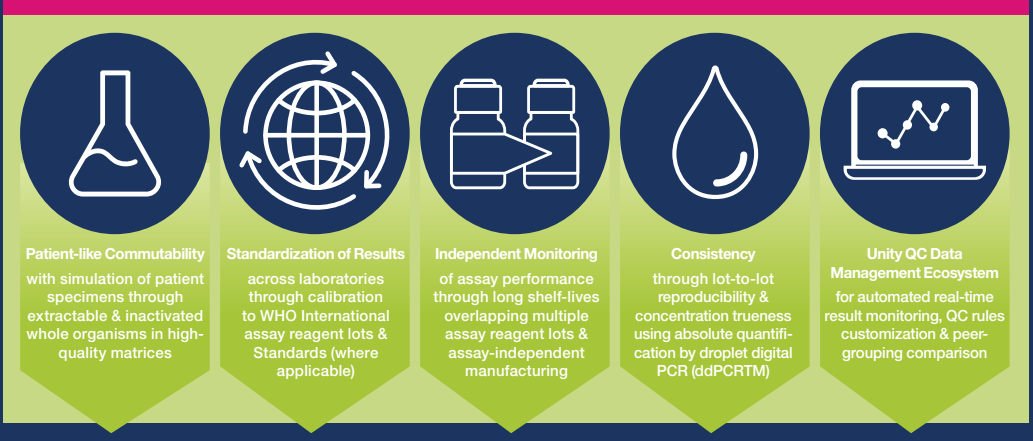

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ABSTRACT OF POSTERS

ESTIMATED NUMBER OF COVID-19 DEATHS AVERTED BY CACCINATION IN BELGIUM.

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Background/Aim

Vaccination campaigns have been rolled out globally in order to limit the impact of COVID-19 on severe health outcomes, including mortality. We aimed to estimate the number of averted deaths in the Belgian population aged ≥65 years by COVID-19 vaccination between January 2021 and January 2023.

Methods

Nationwide data on COVID-19 infections, vaccinations, all-cause mortality and demographic characteristics were collected and linked at the individual level. We estimated Vaccine Effectiveness against COVID mortality (VE) in persons having received a vaccine dose in the last 6 months using a Cox proportional hazards model adjusted for age, sex, time since vaccination, previous infection, comorbidities province and income. COVID-19 death was defined as a death within 1-7 weeks of a positive SARS-CoV-2 laboratory-test. Based on these VE estimates, COVID-19 vaccine coverage data and COVID-19 mortality data from the national surveillance, we estimated the number of averted deaths (expected number of deaths without vaccinating minus the reported number of deaths) by age group, and by variant.

Results

By January 31st 2023, 11,033 COVID-19 deaths have been reported through the Belgian surveillance. The average vaccine coverage was 48.2% during alpha, 84.7% during delta and 62.8% during omicron dominance. VE against COVID-19 mortality was estimated at the timepoint of 0-59 days after vaccination, for 65-79 year and ≥80 year-olds respectively, at 64.6% (58.4%-69.8%) and 34.9% (28.0%-41.1%) during Alpha, at 84.3% (81.8%-86.5%) and 79.1% (76.8%-81.2%) during Delta and at 82.6% (80.3%-84.7%) and 70.8% (68.4%-73.0%) during Omicron dominance. We estimated 10,042 deaths averted (range: 8,917-11,188) among the Belgian population aged ≥65 years, representing a 47.7% reduction (range 44.8%-50.4%) in the expected COVID-19 deaths. Out of 10,042 averted deaths, there were slightly more persons ≥80 year (53.6%; 5,382) than between 65-79 years (46.4%; 4,660), and the majority was averted during Delta (46.5%; 4,670) or Omicron dominance (43.5%; 4,366), compared to during Alpha dominance (10.0%; 1,006).

Conclusion

The multiple COVID-19 vaccinations in Belgium led to an important reduction in COVID-19-related mortality among the Belgian population ≥65 years, in particular during Delta and Omicron dominance, underscoring effectiveness of vaccines against COVID-19 mortality.

EMERGENCE AND SPREAD OF A MUPIROCIN-RESISTANT VARIANT OF THE EUROPEAN EPIDEMIC FUSIDIC ACID-RESISTANT IMPETIGO CLONE OF *STAPHYLOCOCCUS AUREUS* IN BELGIUM, 2013 TO 2023.

Nicolas Yin[1], Charlotte Michel[1], Nadia Makki[2], Ariane Deplano[1], Alisha Milis[2], Benoit Prevost[1], Veronique Yvette Miendje-Deyi[3], Marie Hallin[1,4,5] and Delphine Martiny[1,6].

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Background/Aim

Coresistance to both mupirocin and fusidic acid in *Staphylococcus aureus* affects the treatment of impetigo in Belgium, where they are the only topical treatments available. We investigated resistance to fusidic acid and mupirocin in methicillin-susceptible *S. aureus* (MSSA) strains involved in community-acquired skin and soft tissue infections (SSTIs).

Methods

The 10-year variation in fusidic acid and mupirocin resistance in outpatients with SSTI-associated MSSA was studied in two large laboratories (AML, Antwerp and LHUB-ULB, Brussels). In addition, MSSA strains coresistant to fusidic acid and mupirocin and sent to the Belgian *Staphylococci* Reference Centre were *spa*-typed and analysed for the presence of the *eta* and *etb* virulence genes and the *mupA* resistance gene. In addition, whole-genome sequencing was performed on isolates collected in the last 2 years.

Results

From October 2013 to September 2023, resistance to fusidic acid increased from 3.1% (68/2211) to 11.4% (263/2300) in LHUB-ULB and from 17.7% (153/862) to 25.5% (341/1336) in AML. Meanwhile, resistance to mupirocin increased from 0.5% (10/2211) to 1.7% (38/2300) in LHUB-ULB and from 1.5% (13/862) to 5.6% (75/1336) in AML. Similarly, coresistance to fusidic acid and mupirocin increased from 0.04% (1/2211) to 1.4% (33/2300) in LHUB-ULB and from 0.8% (7/862) to 5.3% (71/1336) in AML. Coresistance in children reached 8.9–10.1% in the third quarter 2023. From 2018 to 2023, 64/70 (91.4%) mupirocin-resistant MSSA strains were coresistant to fusidic acid. Whole-genome sequencing revealed that 29/33 (87.9%) of the isolates were sequence type ST121, clonal and more distantly related to the European epidemic fusidic acid-resistant impetigo clone (EEFIC) observed in Belgium in 2020. These strains carried the *mupA* and *fusB* genes, which confer resistance to mupirocin and fusidic acid, respectively, and the *eta* and *etb* virulence genes.

Conclusion

We highlight the spread of a mupirocin-resistant EEFIC (M-EEFIC) in children, with a seasonal trend for the third quarter. This is of concern because this variant is resistant to the two main topical antibiotics used to treat impetigo in Belgium.

INDIVIDUAL LEVEL ANALYSIS OF DIGITAL PROXIMITY TRACING FOR COVID-19 IN BELGIUM HIGHLIGHTS MAJOR BOTTLENECKS.

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Background

Contact tracing was implemented on an unprecedented scale to control the COVID-19 pandemic. Digital proximity tracing was developed to complement or replace labour-intensive conventional contact tracing interviews. However, studies assessing its real-life performance remain scarce, complicated by the privacy-centred design of the dominant Google-Apple exposure notification framework. In this study, we aimed to compare the comprehensiveness and positive predictive value of manual and digital contact tracing at the individual level.

Methods

Between October 2021 and January 2022, we performed manual contact tracing in a population of higher education students in Leuven, Belgium. In this period, the Coronalert app was active as the national implementation of the Google-Apple exposure notification framework. Students who underwent testing or manual tracing were systematically questioned about their utilisation of Coronalert and receipt of a digital notification. We combined the resulting data on the two contact tracing approaches to determine delays and success rates in each step of the digital notification cascade.

Results

Overall, we estimated that only 4.3% (CI: 2.8-6.1%) of exposed contacts were digitally notified, resulting in 10 times more cases detected through conventional contact tracing. The low comprehensiveness of digital proximity tracing was a result of failures in each of four studied steps in the notification cascade: app use by the case, consenting to notifications, app use by the contact, and technical sensitivity. The infection risk of digitally traced contacts (5.0%; CI: 3.0-7.7%) was significantly lower than for manually traced non-app users (9.8%; CI: 8.8-10.7%; p=0.002). Contrary to common perception as near instantaneous, there was a 1.2-day delay (CI: 0.6-2.2) between case PCR result and digital contact notifications.

Conclusion

Our results show disappointing real-life success rates in the digital notification cascade and a lower positive predictive value of digital compared to manual notifications. Moreover, they challenge the perception of digital proximity tracing as near instantaneous. Overall, this study highlights major limitations of the dominant digital proximity tracing framework.

ASSESSMENT OF COVID-19 CONTACT TRACING NETWORK ACCURACY VIA PHYLOGENETIC ANALYSIS OF COMMUNITY-LEVEL SARS-COV-2 GENOMIC DATA.

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Background/ Aims

Contact tracing is pivotal in identifying and preventing transmission chains and provides critical information to plan and implement targeted medical countermeasures, such as incubation period or secondary attack rate. Nevertheless, the efficacy of contact tracing remains inadequately characterized. Here, we propose an automated methodology leveraging genomic data to assess the reliability of contact tracing in accurately identifying transmission events. We exemplify our approach through the examination of the Omicron BA.1 epidemic wave that impacted the student community of the University of Leuven.

Methods

Our methodology entails evaluating whether infected cases linked through contact tracing indeed cluster together within a time-scaled phylogeny inferred at the community level. To construct the phylogenetic tree, we aligned genomic data consisting of 320 BA.1 sequences from infected cases identified in our contact tracing network, along with 4342 'background' BA.1 sequences from the broader community, selected through subsampling a larger phylogeny.

Results

Our analyses unveil that only 39.91% of close contacts identified through contact tracing share the same phylogenetic cluster. However, 15.96% of close contacts with identical sequences did not cluster together within the phylogenetic tree. Considering these discrepancies, our findings suggest that 53.21% of reported close contacts do not correspond to plausible transmission events.

Conclusion

In summary, our method of employing phylogenetic clustering, augmented by single nucleotide polymorphism (SNP) analysis, to categorize contacts allowed us to assess the efficacy of contact tracing efforts during the Omicron BA.1 surge among university students in Leuven. While contact tracing remains fundamental for early outbreak detection and control, continuous monitoring of its accuracy is vital for guiding effective, targeted public health interventions.

RAPID INCREASE OF VACCINE SEROTYPE 4 INVASIVE PNEUMOCOCCAL DISEASE iN YOUNG ADULYS SINCE 2020 IN BELGIUM

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Background

After introduction of pneumococcal vaccination for children in Belgium in 2007, vaccine preventable serotype 4 IPD almost disappeared (<0.5%). Since 2020, a resurgence was noticed, with serotype 4 responsible for 9.6% of all IPD cases in 2023.

Methods

All IPD cases from 2007 to 2023, collected by the stable surveillance system of the National Reference Center, were used. Serotype 4 strains from 2021 and 2022 (n=140) were characterized by whole-genome sequencing.

Results

Despite overall low numbers of IPD cases during the COVID-19 pandemic, an important increase of serotype 4 infections is observed since early 2020. For 2023, this serotype (9.6%) was ranked third among all serotypes causing IPD, and is even the most prevalent serotype (25.3%) among young adults. The increase of serotype 4 (2020-2023) is mainly pronounced in males (78.8% vs 55.5% for non-serotypes 4) and in the age category 18-64 years (75.6%: 18-49 (46.6%) and 50-64 (29.1%)). Compared to non-serotypes 4, it is more frequently diagnosed in Brussels-Capital region (32.2% vs 9.9%) and Wallonia (35.4% vs 26.9%), compared to Flanders (28.1% vs 60.2%). Six MLST types were identified for the strains of 2022, with 93.2% of strains assigned to ST801 (71.6%) and ST15063 (21.6%), both part of GPSC 162. Geographical differences for these major STs were observed.

Conclusions

The continued increase of serotype 4 IPD since early 2020 is due to an increase in younger adults (18-49 years) mainly in specific regions in the country. Two clones are responsible for this worrisome increase of a vaccine serotype in an age group currently not targeted for vaccination, accounting for 1 in 4 infections for the young adults in 2023. Further characterization of the serotype 4 cases and genomic comparisons are ongoing to better understand this evolution.

INDIRECT EFFECT ON ADULT IPD OF CHANGES IN CHILDHOOD IMMUNIZATION PROGRAMME FROM PCV13 TO PCV10 AND AGAIN TO PCV13 IN BELGIUM (2014-2023).

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2 KU Leuven, Department of Microbiology, Immunology and Transplantation, Laboratory of Clinical Microbiology, Leuven, Belgium.

Background

Belgium has a unique setting to study the potential indirect effect of changes in childhood immunization programs on adult IPD. While different PCVs with high stable vaccine uptake were used for children over time (PCV13 (2011-2015), PCV10 (2015-2019) and PCV13 (2019-now)), vaccination coverage in adults remained low (<15%).

Methods

Capsular typing information (Quellung reaction) of all IPD cases in adults (>18 years), collected by our stable national surveillance system, was used. We compared serotype distribution of IPD cases from 3 periods, each 3 to 4 years after a childhood vaccine switch: 2014-2015 (PCV13-1), 2018-2019 (PCV10) and 2022-2023 (PCV13-2).

Results

In total, 7718 adult IPD cases were detected during the 3 periods. Following childhood PCV implementation, IPD incidence rates have decreased, and COVID-19 containment measures had the largest impact (-56.1%) on IPD incidence in adults. PCV10 serotypes 1 and 7F decreased steadily from respectively 5.3% and 5.0% in PCV13-1 period to almost zero detections in PCV13-2 period. In contrast, serotype 4 increased dramatically in PCV13-2 period (to +9.1%), while only accounting for 0.4% in previous periods. Serotype 19A proportion was highest in PCV10 period (10.5% compared to 6.1% (PCV13-1) and 8.1% (PCV13-2)). Serotype 3 increased steadily over time, while serotype 6C remained stable (the latter only increased in older adults). PCV13 serotype proportion ranged from 33.1 to 34.0% to 39.2%. Regarding PCV20/PCV21 serotypes, the largest decrease was observed for serotype 12F (-9.8%), in contrast to the increase observed for serotype 8 (+6.2%) over time.

Conclusions

Following childhood PCV implementation, vaccine serotypes 1, 7F and 19F have decreased, in contrast to 3 that continues to increase. In the PCV10 period, serotype 19A infections increased. Despite PCV10/13 use and switch in vaccines, PCV13 serotype proportion remained high (33.1-39.2%).

DOUBLING OF TRIAZOLE RESISTANCE RATES IN INVASIVE ASPERGILLOSIS IN BELGIUM OVER A PERIOD OF 10 YEARS.

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Background

A national prospective surveillance study on invasive aspergillosis (IA) in Belgium dates from 2011 and revealed a triazole resistance rate of 4.6%. The aim of this study is to evaluate if there is a change in the epidemiology of invasive aspergillosis between the years 2011/2012 and 2022/2023.

Methods

All Belgian laboratories could send clinically relevant *Aspergillus species* complex isolates cultured from clinical samples collected in the one-year study period (April 2022 to March 2023) to the National Reference Centre for Mycosis in Leuven for antifungal susceptibility testing. Isolates were included in the study when judged clinically relevant according to consensus definitions on IA (EORTC/MSGERC, (modified) *Asp*ICU, expert case definitions for IAPA and COVID ECMM/ISHAM criteria). Triazole-resistance screening was performed and in case of growth in any triazole-containing well, the EUCAST broth microdilution reference method was used, and CYP51A sequencing was performed to detail the resistance mechanism.

Results

A total of 309 isolates from 297 patients were included in the study, contributed by 29 clinical laboratories located across Belgium. The median age across the whole dataset was 66 years, 61.2% was male and over 90% of included isolates originated from patients living in the northern part of the country. Identification to species level confirmed the predominance of *Aspergillus fumigatus* isolates, for which azole resistance screening showed a triazole resistance rate of 9.7%, nearly double compared to the rate in 2011 (4.6%). Azole resistance was confirmed for all isolates by the EUCAST reference method and Cyp51A sequencing showed that the majority of resistant isolates (76.9%) was characterized by the resistance mechanism TR34/L98H, while 19.2% carried the profile TR46/Y121F/T289A.

Conclusions

A higher triazole resistance prevalence of 9.7% was observed for *Aspergillus fumigatus* complex isolates from patients diagnosed with invasive aspergillosis in Belgium for 2022-2023, nearly double the rate that was reported in 2011. These data strengthen the need to rediscuss local decisions on empirical antifungal treatment regimens.

IDENTIFICATION OF THE NEW HSV-2 VARIANT (HSV-2VNJBJB) IN THREE PATIENTS SUFFERING FROM RECURRENT INFECTIONS UNRESPONSIVE TO ANTIVIRAL THERAPY.

Graciela Andrei[1], Hanna Schalkwijk[1], Sarah Gillemot[1], Elizaveta Padalko[2], Liselotte Coorevits[2], Willy Peetermans[3], Simon Feys[3], Robert Snoeck[1].

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HSV-1 and HSV-2 are prevalent human pathogens (global seroprevalence of 66% and 13.2%, respectively), establishing long-life latency in neurons and reactivating periodically. HSV-1 preferentially infects oral mucosa while HSV-2 more often causes genital herpes, though both viruses can infect either mucosa. As all herpesviruses, they are considered to exhibit low genetic diversity [mean pairwise distance of 0.8% (HSV-1) and 0.1% (HSV-2)].

In the framework of our translational research platform RegaVir for diagnosing herpesvirus drug-resistance, 3 HSV-2 isolates [RV-938 (Patient #1); RV-2760 & RV-2765 (Patient #2)] were recovered from 2 non-immunocompromised Belgium women suffering from recurrent genital herpes under valacyclovir therapy for years. The isolates harbored no known thymidine kinase (TK) or DNA polymerase (DP) drug-resistance mutations but >20 novel changes in the DP, primarily in the thumb domain, when aligned to HSV-2 laboratory strains. Two novel TK changes (P273L & E276K) were also found in the RV-2760 and RV-2765 isolates. All these changes did not affect the sensitivity to anti-HSV-2 drugs, indicating their association with inter-strain variability.

Alignment of the patients' DP sequences to different clinical HSV-2 isolates showed a high similarity to the new HSV-2 variant (HSV-2v) identified in sub-Saharan African patients, characterized by an unexpectedly high DP variability (2.4% divergence). We also found the HSV-2v variant in a man suffering from persistent HSV-2 ocular infections. To our knowledge, these are the first HSV-2v strains isolated in Belgium and their replicative fitness is being investigated in dual infection competition assays.

EPIDEMIOLOGICAL SITUATION OF TICK-BORNE ENCEPHALITIS IN BELGIUM, AN OVERVIEW.

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Background

Tick-borne encephalitis (TBE) is an important zoonotic disease for many countries in central, northern, and eastern Europe, and a gradual increase of notified cases has been reported since 2017. This work describes the epidemiological situation of TBE in Belgium.

Methods

Different sources of information are used to follow-up the epidemiological situation of TBE in Belgium: 1) surveillance of human cases through the National Reference Centre since 2012; 2) seroprevalence studies in animals using enzyme-linked immunosorbent assay, followed by a seroneutralization test for confirmation, since 2009; and 3) PCR-analyses on ticks, since 2017. In addition, a seroprevalence study was conducted in 2019 among staff of the Flemish Agency for Nature & Forests exposed to tick bites during their professional activities.

Results

The number of diagnosed infections in humans by year ranges between zero and seven. The majority of cases are imported. In 2018, two cases probably infected in Belgium were identified and in 2020 there were three persons with a definite autochthonous infection, contracted in different locations of the country.

Depending on the animal species, different prevalence rates have been reported, ranging from 0,11% in dogs in 2009 (Belgium) to 9,27% in wild boar in 2019/2020 (Flanders). The results of the study on wild boar suggest an increase in TBEV prevalence over the last decade.

A total of about 5,000 ticks, removed from humans and animals or collected through flagging, have been tested for TBEV. All were negative.

None of the 195 participants to the seroprevalence study in forestry workers had evidence of a recent or old infection.

Conclusion

Overall, the risk of infection for humans in Belgium is still estimated to be very low. In a context of sporadic autochthonous cases of TBE, which are geographically spread, vaccination against the disease in Belgium is not recommended for the general population, nor for professional or recreational risk groups. However, vaccination is recommended for travellers to high-risk areas in other countries and performing outdoor activities (hiking, camping...).

FACILITATED INTERPRETATION OF METAGENOMICS DATA FOR DETECTION OF BACTERIAL PATHOGENS AND THEIR ANTIMICROBIAL RESISTANCE GENES.

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Background/Aims

Metagenomic sequencing is a promising method that has the potential to revolutionize the world of pathogen detection and antimicrobial resistance (AMR) surveillance. However, the analysis of the huge amount of data obtained requires performant bioinformatics tools and databases, with intuitive and straightforward interpretation. In this study, we present interpretation guidelines, to help with the analysis of the output data generated by KMA, a popular *K*-mer read alignment tool.

Methods

Based on long-read metagenomics data of chicken fecal samples with a spike-in mock community, we proposed confidence levels for taxonomic identification and AMR gene detection. These confidence levels were tested on 28 metagenomics datasets obtained with sequencing of real and spiked samples from fecal (chicken, pig and buffalo) or food (minced beef and food enzyme products) origin.

Results

The results obtained with the detection confidence levels matched the expected data from the metagenomics datasets. Additionally, we demonstrated that the completeness and diversity of the genomes present in the reference databases are key parameters for accurate and easy interpretation of the sequencing data. Finally, we explored whether KMA, in a two-step procedure, can be used to link the detected AMR genes to their bacterial host chromosome, both detected within the same long-reads.

Conclusion

The methodology proposed in this study will facilitate the analysis of metagenomics sequencing datasets for KMA users. Ultimately, this will

contribute to improvements in the rapid diagnosis and surveillance of pathogens and AMR genes as prioritized by the EU.

Funding information

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SURVEILLANCE OF NON-INVASIVE STREPTOCOCCUS PNEUMONIAE INFECTIONS IN BELGIUM TO EVALUATE NATIONAL VACCINATION STRATEGY (2020-2023).

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Background

Streptococcus pneumoniae can cause invasive (IPD) or non-invasive pneumococcal disease (NIPD). Surveillance is largely focused on IPD, making it challenging to assess the full impact of pneumococcal vaccination programs on pneumococcal disease as a whole.

Methods

During a 32-month period (September 2020 – May 2023), NIPD samples and relevant patient data were prospectively collected from the routine practices of 24 hospitals across Belgium. Capsular typing was performed by a validated Fourier-Transform Infrared spectroscopic method (IR Biotyper™, Bruker, Germany). Antimicrobial resistance was assessed with broth microdilution (Sensititre™, ThermoFisherScientific, USA), using EUCAST clinical breakpoints v13.0.

Results

In total 1,008 confirmed non-invasive pneumococcal samples were collected from lower respiratory tract infections (LRTI) (n=760), otitis media (OM) (n=190) and sinusitis (SIN) (n=58) (Table 1). ST3 was the most commonly detected serotype in the LRTI (14.5%) and the OM (28.6%) group and ST23B was the most prevalent serotype among the SIN patients (15.3%). More non-PCV20 serotypes were circulating in the NIPD population when compared with the IPD population (53.1% vs. 34.7%; IPD data published by the National Reference Centre for IPD in Belgium). Furthermore, the NIPD population was associated with higher antimicrobial resistance for the majority of tested antibiotics (e.g. 9.6% of penicillin non-susceptibility in the NIPD population vs. 2.8% in the IPD population; MIC>2 mg/L).

Conclusions

The data presented in this study support the need for surveillance of NIPD along with IPD, to understand fully the contribution of each serotype to pneumococcal disease and to inform future vaccination programs.

Table 1. Summary of the sample metadata retrieved from the clinical samples.

	N (%)
Total number of clinical samples received meeting the inclusion criteria and validated as *S. pneumoniae*	1008
Number of samples with more than one *S. pneumoniae* strain identified	15
Patient and Sample Information	
Region	
Wallonia	603 (59.8)
Flanders	398 (39.5)
Brussels	7 (0.7)
Age	
<16 years	239 (23.7)
16-49 years	177 (17.6)
50-64 years	224 (22.2)
>64 years	368 (36.5)
Sex	
Male	572 (56.7)
Female	435 (43.2)
Unknown	1 (0.1)
Medical Care	
Ambulatory	484 (48.0)
Hospitalised	427 (42.4)
Intensive Care Unit	95 (9.4)
Long-term Care Facility	1 (0.1)
Unknown	1 (0.1)
Comorbidities/Immunocompromised	
Patients with at least one comorbidity or immunocompromised	391 (38.8)
Chronic Obstructive Pulmonary Disease	203 (20.1)
Cancer	53 (5.3)
Diabetes	47 (4.7)
Other Pathogens	
Specimens with at least one more viral or bacterial pathogen detected	419 (41.2)
H. influenzae	247 (24.5)
SARS-CoV-2	52 (5.2)
Clinical Specimen	
Sputum	517 (51.3)
Middle ear fluid	173 (17.2)
Endotracheal/Bronchial aspiration	152 (15.1)
Bronchalveolar lavage	65 (6.4)
Sinus	38 (3.8)
Nasopharyngeal aspirate/swab	20 (2.0)
Nasal swab	15 (1.5)
Respiratory pus	10 (1.0)
Pus (Ear)	7 (0.7)
Other	11 (1.1)
Clinical Diagnosis	
Lower respiratory tract infection	760 (75.4)
Otitis media	190 (18.8)
Sinusitis	58 (5.8)
Vaccination Status	
Unknown	783 (77.7)
Not vaccinated	126 (12.5)
Vaccinated	99 (9.8)
Prevenar 13	42
Pneumovax 23	21
Synflorix	5
Prevenar 13 and Pneumovax 23	1
Unknown vaccine	30

GENOMIC SURVEILLANCE OF *STAPHYLOCOCCUS AUREUS* IN BELGIAN HOSPITALS, 2022-2023.

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Background/Aims

The National Reference Centre for *Staphylococcus aureus* has been performing regular surveillance of *S. aureus* isolated in hospitals since 1992. With the development of next-generation sequencing, it is now possible to perform genome-based surveillance, investigating not only the clonal diversity of circulating strains, but also their resistome and virulome. This study presents the results of the first genomic surveillance of *S. aureus* in Belgian hospitals in 2022-2023.

Methods

Belgian hospital laboratories were invited to collect up to 3 methicillin-resistant (MRSA) and 2 first methicillin-susceptible *S. aureus* (MSSA) isolates from clinical specimens for each hospital they serve in 2022-2023. The isolates were subjected to whole-genome sequencing on a NovaSeq 6000 machine (Illumina Inc., San Diego, CA, USA). Resistome, virulome, multilocus sequence typing (MLST), whole-genome MLST (wgMLST) and *spa* type determination were performed using the BioNumerics 8.1 (bioMérieux, Marcy l'Etoile, France) *S. aureus* genotyping plugin v1.1 (database *S. aureus* Virulence KB 2022.12.05 and database *S. aureus* Resistance KB 2023.10.27), the WGS tools plugin v1.08 and MLST for the WGS plugin v1.0.

Results

Fifty-six MSSA and 123 MRSA were analyzed, mainly isolated from blood cultures (41.3%), skin samples (21.8%) and puncture fluids (13.4%). The more common clonal complexes (CC) were CC5 (35.8%), CC8 (14.6%) and CC45 (14.6%) for MRSA, and CC30 (17.9%) and CC5 (10.71%) for MSSA. All MRSA carried *mecA*. Resistome analysis showed that 71.4% of MSSA were resistant to penicillin, 37.5% to erythromycin and clindamycin and none to ciprofloxacin, while 65.0% of MRSA were resistant to ciprofloxacin, 29.2% to erythromycin, 26.8% to clindamycin and 12.2% to gentamicin. *tst* (coding for toxic shock syndrome toxin-1) was detected in 11 blood culture isolates (7 MSSA and 4 MRSA), while *lukS*-PV(coding for Panton-Valentine leukocidin) was detected in 15 MRSA (5 fluid punctures, 5 skin samples, 2 blood cultures) and in 1 MSSA (fluid puncture).

Conclusion

The establishment of genomic surveillance of *S. aureus* allows a better understanding of circulating strains, their resistome and virulome. Furthermore, the repository of whole-genome sequencing data will allow comparison of data from different countries and with future surveillance.

ADVANCING WASTEWATER SURVEILLANCE: DEVELOPMENT AND VALIDATION OF AMR DETECTION METHODS.

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Background

Antimicrobial resistance (AMR) is currently one of the world's top ten health threats, causing infections to become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death. Accurate surveillance is a key component in the fight against AMR. Wastewater is progressively becoming a new player in AMR surveillance, enabling a cost-effective real-time tracking of AMR and global monitoring of AMR profiles in specific regions. Digital droplet PCR (ddPCR) technology is gaining more interest for wastewater and AMR surveillance, as it provides the advantage of simultaneously detecting and quantifying AMR genes in wastewater. However, there are currently no probe-based ddPCR methods for AMR surveillance that have been validated according international standard performance criteria. Therefore, in this study, we present a workflow in order to develop and validate ddPCR methods responding to these international standard performance criteria, focusing on four duplex assays including a total of eight clinical relevant target genes which confer resistance to the following antimicrobials, extended spectrum beta-lactam (bla_{CTX-M}), carbapenem ($bla_{KPC-2/3}$), tetracycline *(tet(M)*, erythromycin *(erm(B)*), vancomycin *(vanA)*, sulfonamide *(sul2)*, aminoglycoside *(aac(3)-IV)* and an indicator of antibiotic (multi-)resistance and horizontal gene transfer, the class I integron (intI1). These ddPCR methods were successfully assessed for their specificity and sensitivity. In addition, their applicability was tested on fourteen wastewater samples collected by auto-samplers (24-hour composite) at the influent of two treatment plants. The results align with previous scientific literature observations, confirming their reliability. This study proposes a workflow for the development and validation of methods in order to have an harmonized and global AMR surveillance, as requested at the European Union (EU) level.

CLIMPATHIC: ADVANCING GENOMIC SURVEILLNACE OF CLIMATE-LINKED PATHOGENS IN WASTEWATER.

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Abstract

Climate change has a significant impact on the distribution and transmission of pathogens, posing a profound threat to public health. There is a need for an effective surveillance system to monitor the spread of endemic and (re) emerging pathogens within a population, especially in the context of climate change. Waterborne, vector-borne and foodborne diseases are particularly concerning due to the altered environmental conditions. The CLIMPATHIC project has four objectives to address these needs with the norovirus and dengue as study cases. First, it will select and prioritize pathogens that are relevant to climate change based on a comprehensive literature review. Secondly, analysis protocols will be developed and adapted, including qPCR, ddPCR, targeted sequencing and metagenomics, to enable pathogen detection and characterization in wastewater samples. Thirdly, the developed methodologies will be applied on real environmental samples to target norovirus and dengue. Finally, this project wants to emphasize on skill and technology transfer through consortium meetings, training sessions and workshops.

By developing and applying cutting-edge genomic surveillance techniques, this project will obtain crucial insights into pathogen distribution, prevalence and characteristics influenced by climate change. It will strengthen the preparedness for emerging pathogens and improve surveillance capacity thus facilitate a faster diagnostic response. Early detection of pathogens of concern in environmental samples will enhance public health and protect at-risk communities. Additionally, awareness will be raised about the impact of climate change on pathogen prevalence and distribution. Overall, CLIMPATHIC will contribute to advancing public health in the face of a change climate.

RAPID ON-SITE METAGENOMIC SEQUENCING FOR ONE HEALTH PATHOGEN AND ANTIMICROBIAL RESISTANCE SURVEILLANCE.

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Background

Efficient diagnostics are critical for addressing One Health challenges, particularly in monitoring pathogens and antimicrobial resistance (AMR), across human, environmental and food-production domains. As an open approach, metagenomic sequencing offers a promising solution for such surveillance, but its utility has been largely confined to laboratory settings. The advent of miniaturized devices like the Oxford Nanopore Technologies (ONT) MinION enables on-site, real-time analysis of a complete sample at genomic level. However, current sample preparation protocols often require substantial equipment and can be time-consuming, limiting their use. In this study, we developed a rapid, on-site applicable DNA extraction and library preparation approach for ONT sequencing, utilizing portable equipment. We evaluated its performance in microbial and AMR detection, including associating AMR genes to their respective hosts.

Methods

We optimized a portable method for on-site metagenomic DNA extraction, and applied it to chicken fecal samples. By spiking samples with a defined mock community, we compared our methodology with various other workflows from DNA extraction to sequencing, including a currently used laboratory-based approach. Novel bioinformatic methods were employed to compare sequencing data generation, taxonomic profiling and resistance gene detection among the different methods.

Results

Our optimized metagenomic method comprises a portable lysis device followed by magnetic bead-based DNA purification and automated sequencing library preparation, enabling rapid sample processing and sequencing across diverse settings. We observed that our methodology outpaced the current laboratory workflow in terms of time efficiency while maintaining comparable sequencing throughput. Furthermore, we demonstrated the utility of spike-in defined mock communities for workflow comparison, revealing method-specific biases in taxonomic and resistance gene profiles. Long-read sequencing facilitated the identification of full-length resistance genes and their attribution to specific host species based on the additional genomic information these reads contain.

Conclusion

Our method offers a rapid, comprehensive and versatile approach for microbial detection and AMR surveillance within a One Health framework.

The research that yielded these results was funded by in-kind contribution of Sciensano within the context of JRP12-AMRSH5-FARMED and by the EU's Horizon 2020 Research and Innovation program under grant agreement No 773830: One Health European Joint Program.

EMERGENCE OF INVASIVE *HAEMOPHILUS INFLUENZAE* ISOLATES WITH *FTSI* MUTATIONS ASSOCIATED WITH HIGH BETA-LACTAM RESISTANCE IN BELGIUM: IDENTIFICATION AND GENOMIC CHARACTERIZATION.

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Background/Aims

Haemophilus influenzae (Hi) is a significant cause of acute otitis media, meningitis, and sepsis. Hi is becoming increasingly resistant to beta-lactams, which are considered the first-line treatment for Hi-associated diseases. We aim to describe the first blood Hi isolates received at the National Reference Centre (NRC) that show mutations associated with high-level beta-lactam resistance.

Methods

All invasive *Hi* isolates from Belgian laboratories are sent to the NRC. All isolates are identified by MALDI-TOF MS. The antimicrobial susceptibility testing is performed and interpreted following EUCAST v13 guidelines and MIC are determined using e-test for the following molecules: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefuroxime (CXM), cefotaxime (CFX), meropenem (MEM), ciprofloxacin (CIP), trimethoprim-sulfamethoxazol (SXT) and tetracycline (TET). *ftsI* gene sequencing is performed for all isolates showing reduced susceptibility to beta-lactams.

Results

In 2023, 181 blood isolates were sent to the NRC, including 20 with *ftsI* mutations associated with low-levels of beta-lactam resistance. For the first time in Belgium, mutations associated with high levels of resistance (group III) were detected in several invasive strains. These 6 strains were non-typeable and 5 of 6 carried the same *ftsI* mutations (D350N, S357N, M377I, S385T, R517H, T532S). Four of them presented exactly the same phenotypic profile: biotype II, non-typeable, absence of beta-lactamase, resistance to CXM and SXT. Three out of the four, isolated during the first half of the year, belonged to the MLST clonal complex (CC) 3. In addition, all 3 strains were detected in pneumonia patients living in Limburg. WGS is in progress for all 6 strains.

Conclusion

Our study revealed for the first time in Belgium the emergence of six *Hi* invasive strains with mutations associated with high-level beta-lactam resistance, three of which caused pneumonia in Limburg. Their phenotypic profiles and the fact that three strains belong to the same CC indicate a possible cluster. Ongoing WGS for these strains will provide further insights into similarity.

IMMUNOGENICITY OF V116 (21-VALENT PCV) IN PNEUMOCOCCAL-NAÏVE ADULTS 50–64, 65–74, AND ≥75 YEARS OF AGE: SUBGROUP ANALYSIS OF A RANDOMIZED PHASE 3 TRIAL (STRIDE-3).

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Background

V116 is an investigational PCV containing the most prevalent serotypes associated with pneumococcal disease (PD) in adults in regions with established pediatric vaccination programs. In the Phase 3 STRIDE-3 study (NCT05425732) in adults ≥50 years of age (Cohort 1), V116 was non-inferior to PCV20 for the 10 common serotypes and was superior for 10 of 11 serotypes unique to V116, as measured by opsonophagocytic activity (OPA) geometric mean titers (GMTs) 30 days post-vaccination. The safety profile of V116 was comparable with PCV20. This subgroup analysis of STRIDE-3 evaluated immunogenicity in adults ≥50 years by age groups.

Methods

Participants were randomized 1:1 to receive one dose of V116 or PCV20 and were stratified by age (50–64, 65–74, 75–84, and ≥85 years of age). Serotype-specific OPA GMTs were evaluated at baseline and 30 days post-vaccination (Day 30).

Results

V116 was immunogenic for all serotypes included in the vaccine. Serotype-specific OPA GMT ratios at Day 30 for the 10 common serotypes with PCV20 and the 11 unique serotypes across all age groups were consistent with the overall population. A trend toward lower immune responses was observed in adults 65–74 years and ≥75 years compared to adults 50–64 years.

Conclusions

V116 was immunogenic for all 21 vaccine serotypes across all age subgroups, with responses higher than PCV20 for serotypes unique to V116. These findings support V116 as a novel population-specific vaccine for the prevention of PD in older adults.

PROLONGED VIM-PRODUCING PSEUDOMONAS AERUGINOSA OUTBREAK AT A BELGIAN INTENSIVE CARE DEPARTMENT, AN OUTBREAK INVESTIGATION REPORT 2019-2023.

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Background

Outbreaks due to Verona integron-encoded metallo-β-lactamase (VIM)-producing *Pseudomonas aeruginosa* (VIM-PA) frequently stem from sink-drains. Genome analysis facilitates linking nosocomial infections to pathogens found in environmental reservoirs. Understanding hospital epidemiology is fundamental for outbreak management.

Study's aim

Investigate the VIM-PA outbreak occurring at four intensive care units (ICU) of a Belgian tertiary university hospital.

Methods

Between 01/01/2019 and 30/07/2023, data were retrospectively retrieved from the ICUs labeled as Unit 1, Unit 2, Unit 3, and Unit 4. Whole genome sequencing (WGS) of VIM-PA was carried out for available clinical and sink-drain samples with Novaseq6000, Illumina, USA. The core genome multilocus sequencing typing (cgMLST) scheme of Blanc et al. was used to confirm clonality with Bionumerics. Estimation of new case incidence was performed by analyzing the weekly data of at-risk and VIM-PA colonized patients, fitting a Poisson model.

Results

51 patients satisfied the criteria of colonization and 32 (63%) of them met the definition of infection with VIM-PA. The median patient age was 62 years, 55% underwent surgery, and the median days of endotracheal intubation were 36. PA-VIM infection was partially responsible for 7 deaths. The outbreak investigation showed that 19 (53%) of the examined sink-drains grew at least once a VIM-PA.

WGS was performed for 68 VIM-PA, 19 environmental, and 49 clinical isolates. Two clusters were observed by core genome sequencing: Sequence Type (ST) 111 with 57 clones (17 environmental, 40 clinical isolates), and ST 17 with 8 clones (2 environmental, 6 clinical isolates).

The estimated incidence rate of new cases was different between ICUs. Unit 2 had a significantly shorter waiting time before a new predicted case, while Unit 1, and Unit 3 waiting time was longer and comparable.

Conclusions

The ICUs of our center experienced a five-year prolonged outbreak caused by only two VIM-PA clones with minimal mutations over the years, both linked to sink-drains. Statistical modeling showed different estimated incidence rates between units. Ad hoc intervention measures were hence prioritized.

HEALTHCARE-ASSOCIATED INFECTIONS AND ANTIMICROBIAL USE IN BELGIAN ACUTE CARE HOSPITALS: RESULTS OF THE 2022 ECDC POINT PREVALENCE SURVEY.

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Background

Healthcare-associated infections (HAIs) and antimicrobial resistance (AMR) pose significant challenges in healthcare systems worldwide, leading to prolonged hospital stays, increased costs, and elevated morbidity and mortality rates. The 2017 European Centre for Disease Prevention and Control (ECDC) point prevalence survey (PPS) revealed a 5.9% HAI prevalence in European acute care hospitals, with Belgium reporting a higher rate of 7.3%. Additionally, in 2017, a crude prevalence of 28.1% of inpatients receiving at least one antimicrobial was recorded in Belgian hospitals. In 2022, the ECDC PPS was repeated in Belgian acute care hospitals to reassess both HAI prevalence and antimicrobial use.

Methods

Data collection for the 2022 survey was conducted from September to November, following the ECDC protocol (version 6.0) at hospital/ward/patient levels. Modifications from the 2017 protocol included updated infection definitions and microorganism codes for COVID-19, along with the incorporation of HAIs associated with long-term care facilities.

Findings

A total of 57 acute care hospital sites participated, encompassing 10,142 patients. The 2022 survey revealed that 9.2% (95% confidence interval (CI): 8.7-9.8%) of patients had at least one HAI. This prevalence was 8.5% (95% CI: 7.5-9.5%) when excluding HAI associated with long-term care facilities. Predominant infections included pneumonia and lower respiratory tract infections (32.8%, including COVID-19 infections at 8.5%), surgical site infections (13.6%), urinary tract infections (18.5%), bloodstream infections (12.2%) and gastrointestinal infections (9.0%). Microbiological results were positive for 65.6% of HAIs, with *Escherichia coli, Staphylococcus aureus*, and *Klebsiella spp.* being the most common isolates.

Regarding antimicrobial use, the survey found that 29.3% (95% CI: 28.4-30.2%) of patients were receiving at least one antimicrobial, with higher prevalence observed in patients over 65 years (31.1%). Intensive care units (56.3%) and surgical wards (38.7%) demonstrated the highest antimicrobial use prevalence, while psychiatric wards exhibited the lowest (3.0%). Common indications for antimicrobial treatment included community-acquired infections (48.6%) and HAIs (26.1%). Indication for surgical prophylaxis was recorded for 12.4% of prescribed antimicrobials. Notably, 22.7% of surgical prophylaxis courses

lasted more than one day. The top three most prescribed antimicrobial agents were amoxicillin in combination with a beta-lactamase inhibitor, cefazolin, and piperacillin in combination with a beta-lactamase inhibitor.

Conclusions

The 2022 ECDC PPS revealed an increased prevalence of both HAIs and antimicrobial use in Belgian acute care hospitals compared to previous surveys. This emphasizes the ongoing need for rigorous infection prevention and control measures, as well as robust antimicrobial stewardship programs, to address these challenges effectively. Future investigations should focus on prescription attitudes and modifiable practices to optimize patient outcomes and mitigate the spread of AMR.

THIRD DOSE OF COVID-19 MRNA VACCINE CLOSES THE GAP IN IMMUNE RESPONSE BETWEEN NAÏVE NURSING HOME RESIDENTS AND HEALTHY ADULTS.

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Background

Nursing home residents, a frail and old population group, respond poorly to primary mRNA COVID-19 vaccination. A third dose has been shown to boost protection against severe disease and death in this immunosenescent population, but limited data is available on the immune responses it induces.

Methods

In this observational cohort study, peak humoral and cellular immune responses were compared 28 days after the second and third doses of the BNT162b2 mRNA COVID-19 vaccine in residents and staff members of two Belgian nursing homes. Only individuals without evidence of previous SARSCoV-2 infection at third dose administration were included in the study. In addition, an extended cohort of residents and staff members was tested for immune responses to a third vaccine dose and was monitored for vaccine breakthrough infections in the following six months. The trial is registered on ClinicalTrials.gov (NCT04527614).

Results

All included residents (n = 85) and staff members (n = 88) were SARS-CoV-2 infection naïve at third dose administration. Historical blood samples from 28 days post second dose were available from 42 residents and 42 staff members. Magnitude and quality of humoral and cellular immune responses were strongly boosted in residents post third compared to post second dose. Increases were less pronounced in staff members than in residents. At 28 days post third dose, differences between residents and staff had become mostly insignificant. Humoral, but not cellular, responses induced by a third dose were predictive of subsequent incidence of vaccine breakthrough infection in the six months following vaccination.

Conclusion

These data show that a third dose of mRNA COVID-19 vaccine largely closes the gap in humoral and cellular immune response observed after primary vaccination between NH residents and staff members but suggest that further boosting might be needed to achieve optimal protection against variants of concern in this vulnerable population group.

THE PREVALENCE OF PATHOGENS IN TICKS COLLECTED FROM HUMANS IN BELGIUM, 2021 VERSUS 2017.

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Background/ Aim

Ticks can carry a wide variety of bacteria, viruses, and parasites of which some are pathogenic to humans. The current study analyzed the prevalence of such pathogens in ticks biting humans in 2021 and compared the results with 2017.

Methods

Belgian citizens were invited to send ticks removed from their skin by postal mail and to fill in a short questionnaire on the citizen science platform TiquesNet.be. Ticks were microscopically identified to species and life stage level and further screened for the presence of *Borrelia burgdorferi* (s.l.), *Anaplasma phagocytophilum, Borrelia miyamotoi, Babesia spp., Rickettsia helvetica, Neoehrlichia mikurensis* and tick-borne encephalitis virus (TBEV), using multiplex qPCR methods.

Results

Over the tick season 2021, a total of 1,094 hard ticks were collected. As in 2017, the large majority were *Ixodes ricinus* (98.7%) and few *Ixodes hexagonus* (0.8%) and *Dermacentor reticulatus* ticks (0.5%) were identified. Out of 928 nymphs and adults screened for the presence of pathogens, 9.9% (95% CI: 8.2–12.0%) were infected with *B. burgdorferi* (s.l.) which is significantly lower than the prevalence of 13.9% (95% CI: 12.2–15.7%) found in 2017. In contrast, the prevalences of *A. phagocytophilum* (4.7%; 95% CI 3.5–6.3%) and *R. helvetica* (13.3%; 95% CI 11.2–15.6%) were significantly higher in 2021 compared to 2017 (1.8%; 95% CI 1.3–2.7% and 6.8%; 95% CI 5.6-8.2% respectively). Similar to 2017, no TBEV was detected in the ticks. For the other pathogens, prevalences ranged between 1.5% and 2.9% in 2021 and no statistical differences compared to 2017 were found. *R. raoultii* was again detected in *D. reticulatus ticks* (n=3/5 in 2021). Co-infections were found in 5.1% of ticks.

Conclusion

As in 2017, all pathogens except TBEV were detected in the collected ticks in 2021. When comparing prevalences for both years, differences were found for some pathogens, yet all results fell within expectations. Whether it concerns annual fluctuations or trends over time needs further investigation.

GENOTYPING AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA AURIS ISOLATES DETECTED IN BELGIUM FROM 2016 TO 2023.

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Background

Candida auris is considered a serious global health problem Initially discovered in Japan in 2009, it has rapidly spread globally , with its first detection in Belgium in 2016. Genomic epidemiology suggests the simultaneous emergence of six distinct clades of *C. auris* on different continents. This yeast is characterized by a high rate of antifungal resistance, with documented cases of pan-resistance.

Materials/Methods

In the context of epidemiological surveillance in Belgium, *C. auris* isolates received by the National Reference Center (NRC) at UZ Leuven undergo susceptibility testing and genotyping . Antifungal susceptibility testing is performed using the European Committee for Antimicrobial Susceptibility testing (EUCAST) broth microdilution reference method. Due to the absence of established speciesspecific clinical breakpoints for *C. auris* in EUCAST guidelines, precise susceptibility interpretation is difficult. To provide an indication of susceptibility, tentative breakpoints from the CDC are used for interpretation Molecular genotyping was achieved through short tandem repeat analysis, followed by whole genome sequencing using the Oxford Nanopore technology.

Results

Between 2016 and 2023, the NRC received 14 isolates, consisting of six infection and eight colonization cases. Notably, in t hree cases there was no recent exposure to foreign healthcare . The highest number of new isolates, six in total, was detected in 2023. All *C. auris* strains underwent susceptibility testing, and with the exception of one strain, geno typing was also conducted. Out of the 13 strains typed, all but two were classified as clade I (South Asian). The remaining two strains were identified as belonging to clade III (African Based on the tentative breakpoints from the CDC 93% (13/14) of isolates demonstrated resistance to fluconazole, 7% (1/14) resistance to amphotericin B and 14% (2/14) resistance to echinocandins. No pan-resistant isolates were identified.

Conclusion

In Belgium, there is a rising number of *C. auris* cases, with the South Asian clade being predominant. The true incidence is likely underestimated , as some cases lack a clear travel history To date , there have been no reports of outbreaks in our country, nor was resistance to more than two antifungal classes identified . Continuous surveillance is fundamental to prevent further spread. Whole genome sequencing is currently ongoing to characterize all 14 Belgian *C. auris* cases in more detail.

CITIZEN SCIENCE AS AN EFFECTIVE TOOL TO DETECT THE TIGER MOSQUITO (AEDES ALBOPICTUS) IN BELGIUM.

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Background

The tiger mosquito (*Aedes albopictus*) is an important vector of arboviruses. Over the past two decades, the species has invaded and expanded its range in Europe. In countries where it is established, it is responsible for local transmission of chikungunya, dengue and Zika. In Belgium, since 2012, its introduction is followed by active surveillance at points of entry such as parking lots along highways, used tire or lucky bamboo import companies, with tiger mosquitoes detected almost yearly. In order to expand the surveillance countrywide, passive surveillance through a citizen science platform was implemented in 2022. We present the results of the first two years of the surveillance based on citizen science.

Methods

Citizens can notify a tiger mosquito by uploading a picture on the online platform. After passing three filtering questions, the notifier provides the sighting location and his/hers contact details. The species is confirmed by morphological identification. In case of a tiger mosquito, the notifier is contacted and a field inspection for confirmation is scheduled.

Results

In 2022, 177 notifications were made via the platform. Of these, twelve tiger mosquitoes were notified from nine different locations, all except for one found inside houses and gardens in (semi-) urban areas. In 2023, the number of tiger mosquitoes notified doubled, with 27 notified in 18 different locations. There were 3 locations that were notified in both years (Lebbeke, Wilrijk, and Wondelgem), and we could confirm overwintering in two locations (Lebbeke and Wilrijk).

Conclusion

Belgium is currently at the invasion front of the tiger mosquito. Citizen science as a tool for surveillance has proven to be very effective in discovering new locations with tiger mosquitoes which wouldn't have been detected through active surveillance. Promoting the citizen science platform in the coming years will increase our understanding about the presence, introduction and establishment of the tiger mosquito in Belgium.

UNCOVERING GAPS IN KNOWLEDGE: A SURVEY OF BELGIAN GENERAL PRACTITIONERS' AWARENESS OF LEGIONNAIRES' DISEASE DIAGNOSTIC TESTING.

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Background

The epidemiological data from Europe reveals a consistent rise in diagnosed Legionnaires' disease cases over the last decade. Belgian general practitioners (GP) are expected to face a growth in LD's suspicions, a disease once known to be rare. However, their knowledge and understanding about this infection, its treatment guidelines, diagnostic procedures, and access to its diagnostic testing, are not yet proved to be integrated into their routine workflow. This study assessed Belgian GPs' knowledge of LD and the accessibility of diagnostic tests in their practices.

Methods

A questionnaire was distributed to practicing GPs, including primary care trainees, between January 31st, 2022, and March 13th, 2022. This survey targeted approximately 4200 GPs with an estimated population catchment of 22% of the actively working Belgian GPs.

Results

The response rate was estimated at 3%. Overall, the respondents demonstrated a satisfactory understanding of occurrence, risk factors, and clinical manifestations. While 62% preferred the Legionella urinary antigen test as the primary diagnostic method, 75% were unsure about its availability within their laboratories, and 82% never prescribed it within the year. When a strong clinical suspicion persisted, 30% of participants would contact a specialist, while 13% would ask a serology and 12% a PCR on a respiratory sample.

Conclusion

Belgian GPs should evaluate the possibility of conducting UAT testing and/or specific PCR on respiratory samples in their laboratories to enhance LD case management and improve their preparedness. Furthermore, initiatives should be undertaken to boost communication between the LD specialists and Belgian GPs.

RAPID MOLECULAR TYPING OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O157 BY IS629-PRINTING FOR CLUSTER DETECTION AT THE BELGIAN NATIONAL REFERENCE CENTRE.

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In 2019, 125 different STEC strains were referred to the Belgian National Reference Centre (NRC) for Shiga toxin-producing *Escherichia coli* (STEC). Due to limited resources full characterization of these isolates by whole-genome sequencing (WGS) is only performed in batch monthly. Determination of O-serogroups and virulence factors (siga toxin [*stx*] subtype, *eae*A, *aai*C and *agg*R) is sufficient to rapidly identify clusters of non-O157 STEC strains but not clusters of O157 STEC strains. For the latter, representing 65 isolates in 2019, IS629-printing, completed with epidemiological information, is used. Starting from isolated colonies, IS629-printing is a rapid method providing results in the same working day. It has been shown to be accurate in food-born outbreaks with confirmed epidemiological links although not as discriminatory as Pulsed Field Gel Electrophoresis or WGS. In this study, IS629 fingerprints and core genome multilocus sequence typing (cgMLST) data from O157 strains isolated in 2019 at the Belgian NRC were compared in order to analyse the discriminatory capacity of IS629-printing.

All 65 O157 strains isolated at the Belgian NRC in 2019 were typed by IS629-printing and *cg*MLST. IS629 fingerprints were analysed using the BioNumerics software (Applied Maths, BioMérieux). WGS was performed by the Brussels Interuniversity Genomics High Throughput core (BRIGHTcore). Sequencing libraries were prepared using the KAPA Hyper Plus kit (Kapa Biosystems) and sequenced on Illumina NGS instruments. The sequencing data was analysed using the *Escherichia / Shigella cg*MLST typing scheme in EnteroBase. The cluster threshold is set at hierarchical clustering level HC5, i.e. all strains in this cluster have links no more than 5 alleles apart.

Overall, eleven clusters of two to ten cases were identified by IS629-printing. Epidemiological data confirmed relatedness of the involved cases in only three clusters: two familial clusters and one day-care-associated cluster. All three were confirmed as HC5 clusters by cgMLST analysis. Six additional clusters of two to three cases, with indistinguishable IS629 profiles and without epidemiological links, were also identified as HC5 clusters. The last two clusters were not assigned to a same HC5 cluster. It is to be noted that the strains within the latter two clusters belonged to a same HC20 cluster, which means that the strains are closely related but do not meet the HC5 clustering criteria. Regardless of the typing method used, epidemiological information such as epidemiological links between the cases and time interval between the cases should always be used to confirm clusters.
The obtained results stress the utility of IS629-printing for daily surveillance of STEC O157 infections at the Belgian NRC, allowing prompt alerting of health authorities.

COMPARISON OF BELGIAN COVID-19 MORTALITY BETWEEN EPIDEMIOLOGICAL SURVEILLANCE AND DEATH CERTIFICATES FOR THE YEARS 2020 AND 2021.

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Background/Aims

Sciensano, in collaboration with health authorities, set up an ad hoc epidemiological COVID-19 mortality surveillance to monitor the severity of the epidemic in real-time, as the processing of death certificates has a 3-year delay. This study aims to compare COVID-19 mortality data collected from hospitals, long-term care facilities (LTCF), and general practitioners through epidemiological surveillance with that obtained from death certificates.

Methods

Via a one-to-one linkage, a match-mismatch analysis was conducted between the epidemiological surveillance of COVID-19 mortality (SURV) and death certificates (COD) for the years 2020 and 2021. Impact of factors such as region and place of death, case classification (U07.1 and U07.2 as underlying cause of death (UCOD)) and epidemic wave were analyzed.

Results

In 2021, SURV identified 85% (n=8,561) of COVID-19-associated deaths from COD (n=10,052), compared to 2020 (90%, n=19,801). For both years together, there was high coverage via hospital (98%) and LTCF (87%) surveillances, but low coverage for COVID-19 deaths occurring at home (3% with 1,703 missing deaths). However, it's noteworthy that while LTCF surveillance coverage was robust at 90% in 2020, it declined significantly to 69% in 2021. One-to-one matching revealed that 84% of SURV records listed COVID-19 as the UCOD in COD, and 75% of COVID-19 deaths in COD were identified in SURV.

Diagnostic uncertainty, mainly in 2020, and a reduction in LTCF surveillance participation throughout 2021, resulted in lower agreement between databases. Among the 3,646 deaths collected via SURV, not labeled COVID-19 as UCOD in COD, the most frequent UCODs reported in the COD included: other ill-defined and unspecified causes (6.3%), pneumonia (5.5%), unspecified dementia (4.2%), heart failure (4.0%), and other COPD (3.3%).

Conclusion

This comparative analysis shows that the quality of SURV in 2021 was still high, reflecting healthcare professionals' commitment to real-time COVID-19 mortality monitoring. However, there remains room for improvement in refining surveillance strategies for future pandemics.

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MONITORING OF THE IMMUNE RESPONSE TO SARS-COV-2 IN VULNERABLE POPULATIONS: THE COVICO STUDY (2023-2026).

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Background

Vulnerable populations have been characterized to exhibit attenuated immune responses to SARS-CoV-2 compared to the general population. Hence, it is imperative to monitor their immune responses over time.

Methods

This four-years Belgian multicenter prospective cohort study encompasses 106 working-age individuals (WAP) and 145 vulnerable subjects, including 39 nursing home residents (NHR), 30 lung transplant recipients (LTR), 48 kidney transplant recipients (KTR), and 28 hemodialyzed patients (HP). Participants undergo thrice-yearly follow-ups including blood sampling and surveys (vaccination status, COVID-19 infections, and flu-like symptoms) in February (v1), June (v2), and October (v3). Over 2023, SARS-CoV-2 anti-receptor binding domain (RBD) specific IgG concentrations (v1, v2, v3) and neutralizing antibody titres (nAb) against SARS-CoV-2 Wuhan, Delta, and XBB.1.5 (v1,v2) and BA.5 (v1) were assessed. NAb responses against XBB.1.5 and BA.5 were evaluated only when nAb against Wuhan >300 IU/ml. Within-and-between group comparisons were conducted on paired cohorts using non-parametric ANOVA with random effect.

Results

Anti-RBD IgG titres exhibit stability over time across all five groups, with a decline observed for KTR ($p=0.005$) and HP ($p=0.004$). LTR demonstrate significantly lower anti-RBD IgG titres compared to the other groups, with ~10% exhibiting an undetectable response. NAb responses against Delta, BA.5 and XBB.1.5 exhibit a ~3-fold, ~5-fold and ~14-fold decreased respectively compared to the response against Wuhan, with LTR and KTR standing out with lower titres compared to the 3 other groups. No significant evolution was observed in nAb response, except for WAP against Wuhan ($p=0.0003$) and Delta ($p<0.0001$), and HD against Wuhan

($p<0.0001$). Undetectable nAb responses against Wuhan and Delta are mainly observed in LTR (37% and 47% at v2 respectively). High rates of undetectable nAb against XBB.1.5 responses are exhibit across all the groups (range of 28-66% at v2).

Conclusion

The immune response remains stable over time, with slight declines in KTP, HP and WAP. LTR display comparatively weaker humoral response. These findings underscore the importance of ongoing monitoring and potentially additional booster doses, particularly for vulnerable populations, to maintain protection against emerging variants of SARS-CoV-2.

A PHASE 3 CLINICAL STUDY TO EVALUATE THESAFETY, TOLERABILITY, AND IMMUNOGENICITY OF V116 IN PNEUMOCOCCAL VACCINE-EXPERIENCED ADULTS 50 YEARS OF AGE OR OLDER (STRIDE-6).

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Background

Pneumococcal diseases (PD), including non-invasive disease such as pneumonia and invasive disease such as meningitis, cause considerable morbidity and mortality in adults. V116 is an investigational 21-valent pneumococcal conjugate vaccine (PCV) specifically designed to protect adults from pneumococcal serotypes responsible for the majority of residual PD. This phase 3 study evaluated safety, tolerability, and immunogenicity of V116 in pneumococcal vaccine-experienced adults ≥50 years.

Methods

A total of 712 generally healthy adults were vaccinated with a single dose of pneumococcal vaccine as follows: Cohort 1 previously received PPSV23 and were randomized 2:1 to receive V116 or PCV15, respectively; Cohort 2 previously received PCV13 and were randomized 2:1 to receive V116 or PPSV23, respectively; Cohort 3 previously received PPSV23+PCV13, PCV13+PPSV23, PCV15+PPSV23, or PCV15 and all received open-label V116. Immunogenicity was evaluated 30 days postvaccination using opsonophagocytic activity (OPA) geometric mean titers (GMTs) for all V116 serotypes. Safety was evaluated as the proportion of participants with adverse events (AEs).

Results

V116 was immunogenic across all 3 cohorts as assessed by serotype-specific OPA GMTs postvaccination for all 21 serotypes. V116 elicited comparable immune responses to serotypes shared with PCV15 (Cohort 1) or PPSV23 (Cohort 2), and higher immune responses to serotypes unique to V116. The proportions of participants with solicited AEs were generally comparable across cohorts.

Conclusions

V116 is well tolerated with a safety profile comparable to currently licensed pneumococcal vaccines, and generates functional immune responses to all V116 serotypes, regardless of prior pneumococcal vaccine received.

EPIDEMIOLOGY OF *TROPHERYMA WHIPPLEI* IN BELGIUM: INSIGHTS FROM THE NATIONAL REFERENCE LABORATORY

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Background

Whipple's disease, caused by *Tropheryma whipplei*, iis rare. Recent studies reported an estimated incidence of 3-10/1.000.000 people. It predominantly affects middle-aged men. Classical WD manifests as a multisystem disorder marked by joint pain (70-90%) and diarrhea (70-85%). A less common presentation is localized chronic infection in which patients experience symptoms related to the affected location.
Literature on the bacterium and disease is limited and not always consistent. We provide an overview of the epidemiology based on our own data and compare these with literature. Additionally, a new request form was created to enhance future monitoring.

Methods

This retrospective study includes patients with samples analyzed from 2020 until 2022 using PCR fo *T whipplei*. ,The study involves data collection and analysis. Epidemiological data were compared with literature.

Results

On average WD was confirmed in five patients per year, yielding an estimated incidence of 0.43/1.000.000 people in Belgium. This is significantly lower compared to literature, possibly due to incomplete data (only data from the National Reference Laboratory), underdiagnosis or geographical variations. Throughout the study 36 patients tested positive, leading to a diagnosis of WD in 15 patients. In nine patients the diagnosis was not retained and for the 10 remaining individuals information was incomplete. In patients with a confirmed diagnosis, we observed a 2:1 ratio of men-to-women, consistent with literature. Patients without a confirmed diagnosis show an approximately equal male-to-female ratio. The prevalence of confirmed WD was highest among individuals aged 41 to 60 years. Joint pain and fatigue were most commonly reported in both confirmed and unconfirmed diagnosis. Weight loss was significantly more present in patients with a confirmed diagnosis (79%) than without the diagnosis of WD (29%). Also, diarrhea was more common in this group. A new request form was developed, incorporating relevant variables.

Conclusion

Overall, the available data in Belgium are similar to those in literature. However, it's noteworthy that weight loss occurs more frequently in individuals diagnosed with WD. This finding can assist physicians in considering this rare disease. With the introduction of a new application form, we hope to gather more information on the epidemiology of this infectious disease.

IS EQUITABLE PRIORITY VACCINATION OF THE VULNERABLE FEASIBLE IN A REAL-WORLD CONTEXT? THE CASE OF BLEGIUM.

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Background

Belgium is the only country to have implemented a comprehensive, proactive, and equitable strategy to prioritize vaccination at population level during the COVID-19 pandemic . This prioritization targeted individuals with pre-existing health conditions, who were at increased risk of severe COVID-19. More than 1.5 million were identified through both centralized (Health Insurance Funds database) and decentralized (Electronic Health Records from Family Physicians) data as high-risk. We aimed to evaluate whether prioritized groups were vaccinated sooner, and which socio-demographic and -economic characteristics were related to the speed of vaccine uptake.

Methods

We calculated the time to vaccination between the start of the prioritization (1st April 2021) and receiving a first COVID-19 vaccine dose, using this interval as a proxy for evaluating the strategy's early impact. A multivariate regression model, incorporating priority status, age, sex, region of residence, income, and migration background, described the natural logarithm of this time gap. The data was obtained through the LINK-VACC project.

Results

The sample included 4,517,802 individuals vaccinated between 1st April and 31st December 2021, of which 26.3% were prioritized. The results show a 34.7 days earlier vaccination for prioritized individuals versus non-prioritized ones. The time difference between the prioritized and non-prioritized groups was larger in younger age groups compared to older age groups (27.7 days versus 19.3 days). Based on the multivariate model estimates, being prioritized ($\beta_{priority}$= -0.3, 95%CI (-0.38;-0.36)), older age ($\beta_{(55-64)}$= -0.56, 95%CI(-0.57;-0.55)), residency in Brussels or Wallonia $\beta_{\_Brussels}$= -0.18, 95%CI(-0.20;-0.16);$\beta_{\_Wallonia}$= -0.18, 95%CI(-0.19;-0.17)), having a high income ($\beta_{(high\ income)}$= -0.11, 95%CI(-0.12;-0.10)), being a Belgian national ($\beta_{belgian}$= reference) and being female (β_{female}= reference) are associated with a shorter time to vaccination.

Conclusion

Developing and implementing a prioritization vaccination strategy accelerated vaccination for the high-risk population with health conditions, demonstrating its feasibility in promoting equitable access to COVID-19 vaccines.

DETECTION OF MEASLES VIRUS GENOTYPE D8 IN WASTEWATER OF BRUSSELS CAPITAL REGION, BELGIUM, MARCH 2024.

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Background

The European Centre for Disease Prevention and Control (ECDC) issued a threat assessment brief in response to the significant increase in measles case numbers and outbreaks that have been observed since 2023. With a notification rate of 5.94 per 1 million population in 2023, Belgium was among the countries with the highest measles rate of Europe, and it's 2 dose vaccination coverage (~83% in 2022) is below the European average. The high probability of measles importation from regions with substantial circulation combined with the upcoming seasonal peak of the virus and sub-optimal vaccination coverage can likely result in a continued increase in measles cases in Belgium in 2024. To investigate the degree of viral circulation, we examined sewage samples from different locations in Belgium for the presence of MV.

Methods

We initiated measles testing on samples from wastewater treatment plants (WWTP) of Leuven, Brussels and Antwerp. In these WWTPs, samples of 24 hours influent wastewater were obtained with a time proportional sampler. Molecular detection of measles virus RNA was performed by real-time RT-PCR. The WHO guidelines recommend that the sequence of the 450 nucleotides encoding the carboxyterminal 150 amino acids of the nucleoprotein should be used as the minimum amount of sequence data required for determining the MV genotype.

Results

Five samples taken from Brussels North were positive for measles virus with Ct values ranging from 39.6 to 35.6. Leuven, Antwerp and Brussels South remain negative.

Detailed phylogenetic comparison of the partial MV sequence retrieved from the Brussel North wastewater sample to reference sequences (WHO) showed highest similarity to genotype D8, which is currently circulating in Europe. It was most closely related to recent strains from Romania, where an outbreak has been ongoing since mid-February 2023 and a national measles epidemic was declared on December 5th 2023.

Conclusion

The methodology outlined in this paper enables highly sensitive genotyping of MV from wastewater. With this method, we detected the presence of MV genotype D8 in wastewater samples of Brussels North during 3 consecutive weeks, indicating substantial viral circulation in this region. We believe that wastewater testing for measles can offer a valuable surveillance tool to trace hot spots of virus circulation.

CLOSING THE GAP: OXFORD NANOPORE TECHNOLOGIES R10 SEQUENCING ALLOWS COMPARABLE RESULTS TO ILLUMINA SEQUENCING FOR SNP-BASED OUTBREAK INVESTIGATION OF BACTERIAL PATHOGENS.

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Background

Whole-genome sequencing has emerged as the primary approach for investigating bacterial outbreaks, with short-read Illumina sequencing being widely adopted by clinical and public health laboratories. However, long-read Oxford Nanopore Technologies (ONT) sequencing, particularly with the recent advancements in R10 chemistry promising lower error rates, has gained traction as a potential alternative. Nevertheless, the performance of ONT sequencing, especially in SNP-based outbreak investigations, remains insufficiently explored.

Methods

Here, we introduce an open-source workflow, Prokaryotic Awesome variant Calling Utility (PACU), accessible at https://github.com/BioinformaticsPlatform-WIV-ISP/PACU, designed for constructing SNP phylogenies from both Illumina and ONT R9/R10 sequencing data. We evaluated the workflow using outbreak datasets of Shiga toxin-producing *Escherichia coli* and *Listeria monocytogenes*, comparing ONT R9 and R10 results with Illumina data. Our assessment not only examines each sequencing technology separately but also integrates samples sequenced by different technologies/chemistries into the same phylogenomic analysis.

Results

PACU demonstrates accurate identification of outbreak clusters for both species across all technologies/chemistries, with ONT R9 results displaying slight deviations from the Illumina reference results. The ONT R10 results closely resembled the Illumina data, indicating its potential for reliable integration into phylogenomic analyses. Notably, integrating datasets sequenced by either Illumina or ONT R10 for different isolates yields stable and highly accurate phylogenies. Furthermore, our study determined that approximately 20 hours of sequencing with ONT R9 and 8 hours with ONT R10 are sufficient to stabilize the resulting phylogenies for the two outbreak datasets.

Conclusion

This study serves as a proof of concept for the effective use of ONT R10, either independently or combined with Illumina, to facilitate rapid and precise bacterial outbreak investigations.

MOLECULAR CHARACTERIZATION OF SEROGROUP B NEISSERIA MENINGITIDIS CLINICAL ISOLATES COLLECTED IN BELGIUM (2016-2022) AND ASSESSMENT OF THEIR PREDICTED BEXSERO VACCINE COVERAGE.

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Invasive meningococcal disease (IMD) caused by Neisseria meningitidis can result in life-threatening meningitis and septicemia. There are twelve serogroups of N. meningitidis, but most cases of IMD are caused by serogroups A, B, C, W, X and Y. In Europe, serogroup B (MenB) accounts for 51% of the documented cases and 74% of cases in infants under one year of age, as recently reported by ECDC. As a major cause of IMD, particularly in young children, genomic surveillance of circulating MenB strains and assessment of the potential impact of vaccination programs could help inform public health policy. Slide agglutination was used to determine the serogroups of 498 strains collected in Belgium between 2016 and 2022. Afterwards, whole genome sequencing (WGS) was used to characterize these strains, consisting of MenB (n=283), MenY (n=95), MenW (n=86), MenC (n=30), non-groupable isolates (n=2), MenE (n=1) and MenX (n=1). Coverage of the Bexsero vaccine was predicted using Bexsero Antigen Sequence Types (BAST), the genetic Meningococcal Antigen Typing System (gMATS), and the Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) index. Of the 283 MenB strains collected between 2016 and 2022, 82.5% (lower limit – upper limit: 73.5 – 91.5%) were predicted to be covered by the vaccine by gMATS, and 67.1% (95% CI: 61.4 – 72.4%) by MenDeVar. This study highlights the benefits of a pathogen surveillance program and the need for experimental characterisation of continuously evolving antigenic variants.

DESCRIPTION OF THE MEASLES CLUSTERS IN BELGIUM IN 2023-2024

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Background

Numbers of measles cases are rising globally and in the European region. In 2023, a total of 67 cases were observed in Belgium. Here, we will describe the different measles clusters in Belgium in 2023-2024, with a focus on the vaccination status of the cases.

Methods

The Belgian measles surveillance is based on different data sources. For the description of the clusters, data from mandatory notification of suspected and confirmed cases was used, and data from the cluster investigations done by the regions was collated.

Results

The first cluster occurred in Brussels in April 2023. The virus was imported from Afghanistan, contamination happened at the immigration registration office and school. There were 9 confirmed cases, vaccination status was unknown or incomplete.
Three clusters occurred in Wallonia. In November 2023 a family of 2 in Verviers was diagnosed after travelling to Russia and then contaminated 3 others, unvaccinated, in the neighborhood. A second cluster in Stavelot around the same time, started with an index case with unknown source of contamination. Others were infected in family or work setting. Vaccination status of the 5 cases was unknown or incomplete. A third cluster was seen in Namur in January 2024. A Romanian family got infected after receiving visitors from Romania. Nine cases were confirmed, their vaccination status was either non-vaccinated, incomplete, or unknown.
In Flanders a cluster of 15 cases involving 7 families in Sint-Niklaas started in October 2023. The source of infection of the index case remains unknown. The majority of infections happened in the family setting or at school. 73% of the cases was unvaccinated. One case was fully vaccinated, avidity testing documented secondary vaccine failure. In November 2023 there were also 3 family clusters in Limburg (4 cases, unvaccinated, recent travel to Russia), East-Flanders (2 cases, unvaccinated, recent travel to Romania) and Vlaams-Brabant (3 cases, 2 vaccinated for age with 1 vaccine, no source). In March 2024 a new outbreak in Vlaams-Brabant occurred, where a total of 4 people in a Romanian family, all unvaccinated, got sick.

Conclusion

Belgium had several outbreaks of measles in the different regions. They were contained relatively quick (<9 weeks) and contamination was limited. Vaccination status was unknown, incomplete or non-vaccinated in all but 1 case. Therefore we conclude the main priorities remain better registration of vaccinations and increasing vaccine coverage, especially for the second MMR dose, with a focus on the immunity gaps we are still witnessing.

EPIDEMIOLOGY OF *HAEMOPHILUS INFLUENZAE* ISOLATES FROM OTITIS MEDIA IN BELGIUM.

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Background

Haemophilus influenzae (Hi) is a leading cause of otitis media (OM) among children worldwide. Beta-lactams are the first-line treatment in Belgium, but resistance has recently emerged, mediated by various mechanisms. The aim of this study was to describe the profile of HI isolated from ear fluids in Belgium.

Methods

All *Hi* strains transmitted to the NRC between 2017 and 2023 for which clear information was provided suggesting ear infection were included. Identification were performed using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Biotypes and serotypes were determined by biochemical tests (Diatabs, Rosco Diagnostica, Albertslund, Denmark) and Difco Haemophilus antiserum agglutination kit (Becton Dickinson, Erembodegem, Belgium), respectively, and following manufacturer's instructions. Antimicrobial susceptibility testing were performed using e-tests and following EUCAST breakpoints in force at the time. The *ftsI* gene was sequenced for any strain showing reduced susceptibility to beta-lactams. Microbiological data and demographics including age and gender were collected.

Results

Seventy-two strains sent to the NRC during the study period were clearly associated with ear infections. The majority were from children under 5 years of age (83.5%) and boys (58.3%). Most strains were non-typeable Hi (95,8%), with biotype II predominating (52.8%), followed by biotypes I (22.2%), III and V (both 12.5%). Around 20% of strains were beta-lactamase producers, while 41.6% (n=30) showed resistance to ampicillin and 11.1% to amoxicillin-clavulanic acid. Half of the strains were resistant to oral cefuroxime, 29.1% to trimetoprim-sulfamethoxazol and 5.6% to ciprofloxacin. Mutations in the *ftsI* gene associated with low and high levels of betalactam resistance were detected in 37.5% and 6.9% of strains, respectively. The latter has only been observed since 2022. These five strains showed MICs at the cut-off for ampicillin and amoxicilin-clavulanic acid, high cefuroxime MICs, and two of them proved resistant to cefotaxime.

Conclusion

Although strains isolated from ear infections are sent to the CNR on a voluntary basis and are therefore not representative of Belgian epidemiology, our observations suggest that beta-lactam-resistant strains are increasingly emerging, indicating the need for enhanced surveillance and a revision of treatment guidelines in the future.

EPIDEMIOLOGY OF INVASIVE *HAEMOPHILUS INFLUENZAE* INFECTIONS IN BELGIUM: 2018 - 2022.

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Background

Haemophilus influenzae plays a major role in invasive bacterial infections. Resistant strains are emerging, prompting the WHO to include *H. influenzae* on its list of priority pathogens for research and development of new antibiotics. We aimed to describe the serotypes, demographics and susceptibility profiles of invasive strains collected in Belgium over a 5-year period.

Methods

Data on invasive strains referred to the Belgian National Reference Center for *H. influenzae* from 2018 to 2022 were thus analyzed retrospectively.

Results

A total of 608 invasive strains were included. The number of notifications per year ranged from 85 to 165, with a marked decrease between 2020 and 2021. The highest incidence rate was observed in Brussels (1.56 per 100,000 inhabitants). Sex and age distribution were in line with global trends, as was the predominance of the non-typeable *H. influenzae* (NTHI). Beta-lactam resistance varies between molecules: 18.9% for ampicillin, 5.6% for amoxicillin-clavulanate. Mutations in the *ftsI* gene associated with decreased susceptibility to beta-lactams increased from 11.5% to 17.7% over the period studied.

The COVID-19 pandemic significantly influenced the epidemiology of invasive *H. influenzae* infections in Belgium. Despite this, demographic analysis revealed a notable male predominance among infants, highlighting a gender disparity not previously documented in the literature. The continued predominance of NTHI underscores the efficacy of Hib vaccination, although the emergence of Hib in patients younger than 5 years in 2022 suggests an alarming serotype dynamic. The detection of meropenem resistance also highlights the growing threat of antimicrobial resistance, while the increase in *ftsI* gene mutations raises concerns about the efficacy of first-line treatment.

Conclusion

This study provides a comprehensive overview of the epidemiology of invasive *H. influenzae* infections in Belgium, focusing on demographic changes, serotype predominance and antimicrobial resistance trends. Vigilant surveillance and research are essential to address emerging challenges and guide future interventions, including potential vaccine development.

NATIONAL REFERENCE CENTRE FOR SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (NRC STEC): ANNUAL REPORT 2023.

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In 2023, 335 cases of Shiga toxin-producing *Escherichia coli* (STEC) infections were reported to the National Reference Centre (NRC) STEC. These included 331 culture-confirmed cases of STEC. Twenty-nine patients were reported to have developed hemolytic uremic syndrome (HUS): two with serogroup O157, 8 with O26, 15 with non-O157 and 4 without STEC-positive culture. Four cases, including two cases of HUS, died as direct or indirect consequence of STEC infection.

A significant increase in number of STEC strains, non-O157 in particular, was observed in 2023 compared to the other years. Yet, this increase was already observed in 2022 as a probable consequence of the implementation of gastrointestinal molecular panels by a number of clinical laboratories. As seen in previous years, the majority (73/332; 22.0%) of the STEC isolates belonged to serogroup O157. Surprisingly, serogoup O63 was the second most common (43/332; 13.0%) serogroup in 2023. All non-O157 serogroups of the 'top 5' most common serogroups in the European Union/European Economic Area were represented: 34 O26 serogroup, 13 O91 serogroup, 20 O103 serogroup, 21 O145 serogroup and 18 O146 serogroup.

In 2023, 19.0 % (63/332) were *stx*1 positive, 53.9 % of the isolates were *stx2* positive (179/332), and 27.1 % were *stx1* and *stx2* positive (90/332). The majority of the STEC strains were at medium (50.0 %) or high (31.21 %) risk for developing HUS. The predominating Stx subtype profiles were: *stx1*a (51), *stx1*a+*stx2a* (23), *stx1*a+*stx2c* (33), *stx1*c+*stx2b* (20), *stx2a*(56), *stx2c*(23) and *stx2f* (58) (Table 3). Surprisingly, *stx2f* generally associated with mild symptoms, was the most common Stx subtype profile in 2023. The second most common Stx subtype profile, *stx2a* alone, was harboured by fourteen different serotypes, with O26:H11 predominating (n=23/56; 41.1 %). The new Stx2i subtype was identified for the first time in 2023 in an *eae*-negative strain of serotype O30:H25.

Twenty-four molecular clusters, including two to seven cases, were identified by whole-genome sequencing analysis in 2023. Six familial clusters could be identified based on the traditional typing data complemented with epidemiological data. On top of this, two non-HUS cases could be related to one cross-border cluster. As the source of contamination, unpasteurized milk, was known from EpiPulse inquiry 2023-FWD-00029, trace back investigations performed by the health inspection authorities could confirm this finding for one of the cases. Two additional EpiPulse inquiries, 2023-FWD-00078 (6 cases of O157:H7, *stx1a*, *stx2a*, *eae*-positive) and 2023-FWD-00080 (7 cases of O146:H28, stx2b, *eae*-negative), were posted by the NRC STEC. Two countries, Denmark and Sweden, responded positively to the latter inquiry. Yet, the source of contamination of this multi-country cluster could not be identified.

A DIAGNOSTIC FRAMEWORK FOR *TROPHERYMA WHIPPLEI* PROPOSED BY THE BELGIAN NATIONAL REFERENCE LABORATORY

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Background

Whipple's disease, caused by *Tropheryma whipplei*, presents in two main clinical forms. The classic, more common form is a multisystemic process with joint pain, gastrointestinal symptoms (diarrhea and abdominal pain) and weight loss. In severe cases neurological symptoms may be present. In the chronic localized form, the abovementioned characteristic symptoms are typically absent due to the lack of systemic *T. whipplei* infection. Endocarditis is the predominant localized infection.

Most patients present with nonspecific symptoms, making diagnosis difficult and slow. As national reference laboratory (NRL) for *T. whipplei* in Belgium, we aimed to create a guideline to support referring laboratories in diagnosing this disease.

Methods

An extensive literature review was conducted on *T. whipplei*, focusing particularly on studies comparing diagnostic techniques and investigating the value of specific sample types. Studies with sufficient evidence were retained. Based on this evidence, a guideline was developed including testing protocols, recommended sample types, and result interpretation.

Results

The flowchart covers guidelines for both classic and chronic localized forms of Whipple's disease. To diagnose the classic form, start with a molecular test on feces. A negative result suggests an alternative diagnosis. A positive result prompts duodenal biopsies for confirmation, both with PAS staining and PCR. If both tests on duodenal biopsy are positive, the diagnosis is confirmed. If only one of the techniques is positive, a tentative diagnosis is made. Diagnostic testing may be repeated on a new sample. Negative results for both techniques makes the diagnosis unlikely. If there are neurological symptoms, cerebrospinal fluid sampling is essential to rule out neuro-Whipple.

When suspecting the chronic localized form of the disease, ideally, samples should be taken from the presumed site of infection. These may include fluids and/or tissues. Processing of these samples and interpretation of the results is done similarly as described above for duodenal biopsies.

Conclusion

Given the absence of a uniform testing policy in literature, Belgium's NRL has introduced its own guidelines. This way, assistance to referring laboratories in diagnosing this rare disease is provided.

ROTAVIRUS EPIDEMIOLOGY SHIFTS DUE TO PANDEMIC: 14-YEAR EXPERIENCE OF BELGIAN NATIONAL REFERENCE CENTER IN THE COVID-19 CONTEXT.

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Background

The epidemiology of rotavirus infections has historically been characterized by distinct age and genotype distributions, with pronounced seasonality. This study, leveraging data from Belgium's National Reference Center, aims to elucidate the impact of the COVID-19 pandemic on these epidemiological patterns over a span of 14 years.

Methods

In this retrospective nationwide study, we analyzed the available demographic data of 8024 rotavirus positive stool samples collected from children and adults across Belgium from the 2009-2010 to the 2022-2023 seasons. Rotavirus seasons were determined from the 1st of August till 31st of July for next year. Samples were subjected to VP4 and VP7 genotyping by our national reference center. We compared age distribution, seasonality, and genotype frequencies before and after the emergence of COVID-19.

Results

Prior to the pandemic, the majority of rotavirus cases (90-95%) occurred in children under five, with peak incidences in March and April. During the pandemic, there was a notable decrease in the number of samples received: in the 2019-2020 and 2020-2021 seasons, the center received at total of only 314 samples, a stark reduction from the mean of 622 (min:max, 298:991) samples per seasons between 2009-2010 and 2018-2019. After the pandemic, the 2021-2022 season exhibited an expansion in the 2 to 5-year-old age group, which accounted for 33.5% of the cases, compared to the previous 12-year mean of approximately 19%. Additionally, the seasonal peak shifted to December-February, correlating with the relaxation of COVID-19 restrictions, in October 2021. Genotypic analysis revealed a marked post-pandemic dominance of G3P[8], which accounted for 86.2% and 82% of all cases after the pandemic, contrasting sharply with the pre-pandemic years where G3P[8] constituted between 1.6% to 50.8% of genotypes.

Conclusion

The COVID-19 pandemic appears to have significantly influenced the epidemiology of rotavirus in Belgium. Notable changes include age distribution of cases, received sample counts, altered seasonality, and a shift in genotype predominance. These findings highlight the necessity for continuous surveillance and may have implications for future public health strategies and vaccination policies.

RETROSPECTIVE STUDY ON *CAMPYLOBACTER* SPP. BACTEREMIA IN BELGIUM: 2014-2023.

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Background

Campylobacter spp. translocate and cause bacteremia with significant mortality in about 1% of gastrointestinal infections, especially in fragile patients. In literature, *C. jejuni, C. fetus* and *C. coli* together account for over 90% of these episodes. However, new *Campylobater* species are emerging. The aim of this study is to describe the ongoing epidemiological change of *Campylobacter spp.* bacteremia in Belgium.

Methods

All cases of *Campylobacter spp.* bacteremia detected in the pre-/post-COVID-19 period (2014-2018/2019-2023, T1/T2), and transmitted to the NRC from 2014 to 2023, were retrospectively included.

Results

During the 10-year study period, 307 cases were reported, including 22 in children. There were 152 cases during T1 and 155 cases during T2. Interestingly, 51 cases were reported in 2023, compared to the annual average of 28 reported in the preceding 9 years. During T1, *C. jejuni, C. fetus* and *C. coli* accounted for 56, 31 and 10% of the annual results, respectively. In T2, while *C. jejuni* and *C. coli* remained stable, *C. fetus* bacteremia decreased by one third (22% of annual isolates). In contrast, non-*C. jejuni/fetus/ coli*, namely *C. ureolyticus* (n=6), *C. lari* (n=3), *C. rectus* (n=1), *C. upsaliensis* (n=1), *Aliarcobacter butzleri* (n=1) and *Campylobacter spp.* (n=1), increased from 2% in T1 to 7% in T2. Indeed, 13 out of 14 were isolated since 2018. In particular, *C. ureolyticus* was isolated only once in T1 but 5 times in T2. Regarding resistance to amoxicillin/amoxicillin-clavulanate/erythromycin/ciprofloxacin, rates for *C. jejuni*, *C. fetus* and *C. coli* were 44/1/4/61%, 1/1/1/18% and 26/0/14/34% respectively. All non- *jejuni/fetus/coli Campylobacter* isolates were susceptible to amoxicillin, ciprofloxacin, tetracycline and erythromycin, except the three *C. lari* isolates resistant to amoxicillin and ciprofloxacin and one *C. ureolyticus* strain resistant to ciprofloxacin.

Conclusion

C. jejuni, followed by *C. coli* and *C. fetus*, remain the main species responsible for *Campylobacter* bacteremia in Belgium. However, other emerging species have been implicated in invasive infections, particularly *C. ureolyticus* since 2018, suggesting invasive potential for this species. In addition, careful monitoring will also be needed to confirm or refute the significant increase in cases reported in 2023.

ASSESSING THE PREVALENCE AND DYNAMICS OF EMERGING CAMPYLOBACTERALES IN HUMAN STOOL SAMPLES IN BRUSSELS BY FILTRATION CULTURE.

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Background

Since selective media were introduced into routine microbiology in the 1980's, campylobacteriosis has become recognized as the leading bacterial cause of human gastroenteritis worldwide with *C. jejuni/C. coli* accounting for more than 90% of the reported cases in Europe. However, so far the genus *Campylobacter* comprises 45 species, of which 15 have been associated with human infections and are obviously underdiagnosed. This study aimed to assess Campylobacterales diversity in clinical stool samples using filtration culture.

Methods

In our laboratory, stools for *Campylobacter* sp. isolation are cultured in a hydrogen-enriched microaerophilic atmosphere onto both a Butzler selective media incubated for 48h at 42°C and a Columbia agar incubated for 5 days at 37°C after selective filtration through 0.6µm polycarbonate filters. Results of all *Campylobacter* sp. cultures carried out for four hospitals in the pre/post-COVID-19 (2017-2019/2021-2023, T1/T2) periods were included.

Results

51,065 cultures were analyzed over the study period (6 years), with annual positivity rates ranging from minimum 5.9% (2018) to maximum 7.9% (2021). Notably, *C. concisus* and *C. curvus* represented 36.7-50.7% and 4.3-9.4% (min-max), respectively, of the annual isolates, whereas *C. jejuni* and *C. coli* represented only 21.1-32.8% and 1.8-5.0% (min-max), respectively. *C. upsaliensis* and *Aliarcobacter butzleri* followed, each averaging 1.4% of isolates per year. Another eleven species combined accounted for less than 3.5% of the total. From T1 to T2, mean numbers of *C. jejuni, C. coli* and *C. concisus* isolates decreased by 38%, 54% and 28% respectively ($p < 0.05$), while mean numbers of *C. curvus, C. upsaliensis* and *A. butzleri* isolates remained stable. Finally, *C. ureolyticus*, accounting for only 3.5, 4.1 and 9.0% of the yearly isolates in 2017/2018/2019, respectively, accounted for 20.0, 18.7 and 23.2% of the yearly isolates in 2021/2022/2023, with peaks in spring.

Conclusion

This is the first report of an increase in *C. ureolyticus* in human samples coinciding with a decline in *C. jejuni-C. coli* infections since the COVID-19 pandemic, raising concerns about its zoonotic potential and highlighting the need for accurate diagnostic tools and surveillance.

COMPREHENSIVE EPIDEMIOLOGICAL AND GENOMIC ANALYSIS OF ENTERIC CAMPYLOBACTER JEJUNI STRAINS IN BRUSSELS: INSIGHTS INTO ANTIMICROBIAL RESISTANCE AND VIRULENCE PROFILES.

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Background

Campylobacter jejuni is a leading cause of bacterial gastroenteritis in humans worldwide. The aim of this study is to explore the epidemiology and resistance characteristics of *Campylobacter* circulating in Brussels by analyzing all *C. jejuni* isolated from stool specimens over a one-year period in a multicentric university hospital in Brussels.

Methods

Over 90% of enteric *C. jejuni* isolates from LHUB-ULB in 2020 (n=152) were characterized by phenotypic testing and illumina whole genome sequencing (WGS). Resistome, virulome, multilocus sequence typing (MLST), whole-genome MLST (wgMLST) analyses were conducted using BioNumerics 8.1 tools (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility analysis was performed via disc diffusion tests and/or e-test for ampicillin, amoxicillin-clavulanic acid, erythromycin, tetracycline and ciprofloxacin.

Results

Subtyping of the 152 strains revealed 19 clonal complexes (CC) and 57 subtypes (ST). The most prevalent CCs were CC21 (30.9%), CC353 (11.2%), and CC13 (8.6%). Resistome analysis identified at least one β lactam resistance gene in 91.4% of isolates, the quinolone resistance *gyrA* T861 point mutation in 64.7%, tetracycline resistance *tet*(O) gene in 48% and the *aph* (3')-*III* aminoglycoside resistance gene in 6.5%. Macrolides resistant determinants were rare (<1.5%). A total of 133 virulence genes associated with adhesion, immune modulation, motility, cytotoxicity and invasion were screened. The virulence genes *cadF, cdtABC* cluster and *flaA* are the most reported virulence genes in literature and were respectively identified in 99.3%, 95.3%%, and 11.2% of our strains.

Conclusion

The correlation between WGS-based genotypic predictions and phenotypic resistance was robust, with rates of 98% for erythromycin, 97.3% for ciprofloxacin, 94.6% for tetracycline, and 61.9% for ampicillin. Through comprehensive phenotypic and genotypic characterization, this study provides epidemiological insights into the circulating strains of *C. jejuni* in Brussels in 2020, identifying virulence profiles and potential pathogenicity factors associated with these strains.

Q-NET-ASSESS PROJEC: IMPROVED MOLECULAR SURVEILLANCE AND ASESMENT OF HOST ADAPTATION AND VIRULENCE OF *COXIELLA BURNETII* IN EUROPE.

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The bacteria *Coxiella burnetii* is a zoonotic pathogen responsible for the Q fever disease in both animals and humans. The primary source of human infections is ruminant livestock, while *C. burnetii* can infect a variety of other animals, including wildlife and ticks. Although the majority of infections in ruminants are asymptomatic, *C. burnetii* infection can result in abortion, stillbirth, and poor offspring, particularly in sheep and goats. In humans, the clinical symptoms can vary from common flu-like symptoms to persistent and potentially fatal infections. Considering the range of hosts and the variety of outcomes of infection, the comprehension of the extent to which the genotype of *C. burnetii* influences this diversity is restricted.

The current *C. burnetii* genotyping techniques generates only limited information and are challenging to standardize across laboratories. Whole genome sequencing (WGS) is easily standardized and gives extensive genetic information. However, considering the challenges in isolating *C. burnetii* from field samples, only a small number of strains have been sequenced so far.

The project Q-Net Assess brings together a group of experts with distinct knowledge of *C. burnetii* surveillance and genetics to enable the collection of *C. burnetii* positive samples with precise clinical information from a variety of hosts, including ruminant livestock, wildlife, human, and environmental samples. Optimized isolation methods will be used to isolate *C. burnetii* from these field samples. To create a complete library of annotated *C. burnetii* genomes that includes phenotypic data from fieldwork and in vitro cellular assays, the isolated strains as well as available archived strains will be submitted to WGS. Novel bioinformatics techniques will be used to analyze WGS data to determine the molecular factors influencing *C. burnetii's* pathogenicity and host range. This information can be used to assess the risk and severity of future Q fever outbreaks. The findings from this project will be combined to create a recommended pan-European framework for *C. burnetii* molecular surveillance in the future.

BARTONELLA QUINTANA ENDOCARDITIS COMPLICATED WITH CEREBRAL STROKE: A CASE REPORT.

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Background

Bartonella quintana (BQ) is the causal-agent of trench-fever. The infection is endemic. Outbreaks are associated with poor sanitation and hygiene. It has been rarely associated with encephalopathy. We present a case of endocarditis complicated with a cerebral stroke.

Methods

Serology tests were performed with VIRCLIA®-system and IFA. CSF was tested by the BioFire-CSF-FilmArray-Meningitis/Encephalitis-Panel. CSF, plasma and biopsy samples were tested by a *Bartonella*-qPCR combined with Sanger-sequencing.

Results

A refugee (23-year-old-male) from Afghanistan, residing at the Red-Cross centre for asylum seekers of Fraipont-Belgium, was admitted for persisting fatigue and cough for months. A calcified aortic bicuspid with severe insufficiency and moderate associated stenosis was diagnosed. A transesophageal echocardiogram showed a severe shrinking valve and a mobile mass (0.4x0.9cm) attached to the calcification of the free edge at the aorta. He developed fever, a moderate inflammatory syndrome with a normocytic anemia, a renal failure with hematuria and proteinuria, indicating a probable glomerulonephritis. He had one major and three minor Duke-criteria for infective endocarditis. Repeated sets of blood-cultures were negative (day 17 to 30) but *Bartonella-serology* was positive (days 19, 31). Later (day 26), he developed fever and intense headache. CSF showed moderate pleiocytosis, but a negative-FilmArray. Empiric antibiotic therapy (intravenous ceftriaxone with amoxicillin) was initiated. A neurovascular MR-angiography showed a multifocal ischemic stroke. CSF showed a negative cytology. Doxycycline-gentamycin were added to the treatment (day 30, 31). The aortic valve was replaced (Ross-procedure, day 40). The biopsy showed nodular and degenerative fibro-calcified rearrangements. On day 47, amoxicillin-ceftriaxone were stopped following a positive BQ-result (Cq=35) on CSF from day 31. On day 51 and 53, respectively, BQ-presence was also documented on the blood sample from day 23 (Cq=34) and on the mitral

valve (Cq=26), which confirms the *Bartonella* endocarditis diagnostic. Treatment was shift to rifampicin-minocycline for a total duration of 6 weeks after surgery.

Conclusion

Our report underlines that BQ is a rare but possible imported agent of endocarditis and neurological damages and the necessity and benefits of effective health-care's access, which are unfortunately still often inaccessible in the countries of origin of migrants and even to migrants staying in high-resource countries.

HAVE WE FORGOTTEN HOW TO RIME? A CASE REPORT ON A RARE INFECTION-TRIGGERED MUCOCUTANEOUS COMPLICATION.

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Background

Reactive infectious mucocutaneous eruption (RIME) refers to severe mucositis with or without cutaneous rash, triggered by a bacterial or viral infection. *Mycoplasma pneumoniae* is the predominant pathogen triggering this phenomenon. RIME almost exclusively affects children and adolescents. Here, we present a rare clinical case of RIME in a male adolescent admitted to our hospital.

Case presentation

An otherwise healthy 17-year-old man presented to the Emergency Department of the OLV Hospital Aalst with symptoms of progressive sore throat, dry cough, swelling of mouth and lips, bloodshot eyes and fever. After chest X-ray revealed a consolidation, the patient was admitted to the Pulmonology Department with the suspicion of both a community-acquired pneumonia and (Herpes gingivo)stomatitis. At admission, a nasopharyngeal swab (NPS) tested negative for 17 respiratory viruses and 4 atypical bacteria included in our PCR panel. Furthermore, a local sample of the oral mucositis lesions and a blood sample both yielded negative results for Herpes simplex 1/2 and Varicella zoster virus.

During hospitalization, the mucositis progressed to an impressive epiglottitis threatening the free airway. A bronchoalveolar lavage was then performed, resulting in a lower respiratory tract (LRT) sample. In contrary to the NPS, this LRT sample tested positive for *Mycoplasma pneumoniae* (CT value 30.94 = moderate bacterial load). *M. pneumoniae* could not be detected in the mucositis lesions themselves, supporting the proposed indirect mechanism of action in RIME that local tissue damage results from the immune response triggered by a distant infection.

Finally, the definite diagnosis was "Community-acquired *M. pneumoniae* pneumonia, complicated with RIME manifesting as extensive oral aphthosis, epiglottitis, tracheitis and conjunctivitis". Intravenous clarithromycin was administered for 10 days, which together with a local mouthwash, analgesia and total parenteral nutrition ensured full recovery.

Conclusion

This case report aims to raise awareness for RIME, which is essential in the recognition of similar cases in clinical practice. Besides, if clinical suspicion of a *M. pneumoniae* infection persists, consider obtaining an LRT sample, as NPSs could test negative due to lower sensitivity or inadequate sample collection.

EPIDEMIOLOGICAL EVOLUTION OF SCABIES IN BELGIUM, 2000-2022.

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Background/Aims

Several countries in Europe reported an increase in scabies infestations over the last two decades. However, the full extent of scabies infestations in Belgium remains unknown as the disease is not notifiable. We aimed to characterize the available data sources on scabies and describe the epidemiological trends of scabies since 2000 to better inform public health stakeholders.

Methods

We conducted a descriptive, retrospective analysis of multiple non mutually-exclusive data sources. Using generalized linear models, we analysed yearly trends and seasonality of scabies diagnoses at: (i) general practitioners in Flanders (Intego network), (ii) occupational health services (IDEWE), (iii) shelters for asylum seekers (Fedasil), (iv) consultations with Médecins du Monde (MdM), and (v) student medical facilities in Leuven and Ghent. Additionally, we analysed reimbursement and sales data for permethrin and benzyl benzoate. Finally, we studied clusters notified at Regional Health Authorities.

Results

The incidence of scabies diagnoses in general consultations in Flanders increased yearly by 15% between 2011 and 2022 (p-value < 0.001), after a stable period from 2000 to 2010. This increasing trend was also observed nationwide in recent years in occupational health practices, student medical facilities, shelters for asylum seekers, and consultations at MdM. Scabies diagnoses exhibited seasonality, with higher incidences in autumn and winter. The age group 15-24 years and urban areas were most affected. There was no difference in incidence and trend between males and females (p-value > 0.5).

The most frequently reported settings for clusters were nursing homes, schools and childcare facilities.
Pharmaceutical data showed similar increasing trends. On average the number of permethrin tubes sold in Belgian pharmacies increased by 13,767 per year (p-value < 0.001) between 2012 and 2022.

Conclusion

The investigated sources showed an increasing trend in scabies since 2011, affecting both males and females similarly and being more pronounced in younger age groups. We recommend further studies to investigate the roles of reinfections and treatment failures contributing to this increase, and subsequently adapt scabies surveillance measures in Belgium.

SHOTGUN METAGENOMICS ON AIR: LONGITUDINAL SURVEILLANCE OF VIRAL PATHOGENS IN A DAYCARE CENTER.

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Co-corresponding authors: Emmanuel André & Jelle Matthijnssens

Background

Traditional viral pathogen surveillance relies on testing for a limited number of pathogens among symptomatic patients. These established approaches may overlook asymptomatic and mild infections and miss infections caused by untested or emerging pathogens. We employed Viral Like Particle (VLP) enriched shotgun metagenomics to analyze air samples from a daycare center for children up to three years old. We aimed to identify a spectrum of viruses and provide genomic insights into their presence in air samples, potentially identifying a novel method for environmental surveillance.

Methods

From January 2022 to December 2022, we collected 41 air samples using an AerosolSense active air sampler (Thermo Fisher Scientific) for two hours, during operational hours of the daycare center. VLPs were extracted and random amplified using The Novel Enrichment Technique of Viromes (NetoVIR) protocol1. High-throughput sequencing was performed on an Illumina NovaSeq 6000 system, yielding an average of 16 million reads per sample after quality control and trimming. We identified viruses using EsViritu2, using comprehensive database of human and animal viruses. Consensus sequences were further verified using BLASTN. Subsequently, we have employed de-novo assembly pipeline ViPER3 to identify various divergent viruses.

Results

Alongside the expected respiratory viruses (e.g. rhinoviruses and bocaviruses), our metagenomic analysis revealed the presence of a wide spectrum of viruses, including human-associated, animal-associated, plant and fungi viruses. Polyomaviruses were consistently detected in all but seven samples, with a notable prevalence of WU Polyomavirus during the summer and human polyomavirus 10 (HPyV10) throughout the rest of the year. Human and animal enteric viruses such as Rotavirus A, D, F, G, Sapovirus, Human Mastadenovirus, and astroviruses were identified in 15 out of 41 samples. The retrieved genomic information allowed the species level identification of several viruses - including rhinoviruses.

Conclusion

Beyond the expected detection of respiratory viruses, shotgun metagenomics on air samples has shown potential for the surveillance of respiratory, dermatologic, and enteric viruses. Our results show that untargeted shotgun metagenomics provides a promising framework for analyzing such samples, generating high quality genomic data that can be used for further pathogen

characterization. Further validation of this novel approach should allow complement current surveillance systems focusing on symptomatic patients.

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INFLUENZA LIKE-ILLNESS SURVEILLANCE USING A BELGIAN SENTINEL NETWORK OF NURSING HOMES: RESULTS OF SEASON 2022-23.

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Respiratory tract infections place a burden on vulnerable nursing home (NH) populations. We aimed to develop a sentinel network to monitor influenza-like illness (ILI), including COVID-19, among Belgian NH residents.

Following a two-year pilot study, an observational sentinel surveillance was initiated in October 2022. A geographically representative sample was established for Belgium using the number of NH beds as a proxy for NH residents. Throughout the year, weekly epidemiological/clinical data is collected on the number of ILI cases, ILI-related hospital admissions and deaths at NH level using an online questionnaire. During the flu season (as determined by the WHO: week 40-20), two nasopharyngeal samples are collected weekly per NH for viral typing with RT-qPCR.

During season 2022-23 (October 2022 to September 2023), 34 NH representing 2,943 residents (2.1% of all Belgian NH residents) participated a complete season. We observed two peaks of new ILI cases of 20 per 1,000 residents: one in mid-December 2022, and one in February 2023. The first peak coincided with the ILI increase observed in the network of general practitioners (GP) and the latter could be attributed to one outbreak. Since April 17, 2023 (week 16), the incidence remained below the low threshold (7 per 1,000 residents per week). From August 28, 2023 (week 35) onwards, ILI incidence increased again with the approaching start of the flu season. During season 2022-23, 37 ILI related deaths were reported. Out of 56 nasopharyngeal samples retrieved, 24 were negative and the majority were either positive for SARS-CoV-2 (10) or for seasonal corona (8). One co-infection was reported.

Comparing the results with these of the ILI sentinel network of GP we can conclude that our findings seem to follow the ILI situation among the general Belgian population. A sentinel network is a feasible way to monitor ILI in NH setting. As of season 2023-24, the network is being expanded to generate more robust findings and to enable regional reporting.

EMERGENCE OF EMM3.93 *STREPTOCOCCUS PYOGENES* CLONE IN IGAS INFECTIONS: EPIDEMIOLOGICAL INSIGHTS FROM BELGIUM (2022 - FEBRUARY 2024.

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Background

From mid-2022, several European countries have observed a notable increase in invasive group A streptococcal (iGAS) infections. Initially, the emergence of the $M1_{uk}$ clone was significant. However, by the end of 2023, another *emm*-type, *emm*3.93, began to replace *emm*1 as the dominant type. In this abstract, we present the epidemiological trends of iGAS in Belgium from beginning 2022 to February 2024, along with the genetic characteristics of the currently circulating emm3.93 iGAS strains.

Methods

Between 2022 and February 2024, the Belgian National Reference Centre (NRC) for invasive β-hemolytic streptococci non-group B received 1735 invasive *S. pyogenes* strains. All isolates were subjected to *Emm*-typing through Sanger Sequencing. As of May 2023, the NRC received 87 *emm*3.93 *S. pyogenes* isolates, of which three were sequenced using an Illumina MiSeq device. A wgSNP analysis, which included the three recently sequenced isolates and four *emm*3.93 *S. pyogenes* strains isolated in 2017, along with virulence gene detection, was performed with 1928 Diagnostic Software. Prophages were detected using PHASTEST.

Results

The increase in reported iGAS infections in Belgium aligns with the predominance of *emm*1 and the recent emergence of *emm*3.93 (Figure 1). Out of the iGAS infections (n=87) caused by *emm*3.93, the most common clinical presentations were was sepsis (48%), pneumosepsis (15%), pneumonia (7%), and meningitis (6%). Interestingly, 31% of these infections occurred in individuals younger than 18 years, which is higher than the usual 25% of other *emm*-types within the same age group. The wgSNP analysis showed that the three recent strains are 28 SNPs different from the 2017 strains (Figure 2). Among these, 18 SNPs were found in two hypothetical proteins near the *ssA* virulence gene within prophage Ø315

(Figure 3). All isolates contained the virulence genes *speA*, *speG*, *ssA* and *smeZ* and 6/7 isolates (86%) had the *speK* gene.

Conclusion

The increase in invasive *S. pyogenes* cases from beginning 2022 to February 2024 in Belgium is associated with the introduction of the $M1_{uk}$ and emm3.93 clones. The impact of the new clone *emm*3.93 on virulence and disease severity necessitates further research.

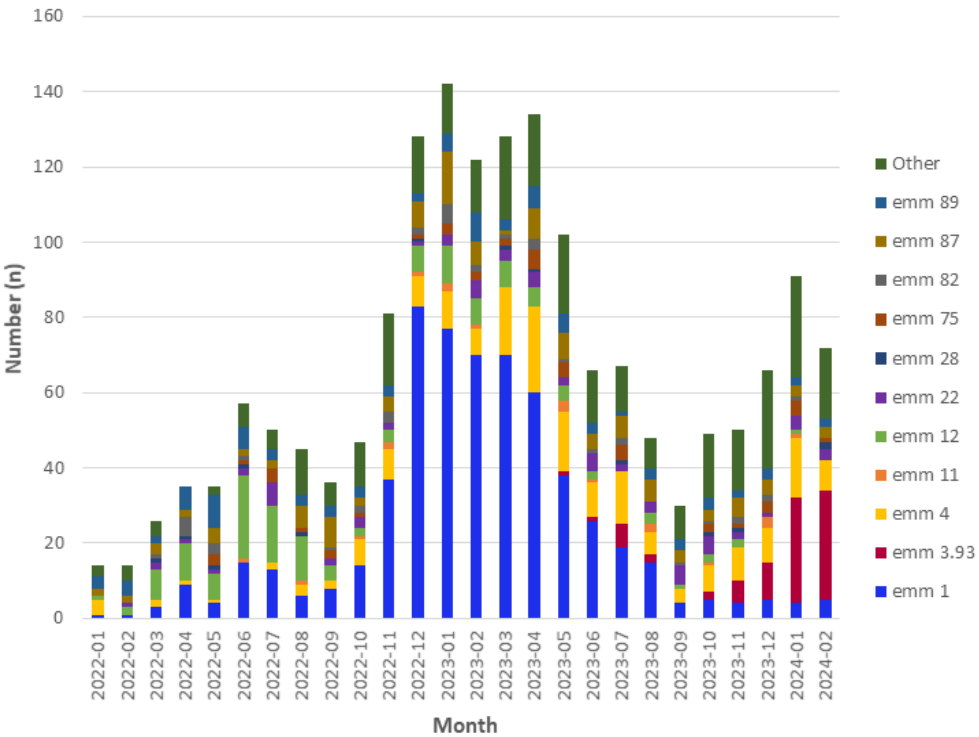

Figure 1: Prevalence of most common emm-types of iGAS infections isolates from beginning 2022 – February 2024 in Belgium received at the National Reference Centre for invasive β-hemolytic streptococci non-group B.

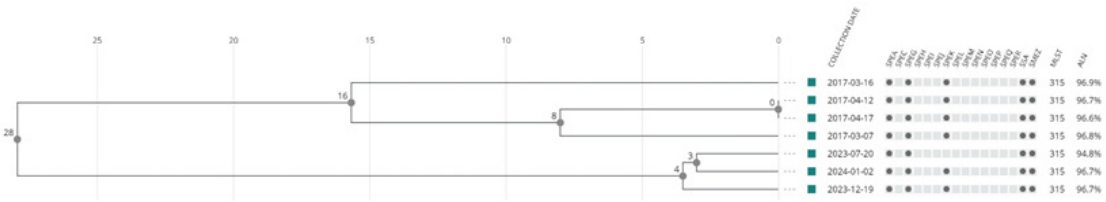

Figure 2: wgSNP analysis of four emm3.93 S. pyogenes isolates of 2017 and three recent emm3.93 S. pyogenes isolates. Figure was generated using 1928 Diagnostic Software.

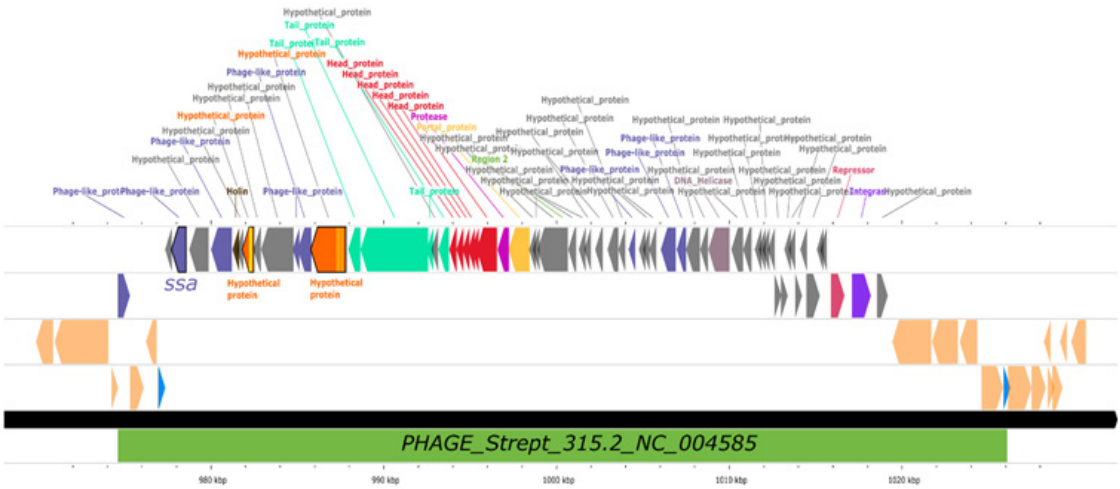

Figure 3: Prophage ø315.2 genomic region. Hypothetical proteins containing SNPs in *S. pyogenes emm*3.93 from 2023 and 2024 compared to emm3.93 isolates from 2017 are indicated in orange, regions containing SNPs are indicated in yellow. Figure was generated using PHASTEST.

WASTEWATER-BASED EPIDEMIOLOGY IN BELGIUM – A COMPLEMENTARY SURVEILLANCE SYSTEM FOR EARLY WARNING OF OUTBREAKS FOR INFECTIOUS DISEASES.

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Wastewater-based surveillance is operated by the Belgian institute of public health, Sciensano, to monitor poliovirus and respiratory viruses (SARS-CoV-2, influenza, and RSV). Over 4.3 million inhabitants representing 38% of the Belgian population are monitored in routine throughout 30 wastewater treatment plants since September 2020. During the past years, test capacities for SARS-CoV-2 on human samples has decreased enormously and circulation of SARS-CoV-2 in the general (symptomatic or asymptomatic) population is measured mainly throughout wastewater. Results obtained from the wastewater samples are integrated in the existing surveillance systems on respiratory viruses. They are weekly communicated to the Risk Assessment Group and depending on their advice the results are communicated to the Risk Management Group, who will decide of the relevant measures to be taken for Public Health.

Since December 2024, a pilot study is conducted to detect the concentration of Influenza and RSV in wastewaters. Preliminary results are similar to results found in human samples.

In January 2024, polio monitoring in wastewaters was integrated in the National polio surveillance system. A National Polio action plan and an emergency plan, in case of a positive sample, were established within the national Polio working group and will be validated by the Health Authorities.

Analytical methods are being developed to monitor other pathogen or target representing a threat to the public health: gastroenteritis, pfas, gonorrhoea, mpox.

In perspectives, wastewater monitoring will be used for antimicrobial resistance and avian influenza. Therefore, wastewater-based surveillance will definitely play an important role in the One-Health approach.

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