

(Table 1). Bacterial DNAs were sequenced with Illumina NovaSeq-6000. Fsa files of reading sequences were uploaded to various online databases and analyzed for SCCmec, ST and spa types, toxins, exoenzymes, and antibiotic resistance genes/mutations.

**RESULTS:** All isolates were CA-MRSA, with SCCmec-type IV (n=4) and SCCmec-type V (n=1) (Table 1). Two isolates were ST-8 (USA-300), others were ST-1, ST-5 and ST-97. Pantone-Valentine leukocidin was detected in one isolate (SMK-164).

Virulence genes such as gamma hemolysins, enterotoxins, serine proteases, genes encoding aureolysin, staphylokinase and complement inhibitor, toxin genes, and global regulators (sae and agr) were detected (Table 1). PVL carrier isolate was also positive for staphylococcal enterotoxin O and enterotoxin P. This strain was isolated from blood and sputum of a patient with bloodstream infection and necrotizing pneumonia.

Resistance genes blaZ and mecA were detected in all. One isolate carried fusC gene, the other carried fusB, mupA and msrA genes and was phenotypically resistant to mupirocin and erythromycin.

**CONCLUSIONS:** Thanks to WGS, very important epidemiological clues have been obtained.

## Respiratory Infections

### IgY and Cyclic peptide novel combination effective against *Pseudomonas aeruginosa* pulmonary infections

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**AIM AND BACKGROUND:** Multidrug-resistant (MDR) *P. aeruginosa* pulmonary biofilm infections exacerbate lung disease outcomes. Passive immunotherapy using avian IgY immunoglobulins (yolk) targeting *P. aeruginosa* and small macrocyclic peptides offers a promising alternative to conventional antibiotics. This study tested the synergistic activity of a novel inhaled treatment combining a macrocyclic peptide with anti-*P. aeruginosa* IgY in vitro and in vivo.

**METHODS:** The synergistic activity of a novel macrocyclic peptide (POPSICAL) combined with anti-*P. aeruginosa* IgY was evaluated against 400 clinical isolates of MDR *P. aeruginosa*. A novel high-throughput assay, coupled with confocal microscopy, was used to assess both bactericidal and antibiofilm activity. The efficacy of inhaled POPSICAL with avian IgY immunoglobulins (yolk) in eliminating acute and chronic (biofilm) lung infections was also tested in a relevant in vivo mouse model.

**RESULTS:** At low concentrations (4 µg/ml), POPSICAL significantly increased the in vitro killing of MDR *P. aeruginosa* in the presence of anti-*P. aeruginosa* IgY (10% v/v). Anti-*P. aeruginosa* IgY appeared to promote bacterial aggregation, resulting in immobilization and increased surface hydrophobicity, thereby enhancing POPSICAL-mediated antibiofilm and bactericidal activity. The inhaled combination of POPSICAL and anti-*P. aeruginosa* IgY achieved over 99% reduction in biofilm and bacterial load in mouse lungs

(1-log<sub>10</sub> kill), accompanied by decreased airway inflammation and protection from mortality.

**CONCLUSION:** Our findings present a novel inhaled therapeutic option using the macrocyclic peptide POPSICAL combined with anti-*P. aeruginosa* IgY for treating pulmonary infections.

### Intranasal phage treatment overcomes the limitations posed by antibody-mediated neutralization in bacteriophage therapy for *Pseudomonas aeruginosa* lung infections

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**Background:** Our recent work discovered that intranasal inhalation of phage (KPP10) and Ca-EDTA substantially re-sensitized multidrug-resistant *P. aeruginosa* to ceftazidime/avibactam. Notably, the route of phage administration was reported to influence the development of antibody-mediated neutralization of phage and thereby reducing its therapeutic efficiency. Therefore, we evaluated the effectiveness of KPP10 regimens utilizing systemic intraperitoneal (IP) and localized intranasal routes to treat chronic *P. aeruginosa* lung infection.

**Methods:** The effectiveness of KPP10 regimens utilizing systemic intraperitoneal (IP) and localized intranasal routes to treat chronic *P. aeruginosa* lung infection, were assessed using mouse lung infection model together with ELISA to measure the production of neutralizing antibodies (IgM, IgG, and IgA).

**RESULTS:** In this study, pre-treatment of mice with KPP10 intraperitoneally induced the systemic distribution of KPP10 to different internal organs, whereas intranasal KPP10 pre-treatment achieved significant KPP10 localization exclusively in alveolar space and elsewhere in lungs. Interestingly, intraperitoneal KPP10 administration regimens in chronic lung infection resulted in decreased survival with significant IgG, IgM, and IgA production. Meanwhile, after giving KPP10 regimens intranasally, anti-phage antibody production was undetectable and survival improved significantly.

**Conclusions:** These results demonstrated that administering KPP10 directly to the lungs via the intranasal route could ensure a higher concentration of KPP10 at the infection site, expediting its bactericidal effects for increased pathogen elimination, while overcoming antibody-mediated phage neutralization and improving clinical outcomes in chronic pulmonary *P. aeruginosa* infections.

### SARS-CoV-2 Still on the Stage: The Need to Pursue an Integrated Respiratory Virus Surveillance

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**AIM:** To demonstrate the importance of an integrated viral surveillance to tackle the changing epidemiology and the continuing disease burden of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

**BACKGROUND:** International institutions such as European Centre for Disease Prevention and Control provide guidance for integrated surveillance of respiratory viruses. However, SARS-CoV-2 is not an integral part of sentinel influenza like illness and severe acute respiratory infections surveillance in Türkiye.

**METHODS:** We utilized the database from the Global Influenza Hospital Surveillance Network project in Türkiye between November 1st, 2022 and May 31st, 2024 over two seasons. Patients admitted to five hospitals with influenza like illness symptoms in the last 7 days and stayed overnight in the hospital were screened three days a week and swabbed within 72 hours of admission in a standardized year around surveillance methodology. An oligonucleotide panel analysis was utilized during November 1st 2022 to October 31st 2023 and multiplex PCR was utilized thereafter to test for influenza A and B, SARS-CoV-2 and respiratory syncytial virus (RSV).

**RESULTS:** A total of 984 inpatients were enrolled and 969 of them, 724 (75%) of whom were adults, had valid laboratory results. RSV was the most prevalent pathogen detected in 4.9% of the samples, followed by SARS-CoV-2 (4.8%) and influenza A and B (3%).

**CONCLUSIONS:** SARS-CoV-2 is circulating and resulting in hospital admissions among all age groups adding onto the influenza and RSV peaks. Integrated respiratory virus surveillance is crucial to guide informed public health policies and effective vaccination programs.

#### Antimicrobial use for influenza-like illnesses in Nha Trang, Vietnam

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**AIM:** To investigate the characteristics of the management of ILI in outpatient and inpatient settings in Vietnam.

**BACKGROUND:** Influenza-like illnesses pose a considerable disease burden and antimicrobial resistance (AMR) is a global concern, driven partly by antimicrobial use among ILI cases.

**METHODS:** We conducted a survey among 407 individuals presenting with ILI symptoms at public community health centers and the pediatric ward of a public hospital in Nha Trang city, Khanh Hoa Province, Vietnam, from December 2022 to March 2023. Health-related quality of life (HRQoL) was estimated from the Vietnamese Short Form (SF)-12 questionnaire using the SF-6D algorithm. In addition to descriptive statistics, we conducted multivariable logistic regression analysis to examine the factors associated with antibiotic prescription for outpatient ILI cases.

**RESULTS:** The study enrolled 198 outpatients and 200 inpatients with ILI. Most of the inpatient cases were children under five, and

experienced longer illness durations and higher costs, with almost all receiving antibiotics. Antimicrobials were prescribed for 79.3% of outpatients and 99.5% of inpatients. During ILI episodes, HRQoL scores averaged 0.796 (IQR 0.674–0.922) in  $\geq 18$  years old. Logistic regression analysis indicated a negative association between a definite diagnosis of viral infection by rapid diagnostic tests and outpatient antibiotic prescription (Odds ratio: 0.20, p value = 0.006).

**CONCLUSION:** This study documents the burden of ILIs in Vietnam, noting a very high proportion of antimicrobial prescribing. Promoting definite diagnosis of viral infections by rapid diagnostic test was suggested to be an effective countermeasure to curtail inappropriate prescription of antimicrobials.

#### Machine Learning for Community-Acquired Pneumonia Diagnosis Using Routine Clinical and Laboratory Data

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**Background:** Community-acquired pneumonia (CAP) is diagnosed based on clinical information, laboratory tests, and chest imaging. However, chest radiography is often inaccessible in primary care, causing variability in clinical diagnosis. This study aims to develop a machine learning model to diagnose CAP using only clinical and laboratory data.

**METHODS:** This study included patients who presented with fever and respiratory symptoms to the outpatient clinic or emergency room of a tertiary care center between 2009 and 2018. A total of 10,707 adult patients were randomly divided into training (70%) and test (30%) sets. We analyzed the model for internal validation on 1,364 patients who visited the same institution between August 2019 and December 2020.

The performance of the machine-learning models was measured using the area under the receiver operating characteristic curve (AUROC).

**RESULTS:** Among the algorithms tested, eXtreme Gradient Boosting (XGBOOST) achieved the highest AUROC (0.936, 95% CI: 0.924–0.947), followed by the gradient boost (0.931, 95% CI: 0.919–0.943) and random forest (0.926, 95% CI: 0.912–0.938) models in the test set. The most significant independent variables for diagnosing pneumonia were the presence of cough, crackle lung sounds, and CRP levels. In the validation set, XGBOOST achieved an AUC of 0.919 (95% CI: 0.886–0.933), with a sensitivity of 82.30%, specificity of 88.92%, and accuracy of 87.90%.

**CONCLUSIONS:** The machine learning model accurately diagnosed community-acquired pneumonia, indicating its potential to assist in primary care settings without relying on chest imaging.