Community-acquired pneumonia - Clinical and laboratory aspects of etiology

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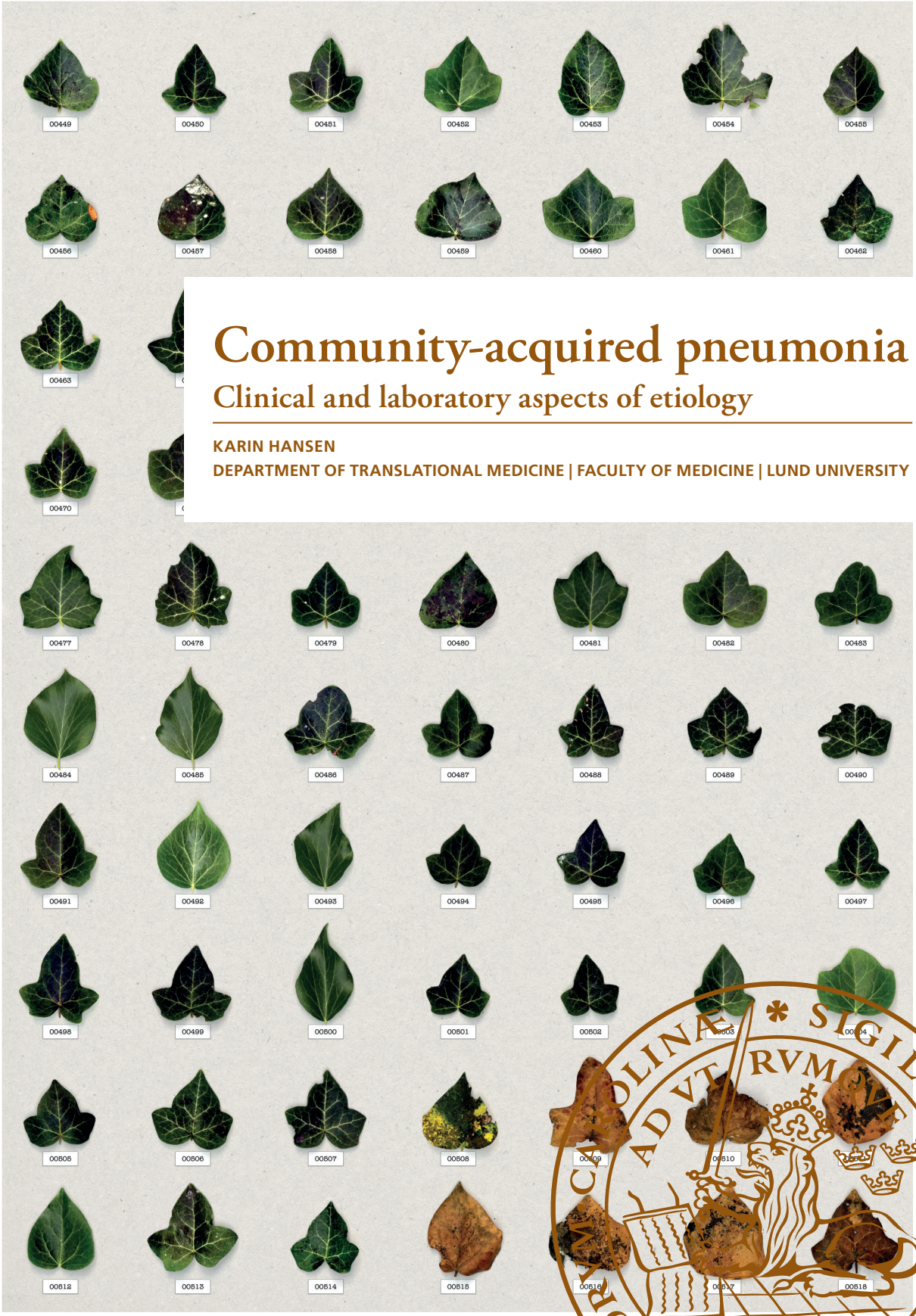

Community-acquired pneumonia

Clinical and laboratory aspects of etiology

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DOCTORAL DISSERTATION

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Abstract:

Community-aqcuired pneumonia (CAP) is one of the most common infectious causes leading to hospitalization. Historically, *Streptococcous pneumoniae* has been the most common etiology in CAP and has therefor led to targeted interventions such as immunization in children, the elderly, and risk groups. Other important pathogens are *Haemophilus influenzae, Mycoplasma pneumoniae* and respiratory viruses.

The aim of this thesis was to investigate the etiology of CAP after introduction of PCV in the pediatric immunization programme as well as diagnostic sampling for respiratory pathogens, with a focus on the upper respiratory tract.

In Paper I, a comparison of nasopharyngeal aspirate (NPA) and flocked nasal swab (fNS) for testing of respiratory pathogens was performed in 50 patients with acute respiratory symptoms, and we discovered that fNS was an adequate and well tolerated alternative to NPA for respiratory viruses.

Paper II-V include different sub-groups of a prospective cohort of 518 patients hospitalized with CAP.

In Paper II, we compared nasopharyngeal (NP) swabs with oropharyngeal (OP) swabs for respiratory pathogens in 484 patients. While NP detected more viruses, OP detected more bacteria (*M. pneumoniae, S. pneumoniae* and *H. influenzae*) but for all pathogens, cases were missed by using only one sample, and combined sampling of NP/OP would give the highest yield for viruses and *Mycoplasma*. For *S. pneumoniae* and *H. influenzae,* more research on the specificity of OP-samples is needed.

In Paper III, results were gathered from routine microbiologic tests, two different urinary antigen tests, and PCR from NP swabs in 518 patients, *S. pneumoniae* was the most common pathogen, followed by *H. influenzae* and viruses. Co-detections between viruses and bacteria was common. No differences was seen in symptoms our outcome when comparing bacterial, viral and viral-bacterial co-detection.

In Paper IV, we investigated the serotype distribution of pneumococcal CAP in hospitalized adults and found that 11% had a serotype included in PCV13 despite it being included in the child immunization vaccine programme. PCV15 and PCV20 would expand the serotype coverage in pneumococcal CAP.

In Paper V, we compared the clinical features of *M. pneumoniae* with *S. pneumoniae*. Clinical symptoms and radiology were insufficient in distinguishing between the to bacteria; inflammatory markers could be of some guidance but most importantly *M. pneumoniae* patients were three decades younger and more often experienced antibiotic failure. Liberal testing for Mycoplasma should be recommended for admitted patients with CAP younger than 50 years.

Key words: *S. pneumoniae, H. influenzae, M. pneumoniae,* CAP

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To my patients

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Preface

The spark for this thesis started when Anna Nilsson, who later became my co-supervisor, suggested I take part in a study on sampling techniques for respiratory pathogens. At the time, I was an infectious disease resident, and I liked the direct implications the study had on changing the recommended sampling technique for influenza virus. Shortly after publication of this article a larger project was proposed by my colleague Jonas Ahl and Professor Kristian Riesbeck who then became my co-supervisor and supervisor. It was a clinical collaboration with Pfizer: they wanted to investigate the pneumococcal serotype distribution in hospitalized patients with community-acquired pneumonia (CAP), of course in relation to their pneumococcal vaccines, and we wanted to explore CAP from a larger context, so, within the scope of the original study we were able to broaden the clinical study protocol and collect a great deal of other data and material to answer far more questions than those originally agreed upon in the collaboration.

Initiating a prospective study with the ambition of seven-day-per-week inclusion while working alongside the industry was an interesting learning experience. After the inclusion of all patients, while waiting for the analysis of the samples, the COVID-19 pandemic arrived, putting everything on hold. As a clinician during the pandemic, the frequent concerns about false-negative results in patients presenting with a typical clinical picture, and shortages of sampling swabs leading to general discussions on optimal sampling locations, encouraged me to continue investigating these very practical aspects for other pathogens.

Over the years of writing this thesis a lot has changed; the turnaround time for an influenza PCR have gone from receiving a result usually the next day, to within four hours, to twenty minutes, and the range of pathogens on the panels have increased. As more diagnostic tests become available, more knowledge is needed on which tests to choose, for whom and when, and hopefully this thesis can contribute to a small part.

List of Papers

This thesis is based on the following papers, which will be referred to in the text as Paper I-V

Paper I

Hansen KB, Westin J, Andersson LM, Lindh M, Widell A, Nilsson AC. Flocked nasal swab versus nasopharyngeal aspirate in adult emergency room patients: Similar multiplex PCR respiratory pathogen results and patients discomfort. *Infectious Diseases (Lond).* **2016**;48, 246-250.

Paper II

Hansen K, Wasserstrom L, Ahl J, Su Y-C, Nilsson AC, Riesbeck K. Combined nasopharyngeal and oropharyngeal sampling improves pathogen detection in community-acquired pneumonia. *Frontiers in Microbiology*. **2026** Apr 8;17:1774447.

Paper III

Hansen K*, Yamba Yamba L*, Wasserstrom L, Rünow E, Göransson T, Nilsson A, Ahl J, Riesbeck K. Exploring the microbial landscape: uncovering the pathogens associated with community-acquired pneumonia in hospitalized patients. *Frontiers in Public Health*. **2023** Dec 13;11:1258981.

Paper IV

Hansen K*, Rünow E*, Torisson G, Theilacker C, Palmborg A, Pan K, Jiang Q, Southern J, Beavon R, Gessner BD, Riesbeck K[#], Ahl J[#]. Radiographically confirmed community-acquired pneumonia in hospitalized adults due to pneumococcal vaccine serotypes in Sweden, 2016-2018-The ECAPS study. *Frontiers in Public Health.* **2023** Feb 17;11:1086648.

Paper V

Hansen K, Wasserstrom L, Ahl J, Nilsson AC, Riesbeck K. Clinical characteristics of *Mycoplasma pneumoniae* compared to *Streptococcus pneumoniae* in hospitalized adults with community-acquired pneumonia- a prospective study. *BMC Infectious Diseases.* **2025** Oct 13;25(1):1292.

*These authors have contributed equally to this work and share first authorship

[#]These authors have contributed equally to this work and share last authorship

List of papers not included in the thesis

Rünow E, Valeur F, Torisson G, **Hansen K,** Theilacker C, Riesbeck K, Ahl J. The incidence of radiologically verified community-acquired pneumonia requiring hospitalisation in adults living in southern Sweden, 2016-2018: a population-based study. *BMC Infectious Diseases.* **2025** Jan 17;25(1):80.

Yamba Yamba L, **Hansen K**, Wasserstrom L, Su YC, Ahl J, Riesbeck K. The importance of Haemophilus influenzae in community-acquired pneumonia: an emerging pathogen in the elderly regardless of comorbidities compared to Streptococcus pneumoniae. *Pneumonia (Nathan).* **2024** Aug 25;16(1):15.

Hansen K, Båtshake Y, Söderholm S, Pettke A, Björkman B, Sondén K. Monkeypox virus-associated meningoencephalitis diagnosed by detection of intrathecal antibody production. *BMC Infectious Diseases.* **2024** Jan 16;24(1):94.

Ek P, Böttiger B, Dahlman D, **Hansen KB**, Nyman M, Nilsson AC. A combination of naso- and oropharyngeal swabs improves the diagnostic yield of respiratory viruses in adult emergency department patients. *Infectious Diseases (Lond).***2019** Apr;51(4):241-248.

Chansa E, **Hansen K,** Gustafsson B. An intraosseous blood transfusion in a critically ill child. *African Journal of Emergency Medicine*. **2014**. 4(2): 83–85.

Hansen K, Johanssson E, Byaruhanga E, K Odberg Pettersson. When to seek care in pregnancy and childbirth: a study in Uganda. *African Journal of Midwifery and Women's Health.* **2009**. 3(3):34-140.

Abbreviations

ARI	Acute respiratory infection
BAL	Broncheoalveolar lavage
CAP	Community-acquired pneumonia
CRP	C-reactive protein
CT	Computed tomography
cfu	Colony forming units
CRP	C-reactive protein
CXR	Chest X-ray
ED	Emergency department
fNS	Flocked nasal swab
HAP	Hospital acquired pneumonia
HFNC	High flow nasal cannula
Hmpv	Human metapneumovirus
HRV	Human rhinovirus
ICU	Intensive care unit
IPD	Invasive pneumococcal disease
LRT	Lower respiratory tract
LRTI	Lower respiratory tract infection
LUAT	Legionella antigen test
MTS	Mid-turbinate swab
NAT	Nucleic acid test
NAAT	Nucleic acid amplification test
NIV	Non-invasive ventilation
NP	Nasopharyngeal
NPA	Nasopharyngeal aspirate
NS	Nasal swab
NTHi	Non-typable Haemophilus influenzae
NVT	Non-vaccine type

OP	Oropharyngeal
PCR	Polymerase chain reaction
PSB	Protected sterile brush
PSI	Pneumonia severity index
PUAT	Pneumococcal urinary antigen test
PVL	Panton-Valentine Leukocidin
RCT	Randomized controlled trial
RSV	Respiratory syncytial virus
RV	Rhinovirus
UAD	Urinary antigen detection
URT	Upper respiratory tract
VAP	Ventilator associated pneumonia
VT	Vaccine type
WBC	White blood cell count

Thesis at a glance

Paper	Aim	Method	Result	Conclusion
I	To compare nasopharyngeal aspirate (NPA) with flocked nasal swab (fNS) for respiratory virus detection and tolerability.	50 patients with suspected respiratory infection were tested with NPA and fNS. Samples were analysed with PCR for 18 respiratory agents. Discomfort scale 1-4.	23 patients positive, 10/11 influenza cases detected by fNS. No significant difference in discomfort.	Flocked nasal swab was comparable to NPA in both pathogen yield and perceived discomfort.
II	To compare nasopharyngeal (NP) and oropharyngeal (OP) swabs for detection of respiratory pathogens.	Hospitalized CAP, 484 patients (subgroup from paper III-IV). NP- and OP -swabs: PCR 14 viruses, 5 atypical bacteria, and *S. pneumoniae* and *H. influenzae.*	Viruses: Concordant in 65%, 21/157 in NP only, 22/157 in OP only. *M. pneumoniae:* 11% NP only, 30% OP only. *S. pneumoniae* and *H. influenzae* both concordant in 53%.	Combined NP and OP sampling is recommended for maximal yield. Specificity is a possible concern for *S. pneumoniae* and *H. influenzae* in OP-swabs.
III	To describe the etiology and clinical features of patients hospitalized with CAP.	518 patients. Standard of care samples, two pneumococcal urinary antigens, NP-swabs: culture and PCR (see paper II) 241 controls: urinary antigens and NP-swabs (culture and PCR).	*S. pneumoniae* 28%, viruses 28%, *H. influenzae* 16%, compared to 5-7% in the controls. Bacterial-viral co-detection in 15%. No difference in symptoms our outcome between groups.	*S. pneumoniae* was the most common, then *H. influenzae* and viruses. Symptoms and outcome was similar in bacterial, viral and mixed detection.
IV	To describe pneumococcal serotype distribution in adults hospitalized with CAP.	518 patients. Blood- and LRT- cultures, were serotyped for pneumococci. Two pneumococcal urinary antigens: BinaxNow and UAD (detects 24 serotypes).	Most frequent serotypes: 3, 8, 11A and 19A. PCV13 serotypes detected in 11% of all CAP, PCV15 in 13% PCV20 in 17% and Non-PCV20 in 14%.	Compared with PCV13, PCV15 and PCV20 expanded the coverage of all-cause CAP.
V	To compare the clinical characteristics of hospitalized CAP caused by *M. pneumoniae* versus *S. pneumoniae.*	Subgroup from paper II-IV, 32 patients with *M. pneumoniae* and 126 patients with *S. Pneumoniae.*	*M. pneumoniae* patients: 30 years younger, more prior antibiotic failure, lower leukocytes but similar symptoms and radiology to pneumococcal CAP.	Liberal testing for *M. pneumoniae* is recommended in younger patients hospitalized with CAP.

Introduction

In 1901, Professor William Osler wrote “The most widespread and fatal of all acute infectious diseases, pneumonia, is now the ‘Captain of the Men of Death’ “ (1). The quote was modified from the 17th century philosopher John Bunyan, who at that time was referring to tuberculosis (2). Since the pre-antibiotic era, many things have changed, but community-acquired pneumonia (CAP) is still the leading infectious cause of death globally (3). Incidence rates of CAP requiring hospitalization span from 216-267 cases per 100,000 person-years (4-7), and some report 645-649 per 100,000, with incidence increasing with age (8, 9).

Defining CAP

Community-acquired pneumonia is an infection of the lung parenchyma contracted outside of a hospital or healthcare setting. Most studies require a radiographic diagnosis in addition to clinical symptoms to confirm pneumonia, usually chest X-ray (CXR) or computed tomography (CT). Without imaging, it is difficult to distinguish pneumonia from lower respiratory tract infections not involving the lung parenchyma. Chest X-ray is still the gold standard due to its availability and lower radiation dose, however, sensitivity compared with CT ranges from 44%-82% (10-12). The overall importance of chest imaging in diagnosing CAP for hospitalized patients in the clinical setting has also been debated (13), but the sensitivity and specificity of clinical judgment are also quite moderate (14-16). Correlation between pneumonia diagnosis made at admission in the emergency department (ED) and a discharge diagnosis of pneumonia is limited (17, 18), and incorrect final diagnoses of pneumonia are not uncommon particularly in the elderly with cognitive impairment (19). One study demonstrated that performing a CT scan in suspected CAP patients increased both diagnostic certainty and adherence to empirical antibiotic treatment guidelines (20). In general, all guidelines recommend chest imaging in some form to establish the diagnosis of CAP and several guidelines updated within the last decade also include lung ultrasound (LUS) (21-23). Lung ultrasound has demonstrated high sensitivity and specificity, but its use is limited by user dependency and the difficulty of re-interpreting and comparing findings across examinations (24).

Defining severity of CAP

There are two different reasons for defining severity in pneumonia: the first concerns the clinician's perspective on level of care, guiding who can safely be treated as an outpatient and stratifying inpatients into non-severe and severe CAP to inform decisions regarding empirical therapy.

The other pertains to defining severity in the research setting when evaluating various aspects of CAP. Severity scores are sometimes used in clinical studies as a definition of severity even though they are prognostic prediction scores. Beyond mortality, intensive care unit (ICU) admission, is another marker of severity; however, it is not a standardised entity and can mean very different things across hospitals and countries. For example, in some countries high-flow nasal cannula (HFNC) therapy is predominantly administered in the ICU, while in others it is administered in regular wards. Furthermore, depending on the availability of HFNC, patients may instead receive high-level supplemental oxygen instead of HFNC. Using invasive mechanical ventilation as marker of severity is more standardised, but pitfalls remain, notably treatment limitations is common in elderly and terminally ill patients.

Severity scores

Generally prediction scores have been developed using 30-day mortality as the primary endpoint, with a focus on identifying which patients can safely be managed as outpatients. Most importantly: all prediction scores are tools, not a replacement for the clinical judgement in each individual case.

Pneumonia Severity Index (PSI)

The PSI is a prediction score developed in 1997 that stratifies patients into five risk classes based on predicted mortality (25). It is the most comprehensive prediction score, validated in approximately 40,000 patients, and incorporates 20 variables divided into co-existing illnesses (congestive heart failure, renal disease, cerebrovascular disease, liver disease and neoplastic disease), clinical findings (altered mental status, respiratory rate >30 per minute, systolic blood pressure <90 mmHg, pulse >125 per minute and temperature <35°C or >40°C) and laboratory results (partial pressure of oxygen <60 mmHg, arterial pH <7.35, sodium level <130 mmol/L, glucose level 14 mmol/L, haematocrit <30%, blood urea 11 mmol/L), additional points are also given for age, being a nursing home resident and having a pleural effusion, maximum score of 395. Female sex means a reduction of points. A prospective study compared an emergency department that had already implemented PSI with another using standard of care as controls. The PSI-guided

cohort treated more patients in PSI class I-II as outpatients without more adverse outcomes (26).

The score's main limitations are the heavy weighting of age, older patients will accumulate a higher score regardless of severity, while younger, otherwise healthy patients at risk of deterioration may be missed. As it is very comprehensive, bedside use is cumbersome, and laboratory values limit the applicability in outpatient settings.

CRB-65

Until recently, it was the recommended score in Sweden. Data from the original publication were derived from three prospective studies with a total of 1068 patients (27), with one point for each parameter (confusion, respiratory rate >30 breaths/minute, blood pressure <90 systolic or <60 diastolic, and age >65 years) (score 0-4). It is simple to apply, but does not account for hypoxemia or comorbidities.

CURB-65

Developed by the British Thoracic Society (28), CURB-65 adds blood **U**rea (>7mmol/L) to the CRB-65 parameters. The blood sample requirement limits outpatient use, and the threshold of urea levels has been debated, as it is more commonly elevated in elderly patients (29). It shares limitations with CRB-65 in excluding hypoxemia and other demographic factors others than age.

DS CRB-65

The current recommended score in Sweden (30), DS CRB-65 extends CRB-65 by adding points for severe comorbidities (**D**: congestive heart failure, renal disease, cerebrovascular disease, liver disease or neoplastic disease) and hypoxemia (**S:** $SPO_2 \leq 90\%$ without oxygen supplementation).

In a prospective study of 4,432 patients, DS-CRB-65 identified the majority of patients with a CRB-65 score of 0 who died or required mechanical ventilation or vasopressors (31). A comparison of all four scores including mortality data is illustrated in Figure 1.

	PSI	CRB-65	CURB-65	DS-CRB-65
	Age, sex, nursing home + Confusion, RR, BP, pulse, temp + 5 co-morbidities + 6 lab values + pleural effusion	Confusion Respiratory rate ≥30 SBP <90 DBP <60 65 > 65 years	CRB-65 + Urea >7mmol/L	CRB-65 + Disease + Saturation <90%
		Risk score (mortality)		
Outpatient	I (0.1-0.3%) II (0.4-0.6%)	0p (0-2%)	0p (0.6-0.7%) 1p (2-3%)	0p (0-0.2%)
Outpatient or inpatient	III (0.4-0.6%)	1p (3-5%)	2p (6-9%)	1p (0.9-1%)
Inpatient	IV (8-9%)	2p (6-13%)	3p (13-15%)	2p (3-5% 3p (9-10%)
Consider ICU		3p (16-33%) 4p (18-34%)	4p (17-40%) 5p (14-43%)	4p (16-28% 5p (20-47%) 6p (29-50%)

Figure 1. Comparison of different severity scores for CAP
Adapted from data on PSI (25, 32), CRB-65 (27, 31, 33, 34), CURB-65 (27, 32), DS-CRB-65 (31, 35).

The severity scores are also used for aiding the choice of empirical therapy. The Swedish guidelines currently recommend DS CRB-65 (36, 37). The infectious disease society of America (IDSA) uses a pragmatic classification: outpatient, non-ICU or ICU patient, with additional recommendations based on co-morbidities (38). The Dutch guidelines recommend either PSI or CURB-65 classification (39).

Other severity scores

The scores above are commonly used for detecting patients at risk of needing for intensive care, but they are primarily validated for assessing safe outpatient treatment. The following scores focus on identifying patients at risk of needing vasopressors and mechanical ventilation.

SMART-COP

SMART-COP incorporates: **S**ystolic blood pressure <90, **M**ultilobar infiltrates, low **A**lbumin, **R**R ≥ 30 **T**achycardia, (pulse ≥ 125), **C**onfusion, poor **O**xygenation and **p**H <7.35, (40), without consideration of age or co-morbidities. A study aiming to detect those needing mechanical ventilation or vasopressor found a sensitivity of 92% for SMART-COP (≥3p) outperforming 74% for PSI class IV-V (75%) and CURB-65 ≥3p (39%) (40).

IDSA/ATS

The Infectious Disease Society of America (IDSA) and American Thoracic Society (ATS) criteria for severe CAP combine clinical, laboratory and radiological features into 9 minor (including clinical parameters, lab values and X-ray findings) and 2 major criteria (vasopressor support and/or mechanical ventilation). The major criteria are not useful as they are outcomes rather than predictors (38), but the 9 minor criteria have shown high sensitivity and negative predictive value for mechanical ventilation, vasopressor support or death within 72 hours from admission. Notably, patients with this endpoint had a mean CRB-65 score of 1.5 and a CURB-65 score of 2.1, highlighting the limited sensitivity of these scores for identifying patients at risk of deteriorating (41).

qSOFA

The quick Sequential Organ Failure Assessment is a simplified version of the SOFA score (42), validated for predicting mortality in sepsis. It shares the same parameters as CRB-65, but with exclusion of age and lower thresholds: confusion/altered mental status, respiratory rate ≥22, and systolic blood pressure ≤100 mmHg. A study comparing qSOFA with CRB-65 and DS-CRB-65 found no significant difference in predicting death or ICU admission. (43).

Lactate

Although not a formal scoring tool, a retrospective cohort study identified lactate >1.8 mmol/L as an independent marker of severity (44).

Etiology of CAP

There is no gold standard in defining etiology in CAP, so authors have adopted two broadly different strategies in presenting their results. The first is to report all microbiological findings without assigning a definite etiology. Others divide their findings into definite and probable etiology.

Most authors agree that *definite* criteria include: culture from blood, pleura, bronchial alveolar lavage or sterile protected brush, or a positive urinary antigen test

for *L. pneumophila* or *S. pneumoniae;* or a positive *L. pneumophila* PCR from sputum (45-49).

The following criteria are considered *definite* by some authors (45, 49) and *probable* by others (46, 48): serology, with elevated serum IgM titres for *M. pneumoniae, C. pneumoniae* or *C. burnetti* or seroconversion for these pathogens, *L. pneumophila* or respiratory viruses. Detection of viruses, *M. pneumoniae* or *C. pneumoniae* by PCR.

Most authors consider a predominant bacterium on high-quality sputum to represent *probable* etiology (45, 47) and some also include nasopharyngeal culture, with varying additional criteria (46, 48).

The most important pathogens in CAP are presented below, but other causes which are either more uncommon in CAP or primarily opportunistic pathogens are not covered.

Streptococcus pneumoniae

A gram-positive facultative anaerobic coccus, usually, but not always, arranged as diplococci under the microscope. It was first isolated by Louis Pasteur in 1881 and the association with pneumonia was reported in 1883. The cell wall is covered by a polysaccharide capsule protecting the pneumococci from phagocytosis and is its most important virulence factor. Classification of different serotypes is based on the antigenic differences in the capsule (50). To date, 108 different serotypes have been described (51). Some pneumococci do not show any capsular expression and are called non-typeable (NT) pneumococci, and more rarely cause invasive disease (52). Different serotypes seem to have different properties, serotype 1 and 7 has high invasive potential mainly infecting younger, healthy adults, and linked to empyema but not associated with a higher case fatality rates (53), while others are more linked to increased mortality (3, 6B, 9N, 11A, 16F, 19F, 19A) (53, 54).

In the pre-antibiotic era, *S. pneumoniae* accounted for an overwhelming majority of CAP cases and preventive measures such as vaccinations have targeted the pneumococci, which has led to a proportional decline, but *S. pneumoniae* still remain the most important etiology of CAP (55, 56).

Pneumococcal vaccination

The polysaccharide vaccine (PPV) was developed in 1977, with expanded coverage of 23 serotypes in 1986, until recently it was the recommended vaccine for the elderly and high-risk groups. It elicits an antibody response that is T-cell independent, relying on marginal zone B-cells (57, 58) and has an effect on invasive disease but not on nasopharyngeal carriage (59, 60). The pneumococcal conjugate vaccine (PCV) was originally developed for children, as marginal zone B-cells are not fully developed before 2 years of age, it induces a T-cell-dependent immune

response, resulting in the development of memory B-cells. The PCV was introduced in the Swedish child immunization programme in 2009 (61) and has been shown to reduce nasopharyngeal carriage of vaccine type (VT) pneumococci but with an increase of non-VT pneumococci (62). This has the potential for herd immunity against the more virulent serotypes, and since PCV introduction, a clear decline of invasive pneumococcal disease (IPD) has been seen in children, but in adults the overall effect has been less prominent, due to an increase in non-VT IPD, especially in the elderly and in patients with chronic medical conditions and immuno-suppression (61, 63).

Haemophilus Influenzae

Haemophilus influenzae was first discovered by the German bacteriologist Richard Pfeiffer in 1892, who mistakenly thought it to be the cause of influenza, and it became known as Bacillus influenza (64). It is a small gram-negative pleomorphic rod-shaped coccobacillus. It belongs to the genus Haemophilus, which includes nine human species, out of which *H. influenzae* is the most important pathogen. The capsular polysaccharide is the major virulence factor in capsulated *H. influenzae,* and these strains are associated with invasive disease with a much larger spectrum than pneumonia ,such as meningitis, cellulitis, sepsis, septic arthritis, and epiglottitis (50). The vaccine targeting the most virulent strain, *H. influenzae* type b (Hib), was introduced in the Swedish child immunization programme in 1993 and has led to a marked decrease of Hib infections. Serotype replacement has not been seen, as other encapsulated strains remain low, and invasive disease in countries with high immunization rates is now mainly caused by the non-capsular strains: the non-typeable *H. influenzae* (NTHi) (65-67). As it is rarely positive in blood culture (68), a lower respiratory culture is preferable, *H. influenzae* PCR is a possible alternative, although challenges exist in distinguishing it from other closely related *Haemophilus* species (69).

Staphylococcus aureus

A gram positive cocci, first described in 1880 by the Scottish surgeon Alexander Ogston, describing staphylococci from an abscess as “the masses looked like a bunch of grapes”, staphyle means “bunch of grapes” in Greek, and kokkos means berry. The German physician Friedrich Julius differentiated the species in 1884 by colony colour: *aureus* derives from the Latin aurum, meaning gold (70). Although not a common cause of CAP, *S. aureus* is more frequent in both hospital-acquired pneumonia (HAP) and in ventilator-associated pneumonia (VAP) (71). Of particular concern is the emergence of MRSA, which, even when appropriate antibiotics are administered promptly, is associated with longer ICU stay than MSSA VAP (72). An important virulence factor in pneumonia is Panton-Valentine Leucocidin (PVL);

PVL-producing strains can cause necrotizing pneumonia with ulceration of the tracheal and bronchial mucosa as well as interalveolar septa, even in immunocompetent adults (73). A lower respiratory tract sample is necessary to diagnose *S. aureus* CAP, as nasal colonization is common in adults (74, 75).

Moraxella catarrhalis

A gram-negative diplococcus; when cultured, the round colonies can be slid along the agar surface, known as the "hockey puck sign". It is common in nasopharyngeal carriage in children, ranging between 55%-68%. Both decreases, no change, and increases in carriage have been reported following the introduction of PCV immunization (76-78). *Moraxella catarrhalis* is a known cause of COPD exacerbations (79), but is a less common finding in CAP (6, 47), and bacteraemia is rare, especially in immunocompetent adults (80, 81). *M. catarrhalis* commonly produces beta-lactamase; however, a study comparing *M. catarrhalis* CAP with pneumococcal CAP found a zero mortality and no difference in length of stay, despite the majority receiving initial treatment with penicillin (82).

Enterobacterales and *Pseudomonas aeruginosa*

Gram-negative bacteria, excluding *H. influenzae*, are generally not included in empirical antimicrobial therapy for non-severe CAP in northern Europe. Prevalence also varies between studies and geographically (83). In a large German cohort, Enterobacterales and *P. aeruginosa* were found in only 1.3% and 0.4% of cases, but mortality was higher in these patients, likely reflecting both the pathogenicity of the bacteria and the fact that affected patients tended to be elderly with multiple co-morbidities (84). In African studies, gram-negative bacteria account for between 15%-40% of CAP cases, with *Klebsiella pneumoniae* being the most frequent, followed by *P. aeruginosa* (85-88). In Asia, the proportion ranges between 9% and 38%, with *K. pneumoniae* again the most common (89-91). Nasopharyngeal carriage of gram-negative bacteria in children in low-resource settings was found to be substantially higher compared to high-resource countries in one study (92).

Atypical pathogens

Historically, "atypical pneumonia" was a descriptive term for an unusual clinical presentation of pneumonia and ineffectiveness of penicillin; since then, the emphasis has shifted to specific bacteria (93).

Mycoplasma pneumoniae

A small bacterium lacking a cell wall, with a typical "fried egg" appearance on agar plates (94). It is a mucosal pathogen that lives as an extracellular parasite on the respiratory epithelial cells of its host (95). First isolated in 1944 by Eaton et al (96), it was not clearly identified as a bacterium until the 1960s. The genus name derives from the Greek mykes (fungus) in and plasma (formed). It is endemic but causes epidemics approximately every 4-7 years, sometimes with shorter intervals of 1-4 years (97), thought to be driven by waning immunity and genetic variation. It primarily causes tracheobronchitis in children and young adults, though some progress to pneumonia. Although *M. pneumoniae* can be cultured, it requires specialised media and takes between 5 days to 6 weeks to grow. Serology has traditionally been used for diagnosis, but has largely been replaced by PCR, which is faster and more sensitive (98, 99).

Chlamydia psittaci

The name of this intracellular gram-negative bacterium derives from the Greek word for parrot, *psittakos*, as it was first observed in these birds. A similar disease in other bird species was given the name *ornithos* (meaning "bird" in Greek), which later became the same condition. Human infections occur through inhalation of dust from faeces or other secretions from infected birds, which may have either asymptomatic or symptomatic infection (50). Human-to-human transmission is rare but has been reported (100). Cases in Sweden ranged from 20 to 77 per year in 2016-2025 (101), and the infection is considered to be underreported (102, 103), as it is not always included in the multiplex PCR panels, only in specific "atypical" panels. Owning birds and cleaning wild bird feeders are known risk factors (104), and when these specific questions prompt sampling and a diagnosis of psittacosis, it is one of the more satisfying moments as an infectious disease physician.

Chlamydia pneumoniae

The Chlamydia complement fixation test had been used since 1935 and patients with pneumonia and a positive result were assumed to have psittacosis. In 1943, Joseph Smadel noted seven cases without bird contact and raised the question that it would be "*desirable to search for a humanized strain (…) transmitted from man to man by upper respiratory route*". It was only over three decades later that a strain (TW-187), previously isolated from the conjunctiva of a child in trachoma vaccine study, was linked to a pneumonia outbreak in Finland and subsequently associated with another strain (AR-39) isolated from patients with respiratory symptoms, ultimately leading to the fusion of the two names to TWAR in 1989 (105-107). Primary infection occurs mostly in childhood, with reinfection in adults. Symptoms are

usually mild, but severe pneumonia can occur, and lower respiratory tract symptoms may be preceded by pharyngitis and hoarseness (108, 109). Infection has been linked to complications such as reactive arthritis, myocarditis and encephalitis. Furthermore, *C. pneumoniae* has been detected in atherosclerotic vessels, suggesting a link between infection and atherosclerosis (110); however, a meta-analysis found no differences in outcomes in patients with coronary artery disease treated with antibiotics (111). Diagnosis is usually made by PCR or serology, culture is both difficult and time-consuming (112).

Legionella

First described in 1977 after a large outbreak among attendees of the American Legion convention in Philadelphia, observing that transmission seemed to be linked to the local environment and person-to-person transmission seemed not to occur, leading to the identification of this gram-negative rod-shaped bacterium causing Legionnaires' disease (113, 114). The bacteria are found in freshwater, moist soil, composted material and contaminated water sources such as showers, air conditioning systems and hot tubs, and human infection generally occurs from inhaling aerosols from such (115). The genus *Legionella* includes 65 species, but *L. pneumophila* is responsible for around 90% of cases and more than 70-80% of these are serogroup 1 (116, 117). *Legionella longbeachae* is the second most common species in Europe, still only responsible for a few percent, while in New Zealand and Australia it is responsible for more cases than *L. pneumophila* and is predominantly found in compost and garden soil (117-119). Clinically, apart from symptoms of pneumonia, gastrointestinal symptoms can be prominent as well as neurological symptoms. While Legionnaires' disease causes pneumonia and potentially severe disease, Pontiac fever is a non-pneumonic, flu-like benign illness (120). Testing includes urinary antigen, which in most cases only detects *L. pneumophila* serotype 1, PCR from a LRT sample is sensitive and can detect several strains, and if not obtainable an OP swab is an alternative, although less sensitive (121). Culture from an LRT specimen is the gold standard, and can detect all Legionella spp, but requires specific culture mediums and lab expertise, and is mainly used in known cases and not as a primary diagnostic tool. Serology is generally not used as it requires paired sera and lacks in sensitivity (120). A Swedish retrospective study found that less than 2% of the patients tested for Legionella had a positive test, but those who did had a 30-day mortality of 13%, and only 15% had received empirical treatment covering Legionella, stressing both the importance and difficulty of identifying such cases (122).

Coxiella burnetii

A gram-negative pleomorphic, intracellular bacterium that causes Q fever, the name coming from the word "query", describing a febrile illness in slaughterhouse workers in Australia before the causative organism was known. It is a zoonotic disease and humans are infected by inhaling infected aerosols from pets and livestock who suffer latent infection, especially as high-level shedding occurs from female animals during parturition (123). It can manifest in different ways and *C. burnetii* pneumonia is one of the acute manifestations. It has been described almost worldwide (123), but is not usually a common cause of CAP requiring hospitalization, ranging from 0.6% in Spain (45), 6% in Israel (124), and 39% in French Guiana (125). Laboratory diagnosis is made from serology or PCR from blood (126).

Colonisation of the nasopharynx

The human nasopharynx is a reservoir for many bacteria, especially *S. pneumoniae, H. influenzae, M. catarrhalis* and *S. aureus.* Colonization of the nasopharynx precedes invasive disease, but only a few of those colonized develop clinical symptoms, in asymptomatic individuals the carriage is still responsible for spreading the bacteria, potentially causing disease in other persons. Specific carriage rates vary between studies, but the pattern is the same, with acquisition very early in life: *S. pneumoniae* and *H. influenzae* carriage rates peak during the first to second years of life (127). *M. catarrhalis* is also frequent in early years, and for all three bacteria, the rates successively decrease with increasing age and are more uncommon in adults (128, 129). The bacteria have a dynamic relationship, which changes in carriage and in infection, with co-colonisation being common between the three bacteria, but for example in acute otitis media (AOM) infection, *H. influenzae* develops a competitive relationship with both *S. pneumoniae* and *M. catarrhalis* (130). After PCV introduction in children, there have been indications of a compensatory rise of *H. influenzae* in both NP carriage and AOM (131, 132). For *S. aureus,* there is a negative association with pneumococcal colonization, as they seem to compete for the nasopharyngeal space (133). In older adults, pneumococcal carriage rates vary between populations. In a meta-analysis of adults over 60 years, carriage rates ranged from 0% to 39% (pooled prevalence of 9%), risk factors for carriage were smoking, contact with children, and living in a nursing home (134). For *H. influenzae*, it has been found in 0.3-3% of older adults (135-137), in contrast to *S. aureus*, which is carried intermittently by approximately 30% and persistently by 20 % of individuals (74, 75). Transmission of the pneumococcus to a new host typically occurs through respiratory droplets from a colonized individual, and is increased by intercurrent viral infection (138). In younger adults, pneumococcal carriage is transient with an average duration of 2-4 weeks (139, 140). Development of infection occurs shortly

after acquisition of *S. pneumoniae,* and in children an inverse relationship between carriage duration and attack rate is observed (141). Infection can also arise from an already present pneumococcal strain following disruption from a viral infection, influenza being the most studied (142).

Viruses

Influenza virus

A member of the family *Orthomyxoviridae* and an enveloped RNA virus with a segmented, single negative-stranded RNA genome encoding the surface glycoproteins of hemagglutinin (HA) and neuraminidase (NA). HA attaches to the cell surface receptor, and NA is the enzyme enabling viral replication and release from the host cell. There are 18 different H-types and 11 different N-types. There are three major influenza virus strains infecting humans, A, B, and C.

Influenza A viruses are characterized by the H and N types. Antigenic shift occurs when a significant change in the surface glycoprotein occurs, caused by a genetic transfer from an animal to a human strain, antigenic drift consists of minor changes that occur commonly, especially in Influenza A, causing yearly epidemics and being the reason for yearly immunization. It can cause severe pneumonia and acute respiratory distress syndrome, both alone and with co-infections (143). It is important to detect, as antiviral treatment with oseltamivir has been shown to reduce mortality if given up to 7 days after symptom onset (144).

Influenza A has likely caused outbreaks since the Middle Ages and since then, there have been at least 15 pandemics since then, the worst was in 1918, when an H1N1 virus referred to as “the Spanish flu” caused around 50 million deaths. Significant for the disease was the W-shaped mortality curve differing from the more usual U-shaped curve, with excess mortality in the youngest and oldest, presenting a high mortality in young adults (145). Much is not known about the pandemic, but we know that the following pandemics were due to antigenic shifts. In 1957, the Asian pandemic was caused by the H2N2 subtype, either due to milder disease or access to antibiotics, the deaths were lower but still around 2 million. In 1968, a recombination of H2N2 virus and an avian strain occurred, resulting in the H3N2, the “Hong Kong” pandemic, causing 1-2 million deaths. In 1977, H1N1 reemerged and caused the “the Russian flu”, not to be confused with the 2009 pandemic virus (“swine flu”), which had changed enough to not allow any cross-protection for those infected with seasonal H1N1 in the prior years, leading to 18,000 deaths globally (146).

All subtypes but two of influenza A can be found in birds, while there are several avian influenzas that have caused sporadic human cases; two variants, H5N1 and H7N9, have caused 861 and 1,500 cases, with a mortality rate of 53% and 39%, respectively (147).

Influenza B, is categorized into two lineages; Yamagata and Victoria, where Yamagata tends to affect older age groups compared to Victoria. The viruses can both co-circulate and alternate in prevalence. Influenza B has a slower antigenic drift than influenza A (148).

Influenza C is a common virus with high seroprevalence in children, it generally causes mild disease (149), however, since it is not included in most respiratory panels, more severe cases would go undetected.

Respiratory syncytial virus

A large enveloped negative-sense RNA virus in the family *Pneumoviridae.* It was first discovered in the 1950s as causing bronchiolitis in infants, and was mostly seen as a potentially severe respiratory disease for very small children (150). In the 1970s, it was recognized as causing severe disease in the elderly, with outbreaks in geriatric facilities (151, 152). Several studies have noticed the impact of RSV on hospitalization in the elderly and high-risk groups (153, 154). Recent years have led to breakthroughs in preventive measures, with approval of two RSV vaccines for pregnant women, the elderly and high-risk groups as well as prophylactic monoclonal antibody treatment for infants (155).

Human metapneumovirus

Human metapneumovirus (hMPV) belongs, alongside RSV, to the *Pneumoviridae* family but in the genus *Metapneumovirus;* it has two genetic classes, divided into subclasses. It was first discovered in 2001 in the Netherlands, but serological studies showed the virus had been circulating for at least 50 years (156). The symptomatology is similar to that of RSV and influenza virus, occurring in seasonal outbreaks (157). In hospitalized patients, it is most commonly detected in infants and adults >40 years (158), and it has been responsible for outbreaks in elderly facilities with a high fatality rate (159). It is an important respiratory virus to keep in mind when the screening for the usual suspects (influenza, RSV and SARS-CoV-2) is negative.

Human rhinovirus and enterovirus

Both non-enveloped RNA viruses are included in the enterovirus genus of the Picornaviridae family and are among the smallest viruses, as indicated by the name *pico,* meaning small (50).

Human rhinovirus (HRV) is the most common cause of upper respiratory infection, the common cold, and is characterized by its diversity, comprising three species and more than 170 genotypes. Despite all preventive measures during the COVID-19 pandemic, levels of HRV infection did not decrease to the same extent as other

respiratory viruses (160) It disrupts the epithelial cell lining in the nose (161, 162) and is frequently detected in etiological studies on lower respiratory infection and CAP with or without bacterial co-infection (6, 163, 164), but is often not screened for in clinical practice. It has been linked to several outbreaks in elderly facilities with both high hospitalization rates and sometimes death (165). In an outbreak in 2003 involving both staff and residents, where HRV was the only detected virus, 12 out of 56 residents died (166).

Enteroviruses can also cause respiratory infections and trigger exacerbations in patients with asthma and COPD, causing LRTI and pneumonia (167) by exacerbating epithelial dysfunction, promoting inflammation and affecting mucociliary clearance (168). In particular, EV-D68 has been responsible for large outbreaks (169).

Diagnostically, several multiplex PCR assays, including the one used in Paper II and III in this thesis, have issues differentiating between enterovirus and HRV (170).

Coronaviruses

Coronaviruses are positive-sense, single stranded RNA-viruses; they are enveloped, with spike proteins on the membrane surface, hence the name corona, meaning crown in Latin. Coronaviruses reside in animal reservoirs, such as birds and various mammals including bats. Four known human coronaviruses are considered endemic: NL63, 229E, OC43 and HKU1, the first two discovered in the 1960s and the latter two in the early 2000s, mainly causing mild respiratory illness (171, 172). Coronaviruses was considered relatively harmless until the outbreak of severe acute respiratory syndrome (SARS)-CoV in 2002, followed by the emergence of Middle East respiratory syndrome (MERS)-CoV a decade later, and then the coronavirus disease 2019 (COVID-19) pandemic.

SARS-CoV

The SARS outbreak of 2002-2003 originated from an animal market in China, and spread to 29 countries, resulting in 8,096 cases and an overall mortality of 9%. Nosocomial transmission was common, and health-care workers accounted for a substantial proportion of cases (173). There have not been any new cases since 2004 (174).

MERS-CoV

The virus first emerged in 2012 in Saudi Arabia, discovered in a man presenting with severe pneumonia who ultimately died. A few other severe sporadic cases were diagnosed before the first family cluster was identified, where half of the infected family members died, confirming human-to-human transmission (175). Dromedary camels are believed to be the primary animal reservoir, and transmission occurs from

saliva or respiratory droplets from infected camels or from ingesting camel milk. It seems not to be easily transmitted between humans and requires close contact. Meanwhile, the virus has caused several large hospital outbreaks, accounting for a third of known cases, associated with mortality rates around 30% (176). Complicating the diagnosis are the unspecific symptoms early in the disease, the lower sensitivity of URT swabs, and the higher viral loads associated with increasing disease severity (177). A unique presentation of MERS-CoV in relation to the other coronaviruses causing severe acute respiratory syndrome is the more common need for vasopressor support (173). The majority of cases occurred between 2014 and 2015, but cases are still detected yearly, primarily in Saudi Arabia (178).

SARS-CoV-2

The virus causing the COVID-19 pandemic was first reported in Wuhan, China ,and rapidly spread worldwide, resulting in 6 million deaths, and causing lockdowns and an enormous strain on health-care systems. The virus first infects the cells by binding to angiotensin II receptors in epithelial cells. The disease is characterized by an early stage of fever, cough and malaise. When some patients fail to eradicate the virus in this stage, it progresses to involvement of the lower respiratory tract and alveolar and endothelial cells, triggering a dysregulated hyperinflammatory immune response causing diffuse alveolar damage, microvascular damage, and coagulopathy (179), and both thrombosis and microthrombosis are relatively frequent complications (180). The SARS-CoV-2 virus has evolved into several genetic variants during the course of the pandemic, with Omicron being the current circulating variant, seemingly causing milder disease but also in a completely different context, with a population that is no longer immunologically naïve, as many have both received vaccinations and undergone infection (181).

Parainfluenza virus

A negative single stranded RNA virus belonging to the Paramyxoviridae family, including 4 known serotypes, each of which occurs with seasonality but with its own regional patterns (182). It is more common in children hospitalized with CAP, but it also occurs in adults, with no distinguishing features at presentation and similar severity, apart from a shorter length of stay compared to other viral causes of CAP (183).

Adenovirus

Causing mainly mild URT infection, gastroenteritis and conjunctivitis in small children. It rarely causes severe infection in immunocompetent adults, but such cases have been reported and can clinically resemble bacterial pneumonia, making it a diagnosis to consider in the culture-negative patient not responding to antibiotics (184).

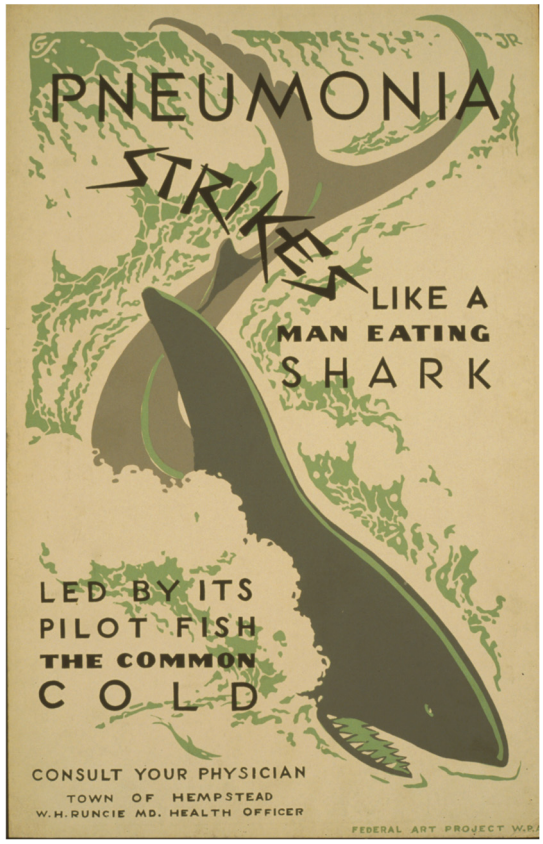


Figure 2. Poster from 1937, encouraging citizens to consult their physician for the treatment of the common cold. By G.S., JR for the Federal Art Project.

Viral-bacterial co-infections

A viral infection with a bacterial superinfection is common in hospitalized CAP patients, and the latest ATS guidelines even recommend antimicrobial treatment for all inpatients with CAP who test positive for a respiratory virus (22). This is, however, highly controversial and not endorsed by IDSA (185).

The pathogenesis differs for different virus-bacterial pairings. Generally, viral infection damages the epithelial barrier, and influenza, RSV, and parainfluenza viruses can bind directly to respiratory bacteria such as *S. pneumoniae, H. influenzae* and *S. aureus*, strengthening their adherence to human respiratory cells (186-188). The interaction between bacteria and viruses is also bidirectional, for example, *S. pneumoniae* makes epithelial cells more susceptible to hMPV infection and enhances RSV infection, while *S. aureus* enhances rhinovirus replication (189-192).

Most deaths in the 1918-1919 influenza pandemic are now considered attributable to bacterial co-infection, with *S. pneumoniae*, *H. influenzae*, *S. haemolyticus* and *S. aureus* the most commonly implicated (193). Many guidelines recommend empirical *S. aureus* coverage in influenza positive patients with suspected bacterial pneumonia, and historically as *S. aureus* has historically been an important co-pathogen alongside *S. pneumoniae,* as reported in both the 1957 Asian influenza pandemic (194, 195) and in the 21st century, where Nordic studies have demonstrated *S. pneumoniae* to be the most co-detected bacterium with influenza, with *S. aureus* or *H. influenzae* in either second or third place (196, 197). For other viruses, these studies showed that hospitalized RSV patients were most often co-infected with *S. pneumoniae*, followed by *S. aureus* and *H. influenzae* while hospitalized SARS-CoV-2 patients had fewer co-detections in general, mostly pneumococci followed by *S. aureus*. The severity of co-infected patients also varies across studies: neither of the two above-mentioned studies found differences in mortality among co-infected patients (196, 197), while others have noticed more severe infections and longer hospital stay, and one also increased mortality (198, 199).

Microbiological diagnostic methods

Globally, guidelines differ in which samples are recommended, which reflects tradition, resources, antibiotic resistance patterns and most importantly, the lack of a gold standard for establishing etiology in CAP; the toolbox of sampling methods is generally the same, but the indications differ somewhat.

In the Swedish guidelines from 2017, all hospitalized patients were recommended sampling with blood- and sputum culture (and nasopharyngeal culture if sputum was not obtainable), and pneumococcal urinary antigen test (PUAT) (36). In the updated guidelines, PUAT has been reserved for severe CAP or for certain situations (23). The Netherlands follow a similar approach, except that NP culture is not mentioned (39, 200). Regarding PCR testing for *M. pneumoniae, L. pneumophila* and viruses, most guidelines agree on testing based on clinical suspicion, outbreak situation and/or severe disease. Gram stain has become less common in Sweden due to its labour intensity in the lab, but is advised in many guidelines for those recommended a sputum sample (21, 38, 201-203).

While northern Europe has perhaps a more liberal testing strategy, other countries are more restrictive and continuous evaluation of the benefits and cost-effectiveness of testing is important.

For example, the IDSA and ATS guidelines recommend obtaining sputum and blood culture only in hospitalized patients with severe CAP (or if empirical treatment covers MRSA or Pseudomonas or hospitalization <90 days) (38).

The Chinese guidelines take on a more individual approach and do not recommend any routine testing in hospitalized non-severe CAP, instead focusing on specific patient groups such as those with immunosuppression, COPD, multilobar infiltrates, and patients with treatment failure (204).

The French guidelines focus especially on patients receiving broader empirical treatment such as cephalosporins, emphasising the importance of guideline adherence in these cases and sometimes recommending extended testing (21). It should be noted that empirical antibiotic recommendations also differ, as IDSA recommends a significantly broader empirical therapy than northern European guidelines (23, 38, 39). An overview of different testing strategies can be found in Table 1.

Table 1. Recommended testing for hospitalized CAP in different guidelines

Country/ organization	**Blood culture**	**Sputum culture**	**NP culture**	**PCR** (virus)	**PUAT**[1]	**LUAT**[2]	**CXR/ CT**[3]
Sweden (2025) (23)	All	All	All (if no sputum)	Consider	Severe	Severe	All LUS[5]
Denmark (2021) (200)	All	All	-[4]	All	-[4]	-[4]	All
Netherlands (2018) (39)	All	All	-[4]	Consider	Severe	Severe	All
France (2025) (21)	Severe + certain cases	All incl gram stain	-[4]	All	Severe	Severe	All LUS[5]
Britain (2009) (201)	Moderate	Moderate incl gram stain	-[4]	Consider	Moderate	Severe	All
IDSA/ATS (2019/2026) (22, 38)	Severe	Severe + certain cases	-[4]	Consider	Severe	Severe	All LUS[5]
South Africa (2017) (202)	Moderate	Moderate	-[4]	Consider	Certain cases	Severe	All
Australia (2021)(203)	All	All incl gram stain	-[4]	All	-[4]	All	All
Saudi Arabia (2025) (205)	Severe	Severe incl gram stain	-[4]	Consider	-[4]	-[4]	All
Korea (2018) (206)	Moderate	All incl gram stain	-[4]	Consider	All	Moderate	All LUS[5]
China (2017) (204)	Severe + certain cases	Severe + certain cases	-[4]	Consider	Severe + certain cases	Severe + certain cases	not spec

[1] PUAT: Pneumococcal urinary antigen test [2] LUAT: Legionella urinary antigen test [3] CXR: Chest X-ray, CT: Computed tomography [4] Not mentioned [5] LUS: Ultrasound, meaning either CXR, CT or LUS is acceptable.

Sample sites

The optimal sample is one with high accuracy and high yield, and which is safe and tolerable for the patient.

Upper respiratory tract sampling

Swab types

One might say "just grab a swab", but of course, nothing is as easy as that: different materials have different qualities. The generally recommended swab for microbial sampling in the URT is the flocked swab consisting of short nylon fibres, due to its superior ability to both absorb and release matter better compared with Dacron (polyester), rayon, and polyurethane foam swabs (207). Studies comparing flocked and rayon swabs have found flocked swabs to yield higher numbers of both total and infected respiratory epithelial cells in NP and nasal swab sampling (208), and to detect more respiratory viruses in both NP and OP sampling compared with rayon swabs (209). During the COVID-19 pandemic, the subject was investigated further due to material shortages. One study found nasal foam swabs to be superior to polyester swabs with sensitivities of 95% and 87%, respectively (210). Another study compared self-collected mid-turbinate swabs (MTS) and found foam swabs to have 10% lower sensitivity than flocked MTS swabs (211), suggesting that although flocked swabs are superior, other swabs are acceptable in these situations.

Figure 3. Comparison of different swab types
Scanning electron micrographs of flocked, cotton and foam swabs. Images kindly provided by Linda Jansson and Sebastian Wasserstrom (Lund Bioimaging Centre).

There are different sampling methods within the nasal cavity, and for swabbing there are different depths of insertion:

Nasopharyngeal swab

Even though this should be the most clear-cut location, different guidelines present the data with subtle differences, but most agree that the swab is inserted parallel to the palate until the nasopharynx is reached (measured by resistance, or at least two-thirds of the distance from nose to ear); in adults, the length to the nasopharynx varies between 8-11 cm (212).

Anterior nasal swab

This usually means swabbing in the nostrils only, inserting it 1-1.5 cm and rotating it against the nasal wall (213, 214).

Mid-turbinate swab and nasal swab

The term nasal swab (NS) can be confusing, as it often represents the same location as mid-turbinate swab, but sometimes an anterior nasal swab is what actually is performed. In the CDC's guidelines, the recommendation is to insert the swab 2 cm parallel to the palate until resistance, then rotate it (214), but a study measuring the mid-turbinate distance endoscopically found the mean length to be 4 cm (212).

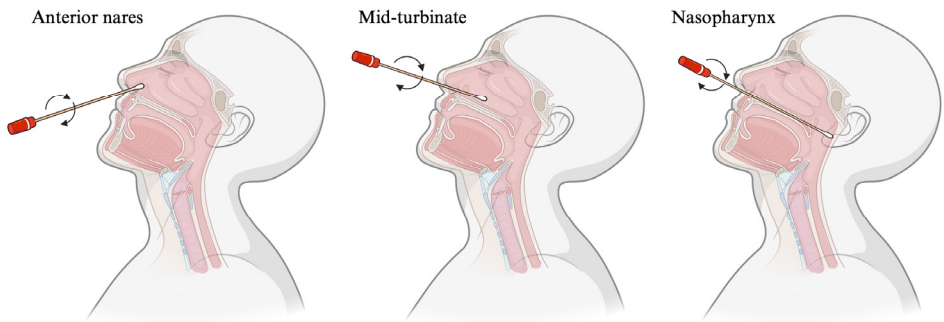


Figure 4. Illustration of different nasal swab depths.
Created in BioRender. Hansen, K (2026)

Nasopharyngeal aspirate

This involves inserting a catheter with a specimen trap through the nostril into the nasopharynx and applying suction while rotating and withdrawing the catheter. The dependence on a suction device is a limitation of the method (214).

Nasal/nasopharyngeal wash

A similar procedure as nasopharyngeal aspirate (NPA), except that before inserting the catheter, drops of normal saline are instilled in each nostril; the tolerability between these two techniques is similar (214, 215).

Oropharyngeal swab

The swab is inserted into the mouth and stroked against the posterior oropharynx, then rubbed over both tonsils while avoiding touching the tongue, gums or teeth (216). Advantages include less discomfort from sampling compared with nasopharyngeal swabbing (217).

Lower respiratory tract sampling

The challenge with all lower respiratory tract (LRT) sampling is achieving representative samples of bacteria while avoiding contamination from the oropharyngeal flora. Below, techniques and challenges for acquiring samples for the detection of bacteria are presented, but detection of viruses can of course also be performed.

Transtracheal aspirate

An invasive procedure introduced in 1959 (218), performed by aspirating secretions from the trachea by inserting a catheter guided by a needle through the cricothyroid membrane. To ensure sample quality, methylene blue can be nebulized into the oropharynx prior to the procedure, and samples showing blue discoloration are disregarded (219, 220). However, complications such as subcutaneous emphysema and pneumomediastinum can occur, although these are generally self-limiting (221). The technique does not offer any advantage over good-quality sputum in initial management of CAP and is no longer used in clinical practice (222).

Sputum

The gram stain, named after Danish physician Hans Christian Gram in 1882 (223), involves staining the slide, fixating the dye, then applying solvent to determine which bacteria's cell walls retain the dye after solvent; those that do and appear purple-blue are gram-positive, and those that do not and appear red are gram-negative. Gram stain of sputa can provide a fast and sensitive diagnosis of pneumococci and *H. influenzae,* but is not standard in all laboratories (224).

A concern with sputum samples is the possible contamination with oropharyngeal flora. The optimal sputum sample is taken in the morning, but this is often not feasible, as antibiotic administration cannot be delayed. Furthermore, the patient should rinse their mouth before sampling. High-quality sputum is defined as more than 25 white blood cells (WBC) per 10 epithelial cells, the WBC count indicating an infectious process and the epithelial cell count indicating contamination with saliva (225). The first hurdle with sputum is, however, collecting the sample, preferably before antibiotics, as it affects both gram stain and culture results (226) but this can be difficult; three different etiological studies obtained high-quality sputum samples in only 12%-37% of cases (6, 47, 227). Markussens et al. managed

to increase sputum sample collection via spontaneous expectoration from 12% to 73% with sputum induction, although only 56% of these were high-quality sputum (228). Saukkorpi et al. studied sputum quality in the elderly in relation to pneumococcal CAP, finding similar specificity in both high- and low-quality sputum challenging the standard of quality screening (229), a finding that has also been disputed by others (230).

Tracheal suction

A procedure involving insertion of a suction catheter through the nares into the trachea, with suction applied before withdrawing the catheter. A randomized trial compared tracheal suction with expiratory sputum sampling, focusing on sample quality, including 141 and 139 patients in each group, respectively. The sputum quality was better in the tracheal suction group, with no difference in adverse events. However, 50% of patients in the tracheal suction group rated the experience as "bad" or "very bad", compared with 12% in the expiratory sputum group (231).

Endotracheal aspirate

The same principle as tracheal suction, but performed in the intubated patient, by inserting a suction catheter through the endotracheal tube and applying suction when withdrawing the catheter (232). It shows only fair agreement with bronchoalveolar lavage (BAL) but is an alternative when BAL or protected brush specimen (PBS) specimen is not available in mechanically ventilated patients (233).

Protected brush specimen

Collected using a telescope catheter that is advanced within the bronchoscope channel: once in place, the brush is pushed out, and after sampling it is retracted into the outer catheter and removed from the bronchoscope. The outer catheter is then cut, the brush advanced and cut off, and the tip is placed in 1 mL of sodium chloride (234). Culture growth of $\geq 10^3$ (colony forming units) cfu/mL is considered significant.

Sampling with a blind brush uses the same telescoping catheter technique, but without the bronchoscope. The sample will be less precise and will originate from more central parts of the bronchial tree. A prospective study of trauma patients with ventilator-associated pneumonia (VAP) comparing sampling techniques found 95% sensitivity compared with BAL and 91% sensitivity compared with bronchoscopic sterile brush (235).

Bronchoalveolar lavage

The term BAL is sometimes used in a wider sense and often used when bronchial washing or mini-BAL would be the more appropriate term. A BAL is a procedure performed by placing the bronchoscope in an appropriate subsegmental bronchus and wedging it to isolate the distal airway. Thereafter, at least 100mL of sodium

chloride is injected and as much as possible is aspirated. The purpose is to sample fluids from the alveoli and distal bronchioles, and it is estimated that 1mL of lung secretions is returned. The BAL fluid can be used for diagnosing various infectious agents. Quantitative culture with a threshold of $\geq 10^4$ cfu/mL is used (234, 236). The main difference between BAL and PBS for microbiological testing is that the latter is used mainly for bacterial diagnostics in pneumonia.

Bronchial wash

Bronchial washing is also a bronchoscopic technique, but involves instilling a smaller volume and sodium chloride (20-40 mL) and not wedging the bronchoscope. This represents bronchial rather than alveolar fluid (234, 236).

Mini bronchial alveolar lavage

A non-bronchoscopic, blind procedure performed in mechanically ventilated patients, involving insertion of a sterile suction catheter through an endotracheal tube or tracheostomy, instillation of a smaller volume of saline (usually 20-80 mL), and collection of the fluid in a collection tube. In both immunocompromised (237) and immunocompetent patients, mini-BAL has shown comparable results to traditional BAL or protected brush sampling (238); a post-mortem investigation found a sensitivity of 78% compared with histological examination and culture (239). The procedure was initially described for diagnosing opportunistic infections in patients with AIDS (240). In that study, the same procedure was also performed in non-intubated patients by inserting the catheter through the nostril; it was reported to be well tolerated by patients but is not used in clinical practice.

Blood culture

In hospitalized CAP patients, blood cultures are positive in 4-14% of patients (241-245), and the added value can be debated. There are several aspects of blood culture aside from its diagnostic value: first, serotype surveillance for pneumococci is based on invasive isolates, another aspect is that the initial diagnosis on admission is not always correct. In a Japanese study, 3% of the included patients with presumed CAP diagnosis had a positive blood culture and a different source of infection (241)

Urinary antigens

Pneumococcal urinary antigen test

Several commercial urinary antigen tests are available for *S. pneumoniae*. The most commonly used are BinaxNOW *S. pneumoniae* (Abbott Diagnostics, Scarborough, Maine, USA) and ImmuView (SSI Diagnostica, Hillerød, Denmark), both of which are lateral-flow immunochromatographic membrane assays detecting C-

polysaccharide antigen. Sensitivity is estimated at 85% for ImmuView and 78% for BinaxNOW *S. pneumoniae,* with specificities of 99% and 100%, respectively (246), however, these figures are based on samples from blood culture-positive patients. A study of BinaxNOW and serotype distribution used samples from patients with positive culture from blood, sputum, BAL or pleural effusion and found an overall sensitivity of 83% in those with proven pneumonia, and 60% in those with probable pneumonia, importantly with great variation in sensitivity across serotypes (247). However, PUAT tests can stay positive several months after respiratory infection (248, 249), can be falsely positive in recently vaccinated individuals and in patients infected with *S. mitis* or *S. oralis,* and are not reliable in young children (250-252).

Several studies have analysed the effect of pneumococcal antigen testing on antimicrobial therapy. A Swedish registry study of hospitalized CAP patients found low adherence to the guidelines, with only 34% undergoing PUAT despite the recommendation to test all hospitalized CAP patients at that time. Furthermore, no overall effect on reducing broad-spectrum antibiotic use was observed; however, among patients with non-severe CAP and a positive PUAT, fewer received broad-spectrum antibiotics or atypical coverage (36, 253). Several other studies have confirmed that a positive result can lead to narrower-spectrum antibiotic treatment but far from all relevant cases, making its overall impact more limited than its potential (254-256).

Serotype specific pneumococcal urinary antigen assay

In Papers III-V in this thesis, this method is referred to as the UAD. It is a serotype-specific urinary antigen assay, based on Luminex technology. It begins with a mixture of colour-coded microspheres, each colour representing a serotype, as the beads are coated with serotype-specific monoclonal capture antibodies. This bead mixture is then combined with the urine sample, and if pneumococcal polysaccharides are present, they bind to their matching capture antibody. Biotinylated serotype-specific detection antibodies are then added, forming an antibody-antigen sandwich. Phycoerythrin (PE)-conjugated streptavidin is subsequently added, binding to the biotinylated detection antibodies. One light identifies the bead by its spectral signature corresponding to its serotype, while a second light quantifies the PE-fluorescence intensity, which is proportional to the amount of bound antigen (Figure 5). This assay is mainly used for research on pneumococcal serotypes, and Pfizer (257, 258), Merck (259, 260) and Public Health England's Respiratory and Vaccine Preventable Bacteria Reference Unit (261) all have such assays.

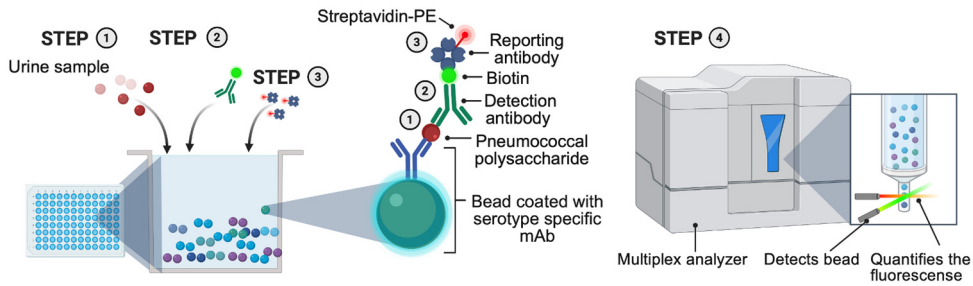


Figure 5. Luminex assay for serotype specific urinary antigen detection
Created in Biorender by Hansen, K (2026)

Legionella urinary antigen test

Legionella urinary antigen tests (LUAT) are lateral-flow tests, and some manufacturers offer a combination kit with PUAT. The different available kits aim primarily to detect *L. pneumophila* serogroup 1, with a sensitivity of approximately 70-89% and high specificity (262, 263), however, they can both be falsely negative early in the disease and remain positive for several months after Legionnaires' disease (264, 265). As it is a point-of-care test and yields fast results, it is an optimal first test when Legionella is suspected, but a negative result does not rule out Legionnaires' disease.

Serology

Serological tests are no longer routine for diagnosing respiratory viruses and atypical agents such as *M. pneumoniae*, *L. pneumophila* and *C. pneumoniae,* but can be an option in certain situations or in searching for specific pathogen. In general terms, raised IgM antibodies or seroconversion with a minimal of fourfold increase in IgG titre from acute and convalescent sera suggest acute or recent infection (266). Compared to PCR tests results for respiratory infections, serology results are usually available retrospectively; other pitfalls include that IgM can remain positive for a prolonged duration in some infections, low sensitivity for viruses, and variability in the performance of commercial kits (267-270).

Viral culture

A procedure involving inoculation of a clinical respiratory specimen into living host cells; once the gold standard, but as it takes up to 10 days to yield results and is less sensitive than PCR, it is no longer used in clinical practice for testing of respiratory viruses (271, 272).

Nucleic acid amplification tests

Nucleic acid amplifications tests (NAATs), are a type of nucleic acid test (NAT), and polymerase-chain reaction (PCR) is the most widely used NAAT.

PCR is a repeated three-step cycle that doubles the DNA each cycle: first, heat separates the DNA into single strands; second, short DNA primers bind to their complementary sequences on each strand; and lastly, a heat-stable polymerase enzyme builds a new complementary strand from each primer, recreating the double strand. The cycle quantification (Cq)-value is a semi-quantitative measure of the amount of virus/bacteria in the sample, meaning how many cycles were required to reach the target threshold of detection: a lower value indicates a higher pathogen load. Reverse transcription PCR (RT-PCR) allows RNA to serve as a starting template for PCR: reverse transcriptase enzyme converts the RNA into a single strand of complementary DNA, a DNA polymerase makes it double-stranded, and from there it is amplified through standard PCR amplification. Quantitative PCR (qPCR) refers to PCR amplification of DNA in real time, measured using a fluorescent probe, which enables quantification of the detected pathogen, by plotting the result against a standard curve of known concentrations (273).

PCR has replaced serology and viral culture for diagnosis of acute viral respiratory infection, *M. pneumoniae, C. pneumoniae, C. psittaci. B. pertussis* and *B. parapertussis* are also included in many panels, as well as several *Legionella* species. It is a highly sensitive method, but as it also detects non-viable pathogens, positivity can represent a recent infection.

For specific "typical" bacteria, most respiratory studies have concentrated on *S. pneumoniae* and *H. influenzae*. As they are closely related to other streptococcus and Haemophilus species, the possibility of cross-detection is therefore a challenge in designing specific PCR targets (274, 275), and it is important to know which specific target is used when considering these PCRs in clinical use and interpreting study results. Some developers of multiplex panels have been transparent in which target genes are used (276) while large commercial distributors such as Biomérieux® (FilmArray®) do not disclose their specific target genes (277).

Diagnostic-guided therapy

Diagnostic tests serve several purposes: the first is guiding antimicrobial therapy through increased knowledge of what is causing the individual patient's symptoms, but this also includes infection prevention and control, antimicrobial resistance monitoring and epidemiologic surveillance of different pathogens. With the increasing availability of tests, especially more expensive NAATs cost-effectiveness in relation to the benefits needs to be evaluated. When deciding which

diagnostic tests to perform on which groups of patients, the first step is to decide the purpose of the test and the context in which it will be used; is the setting one of low microbial resistance and narrow recommended treatment guidelines as in Sweden, or one of higher resistance levels and broad empirical treatments? Is the purpose of testing to de-escalate from a broad-spectrum empirical treatment, or to use narrow treatment in non-severe CAP and escalate if needed, or to use broad diagnostic measures in patients with treatment failure?

Several studies have aimed to investigate whether broad microbial testing benefits patients; endpoints vary across studies, however, with some focusing solely on narrowing the antibiotic spectrum, some on targeted antibiotic use and some on the time to modification of therapy. Most of the studies use the same extended multiplex panel, the FilmArray Pneumonia® panel (FAP), including 18 bacterial, 8 viral targets and 8 resistance genes, offering semi-quantative values for "typical" bacteria. Several studies have retrospectively shown the potential to adjust antibiotics (277, 278), other studies have confirmed shorter time to modification and/or more targeted therapy in patients tested with FAP compared with standard-of-care groups, but not necessarily narrower-spectrum therapy (279, 280) or have found both escalations and de-escalations (281, 282), one study demonstrated shorter antibiotic therapy in the ICU (283).

In an ICU setting, after implementation of broad PCR testing, the main impact was withdrawal of MRSA or- pseudomonal coverage if patients tested negative for these (284), a potential also noticed in a setting with broad empirical coverage including such pathogens (277). A retrospective study of CAP patients found alterations more likely with every additional test, but PCR for atypical agents had the greatest impact (285). A Finnish RCT of viral testing in acute respiratory infections did not find any change in antibiotic days in the intervention group (286), and in another study, clinicians did not alter antibiotics due to sputum results, only on other clinical parameters, the long turnaround time at the lab for culture was cited as a possible contributing factor (227). Two other studies did find more adjustments in antimicrobial therapy in the group with positive sputum results, but only in a quarter of the positive patients (242) or limited by few positive tests overall (287).

One study demonstrated fewer ICU admissions when syndromic testing was applied (280), but no study has, to my knowledge, shown any reduction in mortality based on testing alone; the only variable associated with lower 30 day mortality was being assessed by an infectious disease team (282). A summary of studies on guided therapy is shown in Table 2.

Table 2. Summary of articles on the effect of microbiological testing

Study	Design	Diagnostic test/ Endpoints	Key results
Cano (2026) **(284)** Spain	Retrospective ICU[1] n=213	Before vs after FAP implementation. ->Appropriate antibiotics and adjustments	Fewer antibiotic days in FAP group and more de-escalations (mainly de-escalating pseudomonas/ MRSA coverage)
Riano-Sanchéz (2025) **(282)** Colombia	Retrospective ICU[1] n=304	FAP (LRT) vs SOC, ID-team assessment. ->30-day CFR, LOS, antibiotic duration	No difference in CFR, ICU-days, LOS or antibiotic days. More antibiotic changes (both de- and escalation) in FAP. Lower 30-day CFR with ID assessment
Voiriot (2025) **(283)** France	RCT ICU[1] n=385	FAP (LRT) + PCT vs SOC + PCT. ->Antibiotic free days, 28- and 90-day CFR, LOS	No difference in CFR or total antibiotic exposure but 3 days shorter therapy
Markussen (2024) **(281)** Norway	RCT CAP n=374	FAP (LRT) vs SOC ->Targeted antibiotic, time to targeted treatment	More targeted therapy in FAP group both escalation, de-escalation and initiation
Virk (2024) **(280)** USA	RCT CAP, HAP, VAP. n=1181	FAP (LRT) vs culture. ->Time to antibiotic modification.	Shorter time to both de-escalation and escalation and fewer ICU-admissions in FAP group, more de-escalations (gram+) in controls
Cartuliares (2023) **(279)** Denmark	RCT ED n=294	FAP (LRT) vs SOC ->No/narrow- or targeted antibiotics <4h and <48h.	No increase in no/narrow-antibiotic in FAP group. But more targeted antibiotics at 4h and 48h. No difference in LOS, ICU or CFR
Barreto (2023) **(242)** Portugal	Prospective CAP n=660	Sputum culture ->In-hospital mortality, 30-day CFR, LOS, antibiotic modification	No difference in CFR. More antibiotic changes and more de-escalation in sputum-result group (but only 34% changed)
Cartuliares (2020) **(227)** Denmark	Retrospective ED n=170	Sputum culture ->Correct empirical therapy, antibiotic modification	All antibiotic changes driven by clinical parameters, none by sputum result
Monard (2020) **(278)** France	Retrospective ICU[1] n=159	FAP (LRT) vs SOC +Blinded expert analysis. ->Potential to de-escalate	Proposed modification in 77% in all patients. CAP: possible de-escalation 37%, escalation 8%
Buchan (2020) **(277)** USA	Retrospective inpatients[1] n=259	FAP (BAL/mini-BAL) ->Potential to de-escalate	Potential de-escalation/ discontinuation in 48%; mainly: stopping vancomycin or de-escalating from pip-taz
Wittermans (2019) **(285)** Netherlands	Retrospective CAP n=238	SOC incl sputum PCR atypical ->Antibiotic modification D3	Each additional diagnostic test increased alteration. Biggest impact- atypical PCR
Putot (2016) **(287)** France	Prospective >75y n=217	SOC incl LRT culture, virus PCR + serology -> Antibiotic modification	Antibiotic narrowing when positive test, but positivity rate only 29%

FAP= FilmArray pneumonia® panel, SOC=Standard of care, CFR= Case-Fatality Ratio, LOS= Length of stay. PCT= procalcitonin.[1] Included CAP, HAP and VAP.

Rationale

Paper I

In the emergency department, acute respiratory infection is a common reason to seek care. A rapid and reliable diagnosis of respiratory viruses, particularly during the influenza season, is needed for both infection prevention and control measures, for the administration of antiviral treatment and possibly for discontinuing antibiotic treatment. Nasopharyngeal aspirate had, up to this point been the gold standard for obtaining a sample, but the requirement for a suction device made the procedure less accessible, this investigation therefore aimed to determine whether NPA could be replaced with a flocked nasal swab for the detection of respiratory pathogens.

Paper II

As larger respiratory panels for PCR testing, including viruses and some atypical bacteria, were becoming more widely available and there were indications that nasopharyngeal testing may not be the most sensitive sample site for all pathogens, we wanted to compare NP swabs with OP swabs to determine whether one of them was preferable, or whether a combined approach should be recommended. As a sputum sample is preferred for the diagnosis of CAP but is not always obtainable, and others have suggested that PCR on NP swab for *S. pneumoniae* and *H. influenzae* can serve as an alternative (288) we also included these tests to examine whether an NP or OP-strategy was optimal.

Paper III

The etiology of CAP is not constant, new viruses emerge or are discovered, and diagnostic methods are constantly improving. Findings vary geographically and cannot be assumed to extrapolate across continents (83). As preventive measures such as Hib and, more recently pneumococcal immunization have been implemented there is a need to evaluate whether there has been a change in etiology, as empirical treatment recommendations may need to be updated accordingly.

Paper IV

As PCV was formally introduced into the paediatric immunization program in Sweden in 2009, and as invasive pneumococcal isolates are under surveillance we knew that IPD prevalence had been lowered in children but not in the elderly, mostly due to non-PCV-serotypes. The majority of pneumococcal cases are, however, not

bacteremic and this investigation therefore aimed to investigate the serotype distribution in adult CAP-patients in relation to current and up-coming PCV-formulations.

Paper V

Mycoplasma pneumoniae causes pneumonia in epidemics occurring several years apart with endemic circulation in between, and most often causes mild disease not requiring hospitalization. It can, however, result in more severe disease, and we therefore aimed to compare its clinical features with those suffering from the most common etiology of CAP, *S. pneumoniae*, in order to determine who should be recommended for testing and empirical treatment for *M. pneumoniae*.

Aim

General aim

To explore the microbiological findings and clinical features in adult patients hospitalized with community-acquired pneumonia following the introduction of PCV into the child immunization programme, and to explore optimal diagnostic tests for CAP and acute respiratory infection, with a focus on upper respiratory tract sampling.

Specific Aims

Paper I

To compare nasopharyngeal aspirate to flocked nasal swab for the detection of respiratory pathogens and perceived discomfort.

Paper II

To compare nasopharyngeal and oropharyngeal swabs in adults hospitalized with community-acquired pneumonia for detection of respiratory pathogens.

Paper III

To describe the etiology and clinical features of adults hospitalized with community-acquired pneumonia.

Paper IV

To investigate the pneumococcal serotype distribution in adults hospitalized with community-acquired pneumonia.

Paper V

To compare the clinical characteristics of hospitalized community-acquired pneumonia caused by *M. pneumoniae* with *S. pneumoniae.*

Materials and methods

Overall Methods

Setting

All investigations were performed in Skåne University Hospital in Malmö Sweden, a 600-bed teaching hospital.

Ethical considerations

Paper I. Ethical approval was obtained from the Regional Ethical Review Board at Lund University, no 28/2007, for a study including adult influenza patients in the emergency department for repeated sampling with flocked swabs. Following discussion with the ethical review board, this related study involving the inclusion of patients with suspected influenza in the same setting, but testing with only one swab at the time of inclusion, was included in this approval. Written informed consent was obtained from all participants before enrolment.

Paper II-V. Ethical approval was approved by the Regional Ethical Review Board at Lund University, no 2016/220 and 2016/356. Written informed consent was obtained from all patients or next of kin prior to inclusion. The study was prior to start registered at clinicaltrials.gov (NCT 03606135).

All papers included additional sampling which could lead to discomfort, the samples were not analysed until years after inclusion, and the patients could therefore not gain any possible treatment advantages from the microbiological findings.

Statistical methods

Descriptive data for categorical variables were summarized as counts and percentages, while continuous variables were expressed as medians and interquartile ranges or means and standard deviations. Paired samples were analysed with McNemar's test and kappa statistics. Differences in paired Cq values were estimated using Wilcoxon signed- rank test. For independent groups, categorical data were compared using Pearson's chi-squared test or, when appropriate, Fisher's exact test. Continuous variables were compared using the independent-samples t-test or Mann-Whitney U test when appropriate. When comparing multiple groups, ANOVA or Kruskal-Wallis was applied. A p-value < 0.05 was considered significant. All statistical analyses were performed using IBM SPSS or R.

AI-statement

The AI-tool Claude Sonnet 5 was used to improve the English grammar in the thesis summary. I take full responsibility of all content.

Author contributions

For Paper I, the co-authors were responsible for study design and inclusion of participants, I was responsible for chart review, data analysis, and writing the original draft. For Papers II-V, I was involved in study design and methodology, the writing of the study protocol and constructing the electronic case report form. The ethical process was led by me in collaboration with co-authors. I co-supervised the inclusion process and performed the chart review together with Elisabeth Rünow. For Paper II and V, I performed the data curation, analysis and the original draft, for paper III the original draft and analysis was performed by both me and Linda Yamba Yamba in close collaboration. For Paper IV, I drafted the interim analysis, the manuscript draft was written by Christian Theilacker, apart from the discussion, which was drafted by me, and I held a lead role in editing and reviewing the draft.

Study cohort paper I

The study was performed during the influenza season February 1 to March 10 2009 at the emergency department. Adult patients were screened for symptoms of influenza and enrolled consecutively during the daytime. No exclusion criteria were applied.

Study cohort paper II-V

The study was performed from September 2016 to September 2018. Patients aged ≥18 years was screened through the emergency department's log for a planned or performed chest x-ray or CT within 48 hours of presenting to the emergency room. The patients who had an infiltrate on imaging were further screened for inclusion criteria.

Control group paper III

Asymptomatic controls were screened at the orthopaedic department. The patients were recruited either in the emergency department or admitted less than 48h to the orthopaedic ward. The choice for this particular inclusion site was for practical reasons, these patients could represent all ages but at the meantime were less likely to be infected. Exclusion criteria were respiratory symptoms within the last 14 days and pneumococcal vaccination within the last 30 days. The patients were enrolled during the same period as the study patients.

Microbiology

Paper I

PCR-detection

Nasopharyngeal aspirate and flocked nasal swab (fNS) were collected from all patients. All samples were analysed with a qualitative Influenza PCR at the local microbiology laboratory(289) , and at the microbiology department at Sahlgrenska University Hospital using a multiplex real-time PCR targeting 18 respiratory agents: parainfluenza virus 1-3, influenza virus A/B, hMPV, RSV, rhinovirus, enterovirus, adenovirus, coronavirus (229E, OC43, NL63, HKU1), *M. pneumoniae, C. pneumoniae, H. influenzae* and *S. pneumoniae (290).*

Paper II-V

The microbiological tests included for the whole study cohort are presented below. Not all microbiological tests were included in every paper.

Standard of care

Culture

All microbiological samples that were performed as standard of care in the ECAPS-cohort were registered. Samples were processed at the accredited laboratory of clinical microbiology (Laboratory Medicine in Skåne, Sweden) according to standard procedures. Only samples taken within the first 48 hours after admission were recorded.

Blood culture

Coagulase-negative staphylococci were determined as contaminants and listed as negative.

Lower respiratory tract

Tracheal/bronchial alveolar lavage/ bronchial protected brush. Candida species and coagulase negative staphylococci were determined as negative.

Sputum culture

The cultures do not undergo microscopy and is therefore not qualitative.

Nasopharyngeal culture

Samples were taken from the nasopharynx using a flocked swab eSwab (Copan Italy). Pathogens that primary causes respiratory infections are, according to local practices, reported when cultured: *S. pneumoniae, H. influenzae, M. catarrhali*s and beta-haemolytic streptococci. If other pathogens are detected they are reported as negative.

The results are reported in Paper III. The results were excluded from Paper IV, since NP culture is not recommended in most international guidelines for diagnosing CAP. NP culture also added only one additional case of pneumococci not detected by the other methods included in Paper IV.

Serotyping of S. pneumoniae

All blood cultures were serotyped at the Public Health Agency of Sweden. All nasopharyngeal cultures positive for *S. pneumoniae* were serotyped at the Riesbeck laboratory, Malmö, Sweden. Serotyping was performed using a multiplex PCR, comprising 6 sequential reactions in combination with latex agglutination and the Quellung reaction as described (291). Isolates that were negative twice in PCRs were serotyped with the ImmuLex Pneumotest Kit (SSI Diagnostica, Copenhagen, DK) and Neufeld antisera. Isolates that were negative for *cpsA* in PCR twice or could not be determined through latex agglutination and the Quellung reaction, were considered non-typeable (NT).

PCR-detection for viruses

The clinical microbiology department offered three different panels for viral detection. One including Influenza A/B and RSV, one including Influenza A/B, RSV and hMPV, and a multiplex panel with 14 respiratory viruses (influenza A H1N1, influenza A H3N2, influenza B, RSV A/B enterovirus, rhinovirus, parechovirus, adenovirus, hMPV, and coronaviruses (OC43, NL63, 229E), as well as parainfluenza virus 1 to 3. These samples were registered for Paper III. If a test was positive in routine sampling and negative in study protocol sampling or vice versa, the test was considered positive. If negative in routine sampling and not tested in the study protocol only tests with the multiplex panel were considered tested.

Study samples

Samples were retrieved from both nasopharynx (NP) and oropharynx (OP) and frozen at -80°C. Due to limitations in laboratory capacity, the samples were not analysed at the same time. We therefor chose to analyse the nasopharyngeal samples first and decided not to analyse all 491 samples, to maintain seasonal variety, we chose every other sample, resulting in 241 samples. After publication of Paper III we analysed the oropharyngeal samples.

PCR detection

Viruses

A multiplex panel including 14 respiratory viruses (influenza A H1N1, influenza A H3N2, influenza B, enterovirus, rhinovirus, parechovirus, adenovirus, hMPV, and coronaviruses (OC43, NL63, 229E), as well as parainfluenza virus 1 to 3 and RSV A/B was used (292).

Bacteria

Real-time PCR for *Bordetella pertussis, B. parapertussis, Chlamydia pneumoniae, Mycoplasma pneumoniae.* A separate in-house PCR was used for *S. pneumoniae,* targeting the *Lyt*A gene (292).

An in-house PCR was used for *H. influenzae*. All NP samples were tested with a PCR targeting glpQ (synonym hpd), encoding the surface lipoprotein glycerophosphodiester phosphodiesterase (Protein D) (293, 294). The OP swabs were tested at a later stage, and surprisingly, 51% were positive. Suspecting specificity issues, all OP-samples were tested with an additional PCR targeting another part of the glpQ/hpd gene (hpd#3) which is more specific for *H. influenzae* (295), enabling further discrimination of *H. haemolyticus* from *H. influenzae*. All NP samples with any reactivity with glpQ/hpd were tested with hpd#3 to exclude false-positive signals revealing *H. haemolyticus,* resulting in six cases that were negative, three of which had a reactivity in hpd#3 but above the cut-off Cq-value.

Urine specimens

All specimens were analysed at Pfizer's Vaccines Research and Development Laboratory using two different urinary antigen tests: BinaxNOW S. pneumoniae® (Abbot Diagnostics, Scarborough, ME) and the UAD, a non-commercially available assay using Luminex technology to capture pneumococcal polysaccharides with serotype specific monoclonal antibodies. UAD-1 detects the 13 pneumococcal serotypes included in the PCV13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F 23F) , and the UAD-2 assay detects an additional 11 serotypes (2, 8, 9N, 10A, 11A, 12F, 15B/C, 17F, 20, 22F and 33F).Together, the 24 serotypes cover both PCV20 and PPV23 *(257, 258)*.

Control group Paper III

Urine specimens and two NP swabs were collected from all control subjects. The urine samples were tested with BinaxNOW *S. pneumoniae*® and UAD. One NP swab was cultured for bacteria and the other analysed with the same PCRs as in the study group. For *H. influenzae* it was tested with the PCR targeting *glpQ/hpd* gene.

Results

Paper I

Fifty patients with suspected respiratory infection were included, 50% were female, the median age was 72 years, and 45 patients were admitted. Of these, 23 patients had one or more detected respiratory agents. Of the 11 samples from NPA positive for influenza virus, 10 were concordant with fNS. For all viruses combined, 20 were positive in NPA and 19 were positive in fNS. One case of rhinovirus was only detected in NPA and one case of hMPV was only detected in fNS. For bacteria, 6 patients were positive for *H. influenzae* in NPA, but only 3 were positive in fNS. For *S. pneumoniae,* the situation was reversed, with 3 positive cases in NPA and 6 in fNS.

Thirty-five patients rated the discomfort of the two sampling methods on a scale of 1 (no discomfort) to 4 (great discomfort), and 66% rated fNS as causing little or no discomfort compared with 60% for NPA (Figure 6).

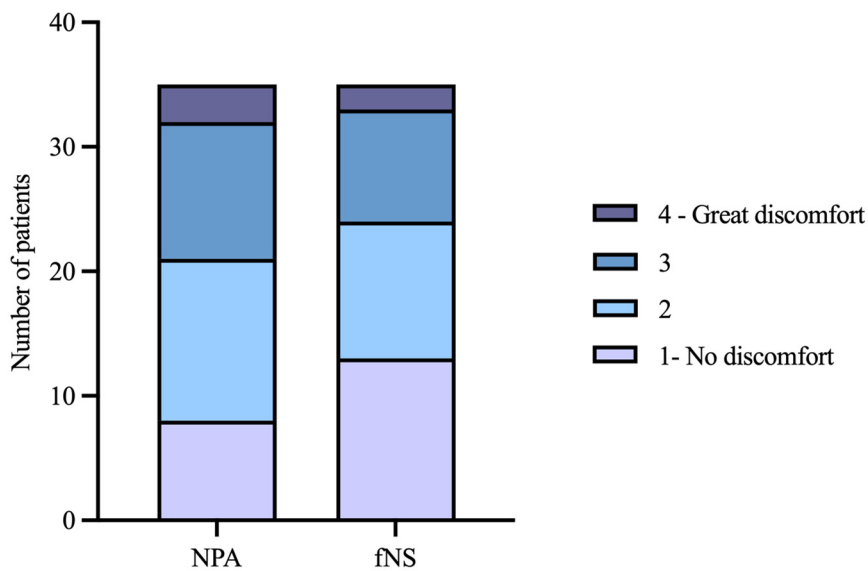


Figure 6. Perceived discomfort between sampling methods

Paper II-V

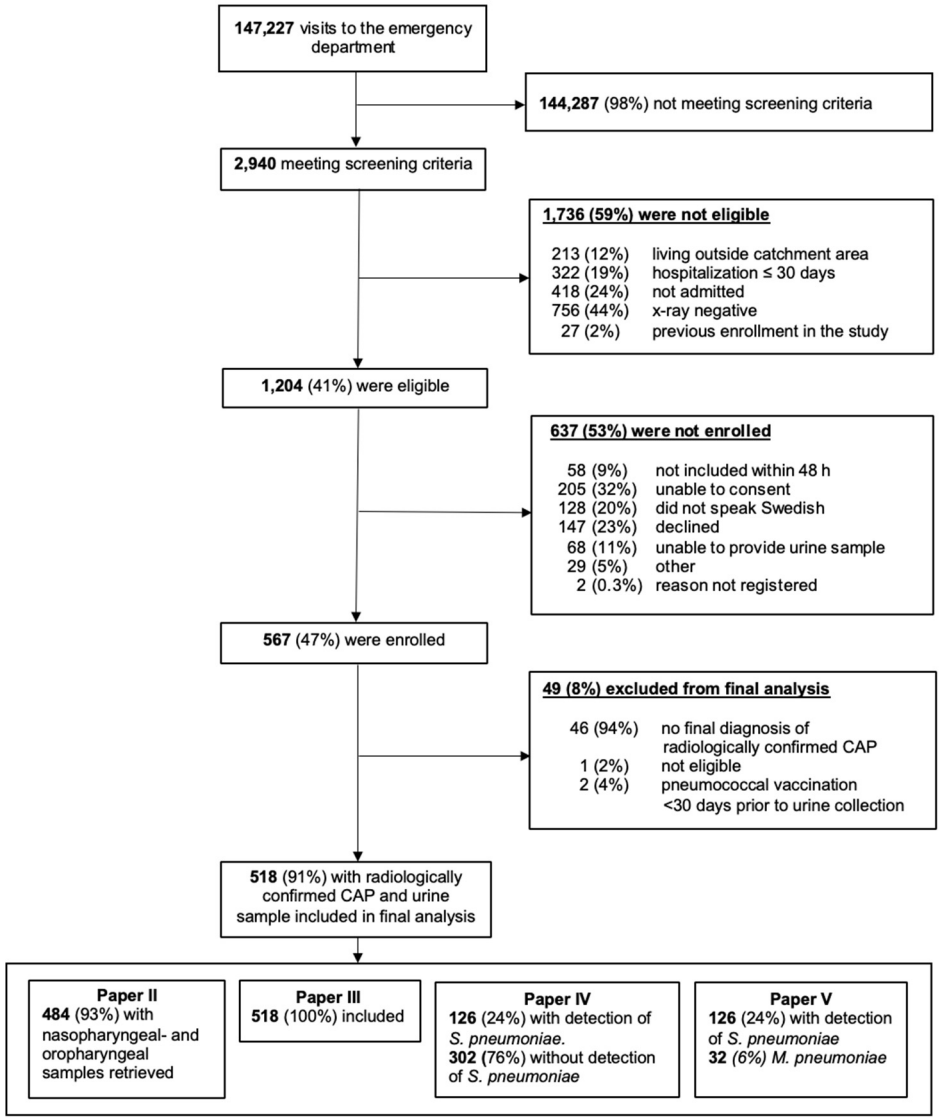


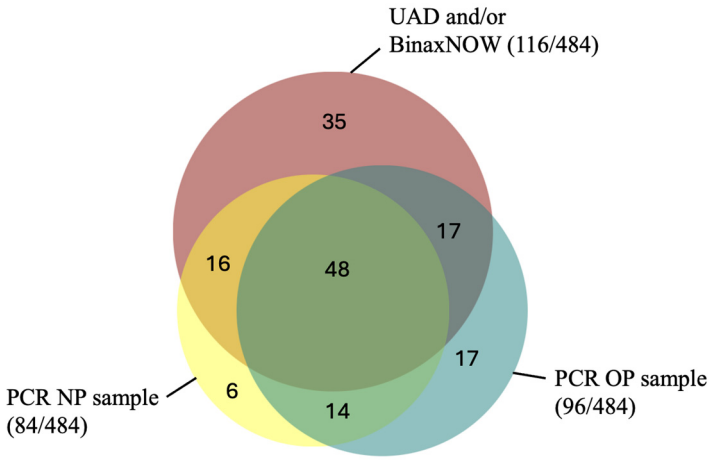
Figure 7. Flowchart paper II-V

Detection of S. pneumoniae

Papers II-V include the same cohort, but the proportion of positive *S. pneumoniae* samples range from 24% to 28% in the different papers depending on whether the URT samples were included. We chose to exclude them from Papers IV-V, since

they are not a recommended sample site in most international guidelines, due to uncertainty regarding colonization. The nasopharyngeal samples were included in Paper III, where we had a control group for comparison. The oropharyngeal samples are only included in Paper II. The overlap of the different pneumococcal tests is illustrated in Figure 5 (modified from Papers II and IV).

A



B

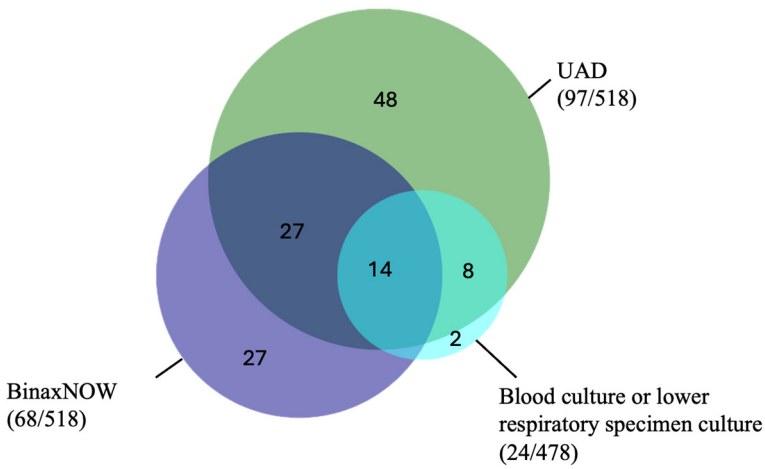


Figure 8. Overview of positive tests detecting *S. pneumoniae*
Fig. A: Only patients with all tests performed are included. NP culture not shown, all but one positive sample were positive in NP PCR.

A total of 420 NP swabs were collected, of which 21 yielded *S. pneumoniae* on culture: of these, all but one were also detected by either UAD/BinaxNOW and/or blood culture. Two of the pneumococci in NP culture were non-typable, 10 were the same serotype as detected by the UAD. Three cases were discordant with other methods: one was serotyped as 15A in NP culture but 17F in the UAD, the second was serotype 3 in NP culture but serotype 4 in both blood culture and UAD, and the third serotype was serotype 12 in NP and blood culture but 12F in UAD. Five of the cultured pneumococci did not have a serotype included in the UAD, four of which were positive in BinaxNOW.

The UAD was concordant with 18 out of 20 blood cultures that had a serotype included in the UAD; one was serotype 12F in UAD and 12 in blood culture, the other was serotype 5 and 8 in UAD but only serotype 8 in blood culture.

Paper II

Paired flocked NP and OP swabs were collected from 484 patients with CAP. With PCR, we detected 157 viruses in 153 individual patients, and 65% of these findings were concordant between NP and OP samples. Twenty-two percent of the detections were made only in NP, and 15% only in OP.

For bacteria, 118 cases of *S. pneumoniae* were found (24%), and only 53% were positive in both NP and OP; there were 19% and 29% detected only in NP and OP, respectively. For *H. influenzae,* 109 patients were positive, and similar to *S. pneumoniae,* 53% were positive in both sampled locations, with only 6% in NP compared with 40% only in OP. The PCR results of *H. influenzae* in relation to NP culture is illustrated in Figure 6 (not included in Paper II).

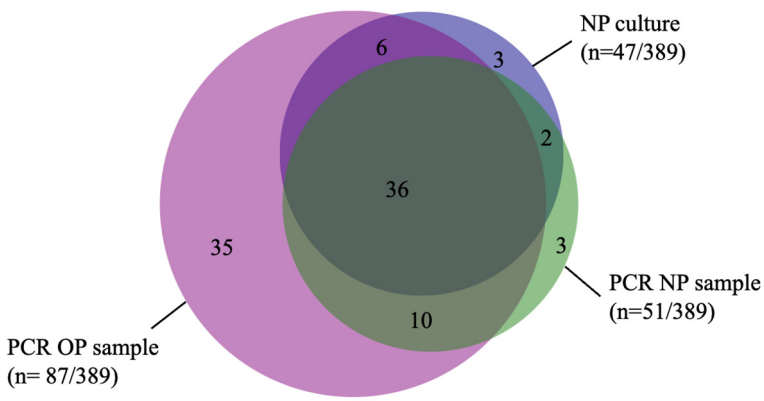


Figure 9. Overview of diagnostic tests for *H. influenzae*
Only patients with all three tests performed are included in the figure.

The Cq-values were lower in NP samples compared to OP for all viruses and bacteria except for *M. pneumoniae*. For all discordant samples, except for *M. pneumoniae*, the Cq-values were generally higher than in the concordant samples, indicating a lower pathogen load in the samples positive only in one location. Patients with discordant results for viruses had a shorter duration of symptoms before sampling, whereas the opposite was true for bacteria, with a longer duration of symptoms.

Paper III

All 518 patients were included in this paper, with a median age of 73 years and 46% female. The 30-day mortality was 4% and 3% were admitted to the ICU. The controls were younger with a median age of 64 years, healthier and 52% were female.

Combining the routine sampling with BinaxNOW *S. pneumoniae*, UAD and PCR in NP samples, at least one potential pathogen was detected in 351 patients (68%). Bacteria were detected in 15%, viruses in 28%, and co-detection of bacteria and viruses in 15%. The most common bacterium was *S. pneumoniae,* detected in 147 patients (28%), followed by *H. influenzae* in 84 patients (16%). Detections in CAP patients and controls are listed in Table 4.

Out of the 518 patients, 105 (20%) had received antibiotics before seeking care at the emergency room. Prior antibiotic treatment in relation to diagnostic method is listed in Table 3 (not included in Paper III).

Table 3. Pathogen detection related to out-patient antibiotic treatment

	Prior antibiotics	No prior antibiotics	*p*-value
NP culture (n=420)[1]	87 (20.7%)	332 (79.0%)	
S. pneumoniae	0 (0%)	21 (6.3%)	0.011
H. influenzae	10 (11.4%)	42 (12.7%)	0.857
M. catarrhalis	6 (6.9%)	33 (10.0%)	0.534
B-streptococci	1 (1.1%)	6 (1.8%)	1.00
PCR (n=490)[1]	97 (19.8%)	392 (80.0%)	
S. pneumoniae	3 (3.1%)	82 (20.9%)	<0.001
H. influenzae	11 (12.8%)	59 (15.6%)	0.350
Urinary antigen (n=518)[1]	105 (20.2%)	412 (79.5%)	
BinaxNOW *S. pneumoniae*	7 (6.7%)	61 (14.8%)	0.028
UAD 1/2	11 (10.5%)	86 (20.9%)	0.015
Blood culture (n=469)	99 (21.1%)	370 (78.9%)	
Any relevant bacteria	1[2] (1%)	33 (8.9%)	0.106

Prior antibiotic use is defined as antibiotic treatment within 14 days. Empirical antibiotic administered at hospitalization was not included, PCR and urinary antigen was collected 0-48h after empirical antibiotic administration. [1] One missing information on antibiotic. [2] Positive for *S. aureus.*

Table 4. Detections in patients with CAP and asymptomatic controls

Analysis	Subjects	Controls	Tested Controls/Subjects
S. pneumoniae UAD	97 (18.7)	8 (2.0)	396/518
S. pneumoniae BINAX	68 (13.1)	4 (1.0)	396/518
S. pneumoniae NP-Culture	21 (5.0)	1 (0.2)	491/420
H. influenzae NP-Culture	52 (12.4)	5 (1.0)	491/420
M. catarrhalis NP-Culture	38 (9.0)	25 (5.1)	491/420
PCR			
S. pneumoniae	85 (17.3)	9 (3.7)	241/490
H. influenzae	70 (14.3)	15 (6.2)	241/490
M. pneumoniae	28 (5.6)	0	241/490
C. pneumoniae	1 (0.2)	0	241/490
B. parapertussis	1 (0.2)	0	241/490
Viruses	145 (29.3)	17 (7.1)	241/495
Rhino or Enterovirus	50 (10.1)	10 (4.1)	241/491[a]
Influenza A	20 (4.0)	1 (0.4)	241/495[a]
Influenza B	21 (4.2)	2 (0.8)	241/495[a]
Human Metapneumovirus	26 (5.3)	0	241/495[a]
RSV A/B	11 (2.2)	0	241/495[a]
Parainfluenza virus 1-3	7 (1.4)	1 (0.4)	241/491[a]
Adenovirus	1 (0.2)	0	241/491[a]
Coronavirus OC43, NL63, 229E	10 (2.0)	3 (1.2)	241/491[a]

There were no cases of parechovirus or *B. pertussis*

Paper IV

There were 518 included patients; 349 of these were ≥65 years of age, and an additional 83 patients had an at-risk condition according to the recommendations on pneumococcal vaccines according to the United States Advisory Committee on Immunization Practices (296), together comprising 83% of all patients. Approximately half (48%) of patients ≥65 years had received the influenza virus vaccine within the last 12 months, but only 16% had any pneumococcal vaccine <5 years. The most frequently detected serotype was serotype 3, accounting for 5% of all-cause CAP, followed by serotype 8, 11A and 19A, each representing 2% of all-cause CAP. Twenty-seven cases were only positive in BinaxNOW and therefore not typable. The serotype distribution in relation to the different vaccines is listed in Table 5, modified from paper IV adding PCV21, which was licensed after the paper

was published, and PPV23. In our cohort, 11% of all CAP patients had serotype included in PCV13, and 13-18% had a serotype included in the higher-valent vaccines (PCV15, PCV20, PCV21 and PPV23)

Table 5. Pneumococcal serotype distribution in the cohort (n=518)

Serotypes	All CAP	PCV13	PCV15	PCV20	PCV21	PPV23
1		X	X	X		X
3	26 (5.0%)	X	X	X	X	X
4	3 (0.6%)	X	X	X		X
5	8 (1.5%	X	X	X		X
6A/6C	3 (0.6%)	X	X	X	X	
6B		X	X	X		X
7F	2 (0.4%)	X	X	X	X	X
9V		X	X	X		X
14	2 (0.4%)	X	X	X		X
18C	2 (0.4%)	X	X	X		X
19A	10 (1.9%)	X	X	X	X	X
19F	1 (0.2%)	X	X	X		X
23F	1 (0.2%)	X	X	X		X
22F	6 (1.2%)		X	X	X	X
33F	3 (0.6%)		X	X	X	X
8	10 (1.9%)			X	X	X
10A	1 (0.2%)			X	X	X
11A	10 (1.9%)			X	X	X
12F	1 (0.2%)			X	X	X
15B/15C	2 (0.4%)			X	X	X
9N	7 (1.4%)				X	X
15A[1]					X	
16F[1]					X	
17F	4 (0.8%)				X	X
20A[1]					X	
23A[1]					X	
23B[1]	1 (0.2%)				X	
24F[1]					X	
31[1]					X	
35B[1]	1 (0.2%)				X	
2						X
20						X
12[1]	1 (0.2%)					
22[12]	1 (0.2%)					
37[1]	1 (0.2%)					
Unknown serotype	27 (5.2%)					
Total detected serotypes		56 (10.8%)	65 (12.5%)	88 (17.0%)	84 (16.2%)	93 (18.0%)

[1] Not in the UAD. Serotypes only detected in blood or sputum, [2] NP only. 4 patients were positive for 2 serotypes, in the vaccine groups, patients are only counted once despite more than one serotype, leading to the total no of detected serotypes may be higher than in the vaccine groups.

Paper V

In the cohort, 33 patients were positive for *M. pneumoniae* in a PCR sample from either NP or OP, while 168 patients were positive for *S. pneumoniae* by any method. To avoid including any patients where the results could represent colonization, only those with a positive UAD/BinaxNOW and/or positive blood culture were included. One patient had a positive test for both pathogens, resulting in 32 patients with *M. pneumoniae* and 126 patients with *S. pneumoniae* remaining for analysis. Patients hospitalized with *M. pneumoniae* CAP had fewer co-existing diseases and the median age was 36 years compared to 70 years for *S. pneumoniae.*

At admission, patients with *M. pneumoniae* had a longer symptom duration, a median of 7 days compared to 3 days for *S. pneumoniae,* and half of the patients had received antibiotic treatment before seeking emergency care, compared to 11% of *S. pneumoniae* patients. Neither symptoms nor radiological findings could discriminate between the two pathogens, although pleuritic pain was more common in *M. pneumoniae* CAP. White blood cell count was distinctly higher in *S. pneumoniae* with a median of 15.2 compared to 9.9 in *M. pneumoniae* at admission. C-reactive protein level was also significantly higher in *S. pneumoniae*, with a median of 201 compared to 157 at admission.

Discussion

Upper respiratory tract sampling for respiratory viruses

Upper respiratory tract sampling is generally recommended for viral detection, and before SARS-CoV-2, influenza virus was the respiratory virus that received the most attention, due to the potential for treatment and the risk of nosocomial transmission. Most studies on URT-sampling have been performed in children, and does not necessarily apply to adults. Studies on adult patients are both more limited in number and complicated by different swab types across studies and the wide range of different sampling types compared: NPA, NS, mid-turbinate, NP and OP swabs, nasal wash, and any combination of these. Furthermore, there is terminological confusion about what NS means.

Flocked nasal swab versus nasopharyngeal aspirate

In Paper I, we compared NPA with flocked NS, inserting the swab 2-3 cm in the nostril, which some would consider a mid-turbinate swab. We found that 19 out of 20 respiratory viruses, and 10 out of 11 influenza cases were positive by fNS. Tthe conclusion that NS is an acceptable alternative to NPA is supported by comparable results in a study comparing NPA with NS (in this case swabbing the nostrils), for influenza detection (297), but negated by a study on immunocompromised adults, which showed insufficient detection for respiratory viruses in NS, however, this study had a small sample size and mostly detected rhinovirus (298). Other studies in adults comparing swabs in different nasal locations for influenza virus have shown lower sensitivity in the anterior nares compared to NP (299), and lower sensitivity but no significant difference between NP and mid-turbinate swabs (299-301), but higher viral load in NP (300). It seems the viral load increases with depth in the nasal cavity but from 2-3 cm to the nasopharynx in adults the sensitivities are still similar, the discomfort also correlates with this depth (299), although we did not see any significant difference in tolerability between NPA and fNS. Our results suggest that a fNS is an acceptable alternative to NPA, but, taking all available knowledge into account, an NP swab is the best alternative.

Nasopharyngeal vs oropharyngeal swab for viruses

The oropharynx is also a potential URT sampling site, and this was investigated in Paper II, comparing paired flocked NP and OP swabs. Although NP-swabs detected more viruses than OP, a substantial number of detections would be missed by only testing with an NP swab. A study with 1400 positive samples, but mainly children, found NP superior to OP swabs for influenza B and hMPV, but OP better for influenza A and adenovirus (302), and another study on influenza found that NP and OP performed similarly, but both missed cases (303). A study with 115 positive influenza patients found a higher yield when combining nasal- and OP foam swabs compared to NPA in all age-groups (304).

The Cq-values in our study were lower in the NP-samples indicating a higher viral load in the nasopharynx than in the oropharynx, a finding consistent with previous reports (217, 305).

Our results indicate that for respiratory viruses, combined NP and OP sampling should be recommended for maximum yield. If only one is possible, NP is likely more sensitive, but if not tolerated, OP is an acceptable choice. This stresses that the result of the swab is only as accurate as the quality of the sample analysed, and for all patients it is important not to blindly trust a negative test result. However, the patients with discordant findings in our cohort had a longer duration of symptoms and higher Cq-values indicating a lower viral load. So, if the test is taken primarily for infection prevention and control reasons, the lower sensitivity of a single swab may not be as important, as the patient is likely less contagious. However, if the results will impact treatment, a combined swab should be prioritized.

Upper respiratory tract sampling for bacteria

Nasopharyngeal culture

Nasopharyngeal culture is rarely mentioned in international guidelines for diagnosing CAP due to concerns of colonisation, but is recommended in the Swedish CAP guidelines if a sputum sample cannot be retrieved (23). Our findings in Paper III, with 21 positive cases of *S. pneumoniae* in NP-culture, of which 20 were also positive in either blood culture, BinaxNOW and/or UAD, while only 0.2% of the control group were positive, support the relevance of pneumococci in NP culture in CAP patients in our setting (288, 306). This may, however, not be applicable to all populations, as pneumococcal carriage in adults varies across studies, from 4-11% (307-310) up to 46% in a Kenyan cohort with a high HIV-prevalence (311).

For *H. influenzae,* we had no alternative detection methods for comparison, as only two patients were positive in blood culture. Nevertheless, it was the most common

finding in NP culture (12%) compared with only 1% of asymptomatic controls, similar to another Swedish study (288). This suggests that finding *H. influenzae* in NP culture is clinically relevant in our context, and interestingly there was no difference in detection for patients who had received antibiotics prior to admission. For *M. catarrhalis,* the asymptomatic carriage rate was higher, and a positive result in CAP patients should be interpreted with caution.

Excluding the positive results for *M. catarrhalis*, NP culture only had a 14% detection rate of relevant bacteria, which should be considered when assessing the cost-effectiveness of this test. Considering antibiotic resistance in *H. influenzae*, NP culture is still relevant when a sputum sample is not obtainable.

Nasopharyngeal vs oropharyngeal swab for bacteria

The benefit of molecular testing is that it can be highly sensitive despite prior antibiotics. For patients treated with antibiotics when seeking care, the detection rate was significantly lower for all pneumococcal tests, not entirely surprising, as the patients experienced treatment failure on antibiotics targeting *S. pneumoniae* and are more likely to represent another etiological cause. While NP and blood cultures were taken routinely before empirical antibiotic administration as routine care, the NP swabs for PCR were taken at inclusion, usually within 24 h, but could be up to 48 h after admission. The challenges of PCR-testing for these pathogens include discriminating between colonisation and infection, and the potential cross-reactivity with other bacteria, especially those that exist in abundance in the upper respiratory tract.

Streptococcus pneumoniae

In Paper III, PCR targeting the gene *LytA* was performed on NP swabs and compared to asymptomatic controls. PCR increased detection in CAP patients but also in the controls (from 0.2% to 5%). Others have concluded that *LytA* PCR in NP swabs or NPA can be a useful diagnostic tool when a sputum sample is not available (288, 312); our findings support this, although some positive samples likely represented colonisation.

In Paper II, the NP PCR results were compared with OP swabs: OP detected more cases, but only 53% were concordant between the two tests. Using the two urinary antigens as a proxy for established pneumococcal CAP, 76% of the positive NP swabs were also positive by UAD/BinaxNOW compared with 67% of positive OP swabs, indicating that the higher yield in OP may reflect lower specificity. Unfortunately, we did not have a control group to assess colonisation frequency for the OP swabs, but a Dutch study of asymptomatic elderly patients detected pneumococci by PCR (*LytA*) in 18% of OP swabs and 10% of NP swabs; the cut-off Cq-value was, however, high (<45) (313). Bjarnarson et al similarly reported

adequate sensitivity and specificity for OP sample PCR, using another gene target *(*Spn9802) (314). A methodological concern is the potential cross-reactivity with non-pneumococcal streptococci, especially in the oral microbiome. Although *LytA* is considered to be highly specific for *S. pneumoniae* (274, 315), studies have shown that OP samples and saliva can show cross-reactivity with non-pneumococcal streptococci (316, 317). Thus, although combining NP/OP sampling for pneumococci increased the yield, there might be a risk of cross-detection in OP samples.

Haemophilus influenzae

In Paper III, we noticed an increase in *H. influenzae* detection by PCR compared with culture: from 12% to 14% in NP swabs from CAP patients, and from 1% to 6% in the controls. After first analysing the OP swabs with *H. influenzae* PCR, we suspected that the *hpd* target gene might be detecting other closely related *Haemophilus spp.*, as there were unreasonably many positive samples. We reanalysed the samples with *hpd#3*, and the number of positive samples in OP decreased, but still yielded significantly more positive results than NP. It is difficult to assess whether that means OP is simply a more sensitive sampling location or whether cross-detection with oropharyngeal flora still occurs. A study comparing sputum with PCR in OP samples, using the omp6 target gene for *H. influenzae,* found that specificity increased when applying a Cq-value cut-off of 35 (314), but the lack of another method to compare with in our study, made establishing an optimal cut-off difficult.

For both *S. pneumoniae* and *H. influenzae,* there is potential for PCR-detection and rapid diagnosis. Combining NP and OP swabs gives higher detection rates, but more research is needed. This should include a control group with both PCR and culture for OP swabs before applying these methods, especially OP sampling, in clinical use.

Etiology of CAP in southern Sweden

This thesis focuses on adults hospitalized with CAP, data was collected prior to the SARS-CoV-2 pandemic. We chose not to divide the findings into definite and probable etiology, instead reporting all findings in relation to a control group without respiratory symptoms. By combining routine sampling with PCR for respiratory pathogens in NP-swabs, and two different pneumococcal urinary antigens, the total diagnostic yield was 68%, which falls within the broad range of other etiological studies (38%-87%) (4, 6, 45, 46, 91, 124, 228, 318-323). There is no gold standard for sampling in CAP, and the wide variation in the number of reported findings across studies reflects variety in diagnostic methods used. A summary of etiology in different geographical regions is presented in Table 6.

Table 6. Summary of etiological studies published after 2010 in comparison to Paper III

	Paper III **(292)**	Europe **(4, 45, 46, 228, 319, 323-326)**[3]	North America **(6, 47, 327, 328)**[3]	Asia **(49, 90, 91, 318, 322, 329-331)**	South America **(125, 320, 332)**	Middle-east **(124, 333, 334)**	Africa **(85, 87, 88, 299, 321, 335)**
Cohort size	518	267-3523	259-2259	76-3403	169-356	120-150	77-459
S. pneumoniae	28%	13-36%	5-12%	4-38%	2-31%	18- 24%	1-21%
H. influenzae	16%	2-40%	1-5%	3-37%	1-15%	3%	1-4%
M. catarrhalis	8%	<1-14%	<1	2-4%	2-9%	7%	1-5%
S. aureus	1%	2-11%	2-4%	1-9%	2-8%	1-8%	<1-6%
β-haemolytic *streptococci*	2%	<1-2	0-<1%	-	<1%	-	3%
α-streptococci	<1%	-	<1%	3%	-	-	7%
Enterobacterales [1]	2%	1-19%	1-4%	<1-38%	1-5%	2-14%	1-40%
M. pneumoniae	6%	<1-12%	0-2%	3-11%	8-9%	18%	1%
L. pneumophila	1%	<1-4%	1%	<1-18%	1-5%	0%	0
C. pneumoniae	<1%	0-3%	0-<1%	<1-6%	8%	21%	<1%
C. burnetti	-	<1%	0	0	39%	6%	-
M. tuberculosis	-	-	<1%	5-8%	<1-1%	18%	1-23%
P. jirovecii	-	-	<1%	2%	4%	n/a	0-4%
Viruses[2]	28%	4-48%	17-23%	15-53%	39-42%	4-45%[2]	32-48%
Viral-bacterial co-infections	15%	5-26%	3-4%	12-27%	17-23%	19%	5-9%

Percentages represent proportions of the whole cohort, some studies have calculated proportions of positive samples, these have been recalculated by me. [1] Incl *P. aeruginosa,* and *Serratia marcescens.* [2] The range of different viruses was not consistent across studies and some studies only tested for influenza. [3]Some studies only reported pneumoccal findings.

Pneumococcal serotype distribution

Streptococcus pneumoniae was the most common pathogen identified in our cohort, detected in 28% of all CAP patients, or 24% when excluding NP-samples. This is slightly lower than other European studies on serotype distribution reporting that 29%-35% of CAP was pneumococcal using either the same UAD or a comparable immunoassay (Bio-plex24), detecting the same serotypes (325, 326), but notably lower than figures from US studies using similar methodology, one of which included a 30-valent urinary antigen, and detected 10%-12% (327, 328, 336). These differences has been speculated to be due to a decline in cigarette smoking and higher immunization rates in the US (56).

In Paper IV, we investigated the serotype distribution of pneumococcal CAP after implementation of PCV in the paediatric immunization programme. In Skåne county, where the study took place, PCV7 was introduced in 2009, switched to PCV10 in 2010, to PCV13 in 2014 and back to PCV10 in May 2018 (61). In our

data, collected in 2016-2018, we observed that 11% of all hospitalized CAP was caused by a serotype included in PCV13, suggesting a limited herd effect on adults from vaccination of children. The most common serotypes were 3, 8, 11A, and 19A; apart from 11A, these were the same as the most commonly reported IPD serotypes in Sweden during the same time period (337). Serotype 3 is of particular concern, as PCV has had less effect on carriage of this serotype (62), and it is associated with complicated disease such as empyema, necrotizing pneumonia and septic shock (54, 338).

Since the paper was published, PCV21 has been licensed replacing PCV15, taking todays available vaccines into account, we found that 16%-18% of all-cause CAP in the whole cohort was caused by a pneumococcal serotype included in PCV20, PCV21 and PPV23. For PCV21, the numbers are likely underestimated, as several serotypes included in this vaccine were not included in the UAD. The proportions were also slightly lower for those actually eligible for vaccinations (patients >65 years and risk groups), possibly explained by a higher number of pure viral infections in that age group and some admissions likely due to frailty rather than disease severity.

A combined approach of PCV20 and PCV21, or PPV23 and PCV21, would expand the serotype coverage most in adults, although the experience of combining two different PCVs are limited. Cost-effectiveness analyses have previously been done for the existing Swedish vaccine recommendations for risk groups and the elderly (339), and focus should be on implementation and follow-up of these recommendations.

Is the epidemiology changing in CAP?

Haemophilus influenzae is a rare finding in blood culture, and only two patients had a positive blood culture in our cohort, so detection from a respiratory sample is needed to establish a diagnosis. In Paper III, we identified *H. influenzae* in 12% using NP-culture, increasing to 16% with PCR. The reported prevalence of *H. influenzae* varies greatly between studies, but there are several indications that *H. influenzae* CAP is increasing, Two Norwegian studies with data collection from 2007-2011 detected 3%-6% *H. influenzae* (46, 324), compared to a later investigation from the same country, including patients from 2020-2023, with a yield of 18% by LRT culture and 31% by adding PCR (FilmArray). Other studies have also observed these trends of increasing *H. influenzae* and decreasing *S. pneumoniae* (340, 341). This is suggested to be due to the pneumococcal vaccination, as changing dynamics have been observed in nasopharyngeal carriage in children (132). This needs to be monitored to possibly adjust future treatment recommendations. However, empirical treatment with benzylpenicillin has not shown adverse outcomes in *H. influenzae* infection (342), suggesting that it is not

as virulent and that we have time to alter antibiotics if we experience antibiotic failure or when culture results arrive.

Clinical features in hospitalized CAP

Apart from malaise, all symptoms decreased with rising age, and fever, chills, cough and sputum were less common in the elderly (>80 years), underscoring that the diagnosis of CAP can be less obvious in this group. Symptoms could not reliably discriminate between bacterial, viral or co-detection cases, but C-reactive protein (CRP) could offer some guidance: median CRP on admission was 79 mg/L in patients with only viral detection, 146 mg/L in patients with viral-bacterial co-detection and 194 mg/L in those with only bacterial detection. Unfortunately, we did not register the maximum level, nonetheless this is what the treating physician in the ED has to rely on.

Viruses and viral-bacterial co-infections

As explored in Paper III, viruses were detected in 28% of patients, in line with other studies using a similar respiratory virus panel, reporting rates of 15%-34% (4, 6, 46, 323). The most common virus was rhino/enterovirus followed by influenza virus, and rhino/enterovirus was more frequently co-detected with bacteria than influenza. Viral-bacterial co-detections did not result in increased mortality in our cohort, however, an increase has been observed by others, particularly for bacterial co-infection with influenza (196, 343) while others have not seen this difference (197, 320, 344). Viral detections were more common in patients older than 65 years, supporting targeted measures in this group such as influenza vaccination. In the whole cohort, male sex was more common (54%), Interestingly, this association was only seen in patients with viral detection in our cohort; both sexes were equally represented when only bacteria was detected. Male sex as a risk factor for pneumonia was described as early as in 1944 (345) and has been noted since (346), in influenza, mortality rates are reported higher in males and especially among 50-64-year olds with underlying myocardial infarction (347). Similarly in patients with COVID-19, male sex was found to be a risk factor for more severe disease and higher mortality (348) whereas females experience more long term symptoms. Differences are speculated to relate to differences in immunological responses, but more research is needed (349).

The patients with unknown etiology

All studies struggle with a proportion of patients with unknown etiology, and as more tests are usually taken in studies, it is a larger issue in clinical reality. In our cohort, 32% of the patients did not have any detection. In Paper III, we did not include the oropharyngeal samples, since we did not have a matched control group. Adding the positive viral detections and *M. pneumoniae* mostly increased the co-

detections, but lowered the undetermined group to 29%. When also adding the PCR-positive detections of *S. pneumoniae* and *H. influenzae* from the OP swabs, which should be interpreted with caution, 25% remained without a finding. Several possibilities exist: we had a very low number of LRT samples (only 8%), and pathogens mostly detected in LRT, such as *S. aureus* and Enterobacterales, would be underestimated. The group of patients with unknown etiology most closely resembled the group with only viral detections, the median age was the same (76 years), CRP-levels were fairly similar (104mg/L compared to 79mg/L), and there was a higher proportion of patients who were non-independent at home; some may have been admitted due to frailty rather than severity of infection. Congestive heart disease was more common in the group without findings, and, as in only CXR was performed in most cases, some patients may have had decompensation misinterpreted as pneumonic infiltrates. Aspiration is also a possible cause. Others have suggested that normal respiratory flora is an underestimated cause of CAP (350). Lastly, it is possible that not all pathogens have yet been discovered; hMPV, for example, was not identified until 2001, despite seroprevalence data dating back decades (156).

M. pneumoniae compared to *S. pneumoniae*

In Paper IV, a comparison was made of the clinical features of *M. pneumoniae* and *S. pneumoniae* during an endemic period of *M. pneumoniae*. Although differences could be seen in symptoms such as more pleuritic pain in *M. pneumoniae* CAP, there were no clinical symptoms that could reliably distinguish between the two pathogens. CRP was higher in pneumococcal CAP but the overlap between the two groups was large, making it of limited use for discrimination. White blood cell count differed more markedly, with higher values in pneumococcal CAP and could be of some diagnostic aid. Radiologically, it was not possible to discriminate between the two pathogens, and the findings were polymorphic, as others have also described (351, 352). The most prominent finding was the difference in age distribution: although *M. pneumoniae* accounted for only 6% of hospitalized CAP, it was responsible for one third of all cases in patients <50 years, although infection in the elderly also occurs (172, 351). As *M. pneumoniae* occurs in epidemics with endemic periods in between, the testing strategy needs to be adjusted accordingly, in endemic periods, it is most important to test younger hospitalized patients.

Conclusion

- A flocked nasal swab is an acceptable alternative to nasopharyngeal aspirate for detection of respiratory viruses, but a combination of a nasopharyngeal and oropharyngeal swab is optimal for maximal yield.
- Nasopharyngeal culture can be used for detection of *S. pneumoniae* and *H. influenzae* in our setting, but findings of *M. catarrhalis* are more difficult to interpret.
- PCR for *S. pneumoniae* and *H. influenzae* in NP swabs increased the diagnostic yield in CAP, but also increased detections in asymptomatic individuals. Combining an NP and OP swab resulted in higher detection but more research on specificity is needed before implementing larger multiplex panels on URT samples.
- *Streptococcus pneumoniae* was the most common finding in hospitalized CAP, followed by viruses and *H. influenzae,* and the latter may be increasing. Symptoms were less prominent in the elderly. Symptoms and outcome were similar regardless of etiology, but both men and the elderly had more viral detections.
- The serotype distribution of *S. pneumoniae* in CAP showed that one in ten adults hospitalized with CAP had a serotype included in PCV13, indicating that herd immunity from child immunization is not fully achieved. The higher-valent PCVs increase the potential preventable fraction of all-cause CAP.
- The most prominent difference between *S. pneumoniae* and *M. pneumoniae* CAP was that *M. pneumoniae* patients were three decades younger and more often had experienced antibiotic failure. In endemic periods, liberal testing for *M. pneumoniae* is warranted in hospitalized patients under 50 years of age.

Methodological aspects

The papers in this thesis are not without limitations. Paper I has a small cohort and a limited number of positive tests, which were distributed across several pathogens. We used 0.9% saline as a transport medium, and the results may have been different if a viral or universal transport medium had been used.

The strengths of the cohort included in Papers II-V were the continuous screening process, seven days per week, for two full years, of which the majority of inclusions were made by one research nurse. The inclusion period also spanned two influenza seasons, and a high proportion of per-protocol samples were collected.

Aspects of the inclusion process

The study cohort was screened from the patient log in the emergency department, so any patients directly admitted to the ward, bypassing the ED, would be missed in the screening process. We had approval from the ethics committee to include patients unable to consent themselves via consent from the next of kin but it was more difficult to include severely ill patients or those suffering from confusion, which would have affected the inclusion rate of this group. We performed a retrospective study identifying CAP patients by ICD codes in the same uptake area and time frame, and found 4% ICU admissions compared to 3% in our cohort, and a mortality rate of 6% compared to 4% (5). Another limitation was that the consent forms were only available in Swedish, limiting the inclusion of patients from other ethnic backgrounds. Having the forms translated into some of the most common languages could have increased inclusion and representation from these groups.

The definition of pneumonia is also somewhat problematic; to fulfil the criteria, radiology must be performed, and patients with a clinical diagnosis of pneumonia who did not undergo any imaging could not be included. Furthermore, as most included patients had only a chest X-ray performed, and given its imperfect sensitivity compared to computed tomography (44%-82%) (10-12), the true number of CAP patients was likely underestimated.

One exclusion criterion was the inability to produce a urine sample; this meant exclusion of dialysis patients with no urine production and patients without a urinary catheter who were unable to control micturition.

Aspects of diagnostic methods

In Paper III, we decided to report findings as detections and not as defined or probable etiology, the reason being that it can be difficult to distinguish nasopharyngeal culture and PCR detection from colonization. To address the issue of colonization, we included a control group without respiratory symptoms.

Complicating matters further, we had taken oropharyngeal swabs from all patients for PCR detection, but for practical reasons could not process these samples until much later. As we did not have oropharyngeal samples for the control group, we chose not to include the OP swabs in the analysis. Since colonization is less of an issue for viral and atypical bacteria, an option could have been to include these in the paper.

We had NP samples for 491 controls in Paper III, but due to resource limitations in the laboratory, we decided to analyse only 241 of these. To preserve the seasonal variability, we analysed every other sample. The control group was, however, healthier and younger than the study population, and we should have taken the opportunity to better balance the two groups. Furthermore, we only collected NP swabs from the control group and should have seized the opportunity to also collect OP swabs.

When we identified issues with the *H. influenzae hpd* PCR, we chose another gene target and reanalysed the samples. We analysed all OP samples, but only the NP samples with any PCR reactivity. This could be concerning for possible false-negative samples, but as our concern was only the specificity of the *hpd* gene target, and none of the negative OP samples became positive with *hpd#3,* we considered this an appropriate strategy.

The radiological findings were interpreted by the radiology specialist on duty, but the grouping was made by me and another infectious disease specialist; a radiology specialist might have categorized some of the images differently.

A major flaw in all etiological studies is the lack of a gold standard, which makes comparisons between studies difficult. Most studies use sputum samples, urinary antigen tests for pneumococci and *Legionella*, and PCR from URT samples for respiratory viruses and atypical bacteria. Which tests are included will of course affect which pathogens are detected. In our study, we had few LRT samples, naturally leading to fewer detections of Enterobacterales and *S. aureus*. Meanwhile, we had several diagnostic tests for detecting *S. pneumoniae.* In Paper IV, the UAD was more sensitive than BinaxNOW and, since the UAD only detects 24 pneumococcal serotypes, the total number of pneumococcal cases, especially serotypes not included in PCV20 or PPV23, is likely underestimated.

Future perspectives

In CAP prevention, things are moving forward. Two higher-valent pneumococcal conjugate vaccines are available, as is an RSV vaccine. In Sweden, it is only in recent years that a vaccine registry has been constructed, and pneumococcal vaccination is now a part of the national immunization programme, including persons at risk and those >65 years (353). The actual implementation of these immunizations needs monitoring, as well as their effects on pneumococcal disease. The serotype-specific urinary antigen test UAD was a sensitive test in these papers, and if commercial kits including additional serotypes become available, it could be a useful tool both in CAP diagnostics and serotype surveillance. Apart from IPD surveillance, etiological studies should be performed to monitor potential changes in CAP etiology and compensatory increases in other pathogens, especially *H. influenzae.* However, before advancing to changes in the empirical treatment recommendations in non-severe CAP, further studies should be performed on the consequences of inadequate empirical treatment for what we consider a less virulent pathogen (68), as existing data suggests that initial treatment with benzylpenicillin in *H. influenzae* infection does not lead to unfavourable outcomes (342).

As more diagnostic tests become available, many tests are stacked on top of each other, sometimes with diagnostic overlap. From a clinical perspective, there are several challenges. First, culture should preferably be collected prior to antibiotic administration, making it difficult to obtain in a later stage if needed, however, sputum and NP culture are inexpensive compared to PCRs. Second, obtaining sputum samples is easier said than done; in our cohort we had very few LRT samples, and one can speculate whether the Swedish strategy of accepting NP culture when a sputum sample is not obtainable possibly leads to lower effort to collect sputum samples. Third, the need for testing of respiratory viruses is affected by the availability of isolation rooms, current epidemiology, and whether the result will lead to a treatment change. All of these factors emphasize the need for diagnostic stewardship.

We studied specific bacterial PCRs for *S. pneumoniae* and *H. influenzae,* but at the moment, the large syndromic testing panels are more likely to find their way to the lab. The struggle with the specificity of the *H. influenzae* PCR in Paper II perhaps made me overly sceptical, but it led to critically reading the literature on the specificity of different PCR methods, especially those not disclosing their target genes. The rapid turnaround time of the syndromic tests is, of course, very

appealing, but at the moment, it is still a complement, not a replacement for culturing respiratory samples for several reasons. First, the panels have limitations in pathogen coverage: what is not on the panel can obviously not be detected, and although some resistance genes are included, it is not a replacement for antimicrobial susceptibility testing. Second, the lack of specificity: one study reported that 40% of detected bacteria in LRT samples were not clinically relevant (354), and another found that 25% of patients had three or more bacterial detections (355). For the more commonly used panels of viruses and atypical bacteria, combining NP and OP swabs would be preferred, but, although OP swabs have been suggested for the larger panels including "typical" bacteria, the high number of gram-negative detections is concerning (356). Studies in asymptomatic patients are needed, along with careful consideration of which patient groups would benefit from such tests and which samples should be accepted. The high cost, in combination with results that can lead to unnecessary escalation of antibiotics, makes implementation of such tests, especially in non-severe CAP, difficult without an antibiotic stewardship accompanying it.

Point-of-care ultrasound is being used more widely instead of chest X-rays and should be accepted as a criterion for CAP diagnosis in studies, in line with the many treatment guidelines recommending it (21-23, 206). One could argue that any radiology as a criterion is dated, as those with a clinical pneumonia or a negative chest X-ray, in many cases, also receive antibiotics with the same indication. Clinical judgement is, however, far from perfect (14), and X-ray-negative CAP has been shown to have a higher frequency of viruses, a lower frequency of pneumococcal findings, and lower inflammatory markers (357). In studies, clinical pneumonia should continue to be referred to as acute or lower respiratory infections. From a clinical perspective, the need for chest imaging can be debated, but abstaining should not come at the cost of broadening antibiotic coverage. In our cohort, we noticed a high rate of cephalosporin use, and I would argue that if patients with non-severe CAP are prescribed broad-spectrum antibiotics, establishing a diagnosis and etiology is more important.

Lastly, a missing piece is the patient's perspective. As the treating physician, I find it more satisfying to know what I am treating and to communicate that to my patients, and in my personal experience, the patients are anxious to know the cause of their infection. Hospitalized pneumonia patients have an increased risk of depression after discharge (358), and it would be interesting to see qualitative follow-up studies on CAP patients' recovery in relation to diagnostic certainty.

Populärvetenskaplig sammanfattning

Lunginflammation är en vanlig anledning till inläggning på sjukhus, och historiskt sett har den vanligaste orsaken till lunginflammation varit pneumokockbakterier. Därför är vaccination mot pneumokocker inkluderad i barnvaccinationsprogrammet sedan 2009, och det rekommenderas till de över 65 år samt till riskgrupper. Det finns dock ca 100 varianter av pneumokockbakterier, vilka kallas serotyper, varav långt ifrån alla ryms i vaccinet, och det finns även flera andra bakterier och även virus som kan orsaka lunginflammation. Ofta kan det också vara en kombination av både virus och bakterier. Provtagning syftar till att ta reda på vilka bakterier och virus som orsakar lunginflammation som blir så pass allvarlig att patienten behöver sjukhusvård. Det är viktigt av flera olika anledningar, både för att ge bättre behandling till den enskilde patienten med rätt antibiotika och för att kunna isolera de med mer smittsamma virus eller bakterier så medpatienterna inte riskerar att blir sjuka, men också för att övervaka vilka bakterier som är vanligast. Mer detaljerat är det också viktigt att veta vilka serotyper av pneumokocker som orsakar mest sjukdom eftersom vaccinet inte skyddar mot alla varianter som finns.

I den första studien jämfördes två olika provtagningsmetoder för testning av luftvägsvirus- och bakterier: nasofarynxaspirat, där en smal sugslang förs in via näsan till nässvalget, jämfört med en provtagningspinne som fördes in ett par centimeter i näsan. Det vi fann var att båda metoderna tolererades förhållandevis väl men att pinnen, som är en lite enklare metod, var tillräckligt bra på att hitta luftvägsvirus och kan användas istället.

De fyra andra studierna utgick från samma datainsamling, där 518 patienter som blev inlagda med lunginflammation blev provtagna med provtagningspinne från nässvalget och bakre svalgväggen och analyserades sedan med PCR-test för både virus och bakterier. I nässvalgsprovet utfördes också odling. Urinprov samlades in för att särskilja olika typer av pneumokockbakterier. Det fanns också en kontrollgrupp, det vill säga patienter utan luftvägsbesvär, som provtogs med urinprov samt och med prov från nässvalget.

I den andra studien i avhandlingen jämfördes pinnprovtagning från nässvalget med bakre svalgväggen och det visade att nässvalget fångade fler virus och svalgväggen fler bakterier men det bästa alternativet är att kombinera svalg-och nässvalgprov. För vissa bakterier fanns det tecken till att provtagningen dock är lite för känslig, så att det behövs mer forskning på dessa.

I den tredje studien sammanvägdes alla resultat. Vi fann att den vanligaste bakterien var pneumokocker, följt av *Haemophilus influenzae* och därefter virus. Hos många patienter sågs både bakterier och virus. Vid jämförelse av lunginflammation orsakad av bakterier, virus eller både och sågs ingen skillnad i dödlighet, längd på sjukhusvistelse eller symtom. Men äldre hade generellt mindre uttalade symtom och virusinfektion var vanligare hos äldre och hos män.

I den fjärde studien tittade vi närmre på de olika serotyperna av pneumokocker, och det var ungefär en tiondel av alla som var inlagda med lunginflammation som hade en pneumokockserotyp som är med i det vaccin som då ingick i barnvaccinationsprogrammet, vilket betyder att detta inte har lett till tillräcklig flockimmunitet hos de äldre. Det har också kommit flera nya pneumokockvaccin som innehåller fler serotyper. En högre vaccinationstäckning hos de äldre med något av de nyare vaccinen hade, i studien, potential att förebygga 13%-18% av lunginflammationerna.

I den femte studien jämfördes lunginflammation orsakad av *Mycoplasma pneumoniae* med *Streptococcus pneumoniae.* Det fanns inga symtom eller röntgenfynd som kunde skilja dem åt men snabbsänkan (CRP) och vita blodkroppar var båda högre vid pneumokockinfektion. Det som var allra tydligast var att Mycoplasma drabbade de yngre patienterna. Trots att det var få fall totalt, stod Mycoplasma för en tredjedel av infektionerna hos patienter under 50 år. När yngre patienter läggs in på grund av lunginflammation bör vi därför vara frikostiga med provtagning för Mycoplasma.

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