Universitätsspital Zürich, Zürich
Institut für Intensivmedizin
Direktor/in: Prof. Dr. med. Reto Schüpbach

Betreuung der Masterarbeit: PD Dr. med. Daniel Hofmänner
Leitung der Masterarbeit: Prof. Dr. med. Reto Schüpbach

Incidence of Bacterial Pulmonary Superinfections in critically ill COVID-19 Patients across different waves on the Intensive Care Unit of the University Hospital Zurich

MASTERARBEIT

zur Erlangung des akademischen Grades
Master of Medicine (M Med)
der Medizinischen Fakultät der Universität Zürich

vorgelegt von
Sarah Schneeberger (Matrikelnummer 17-714-866)

2023

Table of Content

1 Summary

Introduction: The global pandemic of the Coronavirus disease 2019 caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) resulted in millions of severe and critical cases. It is well known that patients hospitalised on the intensive care unit have a higher risk of acquiring bacterial superinfections. In the case of critically ill COVID-19 patients these are still rarely systematically reported and analysed. The aim of this study is to determine the incidence of bacterial pulmonary superinfections in critically ill COVID 19 patients hospitalised on the intensive care unit at the University Hospital Zurich during the different waves in Switzerland and identify the most common causative microorganisms.

Methods: In this retrospective monocentric cohort study, 149 patients were included and assessed for bacterial superinfection by systematic sampling of blood cultures, tracheobronchial secretions and bronchoalveolar lavages as well as evaluation of the clinical status.

Results: Out of the 149 patients, 70 (47%) were diagnosed with a pulmonary superinfection The overall risk of developing a relevant superinfection was lower for the second and later pandemic waves with a subhazard ratio of 0.604 (p = 0.109, 95% CI = 0.33 to 1.12). Among all the superinfected patients the most frequently identified microorganisms were *Enterobacter cloacae* (13.0%), *Klebsiella pneumoniae* (11.3%) and *Pseudomonas aeruginosa* (10.4%). Multidrug resistant bacteria were found in 18 (25.7%) patients with pulmonary superinfection over the course of the pandemic with *Pseudomonas aeruginosa* (2.7%), *Escherichia coli* (2%) and *Burkholderia spp.* (4%) being the most common multidrug resistant pathogens.

Conclusion: The reported incidence of superinfection is high. Compared to the first wave, superinfection rates are declining during the second and later waves. Gram-negative pathogens were prevalent during the first three pandemic surges and are most commonly responsible for causing superinfection. A decrease in multidrug resistant pathogens was noted.

2 List of Abbreviations

ACE	angiotensin-converting enzyme
ARDS	acute respiratory distress syndrome
BAL	bronchoalveolar lavage
BMI	body mass index
CARDS	covid-associated acute respiratory distress syndrome
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
EBV	Epstein-Barr virus
ESBL	extended spectrum beta-lactamase
hMPV	human metapneumovirus
HPV type 1 and 2	herpes simplex virus type 1 and 2
ICU	Intensive care unit
MDR	multidrug resistance
MRSA	methicillin resistant staphylococcus aureus
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus type 2
SAPS-II	simplified acute physiology score II
SOFA	sepsis-related organ failure assessment score
TBS	tracheobronchial secretion
VAP	ventilator associated pneumonia

3 Introduction

The Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) was first described in December 2019 and since then resulted in a global pandemic with millions of cases and a tremendous impact on the health care system(1, 2).

SARS-CoV-2 predominantly enters cells with a high expression of angiotensin converting enzyme 2 (ACE-2) receptors, namely epithelial cells of the respiratory tract, such as alveolar type 2 cells, but also coronary, renal and gastrointestinal cells (3, 4). The clinical presentation ranges from mild symptoms like fever and headaches to more severe courses with dyspnoea and pneumonia (5-8). Further, SARS-CoV-2 can trigger a cytokine-mediated inflammation response that leads to endotheliitis with progressive lung damage and acute respiratory distress syndrome (ARDS) (9, 10). Current therapeutic options are mostly symptomatic and include antiviral drugs for mild cases with high risk of hospitalisation and immunomodulatory drugs such as dexamethasone and tocilizumab (11, 12) for severe or critical courses of illness requiring admission to the intensive care unit (ICU) including the need for non-invasive or invasive mechanical ventilation or further organ support (13, 14). Throughout the duration of the pandemic approximately 21% the the patients who were admitted to hospitals required subsequent admission to the ICU and invasive mechanical ventilation was administered in 69% of the cases (15). Moreover, the utilization of invasive mechanical ventilation increased mortality to up to 43% (15). ARDS, acute kidney failure and bacterial superinfection has been identified as additional risk factors that contribute to ICU mortality rates (15, 16).

Secondary bacterial infection or superinfection in viral diseases such as influenza and COVID-19 is a well-known complication in hospitalised patients, especially in those requiring intensive care (17, 18). On the one hand, those individuals often are in an immunocompromised state due to immunomodulatory treatment and the partly immunosuppressive nature of COVID-19 (19). Additionally, patients on the ICU undergo more invasive procedures leaving them more susceptible to secondary bacterial infection (20, 21). More specifically, a higher incidence of pulmonary superinfection such as ventilator-associated pneumonia (VAP) has been observed in individuals with severe COVID-19 in need for invasive mechanical ventilation compared to those with other viral diseases (22). A similar observation has been reported for bloodstream infections

in critically ill COVID-19 patients, who suffer from an increased risk in contrast to non-COVID-19 patients (23-25). Superinfection is a relevant risk factor leading to a more severe course of illness with worse clinical outcomes especially for critically ill COVID-19 individuals (26, 27). In ventilated patients, it led to a higher mortality rate with an increased duration of ventilation, longer stays on the ICU and prolonged in-hospital stays during the first pandemic wave (16, 28).

Gram-negative bacteria were most commonly reported as causative microorganisms for pulmonary superinfection with multidrug resistance (MDR) being prevalent especially in pathogens isolated from intensive care patients (16). During the first pandemic surge, over 70% of critically ill patients received antibiotic treatment, even though their empirical use is not recommended routinely (26, 29). However, a tendency to less aggressive antibiotic prescription was partly observed as new therapeutic options became available (30). Nevertheless, a higher prevalence of MDR pathogens was noted over the course of the COVID-19 pandemic (31, 32), with possible worse implications for patients.

Despite the fact that superinfection increases the risk for worse clinical outcomes, the present literature regarding this topic in critically ill COVID-19 patients is scarce and focuses more on mild to moderate disease severity (33, 34). The rate of superinfection varies greatly between hospitals of different regions and over the course of the pandemic, which suggests a lack of standardised reporting and consistent evaluation (28, 35). The lack of knowledge regarding the dynamics of the superinfection rates and the corresponding pathogen epidemiology over time is persisting.

Thus, the aim of this study is to determine the incidence of bacterial pulmonary superinfection in critically ill COVID-19 patients hospitalised on the intensive care unit of the University Hospital Zurich during the different pandemic surges in Switzerland, identify causative microorganisms and evaluating their multidrug resistance to gain knowledge for improving patient management and treatment.

4 Material and Methods

4.1 Design

All the data used were gathered as part of the MicrobiotaCOVID cohort study (Kantonale Ethikkommission Zurich BASEC ID 2020 - 00646), a monocentric prospective observational study that was conducted at the Institute of Intensive Care Medicine of the University Hospital Zurich. Patients who met the inclusion criteria (see below under 4.2) received systematic sampling of nasopharyngeal swabs, tracheobronchial secretion (TBS), bronchoalveolar lavage (BAL) and blood cultures.

A BAL with 10-20 ml of saline was conducted at the day of ICU admission if clinically possible and TBS was collected in the case of mechanically ventilated patients. Further sampling of TBS took place at day 1, day 2, day 3, day 5 and after that every fifth day, as previously described (16).

Further testing at the day of ICU admission included the performance of a multiplex PCR for adenovirus, coronaviruses (229E, HKU1, NL63 and OC43), human bocavirus, human metapneumovirus (hMPV), respiratory syncytial virus (RSV), influenza A/B and parainfluenza virus 1-4 in nasopharyngeal swabs as well as a multiplex PCR for atypical respiratory bacteria such as *Legionella pneumophila*, *Chlamydophila spp., Bordetella spp*. and *Mycoplasma spp.* on pharyngeal swabs. Cultures of BAL and TBS were used to identify *Aspergillus spp*. and in selected patients, *Galactomannan* tests were performed from serum or respiratory secretions. These viral and fungal pathogens were sampled and analysed for the initial paper (16) but are not further relevant for this Master Thesis.

The detection of SARS-CoV-2 was carried out by analysing nasopharyngeal and/or pharyngeal swabs, TBS or BAL by real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

The Institute for Medical Microbiology and the Institute for Medical Virology of the University of Zurich analysed the samples by means of standard laboratory procedures (16).

An interdisciplinary team of experts containing ICU and microbiology specialists reviewed the analysed samples. Considering the detected microorganisms as well as the clinical status and laboratory values of the patient, the team decided if the finding was due to a relevant infection, colonization or a contamination of the sample, in analogy to Buehler et al.(16). Not considered relevant for pulmonary superinfection were

Enterococcus spp., coagulase-negative staphylococci, and streptococci other than *Streptococcus pneumoniae* and Streptococcus *anginosus* due to their generally benign nature for lung infections (36).

Demographics, patient characteristics such as comorbidities and risk factors (obesity, smoking, alcohol or addictive drug consumption), medication (long-term medication, antibiotics, immunosuppressive drugs), clinical parameters (vital signs, lab values) and received treatments (prone positioning, further organ support) were acquired by the consultation of electronic health care records using KISIM (CISTEC AG, Switzerland) and PDMS (Dräger, Germany). This information was only partly analysed for this Master Thesis.

4.2 Patients

4.2.1 Inclusion Criteria

Critically ill adult patients (>18 years old) with confirmed COVID-19 RT-PCR and ARDS who were hospitalised on the ICU of the University Hospital Zurich could be included in the study. Additionally, the patient or one of their relatives (in case of legal incapacity) must have signed a written informed consent.

The inclusion criteria are in accordance with the MicrobiotaCOVID Cohort Study (16). Due to the ongoing nature of this cohort, this present study is an explorative analysis of the patients hospitalised between March 2020 and February 2022.

4.3 Definitions

4.3.1 Primary Outcome

The incidence of bacterial superinfection was compared between the first and the second and later COVID-19 waves in Switzerland. As there is no official declaration defining their duration and dating, the beginning of a pandemic surge was defined for this Master thesis based on a rapid increase in the 14-day incidence according to the numbers of the Federal Office of Public Health “BAG (Bundesamt für Gesundheit)” (37) in Switzerland and dated as follows:

1st Wave: March 2020 - June 2020

2nd Wave and later waves: July 2020 – May 2022

Up to the present day, diagnostic criteria for superinfections are not internationally defined and validated. In this study, a team of experts of the MicrobiotaCOVID cohort

study assessed each sample separately and took clinical status into consideration for determining the presence of superinfection (as described above and under Buehler et al (16)). All relevant pathogens were noted, but not further divided if they were due to a first superinfection or reinfection.

4.3.2 Secondary Outcome

The most common causative microorganisms were identified and analysed for multi-drug resistance for each of the five pandemic surges in Switzerland separately. A Pathogen was counted as MDR if it was resistant against three or more different classes of antibiotics. For dating the following waves (I.e. waves after the 2nd wave) the same definition as mentioned above was applied.

1st Wave: March 2020 - June 2020

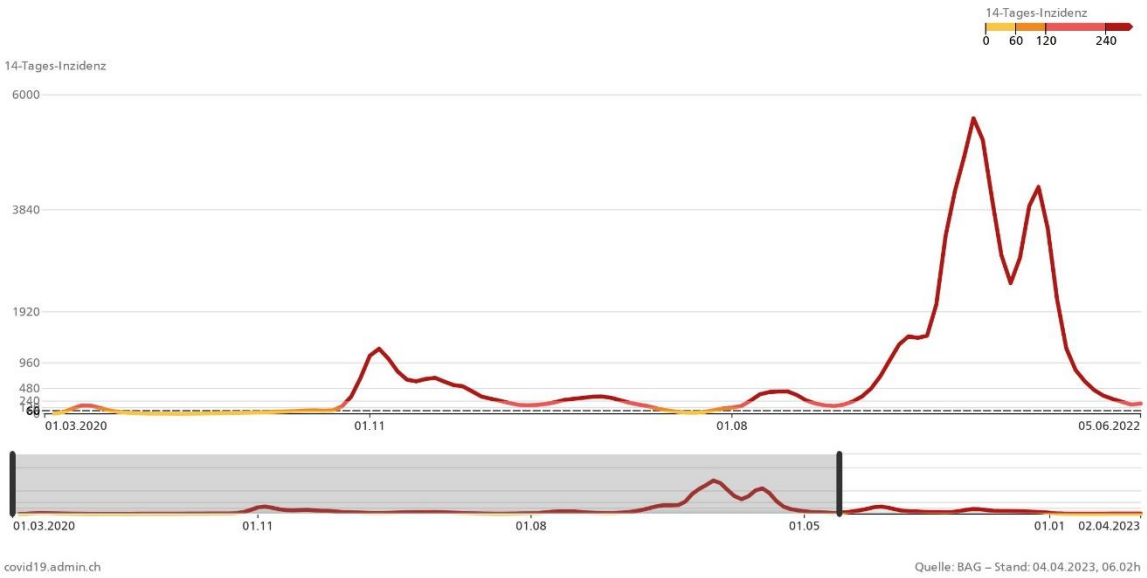
2nd Wave: July 2020 - February 2021

3rd Wave: March 2021 – June 2021

4th Wave: July 2021 – September 2021

5th Wave: October 2021 – May 2022

Figure 1: 14-day incidence of COVID-19 in Switzerland according to the BAG (36)



4.4 Statistics

The collected data were stored in a RedCap Database (Vanderbilt University, Nashville, TN, USA) in accordance with the Clinical Trials center of the University Hospital of Zurich. Data were validated and controlled by different designated team members of the MicrobiotaCOVID study team. A statistician assessed the RedCap data extract. Categorical data are presented as numbers and percentages. All continuous data are reported as mean and standard deviation or as median and interquartile range, as appropriate. A p-value < 0.05 was considered statistically significant.

Using the Kaplan-Meier method, the cumulative incidences of superinfection were modelled up to 30 days for different waves. The risk of developing a relevant superinfection (comparing different COVID-19 waves) was analysed by a statistical model according to Fine and Gray (delivering subhazard ratios), taking death or leaving the ICU as competing risks into account. Statistical analysis was performed using Microsoft Excel, R (R project, (http://www.r-project.org/)) and Stata (StataCorp, College Station, TX, USA).

4.5 Ethics

The study was approved by the cantonal ethics commission Zurich (Kantonale Ethikkommission Zurich BASEC ID 2020 - 00646) as part of the MicrobiotaCOVID cohort and registered at clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT04410263). Patients included in this study gave their full written informed consent (or their relatives in case of legal incapacity).

5 Results

5.1 Patient Characteristics

During the pre-defined inclusion period (March 2020 to February 2022), the data of 151 patients were collected. Two patients could not be included due to inconsistencies in the data validation und analysation, leaving 149 patients fulfilling the inclusion criteria.

Baseline characteristics of the included patients are presented in Table 1.

Table 1: Baseline characteristics, comorbidities, clinical conditions and ICU scores of the study population

	Overall (N = 149)	1st Wave (N = 41)	2nd and later Waves (N = 108)	p value
Baseline characteristics				
Age (year ± IQR)	63 (± 7.5)	59 (± 7.4)	64 (± 8)	0.249
Male sex (n/%)	106 (71.1%)	33 (80.5%)	73 (67.6%)	0.078
BMI (kg/m2 ± IQR)	28 (± 3.6)	27.9 (± 2.5)	27.8 (± 3.8)	0.249
Comorbidity and other clinical conditions				
Current smoking	5 (3.4%)	0 (0%)	5 (4.6%)	0.006
Alcohol abuse	2 (1.3%)	0 (0%)	2 (1.9%)	0.045
Drug abuse	0 (0%)	0 (0%)	0 (0%)	1
Severe Obesity	13 (8.7%)	4 (9.8%)	9 (8.3%)	0.027
Diabetes	46 (31.5%)	17 (41.5%)	29 (26.9%)	0.119
Neurological conditions	14 (9.4%)	5 (12.2%)	9 (8.3%)	0.666
Cerebrovascular disease	20 (13.4%)	6 (14.7%)	14 (13.0%)	0.783
Heart conditions	52 (34.9%)	12 (29.2%)	40 (37.0%)	0.15
Hypertension	76 (51%)	25 (61.0%)	52 (48.1%)	0.725
COPD	14 (9.4%)	2 (4.9%)	12 (11.1%)	0.028
Pulmonary fibrosis	2 (1.3%)	0 (0%)	2 (1.9%)	0.575
Asthma	10 (6.7%)	5 (12.2%)	5 (4.6%)	0.615
Liver disease	9 (6%)	2 (4.9%)	7 (6.5%)	0.153
Chronic kidney disease	27 (18%)	6 (14.7%)	21 (19.4%)	0.777
Immunocompromised state	21 (14%)	6 (14.7%)	15 (13.9%)	0.66
Solid organ transplant	13 (8.7%)	4 (9.8%)	9 (8.3%)	0.408
End organ damage	10 (6.7%)	2 (4.9%)	8 (7.4%)	0.113
Cancer	16 (10.7%)	4 (9.8%)	12 (11.1%)	0.269

Table 1: Continued

Scores				
SOFA-Score	7 (4 - 9)	8 (4.3 - 10)	7 (4 - 9)	0.871
SAPS II	38 (29 - 50)	36 (24 - 47)	38 (31 - 50.3)	0.212

Abbreviations: BMI = Body mass index, COPD = chronic obstructive pulmonary disease, SOFA-Score = sepsis-related organ failure assessment score, SAPS II= Simplified acute physiology score II.
Data are presented as median with interquartile range (IQR) or as number and percentage.

41 (28 %) patients were hospitalised on the ICU throughout the first wave, 78 (52 %) patients in the second wave, 21 (14 %) in the third wave, none during the fourth and 9 (6%) patients in the fifth wave. All in all, out of the 149 patients 108 (72 %) contracted severe COVID-19 after the first surge (i.e. after the 2nd and following waves).
Overall, the median age is 63 (±15) years with a higher percentage being male (71.1%) as listed in Table 1. The body mass index (BMI) averaged at 28 m^2/kg. Regarding risk factors, 45 (30.2%) patients are actively smoking, two patients (1.3%) consume at least a moderate amount of alcohol per day. 21 (14%) patients are in an immunocompromised state either because of prescribed medication or due to illness. The most common comorbidities are the ones affecting the cardiovascular system; in particular hypertension (51%) and other heart conditions (34.9%), as well as diabetes (31.5%) and chronic kidney disease (18%). Overall, patients throughout the first surge compared to those during second and later surges were similar regarding demographics, clinical characteristics and severity of illness at ICU admission as evaluated by the Sequential Organ Failure Assessment score (SOFA) and Simplified Acute Physiology Score-II (SAPS-II).

5.2 Superinfection and Clinical Outcomes

A total of 1475 pairs of blood cultures, 209 BALs, 807 TBS and 131 nasal-/throat-swabs were analysed, accounting for an average of 8 respiratory samples per patient. Out of the 149 patients, 70 (47%) were diagnosed with pulmonary superinfection with 21 (51.2%) patients in the first wave, 41 (52.6%) in the second wave, 8 (38.1%) in the third wave and none in the fourth or fifth wave.
In Table 2, clinical outcomes and superinfections are reported.

Table 2: Clinical Outcomes and superinfection data

Clinical Outcomes and superinfection data	Overall (%)	1st Wave (%)	2nd Wave (%)	3rd Wave (%)	4th Wave (%)	5th Wave (%)
Patients hospitalised on ICU	149	41 (52.6%)	78 (52%)	21 (14%)	0 (0%)	9 (6%)
Patients with bacterial pulmonary superinfection	70 (47%)	21 (51.2%)	41 (52.6%)	8 (38.1%)	0 (0%)	0 (0%)
Patients with pulmonary multidrug resistant pathogens	18 (25.7%)	10 (24.4%)	7 (9.0%)	1 (4.8%)	0 (0%)	0 (0%)
Patients with bloodstream infection	30 (20.1%)	11 (26.8%)	13 (16.7%)	6 (28.6%)	0 (0%)	0 (0%)
Duration of ventilation for superinfected patients (d)	16 (9 -29.8)	24 (11 - 39)	10.5 (7.5 - 27.5)	19 (13.5 - 20)	N/A	N/A
Mortality of patients with superinfection	24.30%	23.80%	24.40%	25%	N/A	N/A

N/A = not available.
Data are presented as median with interquartile range (IQR) or as number and percentage.

Multidrug resistant bacteria were detected in respiratory samples of overall 18 (25.7%) patients. They divided in 10 (24.4%) patients in the first wave, 7 (9.0%) in the second, one (4.8%) in the third wave and correspondingly none during the fourth and fifth wave. Blood stream infection was found in 30 (20.1 %) patients hospitalised on the ICU over the course of the pandemic, with the highest incidence during the third surge with 6 out of 21 patients (28.6%).

Duration of ventilation was the longest for superinfected patients hospitalised during the first wave with a median of 24 days and the shortest duration of ventilation being 10.5 days during the second surge.

The mortality averaged at 24.3% over the course of the pandemic with a slight increase of 1.2% points between the first and third surge.

The overall risk of developing a relevant superinfection was lower for the second and later pandemic waves with a subhazard ratio of 0.604 ($p = 0.109$, 95% CI = 0.33 to 1.12).

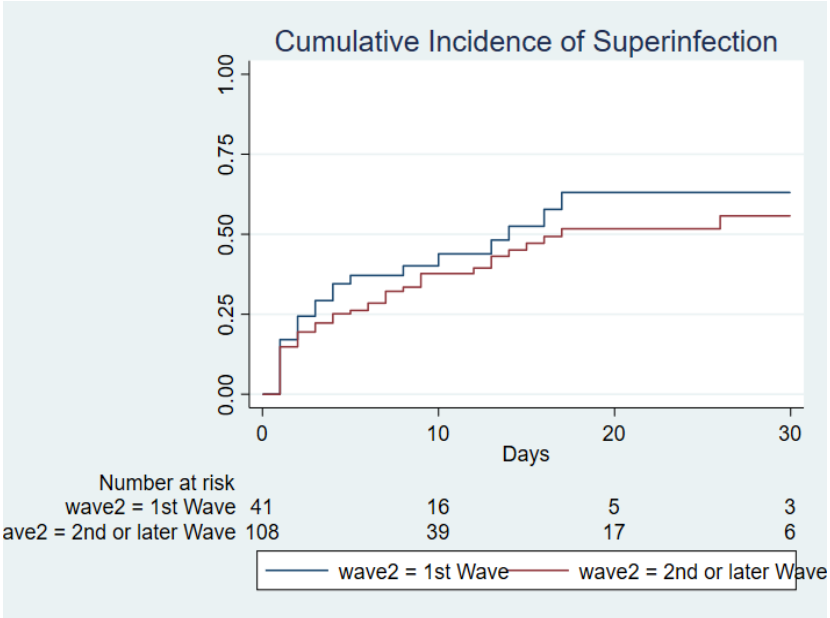


Figure 2: Cumulative 30-day Incidence of Superinfection

5.3 Relevant Microorganisms according to different COVID-19 waves

As shown in Table 3, among the group of superinfected patients, 683 TBS/BALs were collected with 302 (44.2%) samples detecting pulmonary bacteria. Out of these 302 samples, 115 (16.8%) identified pathogens were considered as relevant. Comparing the different waves, the second had the highest rate of overall pathogen detection (54.7%) and relevant pulmonary pathogen detection (27.2%).

Table 3: Microbiology

Detected pathogens	Overall (%)	1st Wave (%)	2nd Wave (%)	3rd Wave (%)	4th Wave (%)	5th Wave (%)
Number of TBS/BAL in superinfected patients	683	376	232	75	0	0
Overall pathogen detection in TBS/BAL	302 (44.2%)	156 (41.9%)	127 (54.7%)	19 (25.3%)	0 (0%)	0 (0%)
Relevant pulmonary pathogen detection in TBS/BAL*	115 (16.8%)	39 (10.4%)	63 (27.2%)	11 (14.7%)	0 (0%)	0 (0%)
Acinetobacter baumanni spp.	3 (2.6%)	1 (2.6%)	1 (1.6%)	1 (7.1%)	0 (0%)	0 (0%)
Acinetobacter berezinae	1 (0.9%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Burkholderia cenocepacia	5 (4.3%)	2 (5.1%)	3 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Burkholderia cepacia complex	4 (3.5%)	2 (5.1%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Chryseobacterium	2 (1.7%)	0 (0%)	1 (1.6%)	1 (7.1%)	0 (0%)	0 (0%)
Citrobacter freundii	2 (1.7%)	2 (5.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 3: Continued

Citrobacter koseri	3 (2.6%)	1 (2.6%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Corynebacterium amycolatum	1 (0.9%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)
Corynebacterium pseudodiphtheriticum	1 (0.9%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter cloacae	15 (13.0%)	6 (15.4%)	7 (11.1%)	2 (14.3%)	0 (0%)	0 (0%)
Escherichia coli	11 (9.6%)	4 (10.3%)	6 (9.5%)	1 (7.1%)	0 (0%)	0 (0%)
Haemophilus influenzae	3 (2.6%)	0 (0%)	3 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Haemophilus parainfluenzae	1 (0.9%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)
Hafnia alvei	2 (1.7%)	0 (0%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Klebsiella pneumoniae	13 (11.3%)	5 (12.8%)	6 (9.5%)	1 (7.1%)	0 (0%)	0 (0%)
Legionella pneumophilia	1 (0.9%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Moraxella catarrhalis	2 (1.7%)	0 (0%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Morganella morganii	4 (3.5%)	1 (2.6%)	3 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Mycoplasma hominis	2 (1.7%)	0 (0%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Pseudomonas aeruginosa	12 (10.4%)	8 (20.5%)	2 (3.8%)	2 (14.3%)	0 (0%)	0 (0%)
Rothia mucilanginosa	1 (0.9%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Serratia marcescens	2 (1.7%)	0 (0%)	1 (1.6%)	1 (7.1%)	0 (0%)	0 (0%)
Staphylococcus aureus	11 (9.6%)	0 (0%)	11 (17.5%)	0 (0%)	0 (0%)	0 (0%)
Stenotrophomonas maltophilia	1 (0.9%)	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)
Streptococcus anginosus	6 (5.2%)	3 (7.7%)	2 (3.8%)	1 (7.1%)	0 (0%)	0 (0%)
Streptococcus pneumoniae	5 (4.3%)	1 (2.6%)	4 (6.3%)	0 (0%)	0 (0%)	0 (0%)
Ureaplasma urealyticum	1 (0.9%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)

* Without repetitive detection of the same pathogen
Data are presented as number and percentage.

Among 28 different isolated microorganisms, the most frequently identified were anaerobic Gram-negative pathogens namely *Enterobacter cloacae* (13.0%), *Klebsiella pneumoniae* (11.3%) and *Pseudomonas aeruginosa* (10.4%). Those bacteria were represented throughout all of the first three waves. The samples taken during the second wave detected *Staphylococcus aureus* (17.5%) and *Enterobacter cloacae* (11.1%) most commonly. During the third wave *Enterobacter cloacae* (14.3%) and *Pseudomonas aeruginosa* (14.3%) were detected the most frequent. In the fourth pandemic wave, no patient was included in the MicrobiotaCOVID cohort and in the fifth wave, no superinfection occurred in the analysed cohort.

Regarding multidrug resistant pathogens, MDR *Pseudomonas aeruginosa* (2.7%), *Escherichia coli* (2.6%) and *Burkholderia spp.* (5.2%) were detected the most frequent as listed in Table 4.

Table 4: Multidrug-resistant pathogens

Detected MDR pathogens	Overall (%)	1st Wave (%)	2nd Wave (%)	3rd Wave (%)	4th Wave (%)	5th Wave (%)
Acinetobacter baumanni	1 (0.9%)	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)
Acinetobacter berezinae	1 (0.9%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Burkholderia cenocepacia	3 (2.6%)	1 (2.6%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Burkholderia cepacia complex	3 (2.6%)	2 (5.1%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)
Chryseobacterium	1 (0.9%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)
Citrobacter freundii	2 (1.7%)	2 (5.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterococcus faecium	1 (0.9%)	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)
Escherichia coli	3 (2.6%)	1 (2.6%)	1 (1.6%)	1 (7.1%)	0 (0%)	0 (0%)
Klebsiella pneumoniae	2 (1.7%)	0 (0%)	2 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Morganella morganii	1 (0.9%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pseudomonas aeruginosa	4 (3.5%)	4 (10.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus aureus	1 (0.9%)	0 (0%)	1 (1.6%)	0 (0%)	0 (0%)	0 (0%)

Data are presented as number and percentage

While *Pseudomonas aeruginosa* (10.3%) and *Citrobacter freundii* (5.1%) were only prevalent during the first wave, *Burkholderia spp.* was isolated in the first two waves. Multidrug resistant *Klebsiella pneumoniae* (3.8%) was exclusively found throughout the second wave. Exactly three multidrug resistant pathogens were detected during the third wave, namely *Acinetobacter baumanni* (7.1%), *Enterococcus faecium* (7.1%) and *Escherichia coli* (7.1%). Although *Staphylococcus aureus* being prominent during the second wave, only one was a methicillin-resistant Staphylococcus aureus (1.6%) (MRSA).

6 Discussion

6.1 Important Results

This study aimed to analyse the incidence of pulmonary bacterial superinfections in critically ill COVID-19 patients hospitalised on the tertiary ICU of the University Hospital of Zurich. Current available literature shows significant heterogeneity in the superinfection rates ranging from 8.5% to 50.2% (38-40). The incidence reported in this study is relatively high over the time period of the first three waves. The MicrobiotaCOVID cohort study aimed at respiratory sampling on a regular and structured basis, which may have resulted in a higher detection rate. Additionally, almost all patients in this cohort suffered from COVID-associated ARDS (CARDS) and were in need of invasive mechanical ventilation, while most other studies are conducted in a mixed setting meaning patients hospitalised on the normal ward were also included (41). Severely ill patients generally require longer hospital stays, which is why they might be exposed to a greater risk of acquiring nosocomial infection. Associated with the evolving pandemic, change of treatment recommendations, the emergence of new virus variants and the implementation of vaccination most likely impacted superinfection rates over time. Furthermore, demographic differences must be taken into consideration as many evaluated studies are of a single-center design (16, 38, 40). As for the moment, neither the World Health Organisation (WHO) nor the European Centre for Disease Control and Prevention have internationally valid criteria for defining superinfection, making comparisons difficult.

In this work, a decline in the incidence of superinfection was noted in the second and later surges. Several reasons might explain this finding. With expanding knowledge about the pathomechanism and the clinical course of the COVID-19 disease, new treatment options became available that could target the needs of CARDS-patients more specifically. The WHO released a report in September 2020 recommending the use of corticosteroids for severe COVID-19 cases, which led to a change of treatment strategies for patients during the second wave (11). Corticosteroids can lower the hyperinflammatory state in patients with CARDS resulting in a better clinical outcome with more ventilator-free days and reduced mortality (43). Moreover, at the beginning of 2021 (corresponding to the end of the second surge in Switzerland), vaccination was already available, decreasing the risk of severe illness and transmission of SARS-CoV-2 (44, 45), which could play an additional role in the shorter duration of ventilation, the

smaller cohort number during the fifth wave, as well as the absence of superinfections in the latter.
The mortality of 24.3% in patients with superinfection is low compared to other reports (33). As a tertiary care facility, the intensive care unit of the University Hospital Zurich has a high resource setting, meaning treatment options were readily available and not as restricted compared to other facilities. Additionally, the medical staff are considerably knowledgeable with a high level of expertise regarding the management of ventilated patients.

The isolated pathogens causing pulmonary superinfection were predominantly Gram-negative bacteria, which is comparable to current literature (22, 38). However, that does not necessarily suggest a predominance of Gram-negative bacteria. Some pathogens like staphylococcus aureus were exclusively present and numerically relevant during the second wave, which raises the question if a local outbreak could have been the reason for this occurrence.

Multidrug resistance of microorganisms is currently one of the most severe threats to the global health system (46). The European Antimicrobial Resistance Surveillance Network reported an increase in MDR pathogens over the last decade (46), raising the concern on how this trend will be affected by the COVID-19 pandemic. Several studies reported more frequent detection of MDR bacteria in COVID-19 patients (32, 47). Navigating the challenges posed by the emergence of a novel disease and its many uncertainties, resulted in chronically overworked health care workers (2, 48). Due to an overwhelming surge in patient numbers and the increased pressure on healthcare facilities, the primary focus shifted to reducing viral transmission of SARS-CoV-2. As a result, certain aspects, such as the strict implementation of regular infection prevention protocols and antibiotic stewardship couldn't be upheld with the same diligence as in pre-pandemic times (23). With the additional challenge of diagnosing superinfection in individuals with COVID-19 and their still unclear impact on clinical outcomes, there is a possibility that antibiotics were prescribed with higher frequency and potentially without a conclusive indication.
The increase in MDR pathogens reported in other studies could not be objectified in this work. However, it must be acknowledged that the analysed cohort was rather small, especially in the third wave, and thus may not represent the multidrug resistance

rate accurately. Nevertheless, multidrug resistance was present throughout all the first three waves, while a decreasing incidence was noted. The study was conducted at a hospital that did not suffer relevantly from health care shortage and has an active antibiotic stewardship protocol in place. The frequent execution of respiratory sampling allowed early detection of superinfection on the one hand and optimisation of antibiotic treatment with shorter usage of broad-spectrum antibiotic on the other hand. This highlights the need for constant sampling procedures during difficult pandemics with a complex and evolving viral disease.

In conclusion, pulmonary bacterial superinfection in ventilated COVID-19 patients was common over the course of the pandemic. However, the incidence was lower during the second and later waves compared to the first. The most common causative microorganisms were Gram-negative bacteria. Worryingly, multidrug resistance was prevalent in all the first three waves. Nevertheless, the increased detection of multidrug resistant pathogens reported in other studies could not be objectified to the same extent in this Master thesis.

6.2 Clinical Implications

The rate of superinfection, even though decreasing over time, is still high. This highlights the importance of gaining a better understanding of their nature. In light of the high clinical burden associated with superinfection especially in critically ill COVID-19 patients, they should be assessed carefully and regularly, in order to achieve early detection and treatment. Better knowledge of the involved pathogens causing pulmonary bacterial superinfection allow a more targeted therapy and earlier de-escalation to support the principles of antibiotic stewardship. As most superinfections are nosocomially transmitted, strict adherence to hygiene protocols might have a potential impact in lowering superinfection rates (23). Nevertheless, a global definition for diagnosing superinfection would be needed. This would help to consistently evaluate and treat critically ill COVID-19 patients.

6.3 Strengths and Limitation of the Study

The MicrobiotaCOVID cohort underwent a systematic sampling process for early detection of pathogens and used very specific definitions for evaluating relevant bacterial pathogens (16), which is a clear strength. However, as a monocentric cohort study with a relatively small number of patients, the results may not be applicable to other regions with different cohort characteristics and microbiological spectrum.

As this research focuses on the incidence of bacterial superinfections, fungal infections and viral coinfections or reactivations were not taken into consideration. Additionally, other clinical outcomes like duration of hospitalisation, complications or received treatments regarding antibiotics or immunomodulatory drugs were not analysed. Future research focusing on the clinical burden of superinfection over time and the impact of antibiotic usage needs to be conducted.

7 References

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Lui Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet.2020 Mar 28;395(10229):1054-1062.

2. Bernacki K, Keister A, Sapiro N, Joo JS, Mattle L. Impact of COVID-19 on patient and healthcare professional attitudes, beliefs, and behaviors toward the healthcare system and on the dynamics of the healthcare pathway. BMC Health Serv Res.2021 Dec 6;21(1):1309.

3. Parasher A. COVID-19: Current understanding of its Pathophysiology, clinical presentation and treatment. Postgrad Med J. 2020;2021 May;97(1147):312-320.

4. Kumar M, Al Khodor S. Pathophysiology and treatment strategies for COVID-19. J Transl Med. 2020;18(1):353.

5. Garcia PDW. Prognostic factors associated with mortality risk and disease progression in 639 critically ill patients with COVID-19 in Europe: Initial report of the international RISC-19-ICU prospective observational cohort. 2021.

6. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet.2020 Feb 15;395(10223):497-506.

7. Wu C, Chen X, Cai Y, Xia Ja, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med.2020 Jul 1;180(7):934-943.

8. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. Gastroenterology. 2020;158(6):1831-3.e3.

9. Pfortmueller CA. COVID-19-associated acute respiratory distress syndrome (CARDS): Current knowledge on pathophysiology and ICU treatment - A narrative review. Best Pract Res Clin Anaesthesiol.2021 Oct;35(3):351-368.

10. Singh SP, Pritam M, Pandey B, Yadav TP. Microstructure, pathophysiology, and potential therapeutics of COVID-19: A comprehensive review. J Med Virol. 2020;2021 Jan;93(1):275-299.

11. RECOVERY T, Group C. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med.2021 Feb 25;384(8):693-704.

12. Salama C, Han J, Yau L, Reiss WG, Kramer B, Neidhart JD, et al. Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia. N Engl J Med.2021 Jan 7;384(1):20-30.

13. Sisó-Almirall A, Zerón PB, Ferrín LC, Kostov B, Moreno AM, Mestres J. Long Covid-19: Proposed Primary Care Clinical Guidelines for Diagnosis and Disease Management. Int J Environ Res Public Health.2021 Apr 20;18(8):4350.

14. [Internet] WHO. Therapeutics and COVID-19: living guideline Jan 2023 [cited March 2023. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-therapeutics-2023.1.

15. Chang R, Elhusseiny KM, Yeh Y-C, Sun W-Z. COVID-19 ICU and mechanical ventilation patient characteristics and outcomes—A systematic review and meta-analysis. PLoS One.2021 Feb 11;16(2):e0246318.

16. Buehler PK, Zinkernagel AS, Hofmaenner DA, Garcia PDW, Aceveco CT, Gómez-Mejia A, et al. Bacterial pulmonary superinfections are associated with longer duration of ventilation in critically ill COVID-19 patients. Cell Rep Med.2021 Apr 20;2(4):100229.

17. Kreitmann L, Monard C, Dauwalder O, Simon M, Argaud L. Early bacterial co-infection in ARDS related to COVID-19. Intensive Care Med.2020; 46(9): 1787–1789.

18. Martín-Loeches I, Sanchez-Corral A, Diaz E, Granada RM, Zaragoza R, Villavicencio C, et al. Community-Acquired Respiratory Coinfection in Critically Ill Patients With Pandemic 2009 Influenza A(H1N1) Virus. Chest.2011 Mar;139(3):555-562.

19. Yang W, Cao Q, Qin L, Wang X, Cheng Z, Pan A, et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19):A multi-center study in Wenzhou city, Zhejiang, China. J Infect.2020 Apr;80(4):388-393.

20. Mohammadnejad E, Manshadi SAD, Mohammadi MTB, Abdollai A, Seifi A, Salehi MR, et al. Prevalence of nosocomial infections in Covid-19 patients admitted to the intensive care unit of Imam Khomeini complex hospital in Tehran. Iran J Microbiol. 2021;2021 Dec;13(6):764-768.

21. Kurt AF, Mete B, Urkmez S, Demirkiran O, Dumanli GY, Bozbay S, et al. Incidence, Risk Factors, and Prognosis of Bloodstream Infections in COVID-19 Patients in Intensive Care: A Single-Center Observational Study. J Intensive Care Med.2022 Oct;37(10):1353-1362.

22. Russo A, Olivadese V, Trecarichi EM, Torti C. Bacterial Ventilator-Associated Pneumonia in COVID-19 Patients: Data from the Second and Third Waves of the Pandemic. J Clin Med.2022 Apr 19;11(9):2279.

23. Bardi T, Pintado V, Gomez-Rojo M, Escudero-Sanchez R, Lopez AA, Diez-Remesal Y, et al. Nosocomial infections associated to COVID-19 in the intensive care unit: clinical characteristics and outcome. Eur J Clin Microbiol Infect Dis.2021 Mar;40(3):495-502.

24. Giacobbe DR, Battaglini D, Ball L, Brunetti I, Bruzzone B, Codda G, et al. Bloodstream infections in critically ill patients with COVID-19. Eur J Clin Invest.2020 Oct;50(10):e13319.

25. Buetti N, Ruckly S, Montmollin Ed, Reignier J, Terzi N, Cohen Y, et al. COVID-19 increased the risk of ICU-acquired bloodstream infections: a case–cohort study from the multicentric OUTCOMEREA network. Intensive Care Med.2021 Feb;47(2):180-187.

26. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet.2020 Feb 15;395(10223):507-513.

27. Brandi N, Ciccarese F, Balacchi C, Rimondi MR, Modolon C, Sportoletti C, et al. Co-Infections and Superinfections in COVID-19 Critically Ill Patients Are Associated with CT Imaging Abnormalities and the Worst Outcomes. Diagnostics (Basel).2022 Jul 3;12(7):1617.

28. Seitz T, Holbik J, Grieb A, Karolyi M, Hind J, Gibas G, et al. The Role of Bacterial and Fungal Superinfection in Critical COVID-19. Viruses.2022 Dec 14;14(12):2785.

29. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and Fungal Coinfection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. Clin Infect Dis.2020 Dec 3;71(9):2459-2468.

30. Galang WAM, Buensalido JAL. Prevalence of Empiric Antibacterial Therapy, Community-Acquired Bacterial Superinfection, and Antibiotic-Associated Adverse Reactions among Patients with COVID-19 Pneumonia Admitted in Makati Medical Center from March 2020 to March 2021. Infect Chemother.2022 Jun;54(2):266-274.

31. Nori P, Cowman K, Chen V, Bartash R, Szymczak W, Madaline T, et al. Bacterial and fungal coinfections in COVID-19 patients hospitalized during the New York City pandemic surge. Infect Control Hosp Epidemiol.2021 Jan;42(1):84-88.

32. Lai CC, Chen SY, Ko WC, Hsueh PR. Increased antimicrobial resistance during the COVID-19 pandemic. Int J Antimicrob Agents.2021 Apr; 57(4): 106324.

33. Omoush SA, Alzyoud JAM. The Prevalence and Impact of Coinfection and Superinfection on the Severity and Outcome of COVID-19 Infection: An Updated Literature Review. Pathogens.2022 Apr 7;11(4):445.

34. Cataño-Correa JC, Cardona-Arias JA, Mancilla JPP, García MT. Bacterial superinfection in adults with COVID-19 hospitalized in two clinics in Medellín-Colombia, 2020. PLoS One.2021 Jul 13;16(7):e0254671.

35. Laethem JV, Piérard D, Allard SD. Beyond Guidelines and Reports on Bacterial Co-/Superinfections in the Context of COVID-19: Why Uniformity Matters. Antibiotics (Basel).2022 Oct 20;11(10):1446.

36. Chastre J, Fagon JY. Ventilator-associated Pneumonia. Am J Respir Crit Care Med.2002 Jan 165: 867–903.

37. [Internet]. covid19.admin.ch Bundesamt für Gesundheit Switzerland [March 2023]. Available from: https://www.covid19.admin.ch/de/epidemiologic/case?geo=CH&epiZoomDev=2020-03-01_2022-09-25.

38. Novacescu AN, Buzzi B, Bedreag O, Papurica M, Rogobete AF, Sandesc D, et al. Bacterial and Fungal Superinfections in COVID-19 Patients Hospitalized in an Intensive Care Unit from Timișoara, Romania. Infect Drug Resist.2022 Dec 1;15:7001-7014.

39. Hughes S, Troise O, Donaldson H, Mughal N, Moore LSP. Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. Clin Microbiol Infect.2020 Oct;26(10):1395-1399.

40. Moreno-Torres V, Mendoza Cd, Fuente Sdl, Sánchez E, Martínez-Urbistondo M, Herráiz J, et al. Bacterial infections in patients hospitalized with COVID-19. Intern Emerg Med.2022 Mar;17(2):431-438.

41. Musuuza JS, Watson L, Parmasad V, Putman-Buehler N, Christensen L, Safdar N. Prevalence and outcomes of co-infection and superinfection with SARS-CoV-2 and other pathogens: A systematic review and meta-analysis. PLoS One.2021 May 6;16(5):e0251170.

42. Jackson S. Musuuza LW, Vishala Parmasad, Nathan Putman-Buehler, Leslie Christensen, Nasia Safdar. Prevalence and outcomes of co-infection and

superinfection with SARS-CoV-2 and other pathogens: A systematic review and meta-analysis. 2021.

43. Wagner C, Griesel M, Mikolajewska A, Mueller A, Nothacker M, Kley K, et al. Systemic corticosteroids for the treatment of COVID-19 (Review). Cochrane Database Syst Rev.2021 Aug 16;8(8):CD014963.

44. Vitiello A, Ferrara F, Trojano V, Porta RL. COVID-19 vaccines and decreased transmission of SARS-CoV-2. Inflammopharmacology.2021; 29(5): 1357–1360.

45. Rotshild V, Hirsh-Raccah B, Miskin I, Muszkat M, Matok I. Comparing the clinical efficacy of COVID-19 vaccines: a systematic review and network meta-analysis. Sci Rep.2021 Nov 23;11(1):22777.

46. ECDC. Antimicrobial resistance surveillance in Europe, 2022 - 2020 data 2022 [April 2023]. Available from: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2022-2020-data.

47. Jeon K, Jeong S, Lee N, Park M-J, Song W, Kim H-S, et al. Impact of COVID-19 on Antimicrobial Consumption and Spread of Multidrug-Resistance in Bacterial Infections. Antibiotics (Basel).2022 Apr 18;11(4):535.

48. Shreffler J, Petrey J, Huecker M. The Impact of COVID-19 on Healthcare Worker Wellness: A Scoping Review. West J Emerg Med. 2020;2020 Sep; 21(5): 1059–1066.

8 Acknowledgments

Firstly, I would like to thank the mentor of my master thesis, Daniel Hofmänner. I am grateful for the guidance and expertise provided throughout the journey of my master's thesis as well as the quick and uncomplicated responses to any of my questions.

I would also like to extend my gratitude to Prof. Dr. med. Reto Schüpbach, Director of the Department for Intensive Medicine and to Prof. Dr. med. Annelies Zinkernagel, Director of the Department of Infectiology well as Prof. Dr. med. Silvio Brugger also from the Department of Infectiology for the possibility of pursuing this study.

Thanks should also go to Dr. med Pascal Frey, Doctor at the Institute of Internal Medicine at Inselspital in Berne for handling the majority of the statistical and analytical work and Dr. med. Thomas Scheier from the Institute of Infectiology at the University Hospital of Zurich for establishing and managing the REDCap Database.

10 Declaration of Authorship

Masterarbeit

Ich erkläre ausdrücklich, dass es sich bei der von mir im Rahmen des Studiengangs

Humanmedizin

eingereichten schriftlichen Arbeit mit dem Titel

Incidence of Bacterial Pulmonary Superinfections in critically ill COVID-19 Patients across different waves on the Intensive Care Unit of the University Hospital Zurich

um eine von mir selbst und ohne unerlaubte Beihilfe sowie *in eigenen Worten* verfasste Masterarbeit* handelt.

Ich bestätige überdies, dass die Arbeit als Ganzes oder in Teilen weder bereits einmal zur Abgeltung anderer Studienleistungen an der Universität Zürich oder an einer anderen Universität oder Ausbildungseinrichtung eingereicht worden ist.

Verwendung von Quellen

Ich erkläre ausdrücklich, dass ich *sämtliche* in der oben genannten Arbeit enthaltenen Bezüge auf fremde Quellen (einschliesslich Tabellen, Grafiken u. Ä.) als solche kenntlich gemacht habe. Insbesondere bestätige ich, dass ich *ausnahmslos* und nach bestem Wissen sowohl bei wörtlich übernommenen Aussagen (Zitaten) als auch bei in eigenen Worten wiedergegebenen Aussagen anderer Autorinnen oder Autoren (Paraphrasen) die Urheberschaft angegeben habe.

Sanktionen

Ich nehme zur Kenntnis, dass Arbeiten, welche die Grundsätze der Selbstständigkeitserklärung verletzen – insbesondere solche, die Zitate oder Paraphrasen ohne Herkunftsangaben enthalten –, als Plagiat betrachtet werden und die entsprechenden rechtlichen und disziplinarischen Konsequenzen nach sich ziehen können (gemäss §§ 7ff der Disziplinarordnung der Universität Zürich sowie §§ 51ff der Rahmenverordnung für das Studium in den Bachelor- und Master-Studiengängen an der Medizinischen Fakultät der Universität Zürich).

Ich bestätige mit meiner Unterschrift die Richtigkeit dieser Angaben.

Datum: 24.09.2023

Name: Schneeberger Vorname: Sarah

Unterschrift: ……(*elektronisch oder eingetippt*)

* Falls die Masterarbeit eine Publikation enthält, bei der ich Erst- oder Koautor/-in bin, wird meine eigene Arbeitsleistung im Begleittext detailliert und strukturiert beschrieben.