The incidence of coinfections and superinfections in COVID-19 patients admitted to the ICU of the Department of Anesthesia, Resuscitation and Intensive Care, University Hospital of Split in 2020

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UNIVERSITY OF SPLIT

SCHOOL OF MEDICINE

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LIST OF ABBREVATIONS

Abbreviation	Meaning
2019-nCoV	2019 novel coronavirus
ACE2	Angiotensin-converting enzyme 2
ARDS	Acute respiratory distress syndrome
CDC	Center for disease control and prevention
COVID-19	Coronavirus disease of 2019
CPIS	Clinical pulmonary infection scale
CRS	Cytokine release syndrome
DALY	Disability-adjusted life year
ECDC	European center for disease prevention and control
EU/EEA	European union/ European economic area
GAS	Group A streptococcus
HAI	Healthcare associated infection
HAP	Hospital associated pneumonia
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IV	Intravenous
LOS	Length of stay
MDR	Multi-drug resistant
MERS	Middle east respiratory virus
RNA	Ribonuclear acid
SARS	Severe acute respiratory syndrome
SARS-CoV2	Severe acute respiratory syndrome coronavirus 2
URI	Upper respiratory infection
VAP	Ventilation associated pneumonia
WHO	World health organization

1. INTRODUCTION

2.1 COVID-19

In December 2019, the first cases of pneumonia of unknown etiology were reported in Wuhan, China (1). It was soon discovered that this was a novel coronavirus. The virus was first named 2019-nCoV; later, the name was changed to SARS-CoV2, and the disease it causes named COVID-19 (2). Early epidemiological studies found a connection between the infected patients and the Wuhan Huanan Seafood Wholesale Market, a wet-marked trading in seafood and live and dead animals. This connection suggested that this could be the epicenter of the pandemic, and that a human-animal interphase was the cause of infection (1). Later retrospective studies found other early cases that were not connected to the Huanan market (1). Coronaviruses similar to SARS-CoV2 are found in bats. Research has shown that the SARS-CoV2 virus is 96,2% identical to coronaviruses found in *Rhinolophus affinis* bats (1, 3). However, these viruses have spike proteins that may not bind effectively to human ACE2 receptors; therefore, it is likely that there has been another animal bridging between the bat and human. A possible bridging animal is the Malayan Pangolin (*Manis javanica*) which carries a coronavirus with spike proteins that are more optimized to attach to human ACE2 receptors, but this coronavirus is genetically more different than the one found in *Rhinolophus affinis* bats (3, 4).

SARS-CoV2 is spread mainly by respiratory transmission; there are a few cases where the virus has been spread by direct contact or fomite transmission. Some cases where the virus has been transmitted transplacentally from the mother to the fetus have also been reported (5). Respiratory transmission is made possible by droplet and aerosol transmission of the virus from the host to the recipient. The droplets/aerosols are formed when the patient, e.g., is speaking, coughing, or sneezing (6, 7).

Coronavirus basis: The coronaviruses are large, enveloped RNA viruses. They have one of the largest genomes among the RNA viruses. Before the SARS-CoV2 virus, six coronaviruses that could infect humans had been identified. (Alpha coronavirus 229E, alpha coronavirus NL63, beta coronavirus OC43, beta coronavirus HKU1, SARS-CoV and MERS-CoV). There is also a wide specter of coronaviruses infecting animals; these primarily infect one species or only a few (8). During replication, there is a high frequency of recombination, and this high frequency increases the chance of mutations (8, 9)

The SARS outbreak in 2003 and the MERS outbreak in 2012 represent novel coronaviruses causing severe respiratory disease(1, 8, 10) The SARS-CoV virus was found in civet cats (*Paguma larvata*) (11) and the MERS-CoV virus in dromedary camels (*Camelus*

dromedarius) (12, 13). Both the SARS-CoV and MERS-CoV had high mortality rates (10). Novel coronaviruses are an example of a zoonotic virus spreading to a human host. Zoonotic viruses spread from an animal host to a human host through what is classified as a spillover-event (8, 10). The virus needs to mutate in such a way that it cannot only spread from the animal to a human; but also, from one human to another human in order to cause widespread disease (14, 15).

At the end of December 2019, China alerted WHO about this possible new virus epidemic. During the next month's China enforced strict epidemiological measures. Hope was that this outbreak would be contained as SARS was in 2003, but sadly this was not the case (1). It soon became apparent that the virus had spread to multiple countries and continents. On 13 January 2020, the first case of COVID-19 outside China was confirmed in Thailand (16). On 30 January 2020 cases were confirmed in 18 countries outside China (16). On 11 March 2020, the WHO declared the spread of SARS-CoV2 a pandemic (17).

The first case in Europe was confirmed on 24 January 2020 in France. The patient had a history of recent travel to China (2). On 22 February 2020, the ECDC (European Center for Disease Prevention and Control) got reports of several cases of COVID-19 in Lombardy, Italy (2). The transmission in these cases appeared to have occurred locally in Italy, as opposed to previous instances in which there was a direct link to travel (2). That was a turning point in the pandemic. As the number of cases increased, Italy enforced strict public health measures on 8 March 2020 in affected regions. On 25 March 2020, all EU/EEA countries were affected (2). As the virus spread through Europe, WHO declared Europe the new epicenter of the COVID-19 pandemic (18).

The first case of COVID-19 was proven in Croatia on 25 February 2020 (19). The first confirmed death due to COVID-19 in Croatia was on 18 March 2020 (20).

On 31 December 2020, a total of 212 091 people had been confirmed infected with SARS-CoV2 in Croatia; of these, there were 110 490 females and 101 601 males (21). In Splitsko-Dalmatinska county, 25 989 people were infected, with the breakdown between females and males being 13 555 (52.2%) females and 12 434 (47.8%) males (21). According to WHO, the total number of deaths in Croatia up to 3 January 2021 was 4 072 (22).

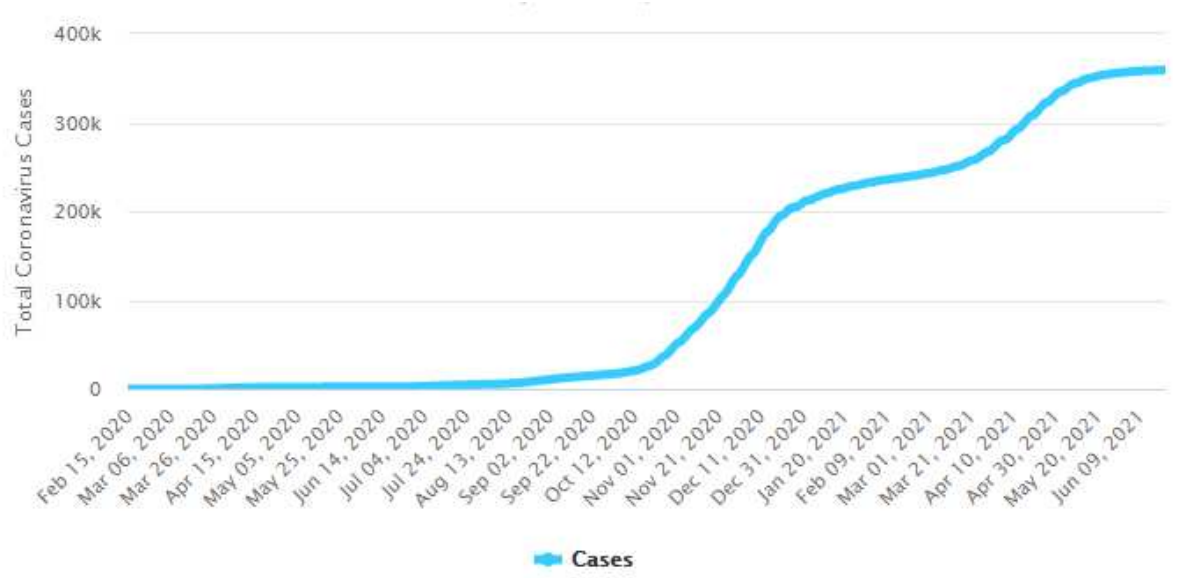

Figure 1. Total cases of COVID-19 in Croatia
Worldometer. Croatia COVID: 359,184 Cases and 8,182 [Internet]. worldometers.info; 2021 [cited 2021 Jun 21]. Available from: https://www.worldometers.info/coronavirus/country/croatia/

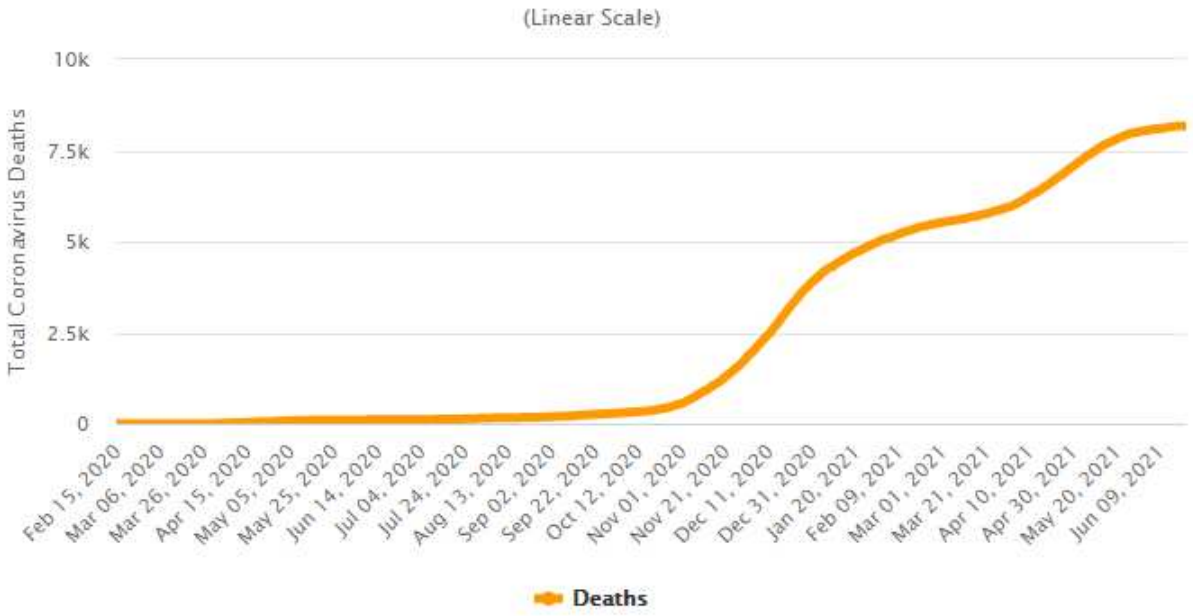

Figure 2. Total deaths caused by COVID-19 in Croatia
Worldometer. Croatia COVID: 359,184 Cases and 8,182 [Internet]. worldometers.info; 2021 [cited 2021 Jun 21]. Available from: https://www.worldometers.info/coronavirus/country/croatia/

According to the WHO, a total of 83 326 479 cases of COVID-19 had been confirmed up to 3 January 2021. The total number of deaths registered worldwide was 1 831 703 on 3 January 2021 (22). This gives a mortality rate of 2.2%. The mortality rate differs from country to country (22).

Shortly after the SARS-CoV2 virus was found responsible for this new pandemic, the race to develop a vaccine started. On 21 December 2020, the first vaccine was authorized for usage in the EU. This vaccine was Comirnaty produced by BioNTech and Pfizer (24). This marks a second turning point in the pandemic.

As expected from a virus having frequent mutations, new subvariants have developed (8, 9, 25). Most of these subvariants have mutations that do not affect the overall pandemic. However, some subvariants have mutations affecting the properties of the virus. Such mutations can make the virus more pathogenic, virulent or reduce the effectiveness of vaccines, therapies, diagnostic tools, or other public health measures. WHO has therefore assessed the different variants of SARS-CoV2 since January 2021. They have made a list of variants of concern and variants of interest. In order to be classified as a variant of concern, the mutation have to have at least one of the following characteristics:

- increased transmissibility or detrimental changes in COVID-19 epidemiology;
- or increased virulence or change in clinical presentation;
- or decreased public health measure response, reduced responsiveness to vaccines, therapies, or diagnostic tools (25).

Variants of concern per 15 June, 2021 are presented in Figure 3 (25).

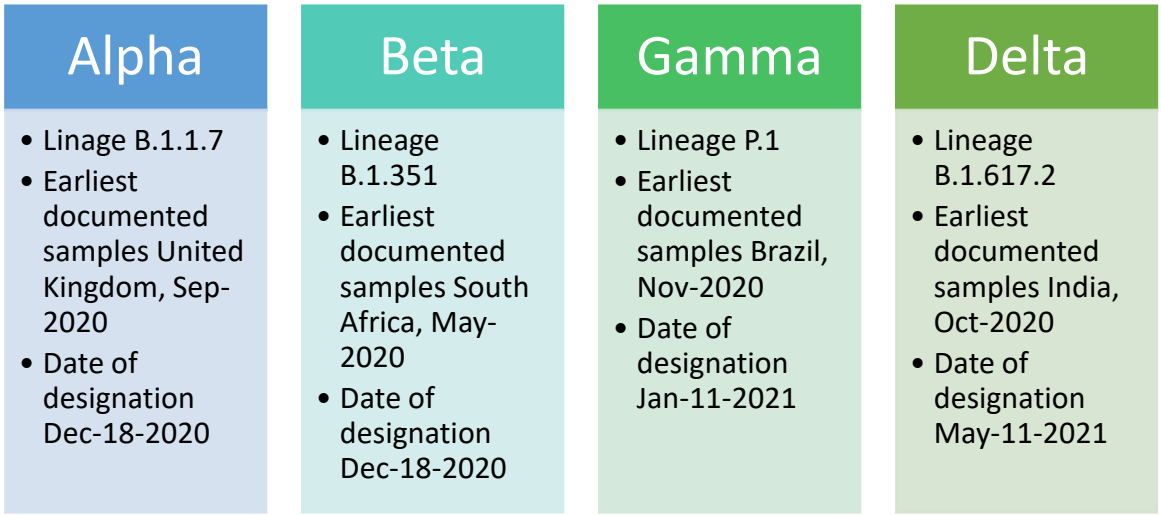

Figure 3. Variants of concerne per 15 June 2021
World Health Organization. Tracking SARS-CoV-2 variants [Internet]. World Health Organization; 2021 [cited 2021 Jun 21]. Available from: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/

The symptoms of COVID-19 are non-specific and are similar to many other viral illnesses (6). The incubation period ranges from 4 to 14 days, but this depends on the mutation of the virus (6, 7). When the symptoms occur, they can range from mild to severe. The most common symptoms are cough, fever, fatigue, anorexia, and myalgias. Anosmia and dysgeusia are also frequently seen and are thought of as characteristic of COVID-19, although they are not exclusively found in COVID-19 patients. Other symptoms of URI, such as sore throat, headache, and rhinorrhea, are also seen. Gastrointestinal symptoms precede respiratory symptoms in up to 10% of patients. The majority of patients with COVID-19 present with mild to moderate symptoms (55%). However, 30% of patients develop dyspnea after 5 days of symptom onset. In patients with a more severe picture of the disease, typical deterioration is seen in the second week. These patients most often need hospitalization on day 7-8 of disease. 75% of the hospitalized patients manifest with hypoxemia and bilateral pneumonia. Most of the hospitalized can be treated at standard level of care. About 20% of hospitalized patients deteriorate quickly after the onset of dyspnea and develop severe respiratory failure, and need to be treated at a higher level of care such as ICU (6).

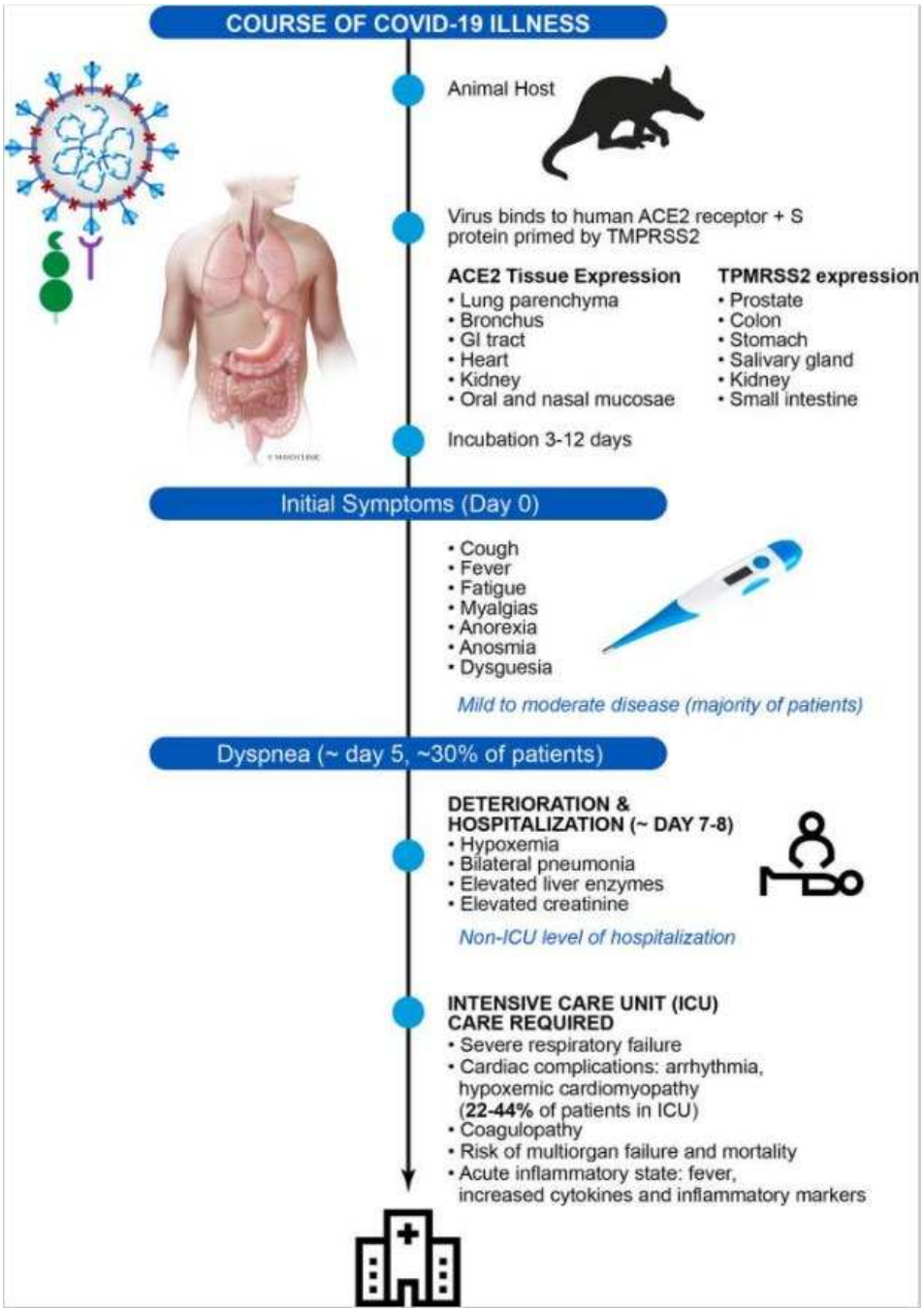

Figure 4. Symptom development in COVID-19
Salian VS, Wright JA, Vedell PT, Nair S, Li C, Kandimalla M, et al. COVID-19 Transmission, Current Treatment, and Future Therapeutic Strategies. Mol Pharm. 202;18:754–71.

Risk factors for developing severe illness are older age (>65y), hypertension, diabetes mellitus, smoking, cardiovascular disease, chronic lung disease, malignancy, and immunosuppression (6).

From the beginning of the pandemic, many trials have been performed in attempts to find effective medications and treatments of COVID-19. Several of these trials have been trying to repurpose other approved drugs for the treatment of COVID-19. The tested drugs

were chosen for their possible ability to inhibit the viral entry mechanism and subsequent reproduction (6).

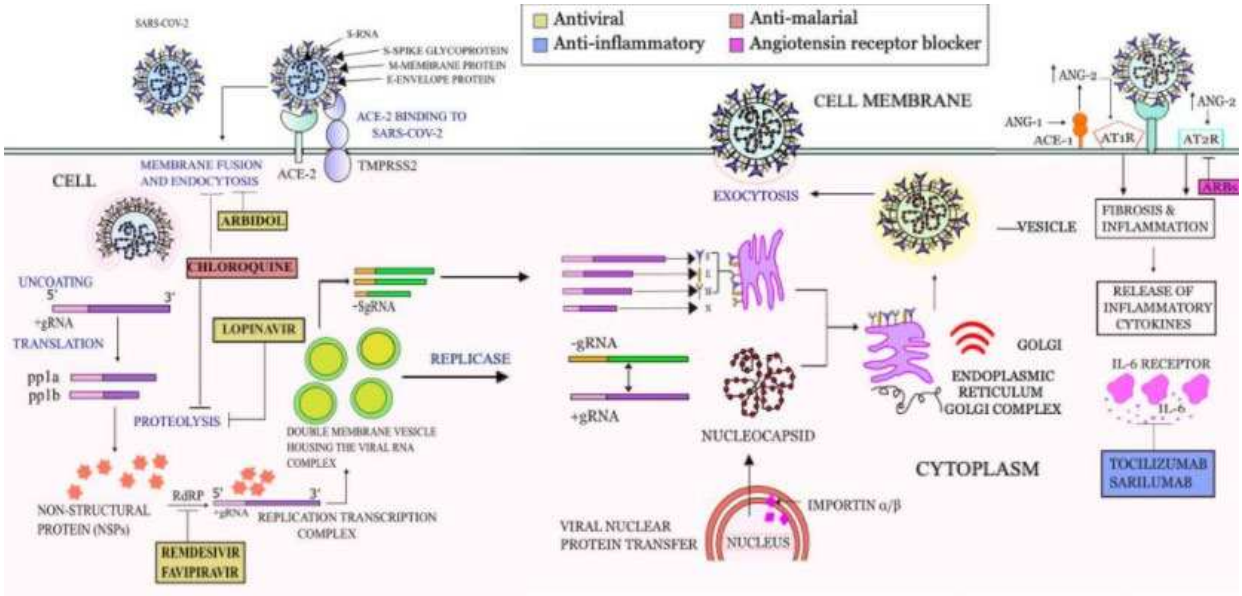

Figure 5. Action of potential medications against COVID-19
Salian VS, Wright JA, Vedell PT, Nair S, Li C, Kandimalla M, et al. COVID-19 Transmission, Current Treatment, and Future Therapeutic Strategies. Mol Pharm. 202;18:754–71.

Multiple groups of therapies have been researched through the pandemic (6). The recommended therapies have also changed during the pandemic as new evidence has been discovered. The process of discovering an effective and safe treatment of COVID-19 is continuing (6, 26). Early in the pandemic, research showed a possible positive effect of Hydroxychloroquine; this was later proven wrong, and the usage is no longer recommended by WHO (6, 26). Lopinavir/Ritonavir was also suggested as a possible treatment, but studies showed no benefits of this treatment, and it is not recommended by WHO today (26). Currently WHO recommends Remdesivir in addition to other usual care in hospital patients. Systemic corticosteroids are the only recommended therapy with strong evidence for its effectiveness and are therefore recommended by the WHO to patients with severe COVID-19 (26).

The spike protein on the SARS-CoV2 virus binds to the ACE2 receptor on human cells. The expression of ACE2 receptors varies significantly from tissue to tissue, and it is expressed more on pneumocyte type 2, heart, kidneys, liver, urinary bladder, and in the GI tract cells more than in other tissues. When the spike protein binds to the ACE2 receptor, this binding is sensed by the Toll-like receptor 7. This leads to secretion of inflammatory cytokines. After entering the cell and reproducing itself it buds off the cell membrane, but it does not directly lyse the cell. When the lung epithelial cell becomes infected, the innate immune system is triggered first. This recruits alveolar macrophages and neutrophils. Then the additive immune system is

triggered by the lung epithelial cell production of interleukins, and T- and B- lymphocytes are activated and complete the immune response. The immune response in COVID-19 patients is complex. Patients with an already weak immune system have an increased risk of severe outcomes, but patients in the ICU who develop ARDS are found to have a high level of circulatory inflammatory cytokines (27). These high levels of circulatory inflammatory cytokines are known as Cytokine release syndrome (CRS) (27, 28). A possible explanation for this is that the immune system becomes damaged and ineffective because of lymphopenia. This triggers compensation from the body that increases the amount of inflammatory cytokines (27). Treatment with systemic corticosteroids tries to prevent CRS development and decrease the complication of ARDS and multi-organ failure (6, 26, 27).

2.2 Coinfections and superinfections

The CDC defines a coinfection as an infection occurring concurrently with the initial infection. A superinfection is an infection developing after the first infection has occurred (29, 30). The term superinfections are also often used to describe infections with microorganisms resistant to antimicrobial therapy (30).

As diagnostical methods and techniques have developed, the number of coinfections detected is increasing. It is easy to assume that all coinfections are bad, but they can, in some instances, be beneficial; this depends on the base infection (31).

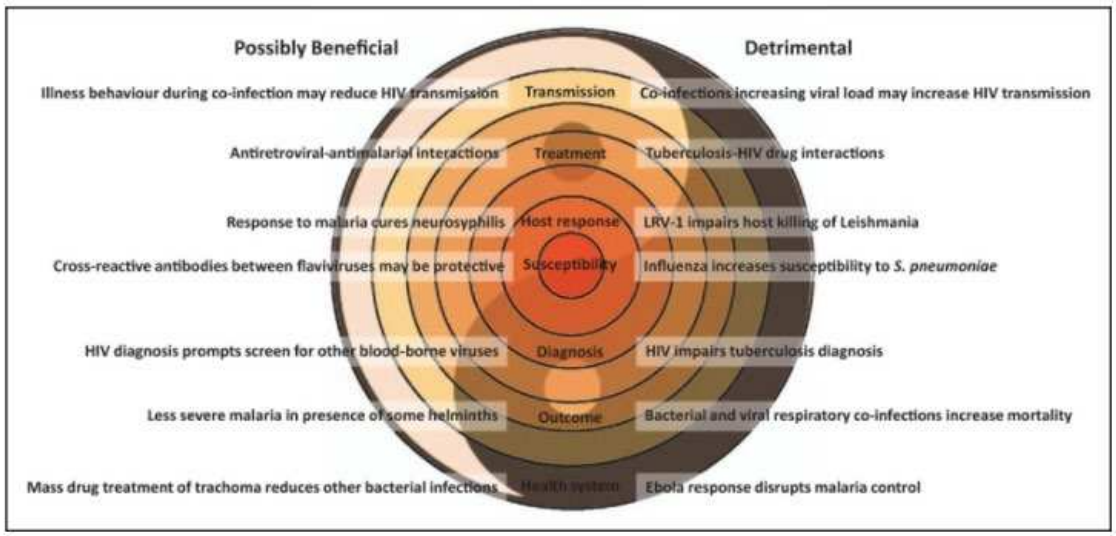

Figure 6. Possible benefits and disadvantages of coinfection
McArdle AJ, Turkova A, Cunnington AJ. When do co-infections matter? Curr Opin Infect Dis. 2018;31:209–15.

In HIV, a coinfection can be detrimental, but a combination of helmitic and tuberculoid infection can reduce the bacterial load in the sputum (31).

There is limited evidence on coinfections with multiple respiratory viruses and their effect. There is established a firm association between respiratory virus and bacteria coinfection, corresponding with more severe illness in the patient (30, 31).

A typical example of negative coinfection is influenza and bacterial pneumonia. Studies show that the influenza infection causes a depletion in alveolar macrophages; this again allows small inoculums of bacteria to establish a productive infection (31, 32).

Another possible cause of a more severe outcome in coinfections between influenza and bacteria is a reduced capacity to repair damage to the alveolar cells. Studies have shown more damage to the alveolar cells when there is a coinfection than without a coinfection (32, 33).

During other viral pandemics, the reported rates of coinfections and pathogens have varied widely. In the 1918 influenza pandemic, a large portion of cases were complicated by bacterial coinfections; these were predominantly *Streptococcus pneumoniae* and *Staphylococcus aureus* (34).

A retrospective study of COVID-19 cases in England found that 32.7% of the patients were affected by a coinfection, with the most common pathogens being *Staphylococcus aureus* and *Streptococcus pneumoniae.* If the patient stayed in the ICU, the prevalence of coinfection increased, and the infection consisted mainly of gram-negative bacteria, especially *Klebsiella pneumoniae* and *Escherichia coli* (34).

Another retrospective study of COVID-19 patients in Spain found that 7.2% of the patients had a coinfection. Community-acquired coinfection was uncommon. The most common causative agents in community-acquired coinfections were *Streptococcus pneumoniae* and *Staphylococcus aureus.* For hospital-acquired bacterial infections the most common pathogens were *Pseudomonas aeruginosa* and *Escherichia coli.* The overall mortality rate was found to be 9.8% (35).

These two studies describe large differences in the number of patients with coinfections. There are many possible explanations for this difference. The studies may have used different definitions of coinfection as neither study defines coinfection. Also, different countries and regions have different pathogens and testing regimes (30, 34, 35). Another critical point is that when a coinfection is diagnosed, it is important to determine if it is a true coinfection or if the

patient is only a carrier of the pathogen; different guidelines for distinguishing between these can also explain the difference between these two studies (30).

Coinfections in COVID-19 infection are associated with more severe course and poorer outcomes (30). COVID-19 patients on invasive mechanical ventilation for a prolonged period have an increased risk of contracting HAP or VAP (36). Therefore, an early diagnosis of coinfection is essential. To detect the coinfection, a method allowing detection of a broad range of pathogens should be used, and the method should also allow for antimicrobial susceptibility testing. Characterizing the coinfection and starting early treatment appropriate for the coinfection will improve antibiotic stewardship and decrease mortality and morbidity (36).

2.3 Ventilation associated pneumonia and Hospital associated pneumonia

According to the CDC (Centers for Disease Control and Prevention), HAP (Hospital associated pneumonia) is defined as an infection occurring more than 48 hours after a patient was admitted to the hospital, and there was no sign of an infection being in the incubation period at the time of addition. VAP (Ventilated associated pneumonia) is defined as occurring more than 48 hours after endotracheal intubation (37).

Nosocomially acquired infections are also known as healthcare-associated infections (HAI). These include infections such as surgical site infections, urinary tract infections associated with urinary catheters, bloodstream infections associated with central or peripheral venous catheters, VAP (ventilation-associated pneumonia), HAP (hospital-associated pneumonia, or infections with *Clostridium difficile* (38).

The development of HAP and VAP are associated with higher mortality and morbidity worldwide (38). The risks of developing hospital infections are many and complex. The risk depends on the patient's status, control measures to prevent the spread of infections in the hospital, and the type of pathogens in the community and the hospital. Patient risk factors include old age, immunosuppression, time in hospital, underlying comorbidities, a high frequency of visits to healthcare facilities, invasive procedures, mechanical ventilation, and stay in ICU (intensive care unit) (38). The risk of developing multi-resistant infections is increased if the patient is treated with IV antibiotics within the last 90 days before acquiring the infection (38). The patients in the ICU have a higher risk of developing an HAI than other patients in the hospital; a prevalence study in Germany shows that 19,5% of patients in the ICU have an HAI (39).

The most common pathogen for VAP and HAP are *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii* (38, 40). Other common causes of HAI are *Klebsiella pneumoniae*, *Enterococcus, Enterobacteriaceae, Proteus mirabilis, E. coli,* and different types of *Candida* (38).

Due to the increased mortality and morbidity of VAP and HAP, it is crucial to prevent such infections. When trying to prevent the infections, we have to look at the modifiable risk factors such as endotracheal tube intubation, nasogastric tube, tracheotomy, reintubations, enteral nutrition, corticosteroid administration, modifications of gastric content pH, the position of the patient, and previous antibiotic usage (41).

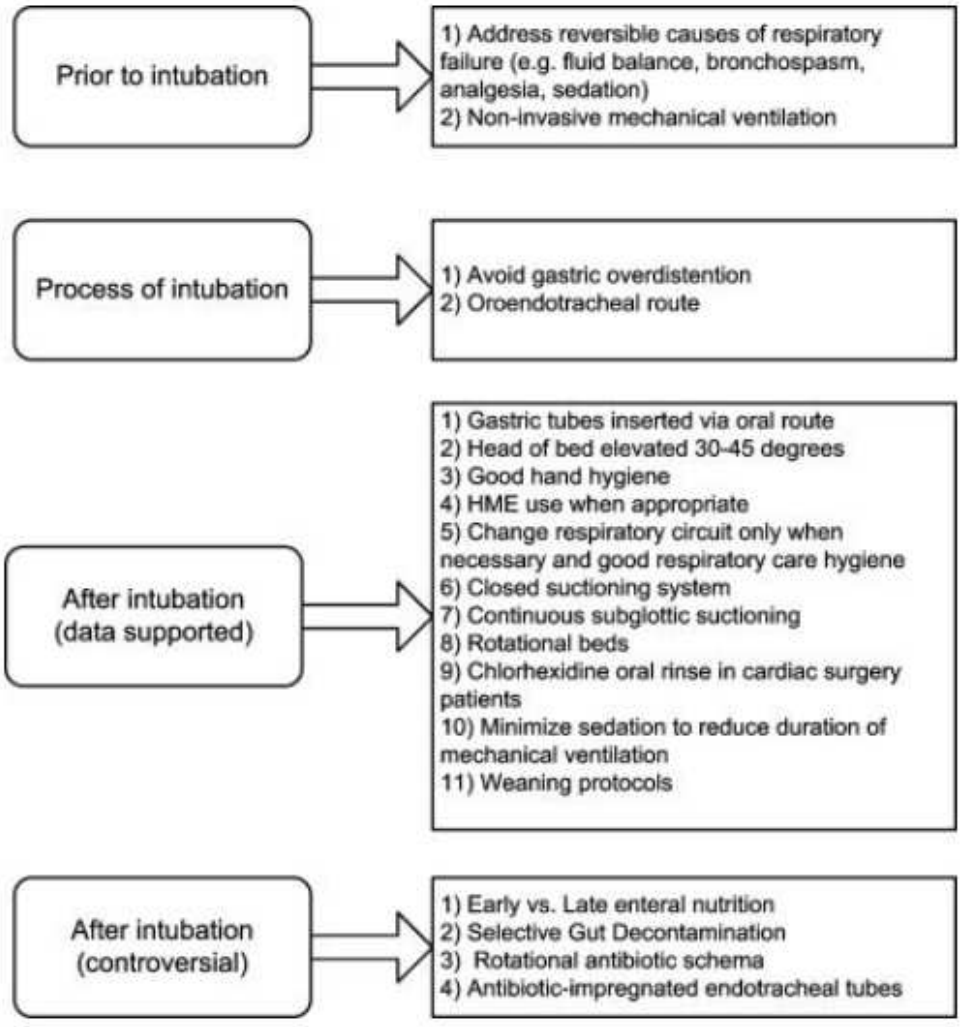

Figure 7. Potenital strategies to prevent VAP
Koenig SM, Truwit JD. Ventilator-associated pneumonia: Diagnosis, treatment, and prevention. Clin Microbiol Rev. 2006;19:637–57.

The areas of preventing VAP can be divided into Pre-intubation, Para-intubation, and Post-intubation. The pre-intubation strategies are trying to prevent intubation in the first place by reversing reversible causes and trying less invasive ventilation techniques. The Para-intubation strategies focus on preventing complications of the intubation process, such as the prevention of aspiration of gastric content or oropharyngeal secretions. The post-intubation strategies can be divided into controversial and non-controversial strategies. The non-controversial strategies are supported by data and include oral gastric tubes, elevation of the head, good hand hygiene, reducing time on ventilation, etc. There is less data supporting the controversial strategies, such as selective gut decontamination, rotational antibiotics, and antibiotic-impregnated endotracheal tubes (41).

Invasive procedures such as intubations, placement of venous catheters, or urinary catheters bypass the body's normal preventive mechanisms rooted in anatomy and physiology. Keeping the invasive equipment free of microorganisms is therefore important. Unfortunately, the endotracheal tube is colonized within hours of placement, and studies have found that 87.5% of endotracheal tubes are covered with a biofilm after 7-10days (42). These biofilms consist of a mix of bacteria, with gram-negative bacteria being the most common (41, 42).

The endotracheal tube pathogenesis in VAP consists of several mechanisms. The biofilm can disperse and passively move into the lungs, cells in the biofilm can be aerosolized and then be blown into the lungs by the air from the mechanical ventilation, or cells from the biofilm can dislodge from the biofilm and move into liquids in the endotracheal tube and by that be transferred deeper into the lungs (42). There is evidence suggesting that the presence of an endotracheal tube is a greater risk for VAP than mechanical ventilation in itself (41–43).

In HAI, especially HAP and VAP, there is a significant increase in Multidrug-resistant pathogens compared with non-hospital acquired infections. This is especially true for patients in ICU (38).

HAP is considered to be the most common nosocomial infection, with a rate of 5-10/1000 hospital admissions in Europe and the USA (40). The symptoms and signs of HAP are cough, often with expectorate, fever, dyspnea or chest pain, tachypnea, crackles, or consolidations on chest x-rays (40). There is no superior established method in the diagnosis of HAP (40, 44, 45). In the guidelines from the Infectious Diseases Society of America/American Thoracic Society of 2016, the diagnosis is based on the presence of a new lung infiltrate and clinical evidence that the infiltrate is infectious in origin (signs such as purulent sputum, leukocytosis, fever, e.g.) (44). Another diagnostic tool is the Clinical

pulmonology infection score (CPIS), which includes clinical and radiological criteria that suggest the likelihood of pneumonia (41, 45).

Table 1. Clinical pulmonary infection scale (CPIS)

Day	Parameter	Value for score of:		
		1 Point		2 Points
1	Temp (°C)	38.8 to 38.9		≥39 or ≤36
	White blood cells/mm^3	<4000 >11 000	or	<4000 or >11 000 and ≥50% bands
	Secretions	Nonpurulent		Purulent
	PaO_2/FiO_2			≤240 and no ARDS
	Chest X-ray infiltrates	Diffuse patchy	or	Localized
2	Temp (°C)	38.5 to 38.9		≥39 or ≤36
	White blood cells/mm^3	<4000 >11 000	or	<4000 or >11 000 and ≥50% bands
	Secretions	Nonpurulent		Purulent
	PaO_2/FiO_2			≤240 and no ARDS
	Chest X-ray infiltrates	Diffuse patchy	or	Localized
	Progression of chest X-ray infiltrates			Yes (no ARDS or congestive heart failure)
	Sputum	Culture >1+		Culture >1+ and same organism on Gram staining

Koenig SM, Truwit JD. Ventilator-associated pneumonia: Diagnosis, treatment, and prevention. Clin Microbiol Rev. 2006;19:637–57.

The choice of antibiotic should be based on local guidelines. These guidelines are often divided into community-acquired infections and nosocomial-acquired infections. They take into account local microbial susceptibility and vary from place to place as the level and type of antimicrobial resistance depend on location. The choice of empirical therapy will therefore vary. When antimicrobial susceptibility tests are ready, the choice of treatment can be tuned to the specific case (38, 40, 41, 46, 47).

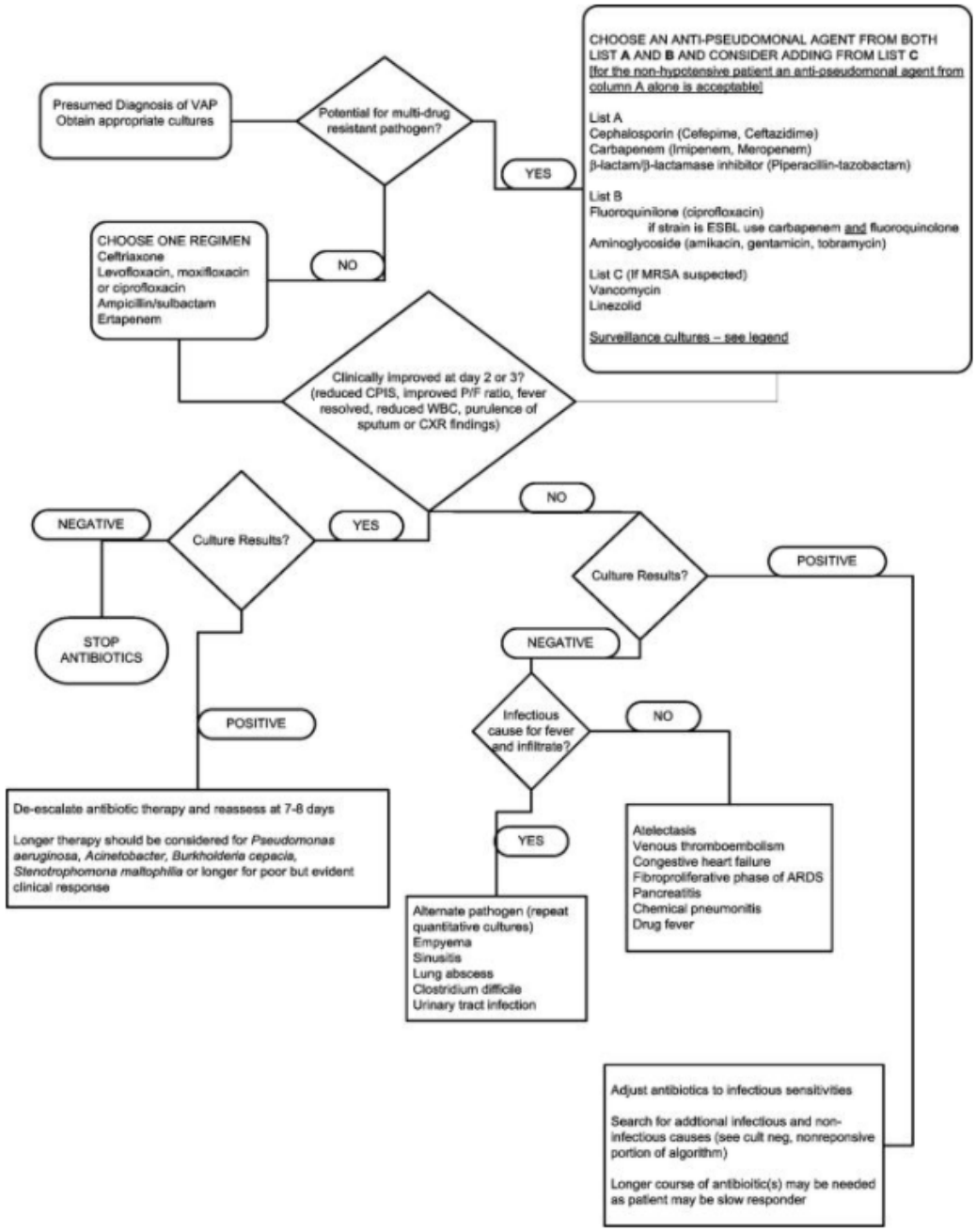

Figure 8. Algorithm for diagnosis and treatment of VAP
Koenig SM, Truwit JD. Ventilator-associated pneumonia: Diagnosis, treatment, and prevention. Clin Microbiol Rev. 2006;19:637–57.

2.4 Antibiotic resistance

Today medicine can be divided into two different areas: the pre-antibiotic area and the post-antibiotic area. The antibiotic area started with Ehrlich's hunt for a "magic bullet" to cure syphilis. His systematic search became a cornerstone in today's development of medications.

During the early days of antibiotic development, J. Klarer and F. Mietzsch discovered the sulfa-drug sulfonamidochyrsoidine antibacterial effects. This was the precursor of sulfonamide (48).

On 3 September, 1928 A. Fleming made the first discovery of penicillin. He found that a mold had started to grow in one of his forgotten petridishes. Others had seen the activity of penicillium earlier but had not taken action on their discovery. A. Fleming did persist in working on the purification and stabilization of the active component during the next 12 years, in 1940 he gave up. He sent samples of the *penicillium* strain to anyone interested. In Oxford, H. Florey and E. Chain had worked on the purification. In 1940, they published a paper describing the purification in sufficient quantities for clinical testing (48). They later started cooperating with A. Fleming to develop the technique further. Thanks to the second world war, mass production was developed by the American government. A huge problem during the war was that soldiers died of infections in injuries that would otherwise not be deadly. The government, therefore, funded the mass production of penicillin, and it was ready for mass distribution in 1945 (48, 49). After this, the golden age of antibiotics started, and multiple new antibiotics were developed (49).

A. Fleming was among the first to warn against antibiotic resistance (48, 50):

"The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant." A. Fleming. (50).

Ever since antibiotics were developed as medication, the bacteria have developed mechanisms of resistance. Resistance to sulfonamide was reported already in the late 1930s. The same mechanism of resistance is still a problem today (51). Studies have found soil-dwelling bacteria with a myriad of antibiotic resistance that also produces a multitude of different antibiotics. This can be part of the explanation of how the bacteria developed resistance gens so fast (51, 52). The resistance gens of bacteria are often placed on plasmids. The horizontal exchange of plasmids between different bacteria is an important factor in the spread of antibiotic resistance (49, 51).

Today many bacteria carry resistance to multiple drugs. These bacteria are known as multidrug-resistant (MDR) bacteria. MDR *M. tuberculosis* is found both in developing and industrialized countries and is an increasing problem (49, 51). Other MDR bacteria are linked to hospitals such as *Acinetobacter baumannii, Enterobacter* spp., *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa,* and others (51).

Today antibiotic resistance to all groups of antibiotics is found (48, 51, 53). The type of resistance depends on the species of bacteria and the target antibiotic. There are multiple forms of the same resistance, and this is best seen in β-lactamases. There has been an exponential growth in unique enzymes against β-lactams (51).

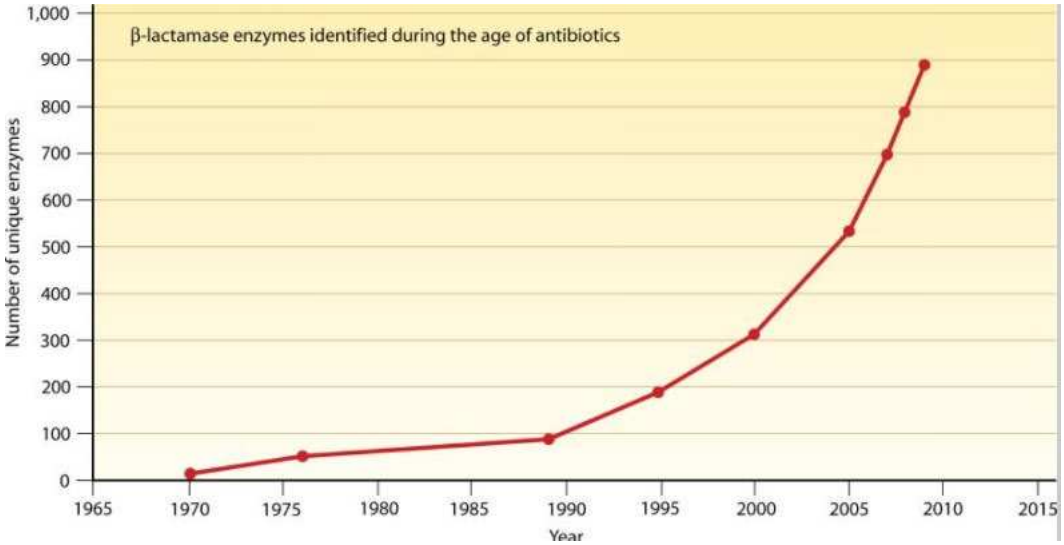

Figure 9. Number of unique β-lactamase enzymes identified since introduction of the first β-lactam antibiotics
Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74:417–33.

In 2015 it was estimated that antibiotic-resistant bacteria caused 671 689 cases of infections in the EU/EEA. A total of 33 110 deaths were attributed to those infections. This corresponds to an incidence of 131 infections per 100 000 people, with a mortality rate of 6.44 per 100 000 (53). Another important overall disease burden measurement is DALY (disability-adjusted life years). The incidents of infection and mortality caused 170 DALYs per 100 000 people (53).

The type of bacteria and their resistance are important contributors to mortality and DALY.

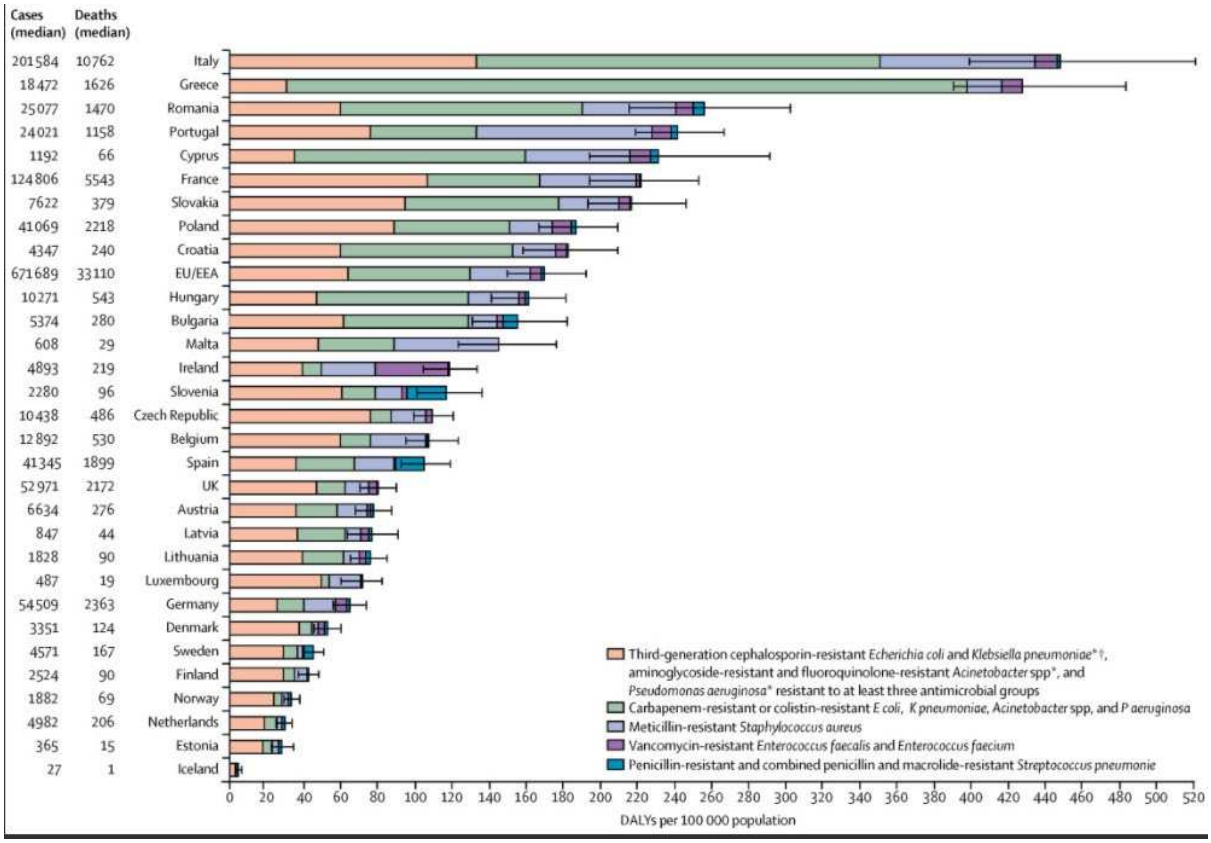

Figure 10. Burden of infections with antibiotic-resistant bacteria in DALY's, EU/EEA, 2015. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19:56–66.

In Europe, the highest prevalence of antibiotic resistance is in the southern and eastern parts. In Croatia, more than 40% of the antibiotic-resistant infections were with carbapenem-resistant or colistin-resistant bacteria, but despite the high resistance the total DALY compared with the rest of EU/EEA were average (53).

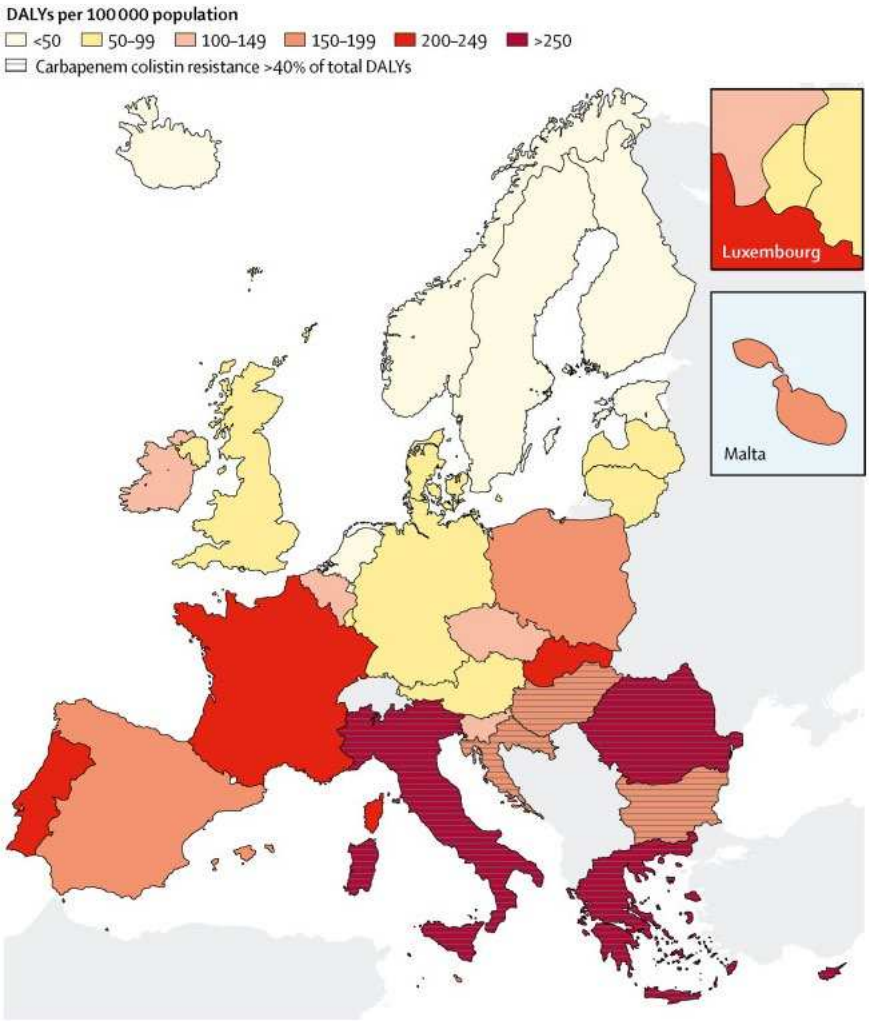

Figure 11. Model estimates of the burden of infections with selected antibiotic-resistant bacteria of public health importance in DALY's per 100 000 population, EU/EEA, 2015. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19:56–66.

A study from 2016 found that the most common respiratory pathogens in Croatia were Group A streptococcus (GAS), pneumococcal species, and *H. influenzae* in decreasing order. Penicillin resistance in GAS was not described. Oral penicillin was effective in 77% of pneumococci infections, while parenteral penicillin effectiveness was dose-dependent ranging from 87%-97%. The resistance to oral amoxicillin was 12% in pneumococcus and 24% in *H. influenzae*. For macrolides, resistance was 7% in GAS species and 36% in pneumococci (54). The most common urinary tract pathogen was *Escherichia coli*, which was resistant to co-trimoxazole in 27%, ciprofloxacin in 19%, co-amoxiclav in 10%, gentamicin in 9%, ceftriaxone in 8%, and nitrofurantoin in 3% of the cases (54). The most common nosocomial

pathogens are *Enterococcus faecium, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus faecium* (54). One of the most significant problems in Croatia regarding antibiotic resistance is the resistance to carbapenems in *Pseudomonas aeruginosa* (20%) and *Acinetobacter baumannii* (87%) (54).

When treating an MDR infection, it is important to choose an empiric antibiotic based on the local risk pathogens and the patient's clinical stability. If sepsis is suspected, antibiotics should be started within 1 hour if possible. If possible, a set of blood cultures should be secured before starting antibiotics, and this should preferably consist of blood cultures taken at two different locations (38). Other appropriate microbiological specimens should also be secured if possible. Taking blood cultures before starting up with antibiotics allows for susceptibility testing. When susceptibility and the microbiological agent are proven, a change in the empiric antibiotics to a more specifically directed antibiotic can be made. Thus, preventing the usage of broad-spectrum antibiotics where more narrow-spectrum antibiotics can be used (38, 46).

Today, in the COVID-19 pandemic, there is a risk of increased antibiotic resistance as hospital capacity has been overwhelmed by COVID-19 patients. Many of these patients have an impaired immune system, and in the later periods of the pandemic, patients with severe COVID-19 have been treated with systemic corticosteroids. Both the impaired immune system and the usage of systemic corticosteroids increase the risk of coinfections and increase the use of antibiotics. From the beginning, there has been a gap between the (comprehending) of the impact of coinfections and comorbidities and the outcome of COVID-19. This has led to rapid changes in the protocols for treating COVID-19. To prevent coinfection antiparasitic, antiviral, antibacterial, and anti-inflammatory drugs have been used. Using these drugs during a prolonged time in the pandemic will have consequences in the future, among these an aggravation of the antibiotic resistance problem (55).

2. OBJECTIVES

Aim:

This study aims to investigate the prevalence of pulmonary superinfections in COVID-19 patients on ventilators and the susceptibility of causative microorganisms to antibiotics during the 2020 pandemic. To investigate the outcome of coinfected or superinfected patients in a 30-day period, LOS (length of stay) in ICU and hospital.

Primary Hypothesis:

- The COVID-19 patient will develop VAP or HAP within 3-5 days after admittance to the ICU.

Secondary hypotheses:

- The COVID-19 patients with coinfections or superinfections had a worse outcome compared with non-coinfected patients
- Most of the superinfections would be caused by gram-negative microorganisms
- The outcome of the patients' length of stay (LOS) in ICU or hospital, mortality is directly connected to coinfection or development of superinfection.

3. MATERIALS AND METHODS

4.1 Study population

To be included in this study the patient had to be admitted to the ICU with proven SARS-CoV2 infection in 2020. Patients who had a lack of data in their medical records on their clinical course and outcome was excluded.

This study includes 18 patients admitted to the COVID-19 ICU of the Department of Anesthesia, Resuscitation, & Intensive Care, University Hospital of Split in 2020 who meet the inclusion and exclusion criteria. All patients had proven infection with SARS-CoV2.

Three patients were excluded from the study due to information missing in their medical records and no discharge letter.

Nine patients included in the study were transferred from other local hospitals and information about the admission date to the local hospital was missing. In these cases, the date of hospital admission was registered as the date the patient was admitted to University Hospital of Split, Split.

4.2 Study design

This study was conducted as an observational retrospective study at the Department of Anesthesia, Resuscitation, & Intensive Care, University Hospital of Split.

The study protocol was approved by the Ethics Committee of the University of Split School of Medicine.

4.3 Method of collecting and analyzing data

Medical data of eligible patients were collected by reviewing the history and discharge papers in medical files stored in the archive. Microbiological data in medical records were compared with microbiological data registered in the Microbiological department. The collected data were organized in Microsoft Excel.

4.4 Statistical analysis

By using the medical history and discharge papers of the patients, the parameters needed were analyzed and results are shown in figures and tables. Microsoft Excel and Microsoft Word were used to make the tables and figures. The Chi-square test was used for categorical data (56). To determine whether differences in data series could be judged as statistically significant or not, the P-value was determined. If $P<0.05$ the differences can be judged as statistically significant. Continuous data such as time were analyzed with “box and whisker plots” in order to visualize the mean, median and spread of the data. The “box and whisker plots” were made using Microsoft Excel.

4. RESULTS

5.1 Demographics

A total of 181 patients were included in the study. Table 2 shows the gender and age distribution. The highest number of patients were found in the age group of 70-74. There was a significant difference between the number of females (25.4%) and males (74.6%), where approximately ¼ of the included patients were female. The difference between females and males is shown in Figure 12.

Table 2. Distribution of patients admitted to ICU with COVID-19

Age (years)	Females N, (%)	Male N, (%)	Total N, (%)
<40	0	1	1 (0.6)
40-44	0	2	2 (1.1)
45-49	1	2	3 (1.7)
50-54	1	8	9 (5.0)
55-59	2	13	15 (8.3)
60-64	2	13	15 (8.3)
65-69	9	24	33 (18.2)
70-74	10	35	45 (24.9)
75-79	14	22	36 (19.9)
80-84	3	9	12(6.6)
85-89	2	4	6 (3.3)
90-94	1	2	3 (1.7)
95-99	1	0	1 (0.6)
Total	46 (25.4)	135 (74.6)	181

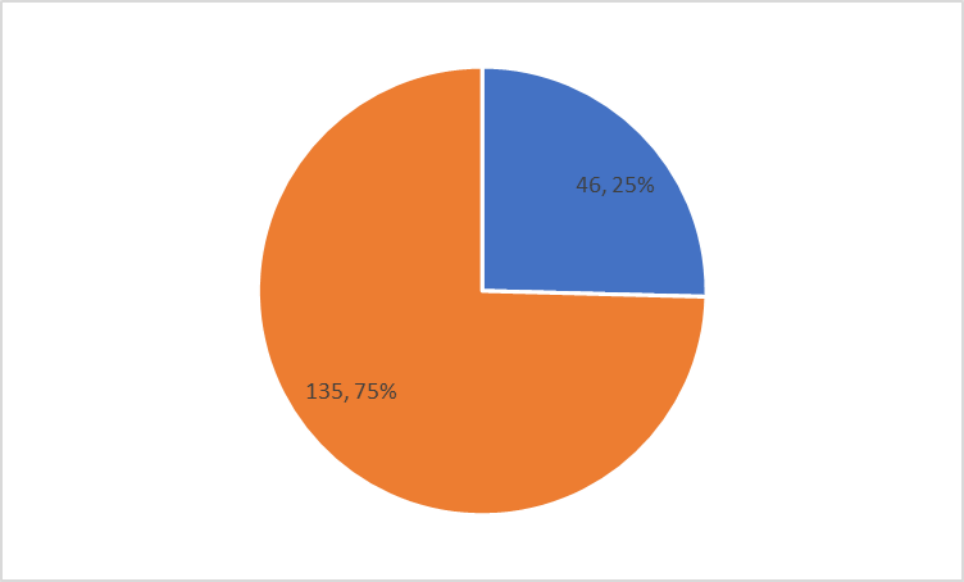

Figure 12. Female-Male distribution
Number, %

5.2 Treatment

Of the 181 patients, 127 patients were intubated (70.2%). Thirty-six patients were treated with noninvasive ventilation support. One patient was treated with extracorporeal membrane oxygenation (ECMO). Four patients had no ventilation support or oxygen supplementation. For 5 of the patients, there was no information in their medical record about ventilation support. Totally 164 of the 181 patients had some sort of ventilation support (90.6%). The breakdown of ventilation support is shown in Figure 13.

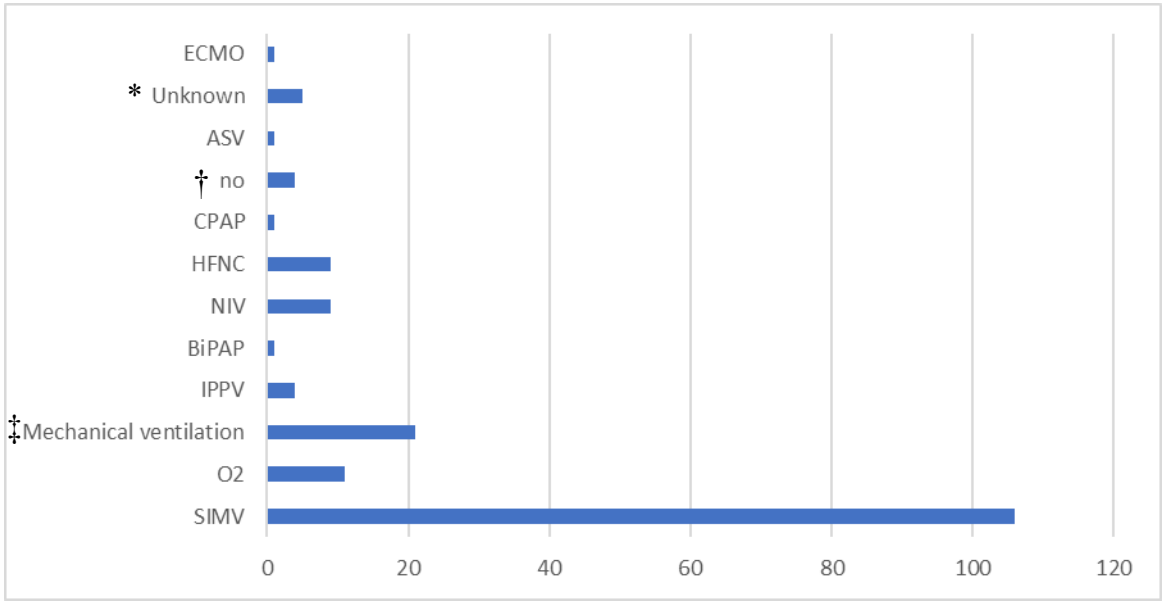

Figure 13. Type of ventilation.
ECMO = extracorporeal membrane oxygenation.
ASV = Adaptive support ventilation mode.
CPAP = Continuous positive airway pressure.
HFNC = High flow nasal cannula.
NIV = Non-invasive ventilation.
BiPAP = Bilevel positive airway pressure.
IPPV = Intermittent positive pressure ventilation.
O2 = oxygen supplementation, either on a normal nasal cannula or on a mask.
SIMV = Synchronized intermittent mandatory ventilation.
* Patients with missing information about ventilation support.
† Patients received no form of supportive oxygenation or ventilation.
‡ Patients intubated on mechanical ventilation, but no specific form of ventilation was defined in the documentation.

Figure 14 shows the use of medication in treating patients with COVID-19 in ICU. A total of 138 patients were treated with systemic corticosteroids. Hundred and ten patients were given treatment with vasoactive drugs due to hemodynamic instability; this was documented in the medical records. In the early period of the pandemic, chloroquine-containing drugs were given to 16 patients; in the later stages of the pandemic, 58 patients were treated with antivirals against SARS-CoV2. Of these, 57 were treated with remdesivir and one with lopinavir/ritonavir.

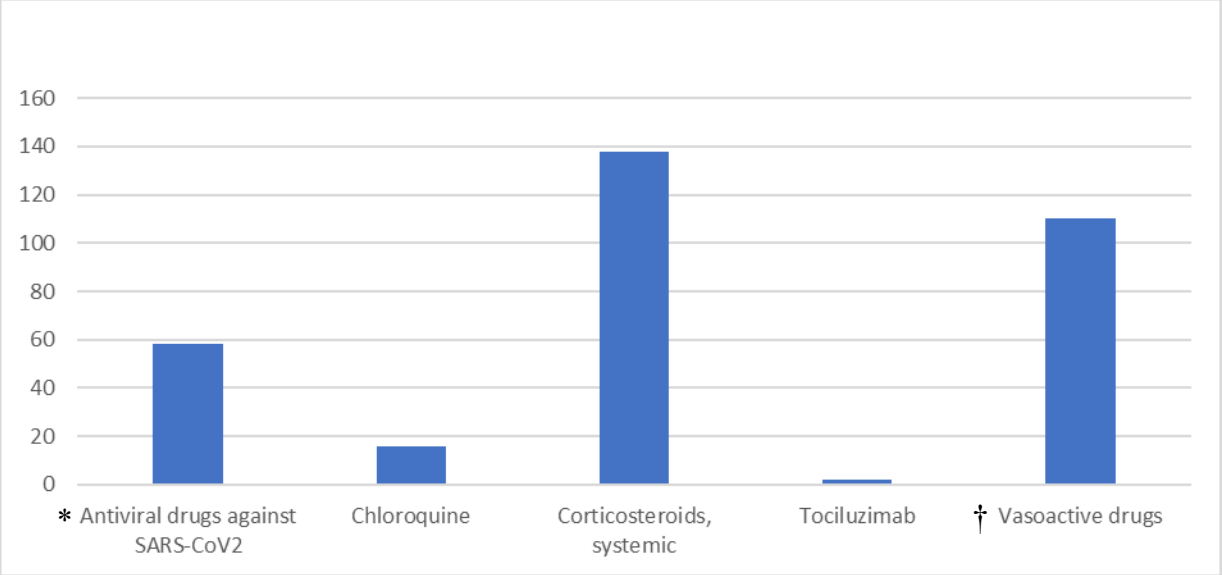

Figure 14. Medical treatments
* Remdesivir, and lopinavir/ritonavir
† Dopamine, adrenaline, levosimendan, noradrenaline, and vasopressin. Vasoactive drugs do not include adrenaline used during resuscitation.

5.3 Prevalence of Ventilation associated pneumonia/ Hospital associated pneumonia

Of 181 patients, 14 were directly admitted to the ICU. Nine patients were transferred from other hospitals (Makarska, Šibenik, Dubrovnik, or Zadar) to Split ICU; and the admission date to the local hospital was not admitted into transfer papers. Twenty-one patients were admitted to the infectiology department in Spilt, and transferred to ICU later on the same day. These are shown as spending 0 days in the infectiology department before admission to ICU in Figure 15.

Figure 15 shows the distribution of time spent in the infectiology department before ICU. The patients spent an average of 5.8 days in the infectiology department before being transferred to ICU. The median of the time spent was 3 days. The mean is significantly larger than the median because there are some patients who spend a long time in the infectiology department. This causes the mean to be much larger.

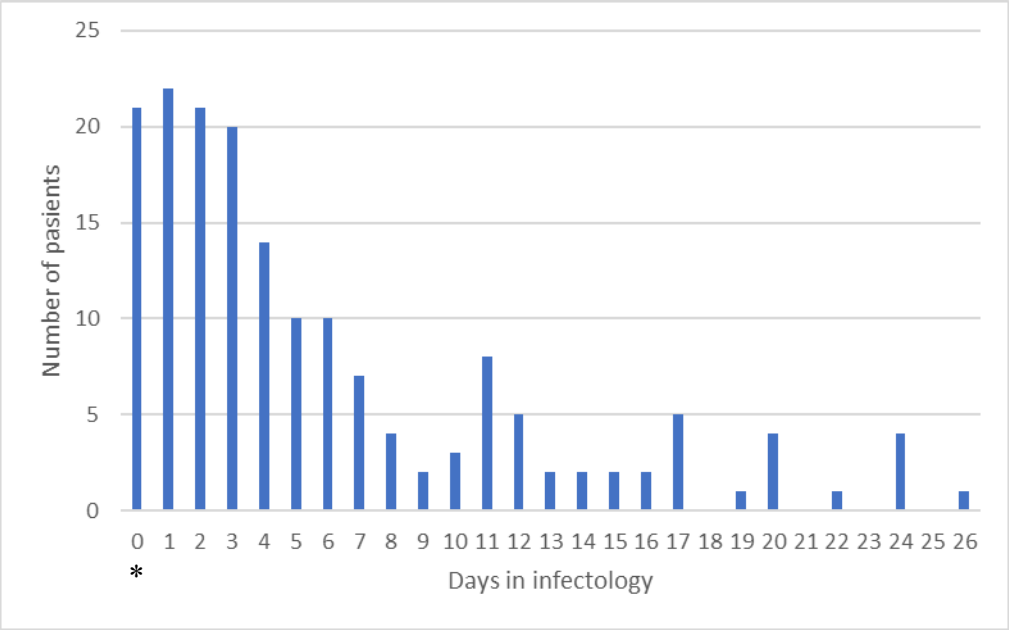

Figure 15. Days spent in the Infectiology department before admission to ICU
* Day 0 is the day of admission.

Of 181 patients, 102 were tested for respiratory infection with tracheal aspiration. Of these, 87 patients had positive samples, and 15 had a sterile sample at all samples taken.

The prevalence of coinfection/superinfection is 48.1% in the respiratory samples.

Figure 16 shows the number of identified respiratory coinfections/superinfections in relationship to time after being admitted to the hospital. Six patients were admitted with coinfections, and 81 patients developed a respiratory superinfection during their hospital stay. 3.3% of the patients had respiratory coinfection at the time of admission, and 44.8% developed a superinfection.

The mean time from admittance to the hospital to the development of respiratory superinfection was 10.9 days, with a median of 10 days.

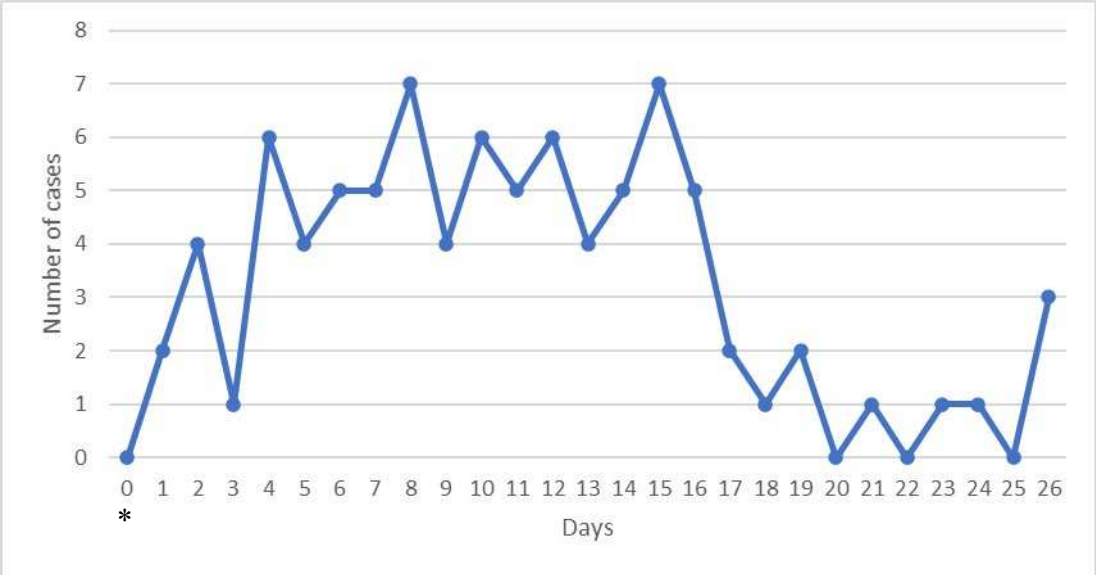

Figure 16. Number of positive respiratory cultures in days after admittance to the hospital.
* Day 0 is the day of admission.

Figure 17 shows the number of developed respiratory coinfection/superinfection as a function of days after admittance to the ICU. Two patients developed positive respiratory cultures after admittance to the hospital but before being admitted to the ICU. The highest number of new coinfection/superinfections was after 2 days in the ICU.

The mean time from admittance to ICU to development of respiratory superinfections was 5.2 days, with a median of 4 days.

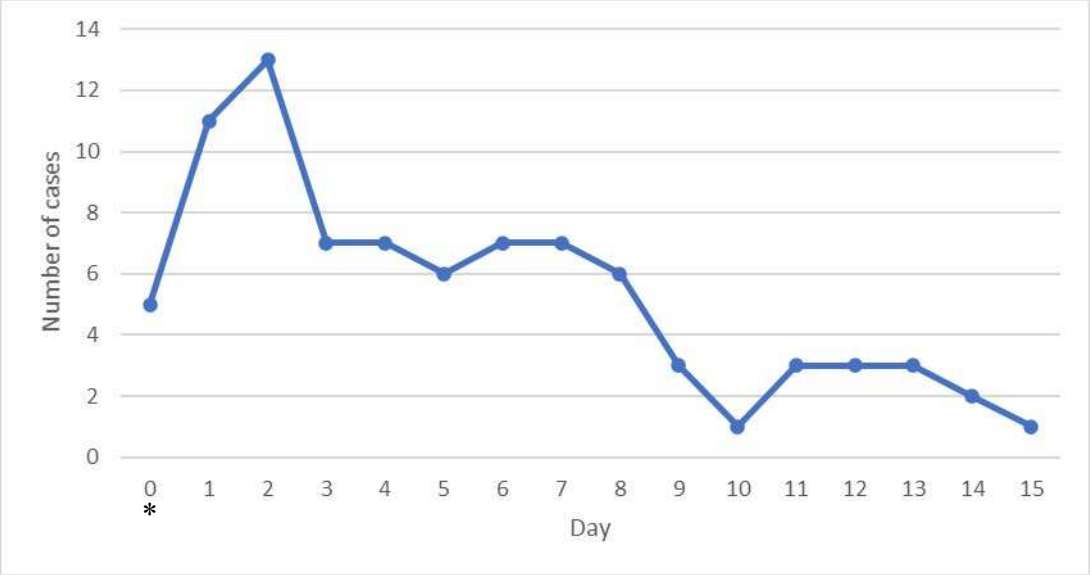

Figure 17. Number of positive respiratory culture, days after admittance to ICU
* Day 0 is the day of admission.

Of 181 patients, 140 had blood cultures taken. Of these, 75 were positive, while 65 patients remained sterile during the entire course of the disease.

The prevalence of coinfection/superinfection is 41.4% in blood cultures.

Figure 18 shows the number of positive blood cultures on days after being admitted to the hospital. Two patients are not included in Figure 18; one had a positive blood culture after 41 days, and the other had a positive blood culture after 50 days. The highest number of positive blood cultures is 4 days after being admitted to the hospital. Two patients had positive blood cultures on admission to the hospital. A total of 6 patients had positive blood cultures in the time period of coinfection; and 69 patients developed a superinfection which resulted in a positive blood culture. This gives a coinfection prevalence of 3.3% in blood culture samples and a superinfection prevalence of 38.1%.

The mean time to develop a positive blood culture in patients admitted to the hospital is 10.6, with a median of 9.

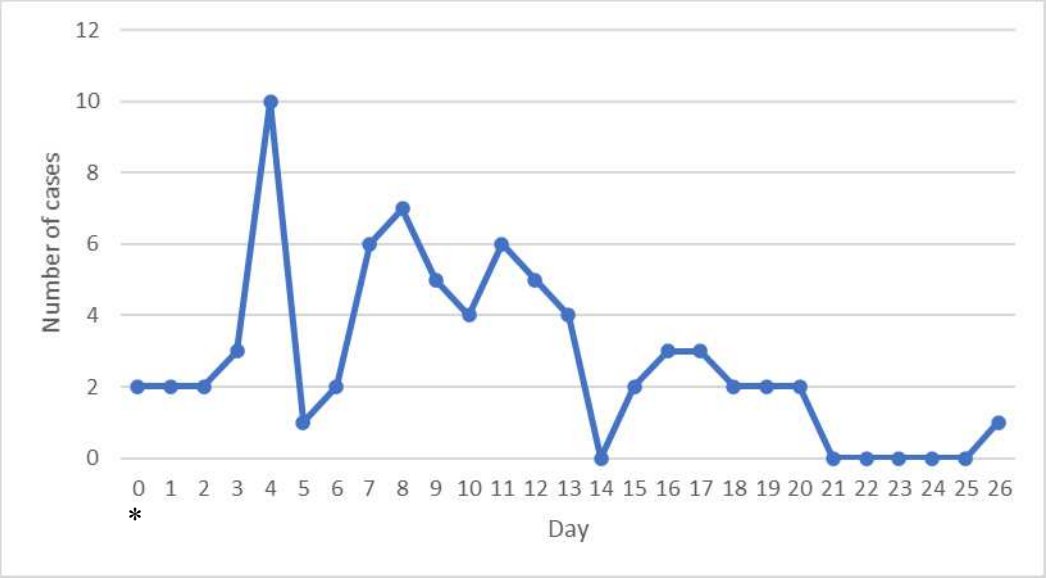

Figure 18. Number of positive blood culture cases, days after admittance to hospital
* Day 0 is the day of admission.

Figure 19 shows the number of positive blood cultures related to time in ICU. One patient is not included in Figure 19; this patient tested positive 39 days after being admitted to ICU. The highest number of positive blood cultures are found 1 day after being admitted to ICU.

The mean time to develop a positive blood culture after admittance to ICU is 4.8 days, with a median of 2.

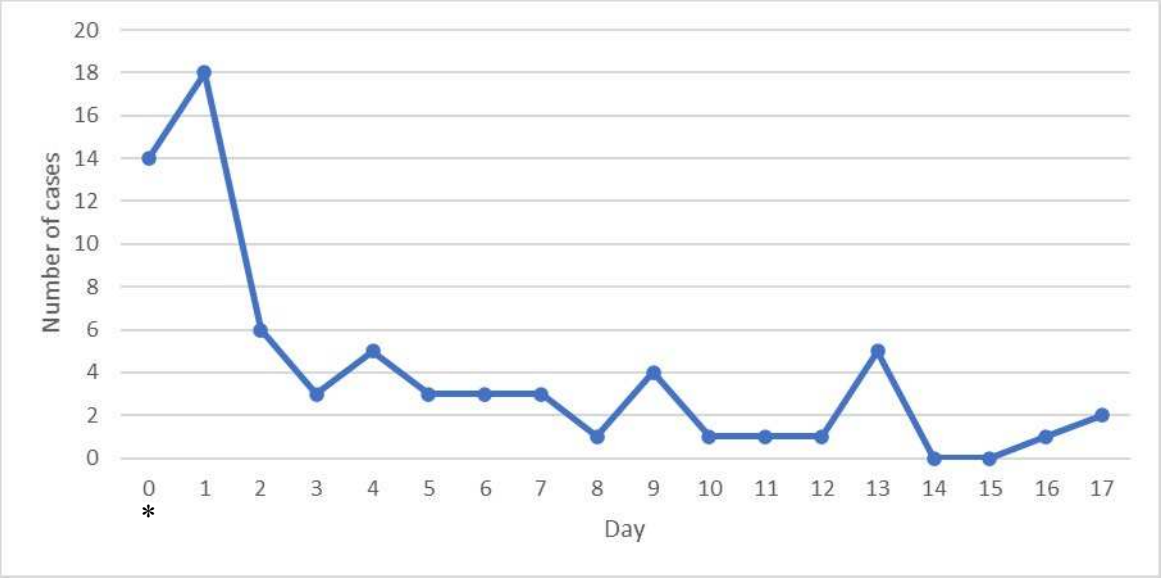

Figure 19. Number of blood culture positive cases on days after admittance to ICU
* Day 0 is the day of admission.

5.4 Outcome of patients (LOS, mortality)

Looking at all patients, they spent an average of 15.9 days in the hospital, with a median of 14 days. The average number of days spent in the ICU was 10.1 days, with a median of 7. The overall mortality rate was 68.5%, with 124 of 181 patients dying.

The difference between the patients who had no respiratory samples, who had a positive respiratory sample, and those with negative respiratory samples are shown in Table 3. There was a statistically significant difference ($p = 0.002$) in mortality in the patients with coinfection/superinfection proven by respiratory sample culture, compared to patients not tested with respiratory samples and patients who had only negative respiratory sample cultures.

Table 3. Respiratory sample culture outcome

		All patients with no respiratory cultures	**Respiratory culture positive patients**	**Respiratory culture negative patients**	***P****	**Chi-square statistic**
Number of pas N		79	87	15		
Time in hospital Day, D	Mean	10.4	21.5	13.5		
	Median	10	18	12		
Time in ICU Day, D	Mean	4.4	15.4	9.6		
	Median	3	14	10		
Time in hospital before death, D	Mean	10.3	20.2	11.9		
	Median	9	17	12		
Time in ICU before death D	Mean	3.8	14.1	8.8		
	Median	3	12	10		
Mortality N, [χ^2]	Diseased	43 [2.29]	69 [1.48]	12 [0.29]	0.002	12.9
	Survived	36 [4.97]	18 [3.22]	3 [0.63]		
Mortality rate		54.4%	79.3%	80.0%		

* P-value from chi-square test
N = number
D = days
[χ^2] = chi-square value

All patients with a positive respiratory culture sample spent a significantly longer time in the hospital than all patients who had either not been tested or had negative respiratory samples. The statistical analysis of time spent in the hospital is shown in Figure 20.

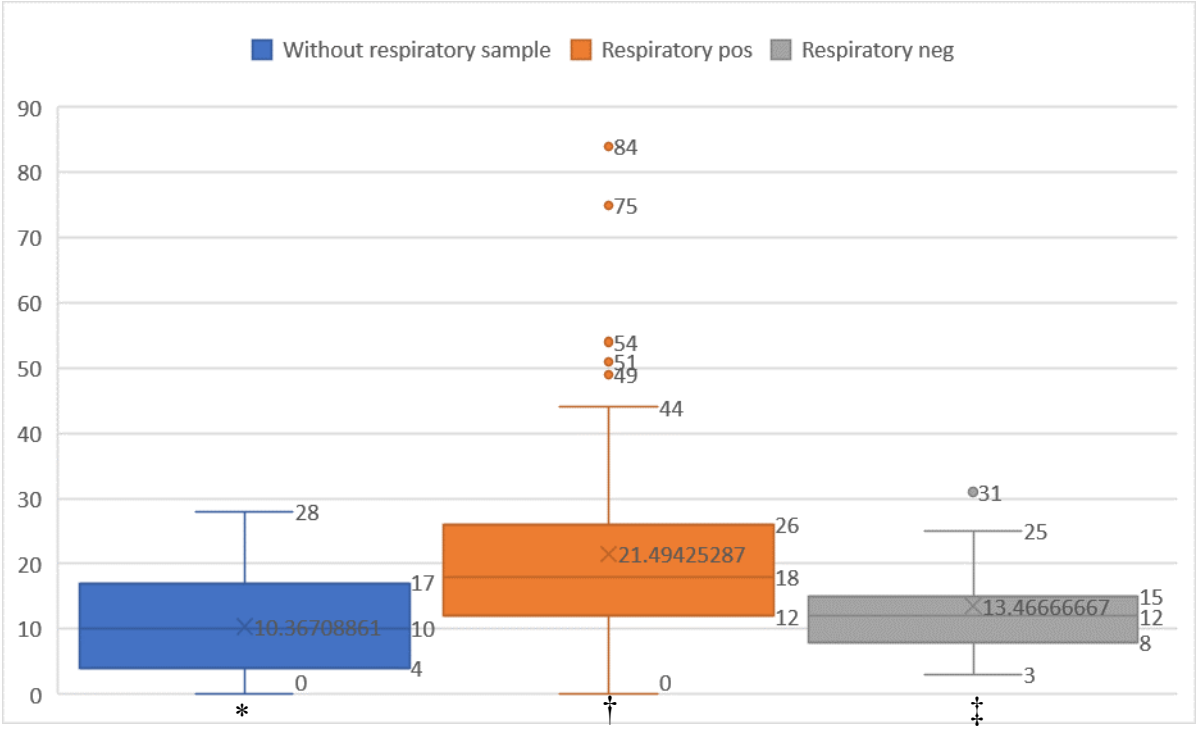

Figure 20. Days in hospital, respiratory sample, all patients
* Median 10, 1. quartile 4, 3. quartile 17
†Median 18, 1. quartile 12, 3. quartile 26
‡ Median 12, 1. quartile 8, 3. quartile 15
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

All patients spent a significantly longer time in ICU if they had a positive respiratory sample compared to all patients who were not tested or had negative respiratory culture samples. The statistical analysis is shown in Figure 21.

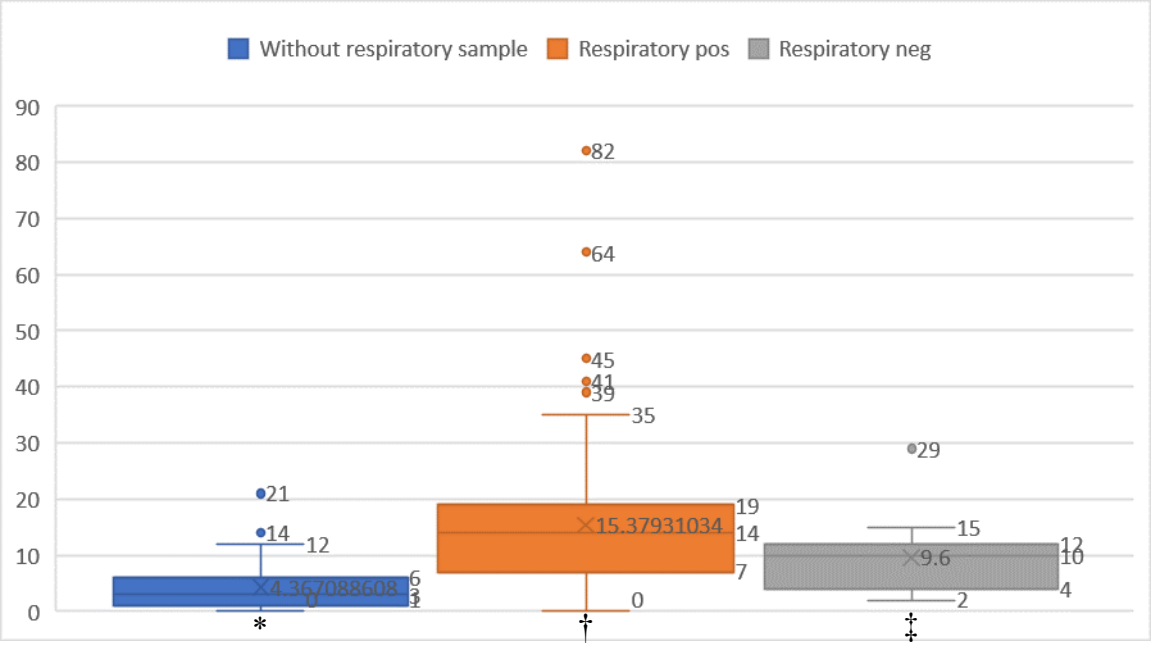

Figure 21. Days in ICU, respiratory samples
* Median 3, 1. quartile 1, 3. quartile 6
† Median 14, 1. quartile 7, 3. quartile 19
‡ Median 10, 1. quartile 4, 3. quartile 12
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

The stay in hospital ended with either the patient being discharged or dying. The results for the patients dying are reported separately. The patients who died who had a positive respiratory sample stayed significantly longer in the hospital than those who died with negative respiratory samples and those who died who were not tested. The statistical analysis is shown in Figure 22.

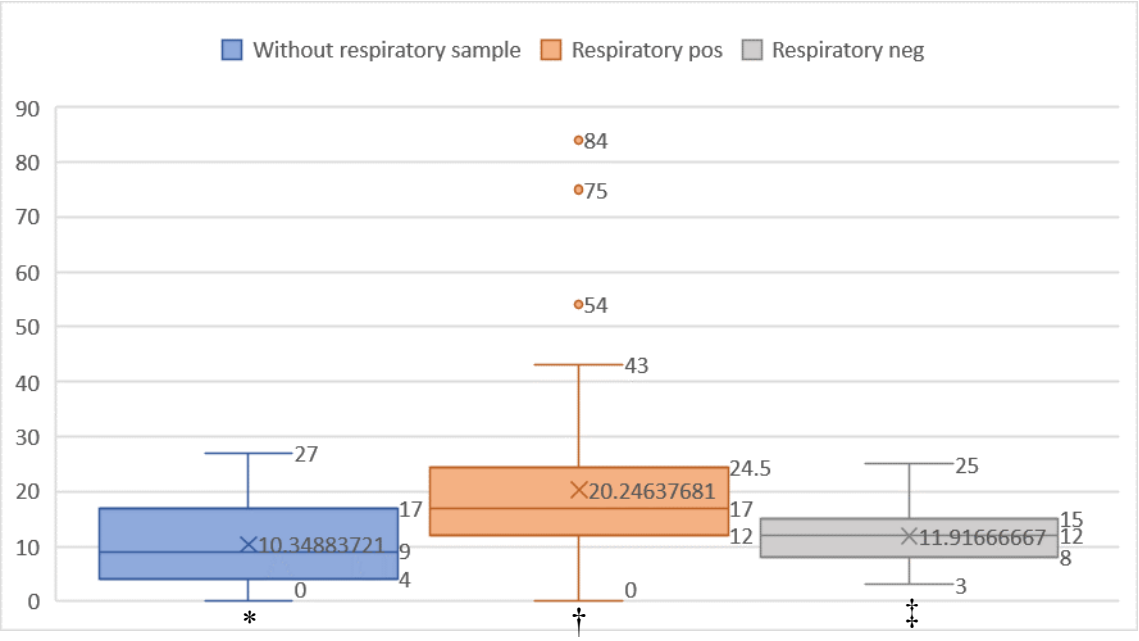

Figure 22. Days in the hospital before death, respiratory samples
* Median 9, 1. quartile 4, 3. quartile 17
† Median 17, 1. quartile 12, 3. quartile 24.5
‡ Median 12, 1. quartile 8, 3. quartile 15
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

Patients who died who had positive samples stayed significantly longer in ICU than those who died who were not tested or had negative tests. The statistical analysis is shown in Figure 23.

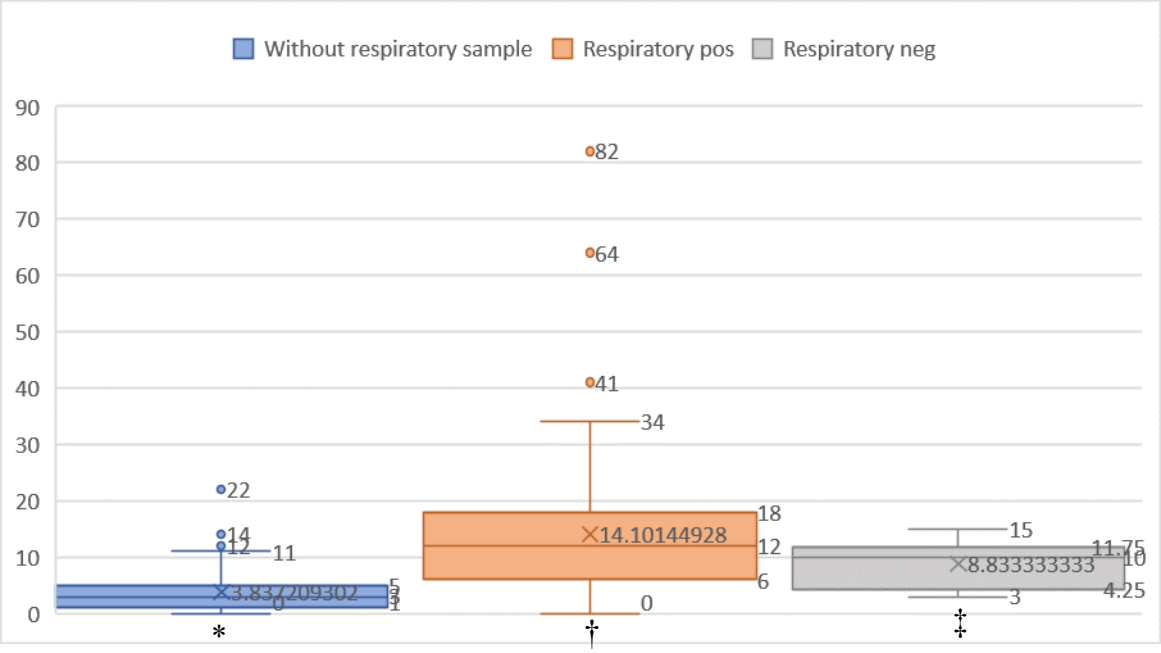

Figure 23. Days in ICU before death, respiratory samples
* Median 3, 1. quartile 1, 3. quartile 5
† Median 12, 1. quartile 6, 3. quartile 18
‡ Median 10, 1. quartile 4.25, 3. quartile 11.75
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

The difference between the patients who had no blood cultures taken, who had a positive blood culture, and those with negative blood cultures are shown in Table 4. There was no statistically significant difference (p = 0.625) in mortality between the patients with blood cultures proving coinfection/superinfection and the patients who had no blood cultures taken and patients who had only sterile blood cultures.

Table 4. Blood culture outcome

		All patients without blood cultures	**Patients with positive blood culture**	**Patients with negative blood culture**	***P****	**Chi-square statistic**
Number of pas		41	75	65		
Time in hospital, D	Mean	9.3	21.5	13.5		
	Median	7	18	12		
Time in ICU, D	Mean	2.4	15.4	8.9		
	Median	2	13	7		
Time in the hospital before death, D	Mean	9.1	20.5	14.6		
	Median	7.5	17	14.5		
Time in ICU before death, D	Mean	2.5	14.2	9.3		
	Median	2	11.5	7.5		
Mortality N, [χ^2]	Diseased	26 [0.63]	54 [0.13]	44 [0.01]	0.625	0.937
	Survived	15 [0.34]	21 [0.29]	21 [0.01]		
Mortality rate		63.4%	72.0%	67.7%		

* P-value from chi-square test
N = number
D = days
[χ^2] = chi-square value

All patients with a positive blood culture spent a significantly longer time in the hospital than all patients who were not tested or had only negative blood cultures. The statistical analysis is shown in Figure 24.

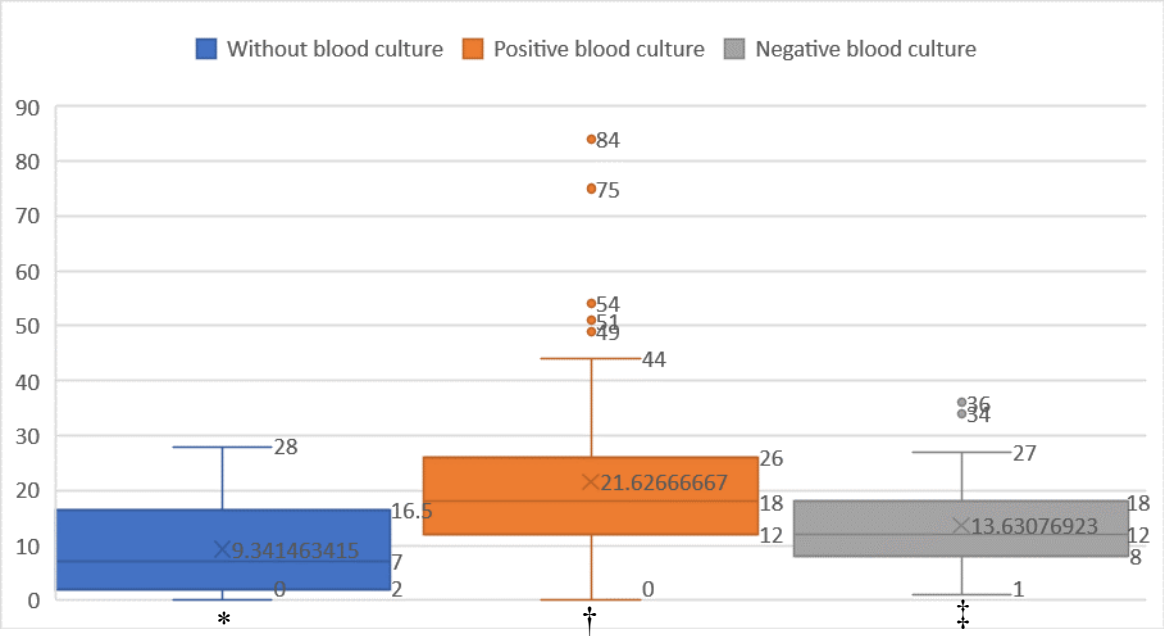

Figure 24. Days in hospital, blood culture
* Median 7, 1. quartile 2, 3. quartile 16.5
† Median 18, 1. quartile 12, 3. quartile 26
‡ Median 12, 1. quartile 8, 3. quartile 18
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

All patients with a positive blood culture spent a significantly longer time in ICU than all patients who were not tested. They spent longer time than those with a negative blood culture, but this may not be statistically significant. The statistical analysis is shown in Figure 25.

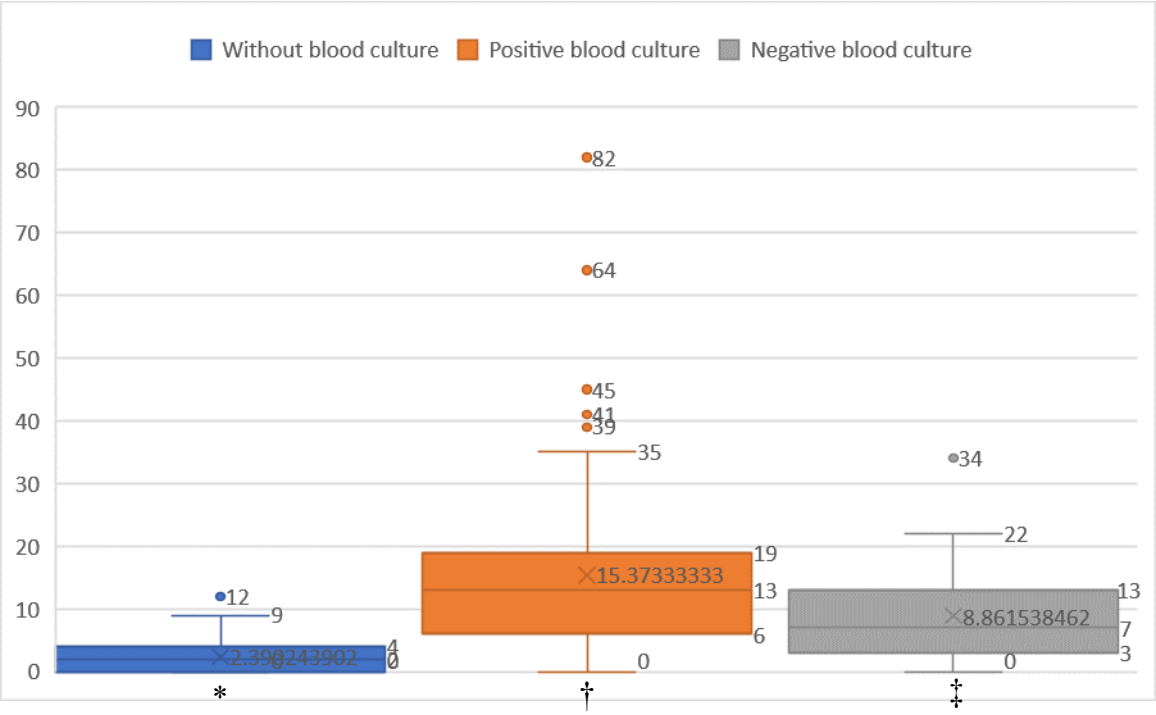

Figure 25. Days in ICU, blood culture
* Median 2, 1. quartile 0, 3. quartile 4
† Median 13, 1. quartile 6, 3. quartile 19
‡ Median 7, 1. quartile 3, 3. quartile 13
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

Deceased patients who had a positive blood culture stayed significantly longer in the hospital than those who died and was not tested. There was no statistically significant difference in the length of stay between those who died with a positive blood culture and those who died with negative culture. The statistical analysis is shown in Figure 26.

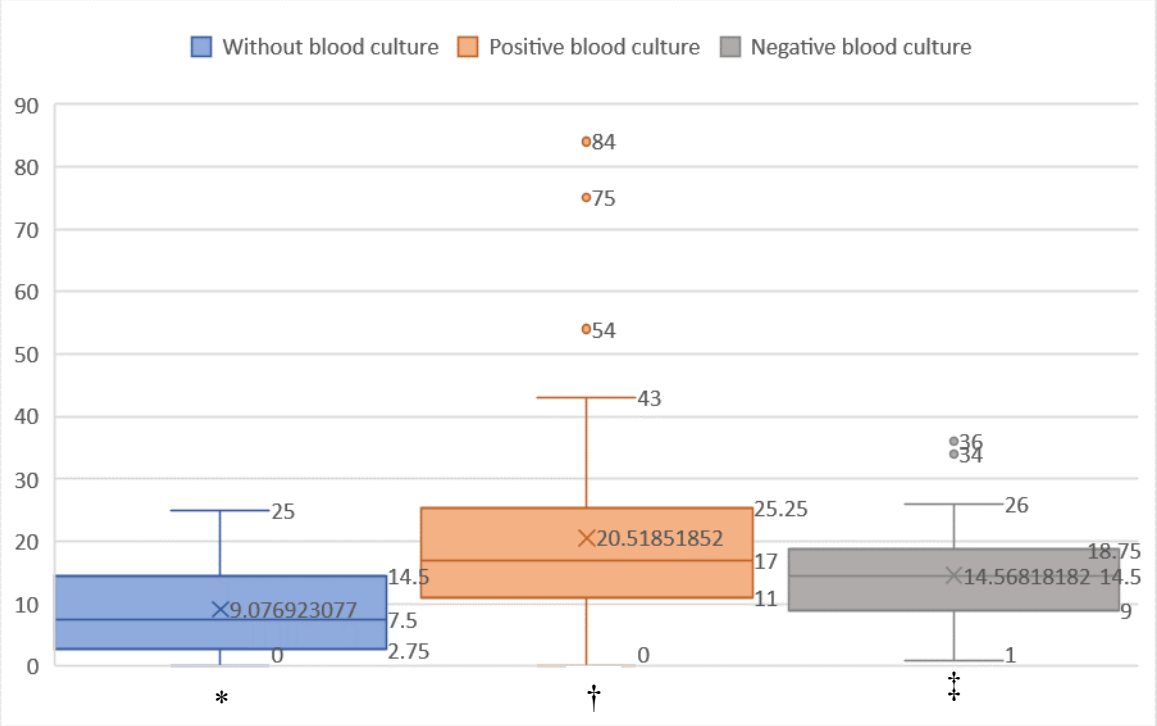

Figure 26. Days in the hospital before death, blood culture
* Median 7.5, 1. quartile 2.75, 3. quartile 14.5
† Median 17, 1. quartile 11, 3. quartile 25.5
‡ Median 14.5, 1. quartile 9, 3. quartile 18.75
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

Patients with a positive blood culture who died stayed significantly longer in ICU than those who were not tested. There was no statistically significant difference in the length of stay between those with a positive blood culture who died and those with negative blood cultures who died. The statistical analysis is shown in Figure 27.

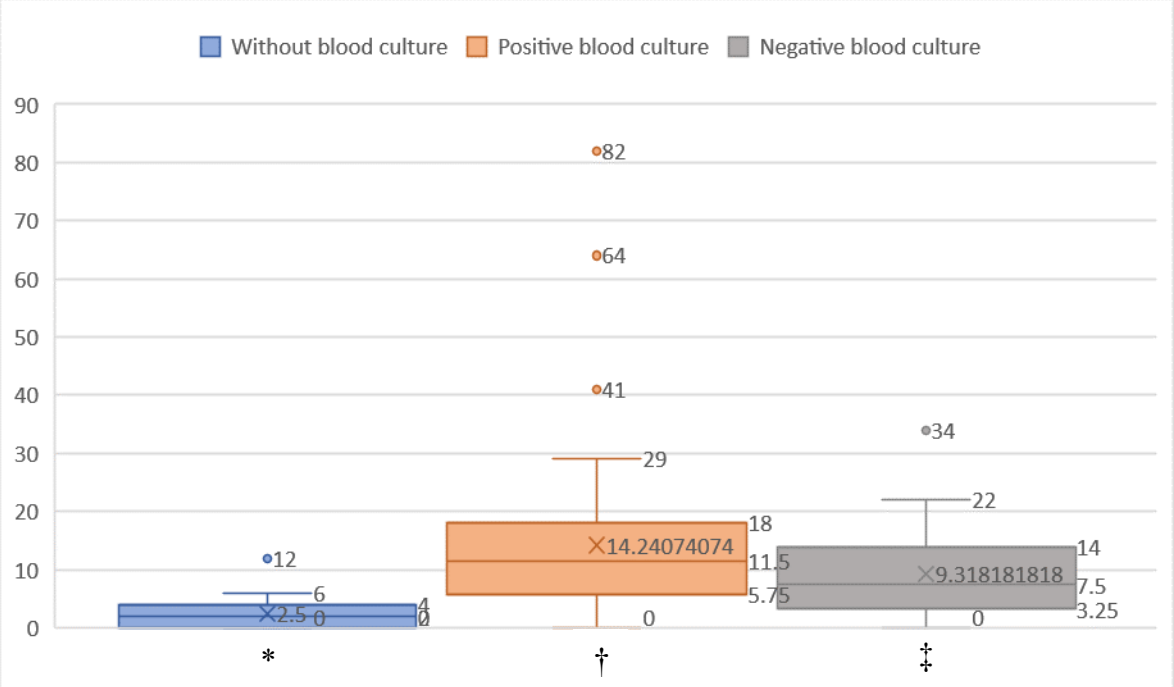

Figure 27. Days in ICU, before death, blood culture
* Median 2, 1. quartile 0, 3. quartile 4
† Median 11.5, 1. quartile 5.75, 3. quartile 18
‡ Median 7.5, 1. quartile 3.25, 3. quartile 14
The maximum whisker in the plots is the smallest value of the maximum value and the largest value less than 3. quartile plus 1.5 times the distance between 1. and 3. quartile. The data points exceeding that limit are shown as separate points in the plots.

5.4 Type of bacteria, respiratory samples, and blood cultures

Table 5 shows the etiology of positive respiratory cultures. The most common agent in respiratory infection was *Acinetobacter baumannii;* this was the causative agent in 40.9% of cases. The second most common cause was *Candida albicans* (16.4%), the third most common agent was *Pseudomonas aeruginosa* (14.5%), followed by *Klebsiella pneumoniae* (7.5%).

Some patients tested positive in multiple rounds and with multiple agents.

Table 5. Etiology in respiratory samples

Agent	Respiratory culture (N)	Respiratory culture (%)
Acinetobacter baumannii	65	40.9
Candida albicans	26	16.4
Pseudomonas aeruginosa	23	14.5
Klebsiella pneumoniae	12	7.5
Staphylococcus aureus	7	4.4
Stenotrophomonas maltophilia	6	3.8
Elizabethkingia anophelis	4	2.5
Proteus mirabilis	3	1.9
Klebsiella variicola	2	1.3
Enterobacter cloacae	2	1.3
Serratia sp	1	0.6
Aspergillus sp	1	0.6
Asprergillus fumigatus	1	0.6
Morganella morganii	1	0.6
Klebsiella aerogenes	1	0.6
Candida glabrata	1	0.6
Streptococcus pneumoniae	1	0.6
Burkholderia gladioli	1	0.6
Providencia stuartii	1	0.6
Total	159	100.0

N = number
% = Percentage of total number of positive respiratory cultures

Acinetobacter baumannii bacteria isolated in respiratory samples in the study show a high resistance rate to almost all investigated antibiotic agents represented in Table 6. It is resistant to all antibiotics investigated except ampicillin-sulbactam (sensitivity of 96.2%) and colistin (sensitivity of 95.9%).

Table 6. Antibiotic sensitivity *Acinetobacter baumannii* in respiratory samples

Antibiotic	**Sensitive N, (%)**	**Intermediate N, (%)**	**Resistant N, (%)**	**Total number N, (%)**
Ampicillin-sulbactam	50 (96.2)	2 (3.8)	0 (0.0)	52 (100.0)
Imipenem	0 (0.0)	0 (0.0)	53 (100.0)	53 (100.0)
Meropenem	0 (0.0)	0 (0.0)	52 (100.0)	52 (100.0)
Gentamicin	0 (0.0)	0 (0.0)	53 (100.0)	53 (100.0)
Tobramycin	0 (0.0)	0 (0.0)	48 (100.0)	48 (100.0)
Amikacin	0 (0.0)	0 (0.0)	52 (100.0)	52 (100.0)
Ciprofloxacin	0 (0.0)	0 (0.0)	53 (100.0)	53 (100.0)
Levofloxacin	0 (0.0)	0 (0.0)	51 (100.0)	51 (100.0)
Sulfamethoxazole-Trimethoprim	3 (6.0)	0 (0.0)	47 (94.0)	50 (100.0)
Colistin	47 (95.9)	1 (2.0)	1 (2.0)	49 (100.0)

N= number
% = Percentage of total

Table 7 shows the antibiotic sensitivity of *Pseudomonas aeruginosa* found in the study. *Pseudomonas aeruginosa* organisms isolated in the respiratory samples display high sensitivity to amikacin (100%), ceftazidime-avibactam (91.7%), and ceftizoxime (91.7%). Only 3 of the 23 samples were tested against colistin, revealing a 100% susceptibility.

Table 7. Antibiotic sensitivity of *Pseudomonas aeruginosa* in respiratory samples

Antibiotic	**Sensitive N, (%)**	**Intermediate N, (%)**	**Resistant N, (%)**	**Total number N, (%)**
Tazobactam-Piperacillin	0 (0.0)	20 (100.0)	0 (0.0)	20 (100.0)
Ceftazidime	0 (0.0)	19 (95.0)	1 (5.0)	20 (100.0)
Ceftazidime-Avibactam	11 (91.7)	0 (0.0)	1 (8.3)	12 (100.0)
Cefepime	0 (0.0)	19 (100.0)	0 (0.0)	19 (100.0)
Ceftizoxime	11 (91.7)	0 (0.0)	1 (8.3)	12 (100.0)
Imipenem	0 (0.0)	9 (45.0)	11 (55.0)	20 (100.0)
Meropenem	6 (33.3)	1 (5.6)	11 (61.1)	18 (100.0)
Tobramycin	10 (52.6)	0 (0.0)	9 (47.4)	19 (100.0)
Amikacin	20 (100.0)	0 (0.0)	0 (0.0)	20 (100.0)
Ciprofloxacin	0 (0.0)	11 (55.0)	9 (45.0)	20 (100.0)
Levofloxacin	0 (0.0)	9 (45.0)	11 (55.0)	20 (100.0)
Colistin	3 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)

N= number
% = Percentage of total

Klebsiella pneumoniae found in respiratory samples showed a 100% sensitivity to ceftazidime-avibactam and cefoxitin. A sensitivity of 87.5% was found to piperacillin-tazobactam, ertapenem, imipenem, and meropenem. A 100% resistance was found to ampicillin and amoxicillin. Colistin was only tested for in 1 of 12 samples and was found to be 100% sensitive. Fosfomycin was also only tested in 1 of 12 samples and was found to be 100% resistant. Sensitivity and resistance to other antibiotics are shown in Table 8.

Table 8. Antibiotic sensitivity of *Klebsiella pneumoniae* in respiratory samples

Antibiotic	**Sensitive N, (%)**	**Intermediate N, (%)**	**Resistant N, (%)**	**Total number N, (%)**
Ampicillin	0 (0.0)	0 (0.0)	7 (100.0)	7 (100.0)
Amoxicillin	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)
Amoxicillin-Clavulanic acid	3 (37.5)	0 (0.0)	5 (62.5)	8 (100.0)
Piperacillin-Tazobactam	7 (87.5)	0 (0.0)	1 (12.5)	8 (100.0)
Cephalexin	3 (60.0)	0 (0.0)	2 (40.0)	5 (100.0)
Cefuroxime axetil	4 (80.0)	0 (0.0)	1 (20.0)	5 (100.0)
Cefuroxime	0 (0.0)	2 (40.0)	3 (60.0)	5 (100.0)
Cefixime	3 (60.0)	0 (0.0)	2 (40.0)	5 (100.0)
Ceftibuten	3 (60.0)	0 (0.0)	2 (40.0)	5 (100.0)
Cefotaxime	4 (57.1)	0 (0.0)	3 (42.9)	7 (100.0)
Ceftriaxone	4 (50.0)	0 (0.0)	4 (50.0)	8 (100.0)
Ceftazidime	4 (57.1)	0 (0.0)	3 (42.9)	7 (100.0)
Ceftazidime-avibactam	4 (100.0)	0 (0.0)	0 (0.0)	4 (100.0)
Cefepime	4 (50.0)	0 (0.0)	4 (50.0)	8 (100.0)
Ceftizoxime	4 (80.0)	0 (0.0)	1 (20.0)	5 (100.0)
Cefoxitin	5 (100.0)	0 (0.0)	0 (0.0)	5 (100.0)
Ertapenem	7 (87.5)	0 (0.0)	1 (12.5)	8 (100.0)
Imipenem	7 (87.5)	0 (0.0)	1 (12.5)	8 (100.0)
Meropenem	7 (87.5)	0 (0.0)	1 (12.5)	8 (100.0)
Gentamicin	4 (50.0)	0 (0.0)	4 (50.0)	8 (100.0)
Amikacin	5 (62.5)	0 (0.0)	3 (37.5)	8 (100.0)
Norfloxacin	3 (75.0)	0 (0.0)	1 (25.0)	4 (100.0)
Ciprofloxacin	6 (75.0)	0 (0.0)	2 (25.0)	8 (100.0)
Levofloxacin	5 (71.4)	0 (0.0)	2 (28.6)	7 (100.0)
Sulfamethoxazole-Trimethoprim	5 (62.5)	0 (0.0)	3 (37.5)	8 (100.0)
Fosfomycin	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Colistin	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)

N= number
% = Percentage of total

Table 9 shows the most common etiology of positive blood cultures. The most common cause of positive blood culture was *Staphylococcus epidermidis* (25.6%), the second most common agent was *Acinetobacter baumannii* (15.0%), the third most common agent was *Staphylococcus hominis* (9.8%), and the fourth most common agent was *Pseudomonas aeruginosa* (6.8%)

Some patients were positive in multiple rounds and with multiple agents.

Table 9. Etiology in blood cultures

Agent	**Hemoculture (N)**	**Hemoculture (%)**
Staphylococcus epidermidis	34	25.6%
Acinetobacter baumannii	20	15.0%
Staphylococcus hominis	13	9.8%
Pseudomonas aeruginosa	9	6.8%
Cutibacterium acnes	9	6.8%
Candida parapsilosis	8	6.0%
Klebsiella pneumoniae	6	4.5%
Staphylococcus haemolyticus	5	3.8%
Stenotrophomonas maltophilia	4	3.0%
Proteus mirabilis	3	2.3%
Enterococcus faecalis	3	2.3%
Staphylococcus capitis	3	2.3%
Candida albicans	2	1.5%
Staphylococcus aureus	1	0.8%
Candida glabrata	1	0.8%
Streptococcus pneumoniae	1	0.8%
Enterococcus sp	1	0.8%
Corynebacterium striatum	1	0.8%
Bacillus licheniformis	1	0.8%
Staphylococcus lugdunensis	1	0.8%
Non-enzymatic gram-negative bacilli	1	0.8%
Coagulase-negative *Staphylococcus* sp	1	0.8%
Enterococcus faecium	1	0.8%
Staphylococcus simulans	1	0.8%
Corynebacterium coyleae	1	0.8%
Escherichia coli	1	0.8%
Providencia stuartii	1	0.8%

N= number
% = Percentage of total

The antibiotic susceptibility of *Staphylococcus epidermidis* found in the study is shown in Table 10. *Staphylococcus epidermidis* found in blood cultures was 100% susceptible to tigecycline, teicoplanin, and vancomycin, and 96.7% susceptible to linezolid, and rifampin. The organisms isolated were highly resistant to mupirocin (73.9%), cefoxitin (69.2%), clarithromycin (66.9%), oxacillin, erythromycin, and azithromycin (all three 66.7%). It was also 100% resistant to amoxicillin and cefuroxime, which were tested in 2 of 34 samples.

Table 10. Antibiotic sensitivity of *Staphylococcus epidermidis* in blood cultures

Antibiotic	**Sensitive N, (%)**	**Intermediate N, (%)**	**Resistant N, (%)**	**Total number N, (%)**
Oxacillin	10 (33.3)	0 (0.0)	20 (66.7)	30 (100.0)
Amoxicillin	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)
Amoxicillin-clavulanic acid	10 (34.5)	0 (0.0)	19 (65.5)	29 (100.0)
Ceftaroline	18 (81.8)	0 (0.0)	4 (18.2)	22 (100.0)
Cefoxitin	4 (30.8)	0 (0.0)	9 (69.2)	13 (100.0)
Cefuroxime	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)
Gentamicin	20 (66.7)	0 (0.0)	10 (33.3)	30 (100.0)
Ciprofloxacin	0 (0.0)	17 (58.6)	12 (41.4)	29 (100.0)
Levofloxacin	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)
Moxifloxacin	16 (66.7)	0 (0.0)	8 (33.3)	24 (100.0)
Sulfamethoxazole-trimethoprim	17 (56.7)	0 (0.0)	13 (43.3)	30 (100.0)
Erythromycin	10 (33.3)	0 (0.0)	20 (66.7)	30 (100.0)
Azithromycin	10 (33.3)	0 (0.0)	20 (66.7)	30 (100.0)
Clarithromycin	9 (32.1)	0 (0.0)	19 (67.9)	28 (100.0)
Clindamycin	16 (53.3)	0 (0.0)	14 (46.7)	30 (100.0)
Tetracycline	24 (80.0)	0 (0.0)	6 (20.0)	30 (100.0)
Tigecycline	29 (100.0)	0 (0.0)	0 (0.0)	29 (100.0)
Linezolid	29 (96.7)	0 (0.0)	1 (3.3)	30 (100.0)
Teicoplanin	29 (100.0)	0 (0.0)	0 (0.0)	29 (100.0)
Vancomycin	29 (100.0)	0 (0.0)	0 (0.0)	29 (100.0)
Rifampin	29 (96.7)	0 (0.0)	1 (3.3)	30 (100.0)
Mupirocin	6 (26.1)	0 (0.0)	17 (73.9)	23 (100.0)

N= number
% = Percentage of total

Acinetobacter baumannii organisms found in blood cultures are sensitive to colistin in 100% of cases and ampicillin-sulbactam in 80% of cases. They are resistant to all other tested antibiotics, as shown in Table 11.

Table 11. Antibiotic sensitivity of *Acinetobacter baumannii* in blood cultures

Antibiotic	**Sensitive N, (%)**	**Intermediate N, (%)**	**Resistant N, (%)**	**Total number N, (%)**
Ampicillin-sulbactam	16 (80.0)	3 (15.0)	1 (5.0)	20 (100.0)
Ertapenem	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Ceftazidime-avibactam	0 (0.0)	0 (0.0)	5 (100.0)	5 (100.0)
Ceftizoxime	0 (0.0)	0 (0.0)	3 (100.0)	3 (100.0)
Imipenem	0 (0.0)	0 (0.0)	20 (100.0)	20 (100.0)
Meropenem	0 (0.0)	0 (0.0)	20 (100.0)	20 (100.0)
Gentamicin	0 (0.0)	0 (0.0)	20 (100.0)	20 (100.0)
Netilmicin	0 (0.0)	0 (0.0)	14 (100.0)	14 (100.0)
Tobramycin	0 (0.0)	0 (0.0)	14 (100.0)	14 (100.0)
Amikacin	0 (0.0)	0 (0.0)	20 (100.0)	20 (100.0)
Nitroreductase	9 (90.0)	0 (0.0)	1 (10.0)	10 (100.0)
Ciprofloxacin	0 (0.0)	0 (0.0)	20 (100.0)	20 (100.0)
Levofloxacin	0 (0.0)	0 (0.0)	20 (100.0)	20 (100.0)
Sulfamethoxazole-Trimethoprim	0 (0.0)	0 (0.0)	19 (100.0)	19 (100.0)
Colistin	15 (100.0)	0 (0.0)	0 (0.0)	15 (100.0)

N= number
% = Percentage of total

The antibiotic susceptibility of the *Staphylococcus hominis* found in the blood culture is shown in Table 12. *Staphylococcus hominis* organisms found in blood cultures are susceptible to tigecycline, linezolid, teicoplanin, vancomycin, and rifampin in 100% of tests. A 100% susceptibility was also found to norfloxacin, which was tested in 1 of 13. They show high resistance to erythromycin and azithromycin with resistance in 83.3% of cases; they were also resistant to clarithromycin in 81.8% of cases. The susceptibility to penicillin was tested in 1 of 13 cases and it was found to be resistant.

Table 12. Antibiotic sensitivity of *Staphylococcus hominis* in blood cultures

Antibiotic	**Sensitive N, (%)**	**Intermediate N, (%)**	**Resistant N, (%)**	**Total number N, (%)**
Penicillin	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Oxacillin	7 (53.8)	0 (0.0)	6 (46.2)	13 (100.0)
Amoxicillin-clavulanic acid	7 (53.8)	0 (0.0)	6 (46.2)	13 (100.0)
Ceftaroline	7 (87.5)	0 (0.0)	1 (12.5)	8 (100.0)
Cefuroxime	8 (61.5)	0 (0.0)	5 (38.5)	13 (100.0)
Norfloxacin	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Ciprofloxacin	0 (0.0)	7 (58.3)	5 (41.7)	12 (100.0)
Moxifloxacin	6 (75.0)	0 (0.0)	2 (25.0)	8 (100.0)
Sulfamethoxazole-Trimethoprim	6 (46.2)	0 (0.0)	7 (53.8)	13 (100.0)
Erythromycin	2 (16.7)	0 (0.0)	10 (83.3)	12 (100.0)
Azithromycin	2 (16.7)	0 (0.0)	10 (83.3)	12 (100.0)
Clarithromycin	2 (18.2)	0 (0.0)	9 (81.8)	11 (100.0)
Clindamycin	7 (53.8)	0 (0.0)	6 (46.2)	13 (100.0)
Tetracycline	6 (54.5)	0 (0.0)	5 (45.5)	11 (100.0)
Tigecycline	13 (100.0)	0 (0.0)	0 (0.0)	13 (100.0)
Linezolid	13 (100.0)	0 (0.0)	0 (0.0)	13 (100.0)
Teicoplanin	12 (100.0)	0 (0.0)	0 (0.0)	12 (100.0)
Vancomycin	12 (100.0)	0 (0.0)	0 (0.0)	12 (100.0)
Rifampin	13 (100.0)	0 (0.0)	0 (0.0)	13 (100.0)
Mupirocin	4 (50.0)	0 (0.0)	4 (50.0)	8 (100.0)

N= number
% = Percentage of total

Patients received an average of 4.0 types of antimicrobials; the median number of types of antimicrobials was 4. As shown in Figure 28, there is a wide spread in how many different types of antimicrobials each patient received. Most patients received 1 to 4 antimicrobials, but some received as many as 15 different types. As shown in Figure 28, most patients in the ICU received an antimicrobial, and the prevalence of prescription was 95.0%.

Of the patients receiving 0 antimicrobial, 2 died the same day as they were admitted.

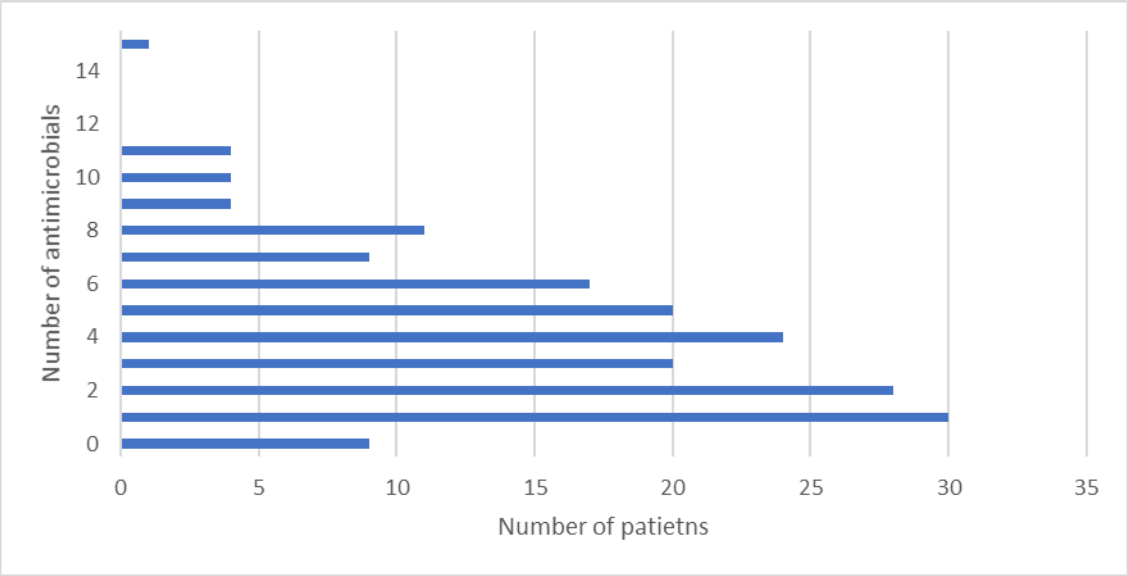

Figure 28. The number of antimicrobials patients received

Antimicrobials used in the treatment of COVID-19 patients are shown in Table 13. The focus of the study is on the coinfection/superinfection so antimicrobials given to treat the COVID-19 infection are not included in this table. The most commonly given antibiotic is meropenem, followed by vancomycin, colistin, and amoxicillin + claviculate acid. The most common antifungal was fluconazole, followed by capsofungin.

Table 13. Types of antimicrobials given

Antimicrobial agent	Total
Meropenem	104
Vancomycin	89
Colistin	66
Amoxicillin + Claviculate acid	61
Ceftriaxone	54
Linezolid	43
Fluconazole	41
Fosfomycin	41
Sulfamethoxazole + trimethoprim	36
Piperacillin tazobactam	25
Torbex	19
Ampicillin + sulbactam	16
Azithromycin	16
Capsofungin	16
Ceftazidime + avibactam	16
Amikacin	13
Ciprofloxacin	12
Clindamycin	9
Metronidazole	9
Levofloxacin	6
Doxycycline	4
Teicoplanin	4
Voriconazole	4
Anidulafungin	3
Gentamicin	3
Moxifloxacin	3
Tigecycline	3
Cefuroxime	2
Micafungin	2
Acyclovir	1
Cefepime	1
Cefixime	1
Ceftazidime	1
Cefpodoxime	1
Clotrimazole	1
Ethambutol	1
Isoniazid	1
Pyrazinamide	1
Trimethoprim	1

5. DISCUSSION

The study demonstrates a statistically significant connection between mortality and respiratory coinfection/superinfection in COVID-19 patients. The prevalence of respiratory coinfection was 3.3%, while 44.8% of the patients developed respiratory superinfection during their stay. The most common etiology of respiratory for coinfection/superinfection was *Acinetobacter baumannii*. This group of *Acinetobacter baumannii* is multi-drug resistant.

About 3 times more males than females were admitted to the ICU. This is consistent with previous studies showing that males have almost 3 times the chance of needing ICU admission, thus having a more severe progression of COVID-19 than females (57). This is consistent with approximately 3 times more males admitted to the ICU than females compared to the number of infected in Splitsko-Dalmatinska county. The infected, as found by testing, were 52.2% females and 47.8% males in Splitsko-Dalmatinska county (21).

70.2% of the patients treated at the ICU were intubated at one point during their treatment, and a total of 90.6% needed some sort of additional oxygen or ventilation support.

A total of 138 patients were given systemic corticosteroids during their stay in the ICU. There were few studies to compare the usage of corticosteroids with, as most of the studies done with corticosteroids in COVID-19 patients, were randomized control trials. One study performed by Wang *et al.* (58) found that 32.05% of severe cases were treated with corticosteroids; the study also found that the proportion of patients treated with corticosteroids were significantly higher in patients treated in the ICU compared with those that were not (58). The proportion of patients treated with corticosteroids in the ICU of the University Hospital of Split was significantly higher (76.2%). This may be since the majority of patients in Croatia were admitted during the second wave after the recommendation of corticosteroid was given (23, 26). One hundred and ten patients were given vasoactive drugs such as adrenalin, noradrenaline, dopamine, levosimendan, or vasopressin during their stay, suggesting a poor condition of these patients (59). The need for vasoactive drugs (60.8%) is consistent with findings in other studies where a need for vasoactive drugs was found to range from 35% to 94%, with an average of 66% in ICU patients (59, 60). Early in the pandemic, it was suggested that treatment with chloroquine was helpful for COVID-19 patients (6, 26), and it was given to 16 patients in this time period. Later in the pandemic, treatment with antiviral therapy such as remdesivir, lopinavir/ritonavir was recommended. The recommendation was later changed so that only treatment with remdesivir remained recommended. Fifty-seven patients were treated with remdesivir (6, 26).

Most of the patients were admitted to the Infectology department at University Hospital of Split before being admitted to ICU. There is a possibility that the patient contracted a superinfection during the time in the infectology department.

Patients developed a respiratory superinfection after a mean of 10.9 days, with a median of 10 days after being admitted to the hospital. This is consistent with findings in the study performed by Garcia *et al.,* who found a mean of 10.6 days, and Baskaran *et al.,* who found a median of 9 days, from admission to hospital to superinfection diagnosis (34, 35). The time to develop a respiratory superinfection after being admitted to ICU is a mean of 5.2 days and a median of 4 days. A study done by Maes *et al.* found that the highest risk of developing VAP was on day 5 after admission to ICU (61). A possible reason for the difference between the times for development of respiratory superinfection as time from hospital admission or time of ICU admission can be that most patients are admitted to hospital and spend some time in another hospital department before being admitted to the ICU. Also, most ICU patients have invasive ventilation support, and intubation in itself is a risk factor for developing VAP (41–43).

Patients who had positive respiratory samples had statistically significant higher mortality than patients who had not been tested and patients who had a negative test. The study performed by Garcia *et al.* found that patients with respiratory coinfection/superinfection had a worse outcome, with increased mortality compared with patients without respiratory coinfection/superinfection (35). The patients who had a respiratory coinfection or developed a respiratory superinfection had a higher mortality rate than patients who were not tested. The mortality rate of patients who were tested for respiratory coinfection/superinfection but were found to be negative was higher than both of the others. This is an unexpected result but may be because of the relatively small number of patients in this category. The rate changes significantly with one more or one less person dying. This number should therefore be considered unsure.

Patients developed a positive blood culture after a mean of 10.6 days, with a median of 9 days after being admitted to the hospital. The time to develop a positive blood culture after being admitted to ICU was a mean of 4.8 days, with a median of 2 days. In the study done by Kokkoris *et al.,* a median of 11 days from admittance to ICU to development of positive blood culture was found (62). A possible explanation for this difference can be that their patients were admitted directly to the ICU and not through a regular hospital department as many of our patients were. Their numbers are comparable to the number of days from admittance to hospital in our study. There could also be another unknown reason.

There is no statistically significant effect on mortality in patients with a positive blood culture compared with those with negative blood cultures or patients who were not tested. The same result was found in a study done by Bayo *et al.* and a study done by Kokkoris *et al.* (62, 63). There is a higher mortality rate in these patients, but the difference is not statistically significant.

Patients with a positive respiratory culture sample spent significantly longer time in hospital and ICU than those who were not tested and those who were tested with negative results. Studies done by Garcia *et al.* and Baskaran *et al.* found the same connection (34, 35). The significance keeps when only looking at the patients who died. One possible cause of this is that patients spending longer time in hospital has an increased chance of developing a superinfection (38).

Patients with a positive blood culture spent significantly longer time in hospital and ICU than patients who were not tested or tested negative. A study done by Kokkoris *et al.* also found an increased duration of ventilation and length of stay in ICU for patients with positive blood cultures (62). The significant difference between the patients who tested positive and those who were not tested remained when only looking at those who died. The significant difference in time spent in hospital or ICU disappeared when comparing those who tested positive and those who tested negative when looking at the patients who died. Increased length of stay in hospital/ICU increases the risk of contracting a superinfection; this may explain the difference in time between the not tested group and the positive group (64). Since the patients who were not tested spent similar time to the patients with negative samples, this may be because these patients had not developed coinfection/superinfection. The lack of tests could be due to lack of symptoms, not prompting a test to be performed.

Looking at blood cultures is important when considering respiratory superinfections; since not all patients had respiratory cultures taken. Positive blood culture in a patient without a respiratory sample can help guide the antibiotic choice (65).

In the respiratory samples, the most common etiology of a positive culture is *Acinetobacter baumannii*, *Candida albicans*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae. Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* are gram-negative microorganisms. *Candida albicans* is a form of yeast infection and is most often opportunistic as it is a part of the normal microbiota of the body (8). The infection with *Candida albicans* suggests that there is some form of immunosuppression in the patients with COVID-19. This could be due to the infection with SARS-CoV2 or a consequence of the systemic corticosteroids, or a combination of these (27). It may also be caused by multi-

antibiotic usage. Some patients tested positive for multiple different causative agents simultaneously or during the length of their stay.

The group of *Acinetobacter baumannii* found in our patients is classified as multi-drug resistant (66). The only antibiotics this group of *Acinetobacter baumannii* were susceptible to, was an ampicillin-sulbactam combination (96.2%) and colistin (95.9%).

The second most common pathogen is *Candia albicans*; there were done no susceptibility testing to antifungal medications. There is, unfortunately, an increase today in resistance to antifungals in *Candida albicans* (67).

The third most common etiology of positive respiratory culture was *Pseudomonas aeruginosa*; the organisms found in our study can be defined as multi-drug resistant (66). The *Pseudomonas aeruginosa* organisms tested were susceptible to amikacin and colistin in 100% of tests; and ceftazidime-avibactam and ceftizoxime in 91.7% of cases. The organisms were 100% resistant to tazobactam-piperacillin, ceftazidime, cefepime, imipenem, ciprofloxacin, and levofloxacin.

The fourth most common etiology of positive respiratory culture was *Klebsiella pneumoniae;* it cannot be classified as MDR (66). This group of Klebsiella is resistant to ampicillin, amoxicillin, cefuroxime, and fosfomycin. And it is 100% susceptible to ceftazidime-avibactam, cefoxitin, and colistin. It is susceptible to carbapenems in 87.5% (ertapenem, imipenem, and meropenem).

In the study performed by Maes *et al.,* the most common pathogen in endotracheal aspiration were *Pseudomonas aeruginosa, Candida albicans,* and *Escherichia coli.* In that study, there was no information about antibiotic sensitivity (61). Both *Pseudomonas aeruginosa* and *Escherichia coli* are gram-negative bacteria. *Pseudomonas aeruginosa* was one of the most common bacteria in our study as well. A study performed by Cultrera *et al.* identified that the most common causes of lower respiratory tract infection were caused by *Candida* sp and gram-negative bacteria (68). These are the same findings as in our study. In the study performed by Garcia *et al.,* the most common cause of VAP was *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Stenotrophomonas maltophilia*; the causes of HAP were equally common and was *Staphylococcus aureus, Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and *Klebsiella pneumonia* (35). That study was also without antimicrobial susceptibility analysis. Both *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were the most common pathogens in our study too. They are among the most common causes of HAP and VAP (38, 40). Our study also found *Stenotrophomonas maltophilia* as a causative agent but to a lesser degree than they did in Garcia *et al.* (35). The previous studies mentioned

in this discussion looking at pathogens in respiratory infection have found results that compare well with ours.

In the positive blood cultures, the most common etiologies are *Staphylococcus epidermidids, Acinetobacter baumannii,* and *Staphylococcus hominis.*

The most common agent found in the blood culture was *Staphylococcus epidermidids*. Earlier *Staphylococcus epidermidis* was seen as an innocuous commensal microorganism of the human skin. It is now seen as an important opportunistic pathogen. Infection with *Staphylococcus epidermidis* does rarely cause life-threatening disease by itself, but it adds to the total burden in the organism (69). The group of organisms found in our patients was resistant to amoxicillin, cefuroxime, ciprofloxacin, and levofloxacin. A 100% susceptibility to teicoplanin, tigecycline, and vancomycin was found. The group of organisms in our sample were resistant to oxacillin in 66.7% of cases. The susceptibility to oxacillin is an important indicator of methicillin resistance (70). Oxacillin is used to measure methicillin resistance today since methicillin is rarely used, and oxacillin and methicillin has similar ways of action (70).

The second most common pathogen in blood cultures was *Acinetobacter baumannii*. This group of pathogens was the most common etiology in respiratory cultures in our study. The susceptibility results were similar but not identical. The *Acinetobacter baumannii* found in blood cultures are resistant to all antibiotics tested except colistin which is 100% susceptible, and ampicillin-sulbactam, which it is 80% susceptible to. It is defined as an MDR (66). A study performed by Kokkoris *et al.* found that gram-negative pathogens dominated their positive blood cultures and that *Acinetobacter baumannii* was the most common; the variants of *Acinetobacter baumannii* found in their study was extensively drug-resistant and pan-drug resistant (62). This is similar to what was found in our study. The study by Kokkoris *et al.* identified *Klebsiella pneumonia, Enterococcus* sp., *Candida albicans*, and *Candida parapsilosis* as other pathogens in their blood samples (62). Our study also found *Klebsiella pneumonia, Enterococcus* sp., *Candida albicans*, and *Candida parapsilosis*, but to a lesser extent.

The third most common etiology of a positive hemoculture was *Staphylococcus hominis*. It is defined as a potential pathogen; the pathogenic mechanisms are not yet determined (71). The organisms isolated in our study were susceptible to norfloxacin, tigecycline, linezolid, teicoplanin, vancomycin, and rifampin in 100% of the tested samples. They were resistant to penicillin, ciprofloxacin in 100% of the tested samples; and there was a resistance to oxacillin in 46.2% of cases.

Most studies have not separated the etiology of coinfection and superinfection depending on origin. The study performed by Baskaran *et al.* had *Klebsiella* sp, *Escherichia coli*, and *Pseudomonas* sp. as the most common causes of coinfection/superinfection overall (34). There was no antibiotic susceptibility testing reported in this study. Our study also has *Klebsiella* sp. and *Pseudomonas* sp. as a common cause of coinfection/superinfection; however, *Escherichia coli* was not a common etiology of coinfection/superinfection in our study. In a study performed by Cultrera *et al.,* it was found that the most frequent isolated bacteria were *Acinetobacter baumannii*, *Enterococcus faecalis, Enterococcus faecium*, *Staphylococcus epidermidis,* and *Stenotrophomonas maltophilia* (68). The frequency and potential burden found by *Staphylococcus epidermidis* are consistent with what is seen in our study. Our study has *Acinetobacter baumannii* among the most common etiologies; other etiologies found in our study are *Stenotrophomonas maltophilia, Enterococcus faecalis,* and *Enterococcus faecium* but not as frequent.

Almost all patients admitted to the study were treated with antibiotics. Not all patients had a positive respiratory sample or a positive blood culture. There is a possibility that antibiotics were given for other infections, such as, e.g., urinary tract infections that were not studied in this study. On average, patients received 4 different types of antimicrobials. A higher number of types may suggest adjustments and de-escalation in the prescribed antibiotic when antibiotic susceptibility testing where completed; this was documented in some of the medical records. The study done by Baskaran *et al.* found that 83.1% of their patients received antibiotics during their stay in the hospital (34). The patients in our study received antimicrobials in 95.0% of cases. The difference in antimicrobial usage per patient could be due to our study focusing on patients in ICU, while their study also looks at COVID-19 patients in the hospital in general.

The most commonly used antibiotics were meropenem, vancomycin, colistin, amoxicillin+ claviculate acid, and ceftriaxone. The most commonly used antifungal was fluconazole. Meropenem is a carbapenem with good activity against gram-negative rods, including *Pseudomonas aeruginosa*; it also has activity against gram-positive organisms and anaerobic organisms. Vancomycin acts mainly against gram-positive bacteria. Vancomycin is poorly absorbed from the intestine and can be used to treat *Clostridium difficile*; if a systemic dose is indicated, vancomycin must be given parenterally (46). Colistin is also known as polymyxin E. Colistin has activity against gram-negative bacteria. Colistin has significant toxicity when administered systemically; therefore, it was primarily used topically before the emergence of MDR *Acinetobacter* sp and *Pseudomonas* sp. Today it is used IV and in an

aerosolized form to treat HAI (46, 72, 73). These antibiotics cover the most common etiologies in both respiratory samples and blood cultures in our study. An alternative to colistin against *Acinetobacter baumannii* is the ampicillin-sulbactam mixture. This mixture has activity against gram-positive, gram-negative, and anaerobe bacteria. It also has activity against organisms producing β-lactamase. This makes it a good choice in patients with mixed infections of gram-negative and gram-positive bacteria. Fluconazole has good activity against *Candida* sp, which was the most common form for fungal infections in our study (74). In the study performed by Cultrera *et al.,* the most commonly used antimicrobials were piperacillin/tazobactam, meropenem, capsofungin, vancomycin, and colistin (68). Meropenem, vancomycin, and colistin were the top 3 in our study; piperacillin/tazobactam and capsofungin were used to a lesser extent. This difference can be from different local guidelines; or local differences in expected susceptibility.

Due to the high prevalence of respiratory superinfections in COVID-19 patients in the ICU, I recommend that there should be a high clinical suspicion of respiratory superinfections in any patient admitted to the ICU with COVID-19. Respiratory samples and blood cultures should be taken early before starting the antibiotic to secure the best possibility of a true positive/negative sample.

Limitations of this study was that it's a retrospective study, so a bias in the medical records is possible. Files were stored in a paper format a t a central storage, and no record was kept in files moving in and out of storage. There is, therefore, a possibility that some files were missing at the time of data collection. The files can also have been incomplete. another limitation is that it done only in one hospital, this is limiting the generalization ability to other hospitals. A third limitation is that there was no systematic testing for coinfections at the time of admittance to the hospital, nor any systematic testing for coinfections/superinfections at a later time. It is, therefore, possible that some of the attending physicians did not order microbiological tests for their patients. Patients may therefore have coinfections or superinfections that were not documented by microbiological testing. A fourth limitations was that some of the subgroups in this study were small, so there was limited data available in this groups. This study did only look at coinfections in respiratory samples and blood cultures, and did not look for other coinfections of these patients. Lastly 9 patients in this study were transferred from other hospitals without the admission date to the local hospital noted. The missing dates was filled out according to methodology.

6. CONCLUSIONS

- The prevalence of pulmonary superinfections in patients in the ICU was 44.8%
- The prevalence of pulmonary coinfection at the time of admittance to the hospital was 3.3%
- The most common causative microorganisms of pulmonary coinfection/superinfection were MDR *Acinetobacter baumannii, Candida albicans,* MDR *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.*
- The most common causative microorganisms in blood cultures were *Staphylococcus epidermidids* (oxacillin resistant in 66.7%), MDR *Acinetobacter baumannii,* and *Staphylococcus hominis* (oxacillin resistant in 46.2%)
- The primary hypothesis is true; on average, a patient develops a VAP or HAP 5 (4 days median) days after being admitted to ICU.
- Patients with a respiratory coinfection/superinfection had statistically significantly higher mortality than patients without respiratory coinfection/superinfection.
- Most of the causative microorganisms found in respiratory samples were gram-negative.
- In blood cultures, the main 3 agents were 2 gram-positive and 1 gram-negative.
- Patients with positive respiratory samples stayed significantly longer in the hospital.

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8. SUMMARY

Objectives: Investigate the prevalence of pulmonary superinfections in COVID-19 patients on ventilators and the susceptibility of causative microorganisms to antibiotics during 2020 pandemic. To investigate the outcome of coinfected or superinfected patients in a 30-day period, LOS (length of stay) in ICU and hospital

Materials and methods: During 2020, 184 patients were admitted to the COVID-19 ICU of the Department of Anesthesia, Resuscitation, & Intensive Care, University Hospital of Split; of these, 181 was included in this study. Three patients were excluded due to incomplete files. The study was conducted as an observational retrospective study. Medical data were collected by reviewing the history and discharge papers in medical files stored in the archive.

Results: Out of 181 patients, 102 patients were tested for respiratory coinfection/superinfection. 3.3% of patients had a respiratory coinfection upon admittance, and 44.8% developed a respiratory superinfection. The mean time to develop a respiratory superinfection was 10.9 days, with a median of 10 days after admittance to the hospital. The mean time to develop a respiratory superinfection was 5.3 days, with a median of 4 days after admittance to ICU. Patients with a positive respiratory sample had a statistically significant increase in mortality (p=0.002); they also had a significantly longer stay in Hospital and ICU compared with noninfected patients. The most common etiology of positive respiratory samples was *Acinetobacter baumannii, Candida albicans, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.*

Conclusion: The prevalence of pulmonary superinfections in patients in the ICU was 44.8%. The most common causative microorganisms of pulmonary coinfection/superinfection were MDR *Acinetobacter baumannii, Candida albicans,* MDR *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* Patients with a respiratory coinfection/superinfection had statistically significantly higher mortality than patients without respiratory coinfection/superinfection.

9. CROATIAN SUMMARY

Naslov: Incidencija koinfekcija i superinfekcija u bolesnika s COVID-19 primljenih u JIL Klinike za anesteziologiju, reanimatologiju i intenzivno liječenje Kliničkog bolničkog centra Split tijekom 2020. godine

Ciljevi: Istražiti prevalenciju plućnih superinfekcija u bolesnika s COVID-19 na strojnoj ventilaciji i osjetljivost uzročnih mikroorganizama na antibiotike tijekom 2020. U cilju istraživanja ishoda bolesnika zaraženih korona virusom ili bolesnika sa superinfekcijama u razdoblju od 30 dana. Također je istraživana duljina boravka/liječenja (LOS) na intenzivnoj njezi i u bolnici.

Materijali i metode: Tijekom 2020. godine na intenzivnoj njezi COVID-19 Klinike za anesteziologiju, reanimatologiju i intenzivno liječenje KBC-a Split primljena su 184 pacijenta; od toga je 181 bio uključen u ovu studiju. Tri bolesnika su isključena zbog nepotpunih podataka. Istraživanje je provedeno kao opservacijska retrospektivna studija. Medicinski podaci prikupljeni su pregledom povijesti bolesti i otpusnim pismima u medicinskim datotekama pohranjenim u arhivi.

Rezultati: Od 181 bolesnika, 102 bolesnika testirana su na respiratornu coinfekciju/superinfekciju. 3.3% bolesnika imalo je respiratornu infekciju nakon prijema, a 44.8% respiratornu superinfekciju. Srednje vrijeme za razvoj respiratorne superinfekcije bilo je 10.9 dana, s medijanom od 10 dana nakon prijema u bolnicu. Srednje vrijeme za razvoj respiratorne superinfekcije bilo je 5.3 dana, s medijanom od 4 dana nakon prijema na intenzivnu. Bolesnici s pozitivnim respiratornim uzorkom imali su statistički značajno povećanje smrtnosti (p=0.002); također su imali znatno duži boravak u bolnici i intenzivnoj njezi u usporedbi s nezaraženim pacijentima. Najčešća etiologija pozitivnih respiratornih uzoraka bila je *Acinetobacter baumannii, Candida albicans, Pseudomonas aeruginosa* i *Klebsiella pneumoniae*.

Zaključak: Prevalencija plućnih superinfekcija u bolesnika na intenzivnoj njezi iznosila je 44,8%. Najčešći uzročni mikroorganizmi plućne coinfekcije/superinfekcije bili su MDR *Acinetobacter baumannii, Candida albicans*, MDR *Pseudomonas aeruginos*a i *Klebsiella pneumoniae.* Bolesnici s respiratornom coinfekcijom/superinfekcijom imali su statistički značajno veću smrtnost od bolesnika bez respiratorne coinfekcije/superinfekcije.