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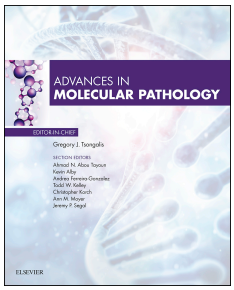

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PII: S2589-4080(22)00010-2

DOI: https://doi.org/10.1016/j.yamp.2022.07.003

Reference: YAMP 94

To appear in: *Advances in Molecular Pathology*

Please cite this article as: Weidmann MD, Berry GJ, Green DA, Wu F, Prevalence and clinical disease severity of respiratory co-infections during the COVID-19 pandemic, *Advances in Molecular Pathology* (2022), doi: https://doi.org/10.1016/j.yamp.2022.07.003.

Prevalence and clinical disease severity of respiratory co-infections during the COVID-19 pandemic

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Disclosure statement

The authors have nothing to disclose.

Key words

Co-infection, superinfection, COVID-19, respiratory virus, SARS-CoV-2, Influenza

Key Points

- Increased use of multiplex PCR-based assays has challenged the notion of a single respiratory pathogen always being responsible for a given clinical disease.
- Rates of co-infection between SARS-CoV-2 and other respiratory viruses is highly variable based on etiology and patient population, but higher overall than rates of bacterial and fungal co-infection.
- Rates of co-infection or super-infection with bacteria are generally lower in patients with SARS-CoV-2 than for those with influenza or respiratory syncytial virus.
- Higher rates of bacterial and fungal pneumonia in ventilated ICU patients with COVID-19 warrants increased diagnostic testing and empirical antibiotic therapy in these patient populations.
- Low rates of community-acquired respiratory co-infections, and higher rates of antimicrobial resistance, warrant greater antibiotic stewardship for patients with non-severe COVID-19.

Introduction

Respiratory tract infections represent a major global health concern. Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in more than 470 million confirmed cases and about six million deaths worldwide [1]. Since the beginning of COVID-19 pandemic, the presence of more than one pathogenic organism in the respiratory tract of COVID-19 patients has been widely recognized due to the availability of advanced molecular detection technologies, such as multiplex polymerase chain reaction (PCR) and next-

generation sequencing (NGS). However, the association between the occurrence of multiple-pathogen infections and clinical disease severity is still unclear. In the study of disease evolution, multiple infections fall into two broad categories: co-infection and superinfection. Co-infection occurs when a person simultaneously becomes infected with more than one microorganism, while a superinfection is an infection following a previous infection. A substantial number of patients with COVID-19 were also co-infected with various pathogens including other respiratory viruses, bacteria, and fungi, which can influence infectious disease dynamics. This review presents an overview of the prevalence and clinical disease severity of respiratory co-infections and superinfections (Table 1) and discusses possible mechanisms of the interactions between viral infections, including SARS-CoV-2, and bacterial infections during the COVID-19 pandemic.

Respiratory Viral Co-infections

In late 2019, an acute respiratory disease, COVID-19, emerged in humans caused by a novel coronavirus, SARS-CoV-2. Unlike previously known coronaviruses, SARS-CoV-2 combines greater pathogenicity with high transmissibility during the pre- or asymptomatic period [2]. COVID-19 has spread rapidly across the globe and has become a major pandemic disease. The increasing number of infections prompt the need to utilize rapid antigen-based assays and real-time reverse transcription–polymerase chain reaction (rRT-PCR) technology to rapidly detect SARS-CoV-2 RNA and confirm the clinical diagnosis of COVID-19 [3].

Respiratory virus infections are associated with a wide spectrum of clinical symptoms. This classical thinking that a single virus is associated with clinical symptoms has been challenged by the more advanced multiplexed PCR-based assays, which may simultaneously identify multiple pathogens associated with one clinical syndrome [4]. Broad respiratory syndromic testing is widely utilized to identify viral pathogens that co-circulate with SARS-CoV-2. Co-infections involving viruses are recognized to influence the disease dynamics that occur relative to a single infection [5].

Prevalence of respiratory viral co-infections

Respiratory viral co-infection with SARS-CoV-2 refers to the simultaneous infection of other respiratory viruses at the time of COVID-19 diagnosis. Previous reports have shown viral co-infection and superinfection are rare [6]. This chapter focuses on the prevalence and outcome of viral co-infection.

Viral co-infection in adults. The overall rate of co-infection of SARS-CoV-2 and other respiratory viruses in adults is considerably variable as reported.

The rate of viral co-infection depends on the co-circulation of respiratory viruses in the specific time of the year and the geographical region where the study was conducted. In the beginning of COVID-19 pandemic, Yue et al. carried out an epidemiological study in Wuhan, China between 12 January to 21 February 2020, and reported that the prevalence of SARS-CoV-2 and influenza co-infections was as high as 57.3% (176/307 cases), though the diagnosis of influenza was based on IgM serological testing alone. [7]. From March 3 through 25, 2020, Kim et al. conducted a study to assess the prevalence of co-infections in Northern California, USA. [8]. A total of 1217

nasopharyngeal swabs of symptomatic adult patients were tested by multiplex rRT-PCR assays for SARS-CoV-2 and other respiratory pathogens. Of the 116 specimens positive for SARS-CoV-2, 24 were positive for one or more additional pathogens including influenza, rhinovirus /enterovirus, respiratory syncytial virus, and non–SARS-CoV-2 coronaviridae. The rates of co-infection between SARS-CoV-2 and other respiratory pathogens were 20.7%.

. When the California Department of Public Health assessed influenza activity in California during the 2020–21 influenza season, their analyses revealed that among the 255 positive influenza cases, 58 persons were co-infected with SARS-CoV-2. The occurrence of influenza and SARS-CoV-2 co-infections was 23%, with influenza B being the predominant type (39/58; 67%) [9]. The report indicated that SARS-CoV-2 testing was prioritized over influenza virus testing and infrequent influenza testing might have contributed to underestimates of influenza transmission [9]. Since the highly transmissible SARS-CoV-2 virus became the predominant circulating respiratory virus in humans, its impact on the circulation of other respiratory viruses has been evaluated. [10] The majority of co-circulating respiratory viruses appear to be less prevalent since the onset of the COVID-19 pandemic. This might be attributable to both intrinsic viral characteristics and viral interference [10].

Viral coinfection in Children. The prevalence of viral co-infections is significantly higher in children than adults. A study from six U.S. children's hospitals located in areas with high COVID-19 incidence during July–August 2021 found viral co-infections with SARS-cov-2 was common (113/713, 16%) in pediatric patients aged <18 years. The

coinfection rate was even higher among infants (32.4%) and young children aged 1–4 years (36.1%). The predominant (>65%) viral coinfections were caused by respiratory syncytial virus (RSV) [11].

Children with COVID-19 present with distinct epidemiological and clinical characteristics that differ from adults. They may be asymptomatic or symptomatic with generalized clinical manifestations, like fever, cough, and nasal congestion. This makes it difficult to distinguish children with COVID-19 from those with infection of other common respiratory viruses. A retrospective study of previous healthy children exposed to SARS-Cov-2 in their household, who subsequently tested positive for SARS-CoV-2, reported 19/34 (51%) children co-infected with other respiratory pathogens [12].

Clinical outcomes of respiratory viral co-infections

COVID-19 pandemic has caused great challenges for healthcare system globally. Viral co-infections are commonly seen in hospitalized patients [13]. A retrospective study found even though viral co-infection was common at 30.5% in hospitalized COVID patients, co-infection was not an independent risk factor for severity of hospital admission, need for high-flow oxygen therapy or mechanical ventilation, and mortality [14]. Another study analyzed inpatient data and revealed that patients positive for SARS-CoV-2 had a lower rate of coinfection with other respiratory viruses compared to those positive for other respiratory viruses (1.4% vs 4.8%). COVID-19 patients testing positive for other viruses including rhinovirus, parainfluenza, and seasonal coronavirus presented with mild disease and did not progress to pneumonia [15]. The proportion of SARS-CoV-2 co-infected patients requiring ICU care (4/21,19%) was significantly lower

than the proportion of those infected with SARS-CoV-2 alone (113/280, 47%), indicating that co-infection with other respiratory viruses might not worsen the outcome of SARS-CoV-2-associated respiratory disease. [16].

The clinical disease severity of respiratory viral co-infections in COVID-19 patients could be impacted by the specific organism(s) causing of co-infection.

Co-infection with Influenza. Influenza virus is a frequent cause of community-acquired pneumonia. Co-infection with SARS-CoV-2 may worsen COVID-19 symptoms and increase mortality, especially for the elderly [9]. Patients co-infected with SARS-CoV-2 and influenza B virus have presented with symptoms of fatigue (13%), abnormalities on chest CT (100%) or decreased lymphocytes and poor prognosis compared to SARS-CoV-2 single positive patients [7]. Ozaras et al. reported six COVID-19 patients that were coinfected with influenza and suffered from pneumonia. Thoracic computed tomography scan showed multiple lesions in the lung in 2 cases, bilateral lungs in 3 cases and single central lesion in one case. [17]. A recent research study using an animal model for SARS-CoV-2 infection demonstrated that co-infection with influenza A virus causes more severe and prolonged pneumonia in SARS-CoV-2-infected hamsters [18]. The research team examined the respiratory organs of co-infected hamsters using immunohistochemical stain and found the infected regions were clearly divided into ‘SARS-CoV-2-patterned and influenza-patterned areas’ and both viruses never co-existed at the same site in these organs, indicating each virus may have distinct cell tropism and can efficiently spread in the respiratory organ without interference on each other [18].

Co-infection with RSV. RSV is the most common cause of bronchiolitis and pneumonia in children younger than 1 year of age. Patients co-infected with SARS-Cov-2 have been reported and hospitalized [11]. A retrospectively study found that 6/32 (18.7%) hospitalized children under 24 months of age co-infected with SARS-CoV-2 and RSV, and had a significantly longer length of stay (7 vs. 3 days) than those without [19]. However, there was no difference in the need for intensive care or mechanical ventilation or mortality [19]. Similar findings were presented by Giannattasio et al. among six infants aged 18 days to 9 months co-infected with both SARS-CoV-2 and RSV. The infants experienced mild fever and respiratory symptoms with an uncomplicated resolution [20]. RSV, which can cause more severe disease in infants, caused relatively mild disease in co-infected infants in this study of limited size. The authors speculate that this could be due to increased infectivity and replication of SARS-CoV-2, which may suppress RSV replication and clinical expression [20]

Virological mechanisms that determine viral persistence/exclusion during co-infections

1) Viral competition for resources in the respiratory tract:

Molecular methods of viral detection and their increased sensitivity over culture have led to increased diagnosis of viral respiratory infections. This increase has also led to a greater frequency of viral co-infections being detected. When looking at the interactions between individual viruses during viral-viral respiratory co-infections, there appear to be both cases of inhibition and enhancement of one virus over another.

In the case of inhibition, it has been demonstrated through mathematical modeling that one virus can block infection with another virus by being the first to infect a cell [21]. In addition, the replication speed of the virus played a role with a rapidly replicating virus, such as rhinovirus, reducing the replication of other viruses and a slow-growing virus, such as parainfluenza virus, being out-competed and subsequently suppressed in a viral-viral co-infection model [21]. This pattern is consistent with reports of viral co-infections seen during the pandemic, with one study reporting 8% of cases as co-infected, with rhinovirus comprising 6% and influenza virus comprising 2% of SARS-CoV-2 co-infections [22].

Ferret models of SARS-CoV-2 and influenza A virus co-infection showed that influenza A outcompeted SAR-CoV-2 in both viral titers and infectivity when co-infected ferrets were co-housed with uninfected ferrets [23]. Additional animal studies in murine models also showed increased influenza viral loads in co-infections and increased mortality, which could be ameliorated by prior immunity to influenza [24].

Studies in humans have been more ambiguous, with some studies showing similar clinical outcomes in patients infected with SARS-CoV-2 alone vs with a viral co-infection [25] and others showing higher rates of viral-viral co-infections in patients with severe cases of COVID-19 [7, 26, 27] or who had died [28]. Published data prior to the pandemic looking at influenza viral co-infections suggested that detection of more than one virus correlated with less severe disease [29] or had no difference in clinical disease severity [30]. This finding will need to be investigated in the context of SARS-CoV-2 co-infections. One large observational study in over 21,000 positive specimens and found that while viral co-infection was not associated with an increase in viral load when compared to single-virus

infections, there was evidence that was suggestive of viral competition that reduced viral load [31].

2) Epithelial and organ damage

Although the significance of viral coinfection is unknown, the mechanisms of co-infection include virus-induced airway damage, reduced mucociliary clearance, and damage to the immune system [32].

Animal models of SARS-CoV-2 and influenza A virus co-infection led to significantly more weight loss and more severe inflammation in both the nose and lungs when compared to animals singly infected with each virus [23, 33]. In addition, influenza A virus pre-infection led to an increase in SARS-CoV-2 infectivity in cell-based models and also led to more lung damage and higher viral loads in mouse models [34]. Other models of epithelial, endothelial and mononuclear cells have also shown infectivity with high viral loads, leading to cell damage and deterioration of barrier function [35].

3) Impact on the pattern of immune responsiveness

Because of the damage to the immune system caused by the coinfection, the presentation of patients who are positive for both SARS-CoV-2 and other viruses may be more severe [36]. It has been shown that SARS-CoV-2 infection leads to epithelial cell damage and the release of interferon [35]. Elevation of pro-inflammatory cytokine levels early in infections have been linked to worse outcomes, with severe COVID-19 being linked with an elevated cytokine response throughout disease progression [37].

When looking at influenza A and SARS-CoV-2 co-infection, one study showed an increase in serum cytokines (interleukin-2R [IL-2R], IL-6, IL-8, and tumor necrosis factor-

α), cardiac troponin I, and a higher incidence of lymphadenopathy in patients solely infected with SARS-CoV-2 [25]. A hamster model of SARS-CoV-2 and influenza co-infection also showed a higher histology score for pulmonary edema, vasculitis and inflammatory cell infiltration than single-virus infected animals [33]. Additionally, looking at the impact of influenza A and SARS-CoV-2 co-infections in adult inpatients showed that co-infection led to an increased odds of acute heart failure, multilobar infiltrates, acute kidney injury (AKI), secondary bacterial infection, and ICU admission. Co-infected patients also had a relatively higher SARS-CoV-2 viral load based on relative cycle threshold values than patients singly infected with SARS-CoV-2, which significantly correlated with AKI and Acute respiratory distress syndrome (ARDS) [38].

Bacterial co-infection in the setting of respiratory viral illness

Respiratory viral infection may predispose hosts to bacterial pneumonia, but the degree to which this occurs varies widely based on the viral pathogen. We will examine three commonly occurring respiratory viral pathogens – influenza virus, Respiratory Syncytial Virus (RSV) and SARS-CoV-2 – to understand how each affects risk for bacterial co-infection, outcomes associated with co-infection and mechanisms underlying these associations.

Influenza-associated bacteria co-infection

As a long-known cause of seasonal epidemics and occasional pandemics, the association of influenza with bacterial superinfection has long been established, with review of autopsy findings from the 1918 “Spanish Flu” pandemic displaying that

bacterial superinfection was likely a central risk factor for mortality [39]. A variety of studies have attempted to characterize the rates of bacterial co-infection or superinfection in the setting of influenza, yet many of these have been limited to a single influenza season [40, 41], A metanalysis found rates varying from 2% to 65%, however, when the studies contributing the majority of heterogeneity were removed the range was 11%-35% [42]. More recently, a prospective multicenter study from Spain has assessed rates of bacterial pneumonia from 2009-2015, including years of both pandemic and seasonal epidemic influenza [43]. These authors found lower rates of bacterial co-infection during 2009 (11.4%), associated with the H1N1 pandemic during that year, relative to increased rates in subsequent years (17.3-23.4%) [43]. A metanalysis focusing on pandemic H1N1 influenza during 2009 found that 19% of hospitalized patients were found to have secondary bacterial pneumonia, and that this rate increased to 23% when looking at autopsy specimens from fatal influenza cases [41].

Outcomes in patients with influenza have been found to be consistently worse in the setting of bacterial co-infection. Authors of the 2009-2015 Spanish study finding an adjusted odds ratio (aOR) of 1.9 [1.5–2.5] for hospital mortality, while another retrospective study of 209 hospitalized influenza patients from the Netherlands, 41 of whom had community-acquired bacterial co-infection (19.6%), found an adjusted hazard ratio (aHR) of 2.6 (95%CI 1.252-5.480) for mortality associated with bacterial co-infection [44]. Of note, this latter study found a large difference in mortality by bacterial species responsible for co-infection, with *S. aureus* producing a mortality (aHR) of 6.267 (95%CI 2.679-14.662). A smaller study comparing mortality rates in patients with

bacterial pneumonia in the setting of influenza (VI, n = 57) or other types of respiratory viral illness (NI, n = 77), they found that VI patients had lower rates of bacterial pneumonia than NI patients (23% vs 44%), as well as overall lower length of hospital and ICU stay, but no significant trends associated with bacterial pneumonia status [45].

A large portion of bacterial pneumonia in the setting of influenza has been found to be community-acquired. One study involving 4,313 influenza patients hospitalized between 2010 and 2015 at 179 US hospitals found a community-acquired pneumonia (CAP) rate of 10.6%, with *S. aureus* presenting as the most common etiology, and a mortality aOR of 3.00 (95% CI, 2.17-4.16) [46]. Bacterial isolates identified were also more consistent with community-acquired pneumonia, with the largest study of all types of bacterial pneumonia in influenza patients identifying *S. pneumoniae* as the most common etiology (51.0%) by a large margin [43], and the most comprehensive meta-analysis of this subject has also found *S. pneumoniae* as the most common etiology (35%), though *S. aureus* made a more significant contribution (28%) [42].

There are a number of pathways by which influenza has been identified in both animal and human studies to increase susceptibility to bacterial pneumonia, including: 1) Depletion of alveolar macrophages by 90% during the first week of influenza infection in mice [47], 2) Inhibition of the phagocytic activity of macrophages and neutrophils through induction of Type 1 interferon (IFN) responses [48, 49], 3) Inhibition of Th17 T-cells that modulate the immune surveillance of pathogenic bacteria via Type 1 IFN signaling, as well as 4) An increase in bacterial binding sites in the upper and lower respiratory tract produced by neuraminidase cleavage of sialic acid [50], and upregulation of surface receptors recognized by bacteria from the host inflammatory

response and wound healing [51, 52]. Interestingly, mortality rates for influenza with bacterial pneumonia have been found to be highest in animal studies when bacterial infection occurs during the first week of viral infection [53], which in conjunction with high rates of CAP and the mechanisms listed above, suggest that viral and bacterial infection act synergistically to reduce pulmonary function and inhibit host responses.

RSV-associated bacterial co-infection

Prior to the COVID-19 pandemic, RSV represented the leading cause of lower respiratory tract infection in children, with an estimated 2.7-3.8 million cases in 2015, causing higher rates of hospitalization and mortality relative to influenza in children under 5 years [54]. A wide range of bacterial co-infection rates is associated with RSV. One study of children with bronchopulmonary RSV found 43.6% had a concurrent respiratory culture positive for pathogenic bacteria [55], with the most common pathogens being *H. influenzae* (43.9%)*, S. pneumoniae* (36.6%)*,* and *M. catarrhalis* (29.3%). A larger study of children with RSV pneumonia between 2010 and 2019 found 32.4% had a concurrent respiratory bacterial infection, the most common etiologies of which were *S. pneumonia* (40.8%)*, S. aureus* (40.8%), and *H. influenzae* (25.4%) [56]. This study also assessed the effect of bacterial co-infection on clinical outcomes in children with bacterial co-infection compared to RSV alone, finding significantly higher rates of invasive ventilation (3.0 vs. 0.0%) and ICU care (11.9 vs. 6.4%), though mortality was not reported. One study focusing on 165 RSV patients who were intubated and treated in the ICU found similarly high rates of respiratory culture positivity (42.6%), however they used quantitative culture to separate bacterial co-

infection (21.8%) and low bacterial growth/possible co-infection (20.8%) based on whether their cultures grew greater or less than 10^5 CFU/mL, respectively [57]. While there was no significant difference in mortality rates, this study found a small but significant increase in length of mechanical ventilation in the bacterial co-infection group (6 vs. 4 days).

Interestingly, a retrospective cohort study concluding within the past year has directly compared rates of CAP in admitted adult patients testing positive for Influenza, RSV or SARS-CoV-2 [58]. Hedberg et al. (2022) found that roughly similar proportions of adults with influenza or RSV had concurrent CAP, 27% and 29% respectively, while only 4% of SARS-CoV-2 patients had CAP. For all three viral infections, no significant differences in length-of-stay (LOS), ICU admission or 30-day mortality between patients with or without CAP were seen, except a reduced aHR of being discharged alive for patients with influenza and CAP relative to those without CAP (0.76, 95%CI 0.64-0.90). Of note, this study assessed only adult patients, while most studies of co-infection in the setting of RSV have focused on pediatric patients. Therefore, in adults, RSV infection involves high rates of bacterial pneumonia, similar to or increased in prevalence compared with influenza co-infection, however exacerbation of outcomes by co-infection in pediatric populations is modest in comparison with that of influenza.

SARS-CoV-2-associated bacterial co-infection

The literature to date on bacterial co-infection in the setting of SARS-CoV-2 has shown significant differences from influenza or RSV co-infection. One study of 4267

hospitalized COVID-19 patients in New York City found that only 2.1% had positive respiratory cultures and 1.9% had positive blood cultures, though 71% received at least one dose of an antibiotic [59]. While Hedberg, Johansson et al. reported a CAP co-infection rate of 4% with SARS-CoV-2, other studies assessing CAP have found significantly lower rates. A multicenter study of 1,705 hospitalized COVID-19 patients in Michigan found only 1.5% qualified as having CAP (defined as positive culture <3 days from admission). Another study of 3,796 hospitalized COVID-19 patients in New York City found that only 0.9% of COVID-19 patients had CAP (defined as positive culture <2 days from admission), which only represented 12.2% of bacterial pneumonia cases in COVID-19 patients, whereas the large majority of cases were due to ventilator-associated pneumonia (VAP, 85.7%) [60]. This study also found that a significantly higher proportion of bacterial pneumonia in COVID-19 patients was ventilator-associated relative to non-COVID patients. Rates of bacterial pneumonia amongst ventilated patients were also found to be significantly higher for those testing positive for SARS-CoV-2, was also seen in an independent study of a large cohort in Sweden [61]. While meta-analyses have only looked at overall rates of bacterial co-infection in the setting of SARS-CoV-2, the largest of these found considerable heterogeneity between studies, with subgroup analysis showing rates of 4% in all hospitalized patients and 14% for ICU patients alone, consistent with the largest individual studies described above [59, 62, 63].

While Hedberg et al. reported did not report significant differences in the risk of ICU admission or 30-day mortality for COVID-19 patients with CAP relative to those without, another large observational study looking specifically at clinically confirmed CAP found

significantly increased rates of ICU admission (Rate ratio (RR) = 4.45), mechanical ventilation (RR = 6.74), higher rates of septic shock (RR = 4.60), longer length of stay (mean 7 vs. 5 days) and greater mortality (47.5% vs 18.0%) for patients with CAP [63]. A study specifically assessing hospital-acquired respiratory co-infections with bacterial or fungal pathogens found that they occurred in 6.8% of COVID-19 patients, and more than 10-fold as many positive respiratory cultures relative to community-acquired infections [64]. For both hospital- and community-acquired infections, the vast majority were associated with bacterial etiologies, with only 4% of community-acquired and 2% of healthcare-associated pathogens involving fungal isolates (Aspergillus). Few other studies have directly assessed the rate of fungal respiratory infection in the setting of hospitalized COVID-19 patients. Of these, Nori et al. represents the largest and also found low rates of co-infection with fungal pathogens, with 3/112 (2.7%) positive respiratory isolates of fungal etiology (Candida and Aspergillus spp) [59]. However, high rates of pulmonary aspergillosis have been demonstrated amongst COVID-19 patients admitted to an ICU, with one meta-analysis of 30 studies covering 3,441 patients demonstrating a pooled incidence rate of 14% (95% CI 11-17%) with a case fatality rate of 52% (95% CI 47-56%) [65].

Etiologies of bacterial respiratory infections also reflect a relatively high proportion of healthcare-associated and/or ventilator-associated pathogens in COVID-19 patients, relative to pathogens found in influenza patients. While *S. pneumoniae* represented the most common bacterial pneumonia pathogen for influenza patients, *Staphylococcus*, *Pseudomonas*, and Enterobacterales spp. (such as *Klebsiella*) represented the most common etiologies in COVID-19 patients found by studies looking at both community-

acquired and healthcare-associated pneumonia [59, 60, 64]. In one large observational study looking specifically at CAP in COVID-19 patients, bacterial pathogen profiles were more similar to that of influenza patients, with the most common pathogen being *S. pneumoniae* (28%), albeit far lower than co-infection in influenza patients (56%), and with COVID-19 patients with CAP having a higher proportion of *S. aureus* as the second-most common pathogen (26% vs. 19%) [58].

Despite lower rates of bacterial respiratory co-infection in COVID-19 patients relative to Influenza and RSV patients, high rates of early antibiotic therapy have been observed in these patient populations. The three largest such studies, including 4267, 3028 and 1705 COVID-19 patients from Spring 2020, respectively, found that 71%, 67% and 57% of patients received some form of antibiotic therapy during their treatment [64, 59, 63]. Interestingly, the smallest of these studies found that 54% of those COVID-19 patients started on early antimicrobial therapy had it discontinued within 1 day after SARS-CoV-2 tests returned positive, but of those remaining on antimicrobial therapy, only 7.4% had a community-onset bacterial infection confirmed [63]. A metanalysis of 24 studies including 3338 patients also found that 72% of patients received antibiotics (95% CI 56-88%). However, most of the studies have looked at cases dating from the first or second wave of the COVID-19 pandemic in 2020, when effective antivirals were not available, and it remains to be seen whether such high rates of antibiotic therapy have continued into 2021-2022.

Nori et al. (2021) also assessed antibiotic use in healthcare-associated infections, finding that 79% of patients who had a positive bacterial culture (blood or respiratory) had received antibiotics in the 30 days prior to positive culture, and all 21 patients with

multi-drug resistant pathogens were in this group [59]. Kubin et al. (2021) found increasing rates of antimicrobial resistance (AMR) in isolates recovered later in patients' hospital stay, 33% of *S. aureus* isolates were methicillin-resistant (MRSA), 76% of which were respiratory isolates, 42% of Enterobacterales were cephalosporin resistant and 7% were carbapenem resistant (the majority of which were respiratory isolates) [64]. However, a study from the same institution comparing rates of AMR for bacterial respiratory isolates in COVID-19 and non-COVID patients found no significant increase in any of these categories except for COVID-19 patients with MRSA and overall resistance to penicillin class antibiotics [60]. Thus, AMR rates seemed comparable for COVID-19 and non-COVID patient populations with the exception of MRSA, but increasing risk of resistance with hospital stay further highlights the need for stewardship in early antibiotic treatment, particularly for non-intubated patients. This is corroborated by healthcare-associated infection risk factors assessed in Kubin et al. (2021), the leading of which were invasive mechanical ventilation (OR 6.0, 95% CI 3.7-10.0), ICU care (OR 4.1, 95% CI 2.5-6.8) and steroid treatment (OR 1.9, 95% CI 1.4-2.6).

While there are a host of mechanisms by which influenza has been found to predispose human epithelia to bacterial co-infection, with high rates of CAP to support the manifestation of these mechanisms in real-world settings, there is a paucity of research into which of these mechanisms may also operate in the setting of SARS-CoV-2 infection. However, observational studies have found far lower rates of CAP in SARS-CoV-2 relative to influenza patients, and instead prolonged exposure to healthcare settings and particularly mechanical ventilation appear to be driving the majority of

bacterial and fungal pneumonia cases seen in COVID-19 patients. Respiratory culture ordering and antibiotic use should therefore be primarily considered in severe COVID-19 disease requiring ICU-level care and invasive mechanical ventilation.

References

1. World Health Organization Coronavirus (COVID-19) Dashboard, Available at: https://covid19.who.int/. Accessed on March 22, 2022.
2. Tan J, Liu S, Zhuang L, et al. Transmission and clinical characteristics of asymptomatic patients with SARS-CoV-2 infection. Future Virol. 2020;10.2217/fvl-2020-0087.
3. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020 May 12;323(18):1843-1844.
4. Ramanan P, Bryson AL, Binnicker MJ, et al. Syndromic Panel-Based Testing in Clinical Microbiology. Clin Microbiol Rev. 2017;31(1):e00024-17.
5. Kumar N, Sharma S, Barua S, et al. Virological and Immunological Outcomes of Coinfections. Clin Microbiol Rev. 2018;31(4):e00111-17.
6. Musuuza JS, Watson L, Parmasad V, et al. Prevalence and outcomes of co-infection and superinfection with SARS-CoV-2 and other pathogens: A systematic review and meta-analysis. PLoS One. 2021;16(5):e0251170.
7. Yue H, Zhang M, Xing L, et al. The epidemiology and clinical characteristics of co-infection of SARS-CoV-2 and influenza viruses in patients during COVID-19 outbreak. J Med Virol. 2020;92(11):2870-2873.

8. Kim D, Quinn J, Pinsky B, et al. Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens. JAMA. 2020;323(20):2085-2086.
9. Rizzo KR, Hoover C, Jain S, et al. Influenza and SARS-CoV-2 Co-infections in California, USA, September 2020-April 2021. Emerg Infect Dis. 2021;27(11):2923-2926.
10. Uhteg K, Amadi A, Forman M, et al. Circulation of Non-SARS-CoV-2 Respiratory Pathogens and Coinfection with SARS-CoV-2 Amid the COVID-19 Pandemic. Open Forum Infect Dis. 2021;9(3):ofab618.
11. Wanga V, Gerdes ME, Shi DS, et al. Characteristics and Clinical Outcomes of Children and Adolescents Aged <18 Years Hospitalized with COVID-19 - Six Hospitals, United States, July-August 2021 [published correction appears in MMWR Morb Mortal Wkly Rep. 2022 Feb 04;71(5):185]. MMWR Morb Mortal Wkly Rep. 2021;70(5152):1766-1772.
12. Wu Q, Xing Y, Shi L, et al. Coinfection and Other Clinical Characteristics of COVID-19 in Children. Pediatrics. 2020;146(1):e20200961.
13. Garcia-Vidal C, Sanjuan G, Moreno-García E, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. Clin Microbiol Infect. 2021;27(1):83-88.
14. Ishiguro T, Kobayashi Y, Shimizu Y, et al. Frequency and Significance of Coinfection in Patients with COVID-19 at Hospital Admission. Intern Med. 2021;60(23):3709-3719.

15. Wee LE, Ko KKK, Ho WQ, et al. Community-acquired viral respiratory infections amongst hospitalized inpatients during a COVID-19 outbreak in Singapore: co-infection and clinical outcomes. J Clin Virol. 2020;128:104436.
16. Burrel S, Hausfater P, Dres M, et al. Co-infection of SARS-CoV-2 with other respiratory viruses and performance of lower respiratory tract samples for the diagnosis of COVID-19. Int J Infect Dis. 2021;102:10-13.
17. Ozaras R, Cirpin R, Duran A, et al. Influenza and COVID-19 coinfection: Report of six cases and review of the literature. J Med Virol. 2020;92(11):2657-2665.
18. Kinoshita, T., Watanabe, K., Sakurai, Y. et al. Co-infection of SARS-CoV-2 and influenza virus causes more severe and prolonged pneumonia in hamsters. Sci Rep 11, 21259 (2021).
19. Alvares PA. SARS-CoV-2 and Respiratory Syncytial Virus Coinfection in Hospitalized Pediatric Patients. Pediatr Infect Dis J. 2021;40(4):e164-e166.
20. Giannattasio A, Maglione M, D'Anna C, et al. Silent RSV in infants with SARS-CoV-2 infection: A case series. Pediatr Pulmonol. 2021;56(9):3044-3046.
21. Pinky L, Dobrovolny HM. Coinfections of the Respiratory Tract: Viral Competition for Resources. PLoS One. 2016;11(5):e0155589.
22. Kim, KW, Deveson, IW, Pang, CNI. et al. Respiratory viral co-infections among SARS-CoV-2 cases confirmed by virome capture sequencing. Sci Rep 11, 3934 (2021).
23. Huang Y, Skarlupka AL, Jang H, et al. SARS-CoV-2 and Influenza A Virus Coinfections in Ferrets. J Virol. 2022;96(5):e0179121.

24. Achdout, H., Vitner, E.B., Politi, B. et al. Increased lethality in influenza and SARS-CoV-2 coinfection is prevented by influenza immunity but not SARS-CoV-2 immunity. Nat Commun 12, 5819 (2021).
25. Cheng Y, Ma J, Wang H, et al. Co-infection of influenza A virus and SARS-CoV-2: A retrospective cohort study. J Med Virol. 2021;93(5):2947-2954.
26. Xiang X, Wang ZH, Ye LL, et al. Co-infection of SARS-COV-2 and Influenza A Virus: A Case Series and Fast Review. Curr Med Sci. 2021;41(1):51-57.
27. Stowe J, Tessier E, Zhao H, et al. Interactions between SARS-CoV-2 and influenza, and the impact of coinfection on disease severity: a test-negative design. Int J Epidemiol. 2021;50(4):1124-1133.
28. Hashemi SA, Safamanesh S, Ghasemzadeh-Moghaddam H, et al. High prevalence of SARS-CoV-2 and influenza A virus (H1N1) coinfection in dead patients in Northeastern Iran. J Med Virol. 2021;93(2):1008-1012.
29. Martin ET, Kuypers J, Wald A, et al. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. Influenza Other Respir Viruses. 2012;6(1):71-77.
30. Asner SA, Science ME, Tran D, et al. Clinical Disease Severity of Respiratory Viral Co-Infection versus Single Viral Infection: A Systematic Review and Meta-Analysis. PLoS ONE 2014;9(6): e99392.
31. Burstein R, Althouse BM, Adler A, et al. Interactions among 17 respiratory pathogens: a cross-sectional study using clinical and community surveillance data. Preprint. medRxiv. 2022;2022.02.04.22270474.

32. Vareille M, Kieninger E, Edwards MR, et al. The airway epithelium: soldier in the fight against respiratory viruses. Clin Microbiol Rev. 2011;24:210–29.
33. Zhang AJ, Lee AC, Chan JF, et al. Coinfection by Severe Acute Respiratory Syndrome Coronavirus 2 and Influenza A(H1N1)pdm09 Virus Enhances the Severity of Pneumonia in Golden Syrian Hamsters. Clin Infect Dis. 2021;72(12):e978-e992.
34. Bai, L., Zhao, Y., Dong, J. et al. Coinfection with influenza A virus enhances SARS-CoV-2 infectivity. Cell Res 31, 395–403 (2021).
35. Deinhardt-Emmer S, Böttcher S, Häring C, et al. SARS-CoV-2 causes severe epithelial inflammation and barrier dysfunction [published online ahead of print, 2021 Feb 26]. J Virol. 2021;95(10):e00110-21.
36. Tay MZ, Poh CM, Rénia L, et al. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol. 2020;20(6):363-374.
37. Lucas C, Wong P, Klein J, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature. 2020;584(7821):463-469.
38. Zheng J, Chen F, Wu K, et al. Clinical and virological impact of single and dual infections with influenza A (H1N1) and SARS-CoV-2 in adult inpatients. PLoS Negl Trop Dis. 2021;15(11):e0009997.
39. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: Implications for pandemic influenza preparedness. Journal of Infectious Diseases. 2008;198(7):962-970.
40. Shah NS, Greenberg JA, McNulty MC, et al. Bacterial and viral co-infections complicating severe influenza: Incidence and impact among 507 U.S. patients, 2013-14. J Clin Virol. 2016;80:12-19.

41. MacIntyre CR, Chughtai AA, Barnes M, et al. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza a(H1N1)pdm09. BMC Infect Dis. 2018;18(1):637.
42. Klein EY, Monteforte B, Gupta A, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. Influenza Other Respir Viruses. 2016;10(5):394-403.
43. Martin-Loeches I, M JS, Vincent JL, et al. Increased incidence of co-infection in critically ill patients with influenza. Intensive Care Med. 2017;43(1):48-58.
44. Teng F, Liu X, Guo SB, et al. Community-acquired bacterial co-infection predicts severity and mortality in influenza-associated pneumonia admitted patients. J Infect Chemother. 2019;25(2):129-136.
45. Manna S, Baindara P, Mandal SM. Molecular pathogenesis of secondary bacterial infection associated to viral infections including SARS-CoV-2. J Infect Public Health. 2020;13(10):1397-1404.
46. Bartley PS, Deshpande A, Yu PC, et al. Bacterial coinfection in influenza pneumonia: Rates, pathogens, and outcomes. Infect Control Hosp Epidemiol. 2022;43(2):212-217.
47. Ghoneim HE, Thomas PG, McCullers JA. Depletion of alveolar macrophages during influenza infection facilitates bacterial superinfections. J Immunol. 2013;191(3):1250-1259.
48. Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. Nat Med. 2008;14(5):558-564.

49. Tian X, Xu F, Lung WY, et al. Poly I:C enhances susceptibility to secondary pulmonary infections by gram-positive bacteria. PLoS One. 2012;7(9):e41879.
50. Foster TJ, Hook M. Surface protein adhesins of Staphylococcus aureus. Trends Microbiol. 1998;6(12):484-488.
51. Bucior I, Pielage JF, Engel JN. Pseudomonas aeruginosa Pili and Flagella Mediate Distinct Binding and Signaling Events at the Apical and Basolateral Surface of Airway Epithelium. Plos Pathogens. 2012;8(4).
52. Davison VE, Sanford BA. Adherence of Staphylococcus-Aureus to Influenza-a Virus-Infected Madin-Darby Canine Kidney-Cell-Cultures. Infect Immun. 1981;32(1):118-126.
53. Jamieson AM, Pasman L, Yu S, et al. Role of tissue protection in lethal respiratory viral-bacterial coinfection. Science. 2013;340(6137):1230-1234.
54. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet. 2017;390(10098):946-958.
55. Hishiki H, Ishiwada N, Fukasawa C, et al. Incidence of bacterial coinfection with respiratory syncytial virus bronchopulmonary infection in pediatric inpatients. Journal of Infection and Chemotherapy. 2011;17(1):87-90.
56. Lin HC, Liu YC, Hsing TY, et al. RSV pneumonia with or without bacterial co-infection among healthy children. J Formos Med Assoc. 2022;121(3):687-693.

57. Thorburn K, Harigopal S, Reddy V, et al. High incidence of pulmonary bacterial co-infection in children with severe respiratory syncytial virus (RSV) bronchiolitis. Thorax. 2006;61(7):611-615.
58. Hedberg P, Johansson N, Ternhag A, et al. Bacterial co-infections in community-acquired pneumonia caused by SARS-CoV-2, influenza virus and respiratory syncytial virus. BMC Infect Dis. 2022;22(1):108.
59. Nori P, Cowman K, Chen V, et al. Bacterial and fungal coinfections in COVID-19 patients hospitalized during the New York City pandemic surge. Infect Control Hosp Epidemiol. 2021;42(1):84-88.
60. Weidmann MD, Berry GJ, Zucker JE, et al. Bacterial Pneumonia and Respiratory Culture Utilization among Hospitalized Patients with and without COVID-19 in New York City. 2022:2022.2002.2008.22270591.
61. Hedberg P, Ternhag A, Giske CG, et al. Ventilator-Associated Lower Respiratory Tract Bacterial Infections in COVID-19 Compared With Non-COVID-19 Patients. Crit Care Med. 2022.
62. Lansbury L, Lim B, Baskaran V, et al. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020;81(2):266-275.
63. Vaughn VM, Gandhi TN, Petty LA, et al. Empiric Antibacterial Therapy and Community-onset Bacterial Coinfection in Patients Hospitalized With Coronavirus Disease 2019 (COVID-19): A Multi-hospital Cohort Study. Clin Infect Dis. 2021;72(10):e533-e541.
64. Kubin CJ, McConville TH, Dietz D, et al. Characterization of Bacterial and Fungal Infections in Hospitalized Patients With Coronavirus Disease 2019 and Factors

Associated With Health Care-Associated Infections. Open Forum Infect Dis. 2021;8(6):ofab201.

65. Chen W, Yin C, Zhong M, et al. Incidence and outcomes of patients with COVID-19 associated pulmonary aspergillosis (CAPA) in intensive care units: a systematic review and meta-analysis of 31 cohort studies. Ann Palliat Med. 2022;apm-21-2043.

TABLE TITLE

Table 1: Prevalence of common co-infections in COVID-19 patients