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Co-infection of *Streptococcus pneumoniae* in Respiratory Infections Caused by SARS-CoV-2

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Abstract: Viral respiratory infections are often associated with bacterial co-infections that often lead to increased severity and mortality of the disease. During the recent pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), hospitalized patients reported developing secondary bacterial infections ranging from 0 to 40% of the cases. In the previous influenza pandemics, *Streptococcus pneumoniae* was the most isolated bacterial pathogen causing increased mortality in patients affected by viral pneumonia. Due to the difficulty to detect pneumococcal infection in SARS-CoV-2 patients by a rapid clinical test, the real prevalence of *S. pneumoniae* might be underestimated, and only a few cases have been documented so far. It has been estimated that 90% of patients admitted to the Intensive Care Unit are empirically treated with antimicrobial. The application of more rapid and sensitive diagnostic methods could help with targeted antibiotic therapy. Additionally, pneumococcal vaccination of high-risk individuals could reduce bacterial pneumonia, hospital admissions, and comorbidities associated with serious illness.

Keywords: SARS-CoV-2; *Streptococcus pneumoniae*; bacterial co-infections.

1. Introduction

Bacterial co-infections in viral respiratory disease are clinically well-documented and can aggravate the disease's outcome in infected patients. The ongoing pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is overwhelming the healthcare system and economy worldwide. Although more clinical data on the disease has been collected since the pandemic began to spread globally in January 2020, many aspects remain unclear on what could worsen the disease's outcome.

SARS-CoV-2 can establish in the upper respiratory tract (URT) of the host in asymptomatic form or develop into respiratory disease with a wide degree of symptoms and severity. 80% of cases present cold symptoms up to mild pneumonia. In the remaining 20% of the cases, the infection can progress to the lower respiratory tract (LRT), causing severe pneumonia with multiorgan dysfunction that can be fatal in 5% of cases [1,2]. Incidence and prevalence of SARS-CoV-2 infection are also very variable and strongly depends on the patient's age and health status [1].

The URT constitutes the main door of entry of the virus in the host through droplets containing infective viral particles emitted by carriers or infected people [1]. SARS-CoV-2 can penetrate the host cells by binding the receptor angiotensin-converting enzyme 2 (ACE-2) present in different organs but particularly abundant in the epithelial cells of the URT and LRT. Since SARS-CoV-2 shares different respiratory tract infection sites [3], which are in common with other respiratory bacterial pathogens, it is important to consider possible bacterial co-infections can exacerbate the fatal outcome of the disease.

Diverse viral and bacterial species can colonize the URT, coexisting being part of the normal microbiota [4]. Potential pathogenic agents can also occupy URT niches in an asymptomatic form among the microorganism community without causing damage to the host [5]. Within this complex network of microorganisms, invading pathogens compete with the commensal bacteria for adhesion receptors and nutrients present on the mucosal surface [6–8]. Changes in external environmental conditions or the weakening of the host's immune status can be decisive for the transition to a symptomatic state leading to the onset of severe forms of the disease [5,9]. Thus, viral and bacterial respiratory pathogens can have synergistic interaction, increasing infection complications, for example, through damage to the cellular barrier [10] and dysregulation of immune responses [11].

Streptococcus pneumoniae is usually the primary bacterial agent causing increased morbidity and mortality in virus-associated pneumonia [12]. There is evidence supporting that the influenza virus alters the respiratory tract to enhance the adherence, invasion, and induction of disease by pneumococcus [13]. Furthermore, a viral infection appears to decrease the efficacy of the immune response and phagocytic activity by alveolar macrophages and neutrophils, leading to a reduced suppression of bacterial co-infection [14–17].

In 1918 during the Influenza pandemic, 500 million people were infected by influenza A H1N1 virus. More than 50 million died, of which 85% to 90% of fatal cases developed severe pneumonia caused by *S. pneumoniae* [18]. In the following pandemics caused by a rearranged version of the influenza virus, *S. pneumoniae* showed a lower incidence in fatal cases (approximately 25%) but was still the most common complicating organism in patients with pneumonia [19,20]. The mortality rate of viral pandemics is strongly impacted by secondary bacterial infections, with a high prevalence of fatal cases in the previous pandemics caused by secondary bacterial infections rather than the virus alone.

In the current SARS-CoV-2 pandemic, data on the prevalence of pneumococcal co-infections are limited and irregularly reported. The only diagnosis based on the Ray-X image of lung and clinical symptoms could render it difficult to distinguish between pneumococcal and SARS-CoV-2 infection.

SARS-CoV-2 is diagnosed on nasopharyngeal swab specimens by Real-Time reverse transcriptase-polymerase chain reaction (RT-rtPCR), which is often a sensitive method used in clinical virology [21]. *S. pneumoniae* is usually detected by microbiological culture from the respiratory tract or blood and confirmed for the presence of C-polysaccharide antigen in urine. Although the microbiological method is still a valid screening, the result less sensitive and rapid compared to molecular methods [22]. Moreover, not all health care centers regularly carry out the detection of respiratory pathogens and longitudinal respiratory sampling, especially in Intensive Care Units (UNI) patients, to reduce aerosol-generating procedures.

Therefore, the real incidence of *S. pneumoniae* in severe SARS-CoV-2 pneumonia remains to be defined [23].

2. Materials and Methods

This study was performed using PubMed and Scholar databases on COVID-19 articles published before October 17 2020, and after February 2020. Separate searches were done for Streptococci respiratory infection, years 2006 to 2020.

3. Pneumococcal Co-infection in SARS-CoV-2 Patients

The current literature suggests that the prevalence of the total bacterial co-infection in SARS-CoV-2 patients could range from 0 to 40% [2,24–26]. The secondary infections seem to have a nosocomial origin with a higher incidence in UNI patients (14% - 31%) and no survivors (50%) [2,26]. The difference in prevalence in bacterial co-infection poses a strong debate for the empirical use of antibiotic therapy in SARS-CoV-2 patients prior to and after admission to UNI [27,28].

Among reports of case-cohort studies, few provided details about the pathogens involved in a secondary infection. Pneumococcal infection associated with SARS-CoV-2 pneumonia is reported only in a few studies (Table 1). The difference in incidence is greatly variable and depends on the country, the pandemic period, and the detection method utilized for the diagnosis.

Table 1. Cohort retrospective studies where the pneumococcal infection was reported.

Biblio	Country	Period of study	N° of patients	Median age of patients (years)	Pneumococcal secondary infection	Pneumococcal infection incidence	Diagnosis
[36]	China	22nd Jan - 2nd Feb, 2020	257	51 (2-99)	153	59.5%	RT-PCR
[37]	China (Suizhou)	8th Feb - 15th Feb, 2020	194	45 (1-88)	14	7.2%	qPCR
[38]	Spain	28th Feb - 22nd April, 2020	989	61 (48-74)	12	1.2%	Pneumococcal urinary antigen
[25]	USA (Chicago)	1st Mar - 11th Apr, 2020	321	60 (43-77)	4	1.2%	Pneumococcal urinary antigen
[39]	France	16th Mar - 6th Apr, 2020	47	68 (56-74)	3	6.3%	Bacterial culture plus Multiplex PCR panel
[40]	UK	6th Mar - 7th Apr, 2020	195	69 (59-81)	5	2.5%	Bacterial culture
[41]	France	13th Mar -16th Apr, 2020	92	61 (55-70)	6	6.5%	Bacterial culture plus Multiplex PCR panel
[42]	Italy	21st Jan - 7th Feb, 2020	56	35 (1-85)	1	0.8%	Bacterial culture plus Respiratory panel cartridge
[43]	Italy	25th Mar -10th Apr 2020	168	2.3 (1-17)	1 (infant)	0.5%	NA

The higher incidence of pneumococcal infection in SARS-CoV-2 patients was reported by Zhu *et al.* (Table 1) [29]. In this study, 294 patients were tested by RT-PCR for 24 respiratory pathogens, of which *S. pneumonia* was the most isolated bacteria in bacterial co-infection, followed by *Klebsiella pneumoniae* and *Hemophilus influenza*. Most pathogen species were detected after 1 - 4 days from the onset. The highest rates of co-infections were found in patients aged from 15 to 44 years.

In contrast, in Europe, the prevalence of reported cases of *S. pneumoniae* co-infection is variable, ranging from 0% to 6%. The study conducted in a UNI in France presents a well-documented analysis of mixed co-infection detected using a broad-spectrum molecular diagnostic panel to detect the most common respiratory pathogens rapidly. In 28% of patients diagnosed with bacterial co-infection on ICU admission, *S. pneumoniae* was the third most detected respiratory pathogen before *Staphylococcus aureus* and *H. influenzae*.

Table 2. Single case reports of pneumococcal infection in SARS-CoV-2 patients.

Biblio	Country	Age (years, months) /gender	Symptoms	Comorbidity	X-Ray Image	Diagnostic	Treatment	Outcome
[44]	Spain	8 months/M	Fever, Cough Dyspnea	None	Left lobe opacity, small pleural effusion	Blood culture	Meropenem Linezolid	discharged
[45]	Lebanon	16 months/F	Fever Severe diarrhea	None	Upper lobe consolidation, bilateral lower lobe infiltrates	Blood culture	Ceftriaxone Metronidazole	discharged
[46]	Spain	86 years/M	Fever Cough Dyspnea	Ischemic Heart Disease Aortic Stenosis CKD	Unilateral consolidative infiltrate	pneumococcal antigen in urine	Ceftriaxone Ceftaroline	discharged
[46]	Spain	38 years/F	Fever Cough Dyspnea Arthromyalgia	None	Bilateral interstitial infiltrates	pneumococcal antigen in urine	Ceftriaxone Cefixime	discharged
[46]	Spain	65 years/M	Fever Cough Dyspnea Arthromyalgia	Depression	Bilateral interstitial infiltrates	pneumococcal antigen in urine	Ceftriaxone Levofloxacin	discharged
[46]	Spain	79 years/F	Fever Cough Dyspnea Arthromyalgia	CKD Chronic anemia	Bilateral interstitial infiltrates	pneumococcal antigen in urine	Ceftriaxone Teicoplanin	discharged
[46]	Spain	44 year/F	Fever Cough Dyspnea Arthromyalgia	Obesity Asthma	Bilateral interstitial infiltrates	pneumococcal antigen in urine	Ceftriaxone Cefixime	discharged
[47]	The UK	86 year/M	Fever Dyspnea Fatigue	Alzheimer's disease, hypertension	Abnormality in the lung	Blood culture	Amoxicillin Clarithromycin vancomycin	death
[47]	The UK	82 years/F	Chest pain Fever Cough	Type 2 diabetes mellitus, ischemic cardiomyopathy, hypertension, CKD	Abnormality in the lung	Blood culture	Amoxicillin Co-amoxiclav	discharged
[48]	Brunei	42/M	Fever, cough, dyspnea, rhinorrhea, and myalgia	None	Normal	Sputum	Oseltamivir amoxicillin	discharged
[49]	USA	80/F	Respiratory Distress Cardia arrest	Diabetes mellitus hypertension, asthma, paroxysmal atrial fibrillation, and dementia	Bilateral interstitial and intra-alveolar infiltrates	pneumococcal antigen in urine	ceftriaxone, azithromycin	death

Single case reports of pneumococcal infection have been globally described in SARS-CoV-2 infected patients (Table 2). The clinical conditions of patients often appear critical and are characterized by severe respiratory distress. Antibiotic treatment was associated with antiviral therapy to burden the infection.

Because of these reported cases, we emphasize the application of rapid and broad diagnostic tests. It is estimated that more than 90% of the critically ill patients with severe SARS-CoV-2 pneumonia received an empiric antibiotic therapy upon ICU admission [25,27,30–32]. The use of a panel consisting of multiplex PCR for seasonal respiratory pathogens would support better and faster diagnosis and the choice of appropriate antimicrobial therapy.

For more in-depth information on the development of the infection, advanced molecular methods for detecting a wide range of potential pathogens and antimicrobial resistance could also be considered. For instance, the application of whole-genome metagenomics would generate information on complex mixed infections and antimicrobial resistance in a relatively short period [33,34]. Although whole-genome metagenomics does not yet have a large application in diagnostics, advanced nanopore metagenomics could have the potential to produce a rapid and accurate detection of respiratory pathogens and antibiotic-resistance genes that would help to avoid a high use of broad-spectrum antibiotics [33] and might also provide useful information on the microbiota associated to diseased patients.

4. Conclusions

These cases suggest that pneumococcal infection can occur associated with SARS-CoV-2 infection but be less frequently than in influenza [35]. However, there may be more case reports of pneumococcal pneumonia monitoring SARS-CoV-2 disease over time. The necessity of rapid, sensitive, and broad tests to detect respiratory pathogens, including *S. pneumoniae*, which is the most common bacteria viral-pneumonia associated, is highlighted.

The combination of seasonal influenza and the pneumococcal vaccine could help prevent a portion of secondary infections, especially in high-risk patients.

Future studies should implement standardized sampling and testing to research respiratory pathogens in SARS-CoV-2 patients and indicate which ones are more prevalent in the co-infection correlating morbidity and mortality rates.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Wiersinga, W.J.; Rhodes, A.; Cheng, A.C.; Peacock, S.J.; Prescott, H.C. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* **2020**, *324*, 782–793, https://doi.org/10.1001/jama.2020.12839.
2. Zhou, F.; Yu, T.; Du, R.; Fan, G.; Liu, Y.; Liu, Z.; Xiang, J.; Wang, Y.; Song, B.; Gu, X.; Guan, L.; Wei, Y.; Li, H.; Wu, X.; Xu, J.; Tu, S.; Zhang, Y.; Chen, H.; Cao, B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* **2020**, *395*, 1054–1062, https://doi.org/10.1016/S0140-6736(20)30566-3.
3. Hui, K.P.Y.; Cheung, M.-C.; Perera, R.A.P.M.; Ng, K.-C.; Bui, C.H.T.; Ho, J.C.W.; Ng, M.M.T.; Kuok, D.I.T.; Shih, K.C.; Tsao, S.-W.; Poon, L.L.M.; Peiris, M.; Nicholls, J.M.; Chan, M.C.W. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *Lancet Respir. Med.* **2020**, *8*, 687–695, https://doi.org/10.1016/S2213-2600(20)30193-4.
4. Robinson, C.M.; Pfeiffer, J.K. Viruses and the Microbiota. *Annu. Rev. Virol.* **2014**, *1*, 55–69, https://doi.org/10.1146/annurev-virology-031413-085550.
5. Siegel, S.J.; Weiser, J.N. Mechanisms of Bacterial Colonization of the Respiratory Tract. *Annu. Rev. Microbiol.* **2015**, *69*, 425–444, https://doi.org/10.1146/annurev-micro-091014-104209.
6. Ferrando, M.L.; Fuentes, S.; De Greeff, A.; Smith, H.; Wells, J.M. ApuA, a multifunctional α-glucan-degrading enzyme of Streptococcus suis, mediates adhesion to porcine epithelium and mucus. *Microbiology* **2010**, *156*, 2818–2828, https://doi.org/10.1099/mic.0.037960-0.
7. Ferrando, M.L.; Schultsz, C. A hypothetical model of host-pathogen interaction of Streptococcus suis in the gastro-intestinal tract. *Gut Microbes* **2016**, *7*, 154–162, https://doi.org/10.1080/19490976.2016.1144008.
8. Ferrando, M.L.; Willemse, N.; Zaccaria, E.; Pannekoek, Y.; van der Ende, A.; Schultsz, C. Streptococcal Adhesin P (SadP) contributes to Streptococcus suis adhesion to the human intestinal epithelium. *PLoS One* **2017**, *12*, https://doi.org/10.1371/journal.pone.0175639.
9. Ferrando, M.L.; van Baarlen, P.; Orrù, G.; Piga, R.; Bongers, R.S.; Wels, M.; De Greeff, A.; Smith, H.E.; Wells, J.M. Carbohydrate Availability Regulates Virulence Gene Expression in Streptococcus suis. *PLoS One* **2014**, *9*, https://doi.org/10.1371/journal.pone.0089334.
10. Dijk, I.A.; Laura Ferrando, M.; Wijk, A.; Hoebe, R.A.; Nazmi, K.; Jonge, W.J.; Krawczyk, P.M.; Bolscher, J.G.M.; Veerman, E.C.I.; Stap, J. Human salivary peptide histatin-1 stimulates epithelial and endothelial cell adhesion and barrier function. *FASEB J.* **2017**, *31*, 3922–3933, https://doi.org/10.1096/fj.201700180R.
11. Ferrando, M.L.; de Greeff, A.; van Rooijen, W.J.M.; Stockhofe-Zurwieden, N.; Nielsen, J.; Wichgers Schreur, P.J.; Pannekoek, Y.; Heuvelink, A.; van der Ende, A.; Smith, H.; Schultsz, C. Host-pathogen Interaction at the Intestinal Mucosa Correlates With Zoonotic Potential of Streptococcus suis. *J. Infect. Dis.* **2015**, *212*, 95–105, https://doi.org/10.1093/infdis/jiu813.
12. Madhi, S.A.; Klugman, K.P. The Vaccine Trialist Group. A role for Streptococcus pneumoniae in virus-associated pneumonia. *Nat. Med.* **2004**, *10*, 811–813, https://doi.org/10.1038/nm1077.
13. McCullers, J.A. Insights into the Interaction between Influenza Virus and Pneumococcus. *Clin. Microbiol. Rev.* **2006**, *19*, 571–582, https://doi.org/10.1128/CMR.00058-05.
14. Robinson, K.M.; Kolls, J.K.; Alcorn, J.F. The immunology of influenza virus-associated bacterial pneumonia. *Curr. Opin. Immunol.* **2015**, *34*, 59–67, https://doi.org/10.1016/j.coi.2015.02.002.
15. Braciale, T.J.; Sun, J.; Kim, T.S. Regulating the adaptive immune response to respiratory virus infection. *Nat. Rev. Immunol.* **2012**, *12*, 295–305, https://doi.org/10.1038/nri3166.
16. Mirzaei, R.; Goodarzi, P.; Asadi, M.; Soltani, A.; Aljanabi, H.A.A.; Jeda, A.S.; Dashtbin, S.; Jalalifar, S.; Mohammadzadeh, R.; Teimoori, A.; Tari, K.; Salari, M.; Ghiasvand, S.; Kazemi, S.; Yousefimashouf, R.; Keyvani, H.; Karampoor, S. Bacterial co-infections with SARS-CoV-2. *IUBMB Life* **2020**, *72*, 2097–2111, https://doi.org/10.1002/iub.2356.
17. Meijerink, M.; Ferrando, M.L.; Lammers, G.; Taverne, N.; Smith, H.E.; Wells, J.M. Immunomodulatory Effects of Streptococcus suis Capsule Type on Human Dendritic Cell Responses, Phagocytosis and Intracellular Survival. *PLoS One* **2012**, *7*, https://doi.org/10.1371/journal.pone.0035849.
18. Morens, D.M.; Fauci, A.S. The 1918 influenza pandemic: insights for the 21st century. *J. Infect. Dis.* **2007**, *195*, 1018–28, https://doi.org/10.1086/511989.
19. MacIntyre, C.R.; Chughtai, A.A.; Barnes, M.; Ridda, I.; Seale, H.; Toms, R.; Heywood, A. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza a(H1N1)pdm09. *BMC Infect. Dis.* **2018**, *18*, https://doi.org/10.1186/s12879-018-3548-0.
20. Rynda-Apple, A.; Robinson, K.M.; Alcorn, J.F. Influenza and Bacterial Superinfection: Illuminating the Immunologic Mechanisms of Disease. *Infect. Immun.* **2015**, *83*, 3764–70, https://doi.org/10.1128/IAI.00298-15.
21. Orrù, G.; Ferrando, M.L.; Meloni, M.; Liciardi, M.; Savini, G.; De Santis, P. Rapid detection and quantitation of Bluetongue virus (BTV) using a Molecular Beacon fluorescent probe assay. *J. Virol. Methods* **2006**, *137*, 34–42, https://doi.org/10.1016/j.jviromet.2006.05.028.

22. Blaschke, A.J. Interpreting assays for the detection of Streptococcus pneumoniae. *Clin. Infect. Dis.* **2011**, *52 Suppl 4*, S331-7, https://doi.org/10.1093/cid/cir048.
23. Cox, M.J.; Loman, N.; Bogaert, D.; O'Grady, J. Co-infections: potentially lethal and unexplored in COVID-19. *The Lancet Microbe* **2020**, *1*, https://doi.org/10.1016/S2666-5247(20)30009-4.
24. Hendaus, M.A.; Jomha, F.A. Covid-19 induced superimposed bacterial infection. *J. Biomol. Struct. Dyn.* **2020**, 1–7, https://doi.org/10.1080/07391102.2020.1772110.
25. Lehmann, C.J.; Pho, M.T.; Pitrak, D.; Ridgway, J.P.; Pettit, N.N. Community-acquired Co-infection in Coronavirus Disease 2019: A Retrospective Observational Experience. *Clin. Infect. Dis.* **2020**, *1*, https://doi.org/10.1093/cid/ciaa902.
26. Zhou, P.; Liu, Z.; Chen, Y.; Xiao, Y.; Huang, X.; Fan, X.-G. Bacterial and fungal infections in COVID-19 patients: A matter of concern. *Infect. Control Hosp. Epidemiol.* **2020**, *41*, 1124–1125, https://doi.org/10.1017/ice.2020.156.
27. Rawson, T.M.; Moore, L.S.P.; Zhu, N.; Ranganathan, N.; Skolimowska, K.; Gilchrist, M.; Satta, G.; Cooke, G.; Holmes, A. Bacterial and Fungal Coinfection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. *Clin. Infect. Dis.* **2020**, *71*, 2459–2468, https://doi.org/10.1093/cid/ciaa530.
28. Lai, C.-C.; Wang, C.-Y.; Hsueh, P.-R. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? *J. Microbiol. Immunol. Infect.* **2020**, *53*, 505–512, https://doi.org/10.1016/j.jmii.2020.05.013.
29. Chen, N.; Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y.; Qiu, Y.; Wang, J.; Liu, Y.; Wei, Y.; Xia, J.A.; Yu, T.; Zhang, X.; Zhang, L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* **2020**, *395*, 507–513, https://doi.org/10.1016/S0140-6736(20)30211-7.
30. Townsend, L.; Hughes, G.; Kerr, C.; Kelly, M.; O'Connor, R.; Sweeney, E.; Doyle, C.; O'Riordan, R.; Bergin, C.; Bannan, C. Bacterial pneumonia co-infection and antimicrobial therapy duration in SARS-CoV-2 (COVID-19) infection. *JAC-Antimicrobial Resist.* **2020**, *2*, https://doi.org/10.1093/jacamr/dlaa071.
31. Yang, X.; Yu, Y.; Xu, J.; Shu, H.; Xia, J.a.; Liu, H.; Wu, Y.; Zhang, L.; Yu, Z.; Fang, M.; Yu, T.; Wang, Y.; Pan, S.; Zou, X.; Yuan, S.; Shang, Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet. Respir. Med.* **2020**, *8*, 475–481, https://doi.org/10.1016/S2213-2600(20)30079-5.
32. Guan, W.-J.; Ni, Z.-Y.; Hu, Y.; Liang, W.-H.; Ou, C.-Q.; He, J.-X.; Liu, L.; Shan, H.; Lei, C.-L.; Hui, D.S.C.; Du, B.; Li, L.-J.; Zeng, G.; Yuen, K.-Y.; Chen, R.-C.; Tang, C.-L.; Wang, T.; Chen, P.-Y.; Xiang, J.; Li, S.-Y.; Wang, J.-L.; Liang, Z.-J.; Peng, Y.-X.; Wei, L.; Liu, Y.; Hu, Y.-H.; Peng, P.; Wang, J.-M.; Liu, J.-Y.; Chen, Z.; Li, G.; Zheng, Z.-J.; Qiu, S.-Q.; Luo, J.; Ye, C.-J.; Zhu, S.-Y.; Zhong, N.-S. Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* **2020**, *382*, 1708–1720, https://doi.org/10.1056/NEJMoa2002032.
33. Charalampous, T.; Kay, G.L.; Richardson, H.; Aydin, A.; Baldan, R.; Jeanes, C.; Rae, D.; Grundy, S.; Turner, D.J.; Wain, J.; Leggett, R.M.; Livermore, D.M.; O'Grady, J. Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection. *Nat. Biotechnol.* **2019**, *37*, 783–792, https://doi.org/10.1038/s41587-019-0156-5.
34. Wang, M.; Fu, A.; Hu, B.; Tong, Y.; Liu, R.; Liu, Z.; Gu, J.; Xiang, B.; Liu, J.; Jiang, W.; Shen, G.; Zhao, W.; Men, D.; Deng, Z.; Yu, L.; Wei, W.; Li, Y.; Liu, T. Nanopore Targeted Sequencing for the Accurate and Comprehensive Detection of SARS-CoV-2 and Other Respiratory Viruses. *Small* **2020**, *16*, 2002169, https://doi.org/10.1002/smll.202002169.
35. Hughes, S.; Troise, O.; Donaldson, H.; Mughal, N.; Moore, L.S.P. Bacterial and fungal co-infection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clin. Microbiol. Infect.* **2020**, *26*, 1395–1399, https://doi.org/10.1016/j.cmi.2020.06.025.
36. Zhu, X.; Ge, Y.; Wu, T.; Zhao, K.; Chen, Y.; Wu, B.; Zhu, F.; Zhu, B.; Cui, L. Co-infection with respiratory pathogens among COVID-2019 cases. *Virus Res.* **2020**, *285*, 198005, https://doi.org/10.1016/j.virusres.2020.198005.
37. He, F.; Xia, X.; Nie, D.; Yang, H.; Jiang, Y.; Huo, X.; Guo, F.; Fang, B.; Hu, B.; Jiang, H.; Zhan, F.; Lv, J. Respiratory bacterial pathogen spectrum among COVID-19 infected and non–COVID-19 virus infected pneumonia patients. *Diagn. Microbiol. Infect. Dis.* **2020**, *98*, https://doi.org/10.1016/j.diagmicrobio.2020.115199.
38. Garcia-Vidal, C.; Sanjuan, G.; Moreno-García, E.; Puerta-Alcalde, P.; Garcia-Pouton, N.; Chumbita, M.; Fernandez-Pittol, M.; Pitart, C.; Inciarte, A.; Bodro, M.; Morata, L.; Ambrosioni, J.; Grafia, I.; Meira, F.; Macaya, I.; Cardozo, C.; Casals, C.; Tellez, A.; Castro, P.; Marco, F.; García, F.; Mensa, J.; Martínez, J.A.; Soriano, A.; Rico, V.; Hernández-Meneses, M.; Agüero, D.; Torres, B.; González, A.; de la Mora, L.; Rojas, J.; Linares, L.; Fidalgo, B.; Rodriguez, N.; Nicolas, D.; Albiach, L.; Muñoz, J.; Almuedo, A.; Camprubí, D.; Angeles Marcos, M.; Camprubí, D.; Cilloniz, C.; Fernández, S.; Nicolas, J.M.; Torres, A. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. *Clinical Microbiology and Infection* **2021**, *27*, 83-88, https://doi.org/10.1016/j.cmi.2020.07.041.

39. Kreitmann, L.; Monard, C.; Dauwalder, O.; Simon, M.; Argaud, L. Early bacterial co-infection in ARDS related to COVID-19. *Intensive Care Med.* **2020**, *46*, 1787–1789, https://doi.org/10.1007/s00134-020-06165-5.
40. Adler, H.; Ball, R.; Fisher, M.; Mortimer, K.; Vardhan, M.S. Low rate of bacterial co-infection in patients with COVID-19. *The Lancet Microbe* **2020**, *1*, https://doi.org/10.1016/S2666-5247(20)30036-7.
41. Contou, D.; Claudinon, A.; Pajot, O.; Micaëlo, M.; Longuet Flandre, P.; Dubert, M.; Cally, R.; Logre, E.; Fraissé, M.; Mentec, H.; Plantefève, G. Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU. *Ann. Intensive Care* **2020**, *10*, https://doi.org/10.1186/s13613-020-00736-x.
42. Bordi, L.; Nicastri, E.; Scorzolini, L.; Di Caro, A.; Capobianchi, M.R.; Castilletti, C.; Lalle, E.; On Behalf Of Inmi Covid-Study Group And Collaborating Centers. Differential diagnosis of illness in patients under investigation for the novel coronavirus (SARS-CoV-2), Italy, February 2020. *Euro Surveill.* **2020**, *25*, https://doi.org/10.2807/1560-7917.ES.2020.25.8.2000170.
43. Garazzino, S.; Montagnani, C.; Donà, D.; Meini, A.; Felici, E.; Vergine, G.; Bernardi, S.; Giacchero, R.; Lo Vecchio, A.; Marchisio, P.; Nicolini, G.; Pierantoni, L.; Rabbone, I.; Banderali, G.; Denina, M.; Venturini, E.; Krzysztofiak, A.; Badolato, R.; Bianchini, S.; Galli, L.; Villani, A.; Castelli-Gattinara, G.; Group, t.I.S.-S.P.I.S. Multicentre Italian study of SARS-CoV-2 infection in children and adolescents, preliminary data as at 10 April 2020. *Euro Surveill.* **2020**, *25*, https://doi.org/10.2807/1560-7917.ES.2020.25.18.2000600.
44. Nieto-Moro, M.; Ecclesia, F.G.; Tomé-Masa, I.; De Lama Caro-Patón, G.; Leoz-Gordillo, I.; Cabrero-Hernández, M.; García-Salido, A. SARS-CoV-2 and Streptococcus pneumoniae coinfection as a cause of severe pneumonia in an infant. *Pediatr. Pulmonol.* **2020**, *55*, 2198–2200, https://doi.org/10.1002/ppul.24916.
45. Mansour, A.; Atoui, R.; Kanso, K.; Mohsen, R.; Fares, Y.; Fares, J. First Case of an Infant with COVID-19 in the Middle East. *Cureus* **2020**, https://doi.org/10.7759/cureus.7520.
46. Cucchiari, D.; Pericàs, J.M.; Riera, J.; Gumucio, R.; Md, E.C.; Nicolás, D. Pneumococcal superinfection in COVID-19 patients: A series of 5 cases. *Med. Clin. (Barc).* **2020**, *155*, 502–505, https://doi.org/10.1016/j.medcli.2020.05.022.
47. Toombs, J.M.; Van den Abbeele, K.; Democratis, J.; Mandal, A.K.J.; Missouris, C.G. Pneumococcal co-infection in COVID-19 patients. *J. Med. Virol.* **2020**, https://doi.org/10.1002/jmv.26278.
48. Chauhdary, W.A.; Chong, P.L.; Mani, B.I.; Asli, R.; Momin, R.N.; Abdullah, M.S.; Chong, V.H. Primary Respiratory Bacterial Coinfections in Patients with COVID-19. *Am. J. Trop. Med. Hyg.* **2020**, *103*, 917–919, https://doi.org/10.4269/ajtmh.20-0498.
49. Sohal, S.; Rodriguez-Nava, G.; Khabbaz, R.; Chaudry, S.; Musurakis, C.; Chitrakar, S.; Chundi, V.V.; Friedman, H.J. SARS-CoV2 and Co-Infections: A Review of Two Cases. *Case Rep. Infect. Dis.* **2020**, *2020*, 1–4, https://doi.org/10.1155/2020/8882348.