Are Co-Infections Important in the Outcome of COVID-19?

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ABSTRACT

The current COVID-19 pandemic is the most significant infectious health problem of the last 80 years and it is currently having considerable adverse effects upon individuals, societies and economies, worldwide, due to its high morbidity and mortality, losses in productivity and rapid human to human transmission that has demanded social exclusiveness to contain the problem. A growing body of evidence has shown that co-infections are frequently detected among the respiratory isolates of patients with COVID-19 and in response to this literature, this systematic review sought to explore the extent, types and influence of co-infection upon clinical outcomes in patients with SARS-COV-2. The electronic databases of MEDLINE, EMBASE and CINAHL were searched in June 2020 and articles were limited to publication since December 2019 (emergence of SARS-COV-2) and English language. Studies were selected using a process of title and abstract screening and full-text review and eligible studies were appraised using the tools provided by the Joanna Briggs Institute. The outcomes were analysed using a narrative approach to permit sufficient description of the data pertinent to the research question. A total of 20 studies were deemed eligible for inclusion, which were of case report

(n=7), case series (n=3) and cohort design (n=10). Using the Joanna Briggs Institute frameworks for these designs, the risk of bias tended to be high across most studies given the detection of >=2 systematic biases, which were largely related to the selection and characteristics of subjects. In regard to the outcomes of interest, this review found that co-infections are a frequent problem among patients hospitalised within COVID-19 where the rate was found to vary between 2.9% and 18.3%, equating to a mean frequency of 6.7%. The types of co-infective pathogens varied markedly, although the majority of isolated microorganisms of patients with COVID-19 were either other non-SARS-COV-2 viruses or bacteria with affinity or virulence to infect the upper and lower respiratory tract. The commonest bacteria, viruses and fungi isolated from patients with COVID-19 co-infections were: *Mycoplasma pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Klebsiella pneumoniae, influenza virus A, respiratory syncytial virus, rhinovirus, enterovirus,* non-SARS-COV-2 coronaviruses and Candida species. The presence of co-infection was found to exacerbate the clinical course and outcomes of COVID-19 in most cases where co-infection was associated with more severe host inflammatory responses and in turn, this correlated with reports of pneumonia, acute respiratory distress syndrome, respiratory failure, sepsis, acute renal failure, acute cardiac dysfunction, poor weaning from ventilation and ventilator dependence, protracted lengths of hospital and/or intensive care unit stay and a more rapid deterioration and time to death. The case fatality among patients with COVID-19 co-infection was crudely estimated to be 10%, although few studies were able to report upon the comparative mortality rate between co-infected and non-co-infected cases. Overall, the findings of this review have some implications for clinical practice, guidelines and policy, which include greater diligence and proactivity in protecting immunocompromised patients from co-infection, adherence to infection prevention and control guidelines, proactivity in testing for co-infective pathogens, revisions to guidelines that include greater acknowledge and recommendations for the diagnosis and management of co-infections and additional support at the policy level to support greater laboratory testing of co-infective pathogens. Future research should focus upon developing a large comparative study to explore the impact of co-infection upon COVID-19 outcomes, in order to validate the findings of this review.

Chapter 1. Introduction

1.1 Overview

This research comprised a systematic review of the literature reporting upon cases of severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) co-infection with other viruses, bacteria or fungi, in order to ascertain whether co-infected patients

observe poorer clinical outcomes, when compared to their non-co-infected counterparts. Indeed, given the current pandemic this systematic review represented an important research priority and forms a part of wider academic efforts to help advance knowledge into the virulence and pathogenicity of SARS-COV-2 – information that could inform future management and prevention strategies. The structure of this review follows with an overview of the background of SARS-COV-2 (chapter 1), which is followed by the methods used to search for relevant literature and appraise and synthesise results (chapter 2), the results of study selection, critical appraisal and the outcomes of interest (chapter 3). Finally, a discussion and conclusion section are presented, with evaluation of the findings, detailing of the implications of the review for current clinical practice and recommendations for future research (chapter 4).

1.2 Emergence Extent and Burden of SARS-COV-2

The novel SARS-COV-2 is a type of beta coronavirus that initially emerged within Wuhan – a region located in the province of Hubei in China where the first reported cases occurred during December 2019 among workers and residents of a seafood market that involved the trading of wildlife species(1). The virus was soon recognised to cause severe respiratory disease in some patients, termed COVID-19, which led to the first cases of SARS-COV-2 related pneumonias, respiratory failure and death(1).It is reported that the initial cases in Wuhan were confirmed early in December some weeks before official organisations alerted the World Health Organisation (WHO) on the 31st December, by which time, the virus had already rapidly spread across borders via human to human transmission as a result of global travel, population density, and population proximity (2). The extent of international spread has been reflected in the first month of confirmed cases where 30 days following the WHO alert, there had been more than 80 cases documented across 18 countries and almost 8,000 cases in China alone(3). As a result of the continued spread of the virus and increases in cases on a global scale, the WHO announced that COVID-19 had become a Public Health Emergency and by March 2020, SARS-COV-2 was defined as a pandemic(4). Indeed, the most recent data published by the WHO as of August 2020 shows that there have been more than 24million cases and almost 850,000 deaths across 216 countries and territories, demonstrating that COVID-19 has been the most significant infectious threat to humans since the Spanish flu in 1918(4).

Not surprisingly, the emergence of SARS-COV-2 within China led to the country becoming the first epicentre of the virus with the number of cases rapidly rising to exceed 80,000, although due to unclear reasons but likely a result of poor pandemic preparedness, the epicentre shifted to foci within Europe, the United States, Asia and South America(5). As of August 2020, current reports reveal that Asia has observed 6.7 million cases, which is comparable to that of South America (6.0 million) and notably, Oceania has been largely spared of such a high burden with total cases being 28,000 (6). In contrast, Europe and North America have observed the greatest burden of SARS-COV-2 with total cases having exceeded 3.4 million and 7.1 million, respectively. Countries with the greatest case load as of June 2020 and with the exception of the United Stateswere: Brazil (3.7 million), India (3.3 million), Russia (980,000), Peru (621,000), South Africa (618,000), Colombia (582,000), Mexico (579,000) and Spain, (451,000)(6). The SARS-COV-2 pandemic has been particularly

impactful upon societies, worldwide, due to the high number of deaths secondary to COVID-19 and its related complications – the overall mortality rate has been estimated to range between 3-15% (7). Whilst there have been 4,634 deaths reported in China as of August2020, equating to a mortality rate of 5.5% for its 85,013 cases, there have been concerns about the under-reporting of cases and deaths within the nation and thus, mortality is best explored from the reports of other countries (8). The highest number of deaths has been reported across numerous countries including the UK (41,477), the United States (184,834) and Brazil (118,726), which equateto case fatality rates of 14.1%, 5.4% and 4.9%, respectively(Worldometer, 2020), and these are in keeping with the former estimates noted by Rajgor *et al.* (7).

Whilst the COVID-19 pandemic is ameliorating across some global regions with case numbers and deaths progressively declining from peaks that occurred between April and May 2020, there is an imminent risk that relaxation of current containment measures may lead to a second wave and recurrence of pre-August2020 case-loads and mortality rates(9). In addition, other regions, such as South America and Africa, are observing peak case-loads in the current context and thus, have observed some delays in infection rates as compared to other regions (9). Whilst the high mortality due to SARS-COV-2 is clearly one of the most feared outcomes for nations with confirmed cases, worldwide, COVID-19 also confers considerable morbidity upon infected individuals, due to complications that can lead to permanent ill health and disability, impairments to quality of life and the emotional and psychological distress of experiencing COVID-19 (10). On a societal level, the loss of productivity and life years due to SARS-COV-2 is also having marked socioeconomic effects where the majority of occupations have been forced to cease, at least temporarily, to permit containment of the virus via social distancing(11). Indeed, the estimated cost of COVID-19 upon the global economy has been reported to be the equivalent of $1-2.7 trillion, which represents a 0.5-1.75% reduction in the economy and of concern, is that the effects of the pandemic will continue to impair socioeconomic growth for the foreseeable future, which could induce and protract recessions in almost all affected countries (12).

1.3 Co-Infection as a Factor Affecting COVID-19 Outcomes

Given the recent emergence of SARS-COV-2, research into the factors affected COVID-19 disease severity and related mortality is limited, although there have been a growing number of studies published within the past few months that have attempted to ascertain the major risk factors of poor clinical outcomes. In a retrospective study of 191 adult cases of COVID-19 who had been admitted to Wuhan hospital in China, Zhou *et al.* (13) found that 48% of cases had a co-existing medical problem, including hypertension, diabetes and coronary heart disease, most commonly with rates of 30%, 19% and 8%, respectively. In the multivariate model, the authors also found that older age, a higher organ failure score ascertained using the SOFA tool and a D-dimer of more than 1microgram/ml were significant predictors of in-hospital death (all $p < 0.05$). The respective odds ratios were: 1.1 (95% CI 1.03, 1.17), 5.65 (95% CI 2.61, 12.23) and 18.42 (95% CI 2.64, 128.55). Although the findings of this study were limited by its retrospective design and related biases, such as confounding and missing

data, other evidence has supported age, comorbidities and markers of organ dysfunction as key factors influencing outcomes of COVID-19(14-16). However, less attention has been paid to the risk and impact of co-infections in patients with COVID-19 even despite evidence from previous epidemic coronaviruses, such as MERS-COV and SARS-COV-1, and seasonal and pandemic strains of influenza, where co-infections have been associated with poorer outcomes and higher mortality rates(17, 18).

Co-infection in the context of SARS-COV-2 refers to any respiratory infection that co-exists with COVID-19 disease whether the pathogen is a virus, bacteria, fungus or other microorganism. Such infections may be termed secondary or super-infections as they tend to arise after lung parenchymal injury has occurred in response to SARS-COV infection. This promotes colonisation and infection by other organisms due to altered barrier function and immune protection(19).Indeed, co-infections have been responsible for the majority of cases of pneumonia and related mortality in persons that have been infected with previous seasonal and pandemic strains of the influenza virus, which has been primarily due to secondary bacterial colonisation of the lower respiratory tract with *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* (20). This was evident in the relatively recent influenza H1N1 virus outbreak that occurred in 2009 in the United States where co-infection with respiratory bacteria accounted for 30% of fatalities (21). Co-infection observes a complex pathogenesis with the onset and progression of infection receiving influence from both viral, bacterial, other pathogen and host factors and whilst the presence of co-infection has clear treatment implications, co-infection in patients with COVID-19 and its management may be compounded by the poor detection of co-infective pathogens (18).

In co-infected COVID-19 patients, pro-inflammatory responses can become markedly heightened as a result of multiple antigenic responses, which not only protract pneumonia, respiratory failure and increase the likelihood of death, but it may enhance the risk of sepsis and other infectious complications that are being reported across case reports and series (13). Notably, it appears from published reports that most patients with COVID-19 do receive empirical antibiotic therapy, although this has not been based on the susceptibility profiles of co-infecting bacteria and will do little to tackle co-infections with other respiratory viruses or fungi (22, 23). Importantly, some authors have suggested that co-infection in the context of COVID-19 disease is likely to account for some of the variance in the higher risk of death and morbidity among persons with co-morbid health problems(18). For example, persons with chronic obstructive pulmonary disease are likely to observe colonisation and infection by non-SARS-COV-2 organisms prior to the onset of SARS-COV-2 pneumonia, whilst those with diabetes can have a degree of immunosuppression that can increases the risk of secondary respiratory infections (18).Moreover, all persons admitted to hospital due to COVID-19 are also at risk of developing co-pathogenic pneumonias as a result of the transmission of nosocomial organisms – a problem that may be accounting for morbidity and mortality across all patient-groups(24).Furthermore, as patients who develop clinically significant COVID-19 disease and require intensive care unit admission for mechanical ventilation, the protracted periods of ventilation duration that has a mean of nine days, further increases the risk of nosocomial infections, as

well as ventilator-associated infections, which could also account for SARS-COV-2 co-infections (25).In addition, patients treated in the hospital environment are more likely to acquire multi-drug resistant pathogens, than those in the community, and thus co-infections may be highly difficult to treat and indeed ventilator-related pneumonia in this context has been associated with a mortality greater than 60% (26).

Among previous coronavirus outbreaks and epidemics, the prevalence of co-infection has been reported to be rare for both MERS-COV and SARS-COV-1 with other respiratory viruses being detected in 0% and 3% of all cases, respectively, although for bacterial co-infection, the prevalence rate has been as high as 13% (27, 28). In response to the COVID-19 pandemic and its morbid and mortal impact upon infected individuals, there has been a large body of evidence, that has recently emerged, suggesting that co-infection is more common, than compared to co-infection rates reported for MERS-COV and SARS-COV-1(29).

1.4 Rationale, Research Aim, Question and Objectives

In response to the former statement, this systematic review aimed to explore the impact of co-infection upon clinical outcomes in persons with SARS-COV-2/COVID-19, in order to establish whether management of the virus requires proactivity in screening for other pathogens and in turn, the administration of directed, rather than empirical, therapy. It is hoped and expected that this review of the relevant literature will lead to more informed management of persons affected by COVID-19 and in turn, improve outcomes on a global scale. In keeping with best review practice, the author generated a primary research question to help retain focus upon addressing the former aim and to tailor the methods to assist in achieving this vision(30). The research question described below was derived by compositing the components of the PEO (population, exposure and outcomes) framework as given in Table 1 (31).

Table 1. Primary research question using PEO (31).

PEO	
Population	Humans
Exposure	SARS-COV-2/COVID-19 respiratory co-infection with other pathogens
Outcomes	Morbidity Mortality

What is the impact of respiratory co-infections upon morbidity and mortality in persons with SARS-COV-2/COVID-19?

In order answer the research question to an extent that can inform and influence current practice, a number of objectives were generated as given below (30).

1 Explore the prevalence and types of SARS-COV-2/COVID-19 co-infections reported across the emerging literature to inform comparisons upon clinical outcomes with non-co-infected cases and to ascertain the pathogens contributing to morbidity and mortality rates.

2 Evaluate the impact of co-infections upon morbidity and mortality outcomes in the defined patient group by comparing outcomes with non-co-infected cases of COVID-19.
3 Identify the implications of objectives 1 and 2 for current clinical practice and specifically, the management of COVID-19 and infection and prevention control strategies.
4 Determine ongoing knowledge gaps to generate recommendations for future research.

Chapter 2: Methods

2.1 Methodology and Review Design

Considering the identified research need – to explore the influence of co-infection in persons with SARS-COV-2 and COVID-19 and the abundance of evidence that has recently emerged in response to the pandemic, this research adopted a secondary methodological approach, in order to permit the evaluation of the former evidence. In this regard, secondary methodology took the form of a systematic review of the literature, which is known to generate some of the most impactful and credible evidence to help inform current practice and research – a notable objective of this report(32). Moreover, the novel virulence of SARS-COV-2 and ongoing pandemic of COVID-19 demands the conduct and derivation of knowledge to help manage the problem on a global scale and thus, exploring the data pertaining to co-infection may reveal insight into ideal prevention and management efforts and in risk stratification and prognostication of infected persons. Furthermore, summarising the status of the evidence related to SARS-COV-2 co-infection is useful for the currently pressured academic and clinical professions as it will spare them valuable time in reviewing individual studies (33). A systematic review design was considered the best approach to collectively analysis the primary literature base given that such reviews tend to utilise a stringent set of methods that permit the derivation of valid, reliable and objective findings – evidence that can be used to inform practice and research(34).Thus, as most systematic reviews achieve impactfulness through compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria, the PRISMA checklist was used to inform the methods and reporting of data in this review(35). Justification for conducting this review was also attained after a search of PROSPERO - the International Prospective Register of Systematic Reviews in June 2020, which failed to identify any similar reviews and thus, the evidence herein was deemed novel, insightful, unique and non-repetitive(36).

2.2 Information Sources

The information sources used to inform this review comprised electronic databases that are relevant to health journals and are available online to permit digital searching, retrieval and access to relevant literature. Indeed, electronic databases are the primary source of contemporary reviews as they are known to index all historic, present and in-press research, which was important in identifying emerging studies concerning SARS-COV-2 co-infection (37). Notably, electronic databases also permit the precise searching for literature via the application of syntax and operators, which ensured that all relevant studies could be identified and prevented the retrieval of unmanageable numbers of studies that could have led to delays in generating this review (38).

However, the capacity of databases to identify relevant research depends upon the number and type of databases searched given that each database comprises a unique index of journals and thus, research article coverage. Precluding some databases could have resulted in relevant studies being excluded from this review, which would introduced a type of reporting bias – an issue that the author sought to avoid in order to address the research objectives (39). Therefore, in June 2020 the databases of MEDLINE (PubMed), EMBASE and the Cochrane registry were searched based on the widespread use of these databases among previous systematic reviews and for their high recall accuracies and ability to identify the majority of literature for review constructs (40, 41). Indeed, the searching of MEDLINE and EMBASE together has been demonstrated to provide a recall accuracy of around 95% and thus, the capturing of this proportion of evidence to inform this review was considered sufficient to attain high and impactful credibility (40, 41). However, to ensure all relevant studies were included, the Cochrane library was used to supplement MEDLINE and EMBASE searching and the references of all included articles were screened to permit the recognition of studies indexed to databases other than those searched in this review (31).

2.3 Search Terms

As database searching requires the application of search terms, it was important to develop a series of search terms to ensure all relevant studies would be captured and thus, considered for inclusion in this review. Therefore, the search terms were developed using the PEO/I criteria to ensure that studies identified would be congruent or somewhat relevant to the research question(31). In addition, a scoping search for relevant studies via Google Scholar using PEO/I search terms helped to identify any additional and important terms to complement those derived initially. The search terms were then reviewed and where appropriate, subject to truncation (*), Boolean combination and subject heading mapping, which is a recognised means to developing a precise literature search(31). Specifically, truncation permitted the searching of terms with alternative suffices, Boolean combination using the operators AND and OR facilitated the searching of a string of terms and subject heading mapping enabled some terms to be matched to journal categories – strategies that are known to optimise the validity of literature searches (42). The search strategy used for MEDLINE, EMBASE and the Cochrane registry is summarised in Table 2. Notably, terms pertaining to the outcomes/interest component of PEO/I were precluded from the search as this was judged to increase the risk of missing relevant studies, as has been previously suggested by Aromataris and Riitano (43).

Table 2. Search strategy for MEDLINE, EMBASE and the Cochrane library.

PEO/I	**Population**	**Exposure**	**Outcomes/ Interest**
SearchTerms	1-SARS-COV-2 2-COVID-19	3-Co-infect* 4-Secondary infection	-
Boolean SearchString	1 OR 2	3 OR 4	-

*columns combined with 'AND', *truncation*

2.4 Study Selection

After database searching, the selection of studies suitable for inclusion was facilitated by adopting and utilising the standard process of title/abstract and full-text screening (31). In the first instance, the information given within retrieved studies titles and abstracts was assessed for congruence with the inclusion criteria (see section 3.5) – those meeting the criteria or of unclear relevance entered the second stage, whilst those meeting the exclusion criteria were discarded. For the second stage of full-text screening, the same process was applied to the studies remaining for consideration from the first screening stage – studies remaining after this step were deemed eligible for review.

2.5 Inclusion and Exclusion Criteria

To permit the former process of study selection, various inclusion and exclusion criteria were generated and these related to both research question (PEO/I) and article-specific characteristics. An overview of the restriction criteria applied at each stage of title, abstract and full-text screening is given in Table 3 and justification for this criteria is detailed below. The inclusion criteria was defined as follows: primary research of case report, case series and observational design published in English language since the emergence of SARS-COV-2 in December 2019. This relatively non-selective criteria was chosen to ensure that all possible reports of co-infection among persons infected with SARS-COV-2 were evaluated in this review. It was not deemed necessary to review co-infection related to SARS-COV-1 or other coronaviruses, although such literature was referred to in the discussion section for evaluation purposes. No restrictions were placed upon study setting or methodological quality as this may have precluded important co-infection research being excluded from collective analysis – an additional source of reporting bias. In addition, this systematic review was concerned with appraising the quality of included studies and weighting the reporting of evidence concerning co-infection based on quality and thus, averting the need to apply such restrictions.

Table 3: Summary of inclusion and exclusion criteria by title, abstract and full-text processing.

Research Question	**Is co-infection an important factor influencing the morbidity and mortality of persons with SARS-COV-2/COVID-19?**	
Aim	**To explore the prevalence and types of co-infection in persons with SARS-COV-2/COVID-19 and to evaluate the impact of co-infection upon clinical outcomes.**	
	Inclusion	**Exclusion**
Title Criteria	1) Studies (case reports, case series and observational studies)	1) **Studies in non-English language** 2) **Research of secondary**

	reporting upon SARS-COV-2/COVID-19 co-infections. 2) Publication since December 2019. 3) English text.	**review design** 3) **Studies concerning SARS-COV-1 or coronaviruses other than SARS-COV-2.** 4) **Studies failing to report upon co-infections in persons with SARS-COV-2/COVID-19.**
Abstract Criteria	1) Abstracts discussing the prevalence of co-infection among cohorts of persons with SARS-COV-2/COVID-19. 2) Abstracts discussing the types of co-infections present in persons with SARS-COV-2/COVID-19. 3) Abstracts discussing variances in outcomes between co-infected persons and non-co-infected persons.	1) **Studies failing to report upon the characteristics and outcomes related to co-infection in persons with SARS-COV-2/COVID-19.**
Full-text Criteria	1) Human studies 2) Primary original research. 3) Full-texts discussing abstract inclusion criterion 3 plus or minus criteria 1 and 2.	1) **Animal or laboratory-based studies** 2) **Full-texts failing to discuss abstract inclusion criterion 3.**

2.6 Risk of Bias

The assessment of methodological quality of all included studies was guided by the use of a validated appraisal tool for case reports, case series and observational studies, which comprised the Joanna Briggs Institute (JBI) approach. The JBI tools were selected given their widespread use by academic review researchers and their validation in guiding accurate, robust and reproducible appraisals of research (44).The importance of appraising the quality of research cannot be undermined given that biases and methodological issues can markedly affect the truthfulness of results reported by primary research studies – a problem that could lead to a misinformed review (45). Thus, the JBI tools for the former study designs helped to avert this problem by enabling the author to conduct a guided and systematic process of

detecting and ascertaining the impact of various systematic biases (44). The JBI checklists comprise a series of questions that seek to elicit methodological issues or biases or in confirming their absence and to explore whether such issues impacted the results reported (appendices 1-3). Answers to each of the quality questions were reported as either yes, no, unclear or not applicable (44). However, as the JBI checklists do not permit the scoring of each studies overall quality and risk of bias, a simple system was developed to help avoid relying upon the authors subjective views, which could generate poorly reproducible assessments of study quality. In this regard, studies with two or more biases were categorised as having a high risk of bias, whilst those with less than two biases were rated as having a low risk of bias. Finally, the JBI tool does not permit assessment of external validity and as this can be an important factor influencing the ability to generalise and apply findings to specific populations, a subjective assessment of external validity was performed based on each studies characteristics of the populations evaluated and their geographic setting (46).

2.7 Data Extraction

Whilst all data required for appraisal of study quality and the synthesis of results was available through online access to full-texts, relevant data was extracted to optimise data management and to ensure accuracy and an error-free approach to the former processes. The importance of data extraction is a well-recognised factor influencing the quality of systematic review research given that errors can lead to results that deviate from the truth and this was important to avoid as to generate informative research into the impact of co-infection upon outcomes of persons with COVID-19 (47). Therefore, select data was extracted from each study using a direct transcribing method to translate the information from electronic texts into a central database, which was guided by pre-developed proformas that have been published by the Cochrane library (48).

2.8 Data Synthesis

The collective analysis of findings pertaining to the impact of co-infection upon clinical outcomes in persons infected with SARS-COV-2 was performed using a descriptive and narrative approach, in order to ensure all relevant data and factors influencing outcomes was reported in its entirety. The approach to narrative synthesis employed the method described by Lisy and Porritt (49) as this represents the best practice standard for descriptive reported of review research. Notably, a meta-analysis was not possible to conduct given the nature of the research question and clinical outcomes and the marked level of heterogeneity present among included studies (50).

2.9 Review Permissions and Ethics

Permission to conduct this systematic review was provided by the local university after submission and review of a proposal document. No ethical approval was required due to the secondary methodology employed. In keeping with ethical research practice, all studies included in this review were screened for ethical approval statements and the author of this review complied with guidance set out by the UK Medical Research Council (51).

Chapter 3: Results

3.1 Study Selection

The combined searching of CINAHL, MEDLINE and EMBASE for relevant literature in June 2020 using the search methods defined in sections 2.2 and 2.3 led to the retrieval of 575 studies. Among these studies, there were 28 replicates and these were removed from further inclusion considerations via EndNote referencing management software. Therefore, the remaining 547 studies were exposed to the title and abstract screening process where application of the limiting criteria defined in Table 3 was used to exclude irrelevant studies (n=523) and to retain eligible and potentially eligible studies (n=24) for full-text review. Reasons for exclusion at this stage was largely due to studies reporting data irrelevant to SARS-COV-2 and non-English studies. For the second stage of full text screening for the remaining 24 studies, an additional four studies were excluded for failing to meet the inclusion criteria – the reason for all four studies was the lack of reporting of morbidity and mortality outcomes in the defined patient group. Therefore, 20 studies were retained for inclusion in this review. A summary of the former filtering process is provided in figure 1.

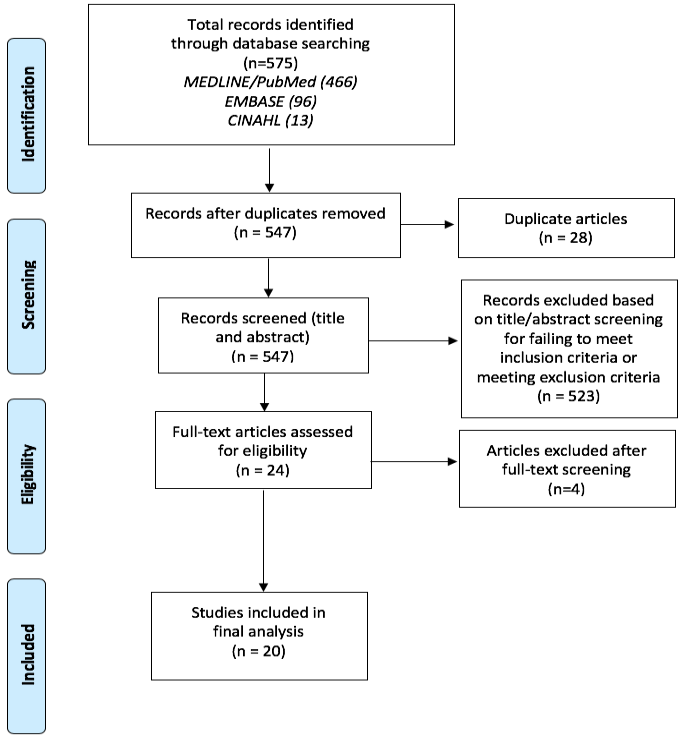

Figure 1. PRISMA filtering diagram showing the processes leading to study eligibility.

3.2 Study Characteristics

Of the 20 studies included in this review, seven were isolated case reports (52-58), three were case series (59-61) and 10 were retrospective observational studies (29, 62-70). A summary of the characteristics of subjects and the co-infective pathogens isolated among those with COVID-19 has been provided in Table 5 in section 3.5.

3.3 Risk of Bias

Whilst the designs of studies included in this review would have imparted implications upon the methodological quality and thus, risk of bias, particularly with case reports, series and retrospective cohort studies being susceptible to numerous systematic biases, as well as observing poor generalisability to larger or more general populations, a systematic appraisal of each study using the JBI frameworks was completed (71). A summary of the methodological quality of each study in accordance with the case report, case series and cohort study frameworks and using the risk of bias categorisation criteria defined in section 2.6, is provided in Table 4 below. Among case reports and case series, the main sources of bias usually arise from issues related to the small sample sizes (either isolated cases or cases of <10 subjects) as this does not permit the valid statistical analysis of an effect size or association (72). In addition, case reports and case series are reported using a descriptive and narrative approach, which can increase the risk of author subjectivity and reporting that deviates from the objective truth. Furthermore, the small sample sizes of the former research designs observe markedly poor generalisability to other larger cohorts or populations, which impairs the utility and influence of such evidence upon clinical practice and related decision making (72). Almost all of the case reports and series included in this review were subject to the former limitations and thus, most were categorised as having a high risk of bias (52-61). For the remaining 10 cohort studies, these were of retrospective design and this type of research, as with case series and reports, also falls towards the lower aspect of hierarchical evidence pyramids, which are ordered in terms of their internal validity and thus, the detection of numerous systematic biases was expected (73).The most common methodological issues with retrospective studies include the following: lack of a control group, retrospective temporality prevents the ability to infer causation, susceptibility to attrition, selection of outcome measurement tools can affect the outcomes and residual confounding that can bias the outcome measures (73). Indeed, the majority of these biases were detected across the cohort studies included in this review and thus, most were rated as having a high risk of bias. Overall, two of seven case reports, two of three case series and three of 10 cohort studies were of a low risk of bias, leaving the remaining 13 studies at high risk of bias.

Table 4. Critical appraisal summary using JBI frameworks (44).

JBI Questions **Studies**	**Case Report (Q1-8)** **Case Series (Q1-10)** **Cohort Studies (Q1-11)**											**Overall Risk of Bias**
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**	**Q9**	**Q10**	**Q11**	

Arashiro *et al.* (52)	Y	Y	Y	Y	Y	Y	Y	Y	-	-	-	LOW
Azekawa *et al.* (53)	Y	N	Y	Y	N	Y	Y	Y	-	-	-	HIGH
Blasco *et al.* (62)	N	Y	N	N	Y	Y	Y	Y	Y	Y	Y	HIGH
Chen *et al.* (63)	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	LOW
Cuadrado-Payan *et al.* (59)	N	Y	Y	Y	Y	N	N	Y	Y	Y	-	HIGH
Fan *et al.* (54)	Y	Y	N	Y	N	Y	Y	Y	-	-	-	HIGH
Kim *et al.* (64)	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Konala *et al.* (55)	Y	N	N	Y	Y	Y	Y	Y	-	-	-	HIGH
Lin *et al.* (65)	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	LOW
Lv *et al.* (66)	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Ma *et al.* (67)	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	HIGH
Nowak *et al.* (29)	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	HIGH
Stochino *et al.* (60)	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	-	LOW
Tadolini *et al.* (68)	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	LOW
Touzard-Romo, Tapé and Lonks (56)	N	Y	Y	Y	Y	Y	Y	Y	-	-	-	LOW
Wang *et al.* (69)	Y	N	Y	N	N	Y	Y	Y	Y	Y	Y	HIGH
Wee *et al.* (61)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	LOW
Wehl, Laible and Rauchenzauner (58)	Y	N	N	Y	Y	Y	Y	Y	-	-	-	HIGH
Wu *et al.* (57)	Y	N	Y	Y	N	Y	Y	Y	-	-	-	HIGH

Zhu *et al.* (70)	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	HIGH

3.5 Evidence of Interest

A summary of the outcomes pertaining to the rate, types and clinical impact of co-infections in persons with COVID-19 has been provided in Table 5 and such outcomes are also described in greater detail in the following text. As estimates of the prevalence of SARS-COV-2 co-infections could only be drawn from studies of cohorts of persons with COVID-19, the data of the 10 retrospective studies was used to evaluate this outcome(29, 62-70). The frequency of co-infections in patients with COVID-19 were reported as follows: 2.9% by Blasco *et al.* (62), 5% by Chen *et al.* (63), 9.5% by Kim *et al.* (64), 3.2% by Lin *et al.* (65), 4.0% by Lv *et al.* (66), 49.5% by Ma *et al.* (67), 3.0% by Nowak *et al.* (29), 18.3% by Tadolini *et al.* (68), 17.2% by Wang *et al.* (69) and by 94.2% Zhu *et al.* (70). Notably, the markedly high rate of co-infection (49.5% and 94.2%) reported by Ma *et al.* (67) and Zhu *et al.* (70) is unlikely to represent a true estimate of the prevalence of co-infection among COVID-19 cases given that the authors adopted methods that actively sought to recruit cases with co-infection. Excluding these anomalies, the crude co-infection rate was calculated using the former rates to generate a mean frequency of 6.7%. The types of pathogens responsible for co-infection across all studies were as follows: bacteria; *Mycoplasma pneumoniae, Acinetobacter baumanii, Klebsiella pneumoniae, Escherichia coli, Staphylococcus haemolyticus, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Enterococcus faecium, Enterobacter clocae, Mycobacterium bovis* and *Mycobacterium tuberculosis*, viruses; influenza virus A, influenza virus B, respiratory syncytial virus, rhinovirus, enterovirus, Boca virus, parainfluenza virus, metapneumovirus and non-SARS-COV-2 coronaviruses, such as coronavirus HKU1 and coronavirus NL63 and fungi; *Aspergillus flavus, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida lustiniae* and *Candida albicans*.

The impact of co-infections upon clinical outcomes was reported more variably across studies included in review and thus, relevant measures have been described in the following body of text. In one of the most insightful studies, Ma *et al.* (67) found that patients with COVID-19 and who were co-infected with influenza virus observed a much greater level of inflammation and organ dysfunction, as compared to those without co-infection. Across the cohort of 93 patients with COVID-19, the authors showed that those with influenza A virus observed a higher incidence of acute renal injury (37% v. 23.4%) and acute cardiac injury (41.3% v. 25.5%), which were approaching statistical significance when compared with the non-co-infected cases ($p=0.11$ and $p=0.15$). However, the co-infected and non-co-infected groups observed comparable incidences of acute respiratory distress syndrome (41.3% v. 42.6%, $p=0.90$) and hepatic dysfunction (17.4% v. 19.1%, $p=0.83$). Among patients who died, the authors also found that the incidence of acute renal injury (68.2% v. 45.5%) and acute cardiac injury (86.4% v. 54.5%) were higher among the co-infected group, than the non-co-infected group, and notably, the between group difference for acute cardiac injury became significant ($p=0.04$). However, the incidence of acute respiratory distress syndrome and hepatic dysfunction remained insignificantly different between

groups (81.8% v. 90.0%, p=0.38 and 22.7% v. 22.7%, p=1.0, respectively). The authors also analysed the laboratory parameters of the cohort to elucidate some of the factors influencing the differences in clinical outcomes between co-infected and non-co-infected cases. The results showed that a significantly higher proportion of patients with co-infection had levels of D-dimer that exceeded 5 micrograms/ml, than compared to those without co-infection (38.6% v. 11.4%, p<0.01). Among the entire cohort there were no significant differences between groups for white cell count, neutrophil and lymphocyte counts or levels of troponin, brain natriuretic peptide, tumour necrosis factor alpha, interleukin-6, creatinine, C-reactive protein, lactate dehydrogenase, alanine transaminase and aspartate transaminase (all p >0.05), although co-infected non-survivors observed significantly higher levels of D-dimer, white cells, neutrophils, creatinine and tumour necrosis factor alpha, than compared to non-co-infected non-survivors (p<0.05). Two other studies reported specifically upon the clinical impact of co-infection. In the cohort study by Zhu *et al.* (70), the rates of viral, bacterial and fungal co-infections among cases of COVID-19 were greater among those who were categorised as having a severe or critical clinical status, although this was not significantly different when compared to those with mild and moderate clinical statuses (p>0.05). In another study,Tadolini *et al.* (68) found that persons with COVID-19 and co-infected with Mycobacterium tuberculosis or Mycobacterium bovis, the median hospital stay was 15 days with most requiring oxygen supplementation and ventilatory support. The overall case fatality rate was 12.3% and notably, those with tuberculosis accounted for the majority of fatalities (10.2%). Among the remaining studies, the authors as active clinicians, reported simple observational accounts of their experience in managing patients with COVID-19 where it was consistently recognised that co-infection tended to be associated with more severe COVID-19 pneumonias, respiratory failure, sepsis and death(14, 23, 29, 62, 64-66).

Among the seven case reports included in this review, clinical outcomes were described as follows below but overall one patient died (52), two survived (53, 58) and four outcomes were unclear (54-57). In the case of the patient who died, Arashiro *et al.* (52) reported COVID-19 co-infection with Legionella pneumophilia and whilst the patient was initially found to have only patchy infiltrates on imaging, the patient later developed respiratory distress and required ventilation on day 13 and died some 10 days later on day 23.Among the other case reports, most patients developed consolidation of pulmonary air spaces on chest x-ray and computed tomography scanning, in keeping with COVID-19 pneumonia, and other complications secondary to SARS-COV-2 infection included acute renal failure and ventilator dependence. Finally, among the three case series, Cuadrado-Payan *et al.* (59) revealed that patients co-infected with influenza A virus observed an early onset of respiratory distress and failure requiring intubation and ventilation, whilst Stochino *et al.* (60) showed that co-infection with Mycobacterium tuberculosis conferred a case fatality rate of 5% but in contrast, Wee *et al.* (61) showed that all co-infected patients survived COVID-19 and did not develop pneumonia or require the need for ventilatory support.

Table 5. Summary of included studies.

Study and Setting	*Design*	*Participants*	*Sample Size*	*Co-Infection*	*Diagnostic Test (SARS-COV-2)*	*Positive/ Negative SARS-COV-2 Tests*	*Main Findings*
Arashiro *et al.* (52) Japan	Case report	An 80 years of age male who tested positive for SARS-COV-2.	1	SARS-COV-2 and Legionella pneumophila serotype 1.	RT-PCR	1/0	Patient was admitted to hospital following a cruise with a cough and fatigue. Developed a positive Legionella urine antigen test on day 7 post admission and a positive SARS-COV-2 test one day 8-9.
Azekawa *et al.* (53) Japan	Case report	A 78 year old woman who tested positive for SARS-COV-2 and influenza A virus.	1	SARS-COV-2 and influenza virus.	RT-PCR	1/0	Patient was admitted with cough and fatigue. After evidence of infiltrates on chest x-ray, she was tested for SARS-COV-2, which was positive and a simultaneous influenza A test was also positive.
Blasco *et al.* (62) Spain	Cohort study	Patients who tested positive for SARS-COV-2 and developed interstitial	183	SARS-COV-2 and influenza A virus, coronavirus HKU1, Mycoplasma pneumoniae and respirator	RT-PCR	183/0	Three patients were co-infected with SARS-COV-2 and respiratory viruses as follows: coronavirus HKU1 (n=1), Mycoplasma pneumoniae

		pneumonia.		y syncytial virus.			(n=1) and influenza virus A plus respiratory syncytial virus (n=1).
Chen *et al.* (63) China	Cohort study	Patients who tested positive for SARS-COV-2 and had pneumonia during Jan 1 – Jan 20, 2020.	99	SARS-COV-2 and Acinetobacter baumanii, Klebsiella pneumoniae, Aspergillus flavus, Candida glabrata and Candida albicans.	RT-PCR	99/0	One patient was co-infected with SARS-COV-2 and a combination of Acinetobacter baumanii, Klebsiella pneumoniae and Aspergillus flavus. One patient was co-infected with SARS-COV-2 and Candida glabrata and three patients were co-infected with SARS-COV-2 and Candida albicans.
Cuadrado-Payan *et al.* (59) Spain	Case series	Patients who were diagnosed with SARS-COV-2 pneumonia.	4	SARS-COV-2 and influenza virus.	RT-PCR	4/0	Four patients were co-infected with SARS-COV-2 and influenza A and B viruses. All had a history of hypertension, two had renal disease and two had type 2 diabetes.
Fan *et al.* (54) Singapore	Case report	A 36 years of age man who was diagnosed with SARS-COV-2 and COVID-19	1	SARS-COV-2 and Mycoplasma pneumonia infection.	Unclear	Unclear	Patient was found to have severe lymphopenia and moderate thrombocytopenia. The peripheral blood smear revealed cold agglutination and roulaeux formation with

		pneumonia.					reactive lymphocytes. Antibody screen demonstrated a Mycoplasma pneumoniae antibody titre of 1:160.
Kim *et al.* (64) United States	Cohort study	Patients who tested positive for SARS-COV-2 in Stanford Hospital, California. Mean age was 46.9 years.	116	SARS-COV-2 and other respiratory viruses (rhinovirus, enterovirus, influenza virus, parainfluenza virus, metapneumovirus and non-SARS-COV-2 coronaviruses)	RT-PCR	116/1217	Of the 116 patients with SARS-COV-2, there were 25 cases of co-infection with influenza A virus (n=1), respiratory syncytial virus (n=6), parainfluenza virus (n=3), metapneumovirus (n=2), rhinovirus/enterovirus (n=8) and other coronaviridae (n=5).
Konala *et al.* (55) United States	Case report	A 66 years of age female who was diagnosed with SARS-COV-2 and COVID-19 pneumonia.	1	SARS-COV-2 and influenza A virus	Unclear	1/0	Patient presented with a fever, cough, shortness of breath and reduced appetite. Multiple comorbidities. Deteriorated to require intensive care unit admission for ventilation.
Lin *et al.* (65) China	Cohort study	Patients who tested positive for SARS-COV-2 within Shenzhen hospital	92	SARS-COV-2 and respiratory syncytial virus, coronavirus HKU1, parainflue	RT-PCR	92/0	Of the 96 patients with SARS-COV-2, six patients were co-infected with respiratory syncytial virus (n=3), coronavirus HKU1 (n=1),

				nza or metapneumovirus.			parainfluenza virus (n=1) and metapneumovirus (n=1).
Lv *et al.* (66) China	Cohort study	Patients with COVID-19 (23% co-infected with other respiratory pathogens)	354	SARS-COV-2 and numerous respiratory pathogens of viral, bacterial and fungal types.	RT-PCR	354/0	Pathogens comprised: Acinetobacter baumanii, Escherichia coli, Candida species, Mycoplasma pneumoniae, Pseudomonas aeruginosa, Staphylococcus haemolyticus and others. Clinical outcomes are reported descriptively.
Ma *et al.* (67) China	Cohort study	Patients with COVID-19.	93	SARS-COV-2 and influenza A virus.	RT-PCR	93/0	A large proportion of the mortality was attributed to patients with co-infections. These subjects were also found to have markedly higher levels of inflammatory biomarkers and markers of organ dysfunction, as compared to non-co-infected persons.
Nowak *et al.* (29) United States	Cohort study	Patients who tested positive for SARS-COV-2 and other respiratory viral pathogens	36	SARS-COV-2 and other respiratory viruses (rhinovirus, enterovirus, influenza and non-SARS-	RT-PCR	36/0	Of the 36 patients who were co-infected with SARS-COV-2 and other respiratory viruses, the pathogens identified included: influenza A virus (n=1), respiratory

		admitted to hospitals within the New York metropolitan area during March-April 2020. Mean age was 60.1 years.		COV-2 coronaviruses).			syncytial virus (n=4), non-SARS-COV-2 coronaviruses (NL63: n=7, HKU1: n=5, 229E: n=4 and OC43: n=1), rhinovirus/enterovirus (n=8), metapneumovirus (n=4) and adenovirus (n=2). There were no reported cases of co-infection with parainfluenza virus or influenza B virus.
Stochino *et al.* (60) Italy	Case series	Patients with COVID-19 and tuberculosis.	20	SARS-COV-2 and Mycobacterium tuberculosis or Mycobacterium bovis.	RT-PCR	20/0	There was a suggestion that co-infection was associated with greater morbidity.
Tadolini *et al.* (68) Europe	Cohort study	Patients with COVID-19 and tuberculosis.	49	SARS-COV-2 and Mycobacterium tuberculosis or Mycobacterium bovis.	RT-PCR	49/0	As above.
Touzard-Romo, Tapé and Lonks (56) United States	Case report	A 57 year old female who presented with cough and dyspnoea who was diagnosed with metapneu	1	SARS-COV-2 and human metapneumovirus.	RT-PCR	1/0	The patient was initially admitted with symptoms suggestive of an infective exacerbation of her pre-existing obstructive sleep apnoea. A nasopharyngeal swab was taken for testing using a respiratory virus

		movirus and later SARS-COV-2.					panel and SARS-COV-2. The first result was positive for metapneumovirus as was the SARS-COV-2 test reported some 24 hours later.
Wang *et al.* (69) China	Cohort study	Patients who tested positive for SARS-COV-2 with a mean age of 56 years.	2,745	SARS-COV-2 and other respiratory viruses.	RT-PCR	2,745/8,274	A total of 104 patients received nucleic acid testing for other respiratory viruses and of these, six were co-infected with respiratory viruses of influenza A virus (n-2), rhinovirus (n=2), influenza A H3N2 (n=1) and other coronaviruses (n=3).
Wee *et al.* (61) Singapore	Case series	Patients with COVID-19.	736	SARS-COV-2 and various respiratory viruses.	RT-PCR	736/3,807	Co-infection rate was reported to be 1.4% and co-infected patients required more ventilation and observed poor respiratory outcomes.
Wehl, Laible and Rauchenzauner (58) Germany	Case report	1 four month old infant with COVID-19.	1	Co-infection with influenza A virus.	RT-PCR	1/0	Observed a mild to moderate course of COVID-19 disease and survived.

Wu *et al.* (57) China	Case report	First reported case of co-infection in a 69 years of age man who was diagnosed with COVID-19 related pneumonia.	1	SARS-COV-2 and Influenza A virus.	RT-PCR	1/0	The patient had been admitted to hospital and was positive for influenza A virus and negative for SARS-COV-2. He was discharged but re-admitted within a few days with fever and dyspnoea. Repeated nasopharyngeal swabs remained negative for SARS-COV-2 but bronchial aspirates taken four days later were positive for SARS-COV-2 using next generation sequencing.
Zhu *et al.* (70) China	Cohort study	Patients with COVID-19 and various respiratory pathogens.	24	SARS-COV-2 and other respiratory viruses.	RT-PCR	24/242	Bacterial co-infections predominated in patients with COVID-19 and were associated with a poorer clinical course of COVID-==========19 disease.

Chapter 4. Discussion

4.1 Summary of Findings

In light of the current COVID-19 pandemic and its highly deleterious impact upon individuals and societies, worldwide, this systematic review sought to explore the extent, types and influence of co-infection upon clinical outcomes in patients with SARS-COV-2. Using a narrative approach to describing the findings of published case reports, case series and cohort studies, this review found that co-infection is a frequent problem among patients hospitalised with COVID-19 where the rate was found to vary between 2.9% and 18.3%, equating to a mean frequency of 6.7%. The types of co-infective pathogens varied markedly, although the majority of isolated

microorganisms of patients with COVID-19 were either other non-SARS-COV-2 viruses or bacteria with affinity or virulence to infect the upper and lower respiratory tract. The commonest bacteria, viruses and fungi isolated from patients with COVID-19 co-infections were: *Mycoplasma pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Klebsiella pneumoniae,* influenza virus type A, respiratory syncytial virus, rhinovirus, enterovirus, non-SARS-COV-2 coronaviruses and Candida species. The presence of co-infection was found to exacerbate the clinical course and outcomes of COVID-19 in most cases. Co-infection was associated with more severe host inflammatory responses and in turn, this correlated with reports of pneumonia, acute respiratory distress syndrome, respiratory failure, sepsis, acute renal failure, acute cardiac dysfunction, poor weaning from ventilation and ventilator dependence, protracted lengths of staying in hospital and/or intensive care units and a more rapid deterioration and time to death. The case fatality among patients with COVID-19 co-infection was crudely estimated to be 10%, although few studies were able to report upon the comparative mortality rate between co-infected and non-co-infected cases.

4.2 Evaluation and Previous Literature

Considering the findings of this review, as summarised above, it is imperative to evaluate the outcomes to ascertain the meaning and impact of co-infection and to discuss the results in the light of the wider literature, in order to help validate the findings and the implications and recommendations drawn from this research. Whilst this systematic review represents the first review to explore the impact of COVID-19 co-infection upon clinical outcomes, the data concerning the frequency and types of co-infections has been previously explored in a systematic review and meta-analysis by Lansbury *et al.* (74). The former authors conducted a search of databases with similar coverage to that used herein and identified 30 studies comprising 3,834 patients. The results showed that the rate of bacterial co-infection in patients admitted to hospital with COVID-19 was 7% (95% CI 3, 12) and notably, the rate of co-infection was much greater in those admitted to intensive care units (14%; 95% CI 5, 26), as compared to general ward settings (4%; 95% CI 1, 9). Indeed, the general co-infection rate of 7% is similar to that reported in this review where the crude frequency was reported as 10%. Moreover, Lansbury *et al.* (74) revealed that the most common bacterial pathogens responsible for co-infection were *Mycoplasma pneumoniae, Pseudomonas aeruginosa and Haemophilus influenzae*, whilst the most common co-infective viral pathogens were influenza A virus and respiratory syncytial virus, which is also similar to this review. However, Lansbury *et al.* (74) showed that the pooled frequency of viral co-infection was only 3% (95% CI 1, 6), whilst this review identified that viral co-infections were as or even more common than bacterial co-infections. The former meta-analysis also revealed that the frequency of co-infection with fungal pathogens was 5.5% but this was only based on the rates of three studies with cohorts susceptible to opportunistic infections and thus, this frequency is likely to be a marked over-estimate of the overall frequency of fungal co-infections in patients with COVID-19. Indeed, the specific fungal organisms (Candida species and Aspergillus species) contributing to co-infections were also identified as the primary fungal pathogens in this review. Whilst the findings of Lansbury *et al.* (74) provide

important insight into the rate and types of COVID-19 co-infections, the informing studies were at moderate to high risk of bias and particularly selection bias, which reduces confidence and certainty that the collective cohort was representative of populations with COVID-19 disease.

This review was primarily concerned with the impact of co-infection upon the clinical course and outcomes of COVID-19 and when comparing the findings to the influenza virus (H1N1) pandemic that occurred in 2009, bacterial co-infections were found to be markedly common with a crude frequency of 23% among patients with severe and fatal respiratory complications(75).The systematic review by MacIntyre *et al.* (75)comprised an evaluation of 75 studies and among 11 of these, co-infection was present in 23% of cases who underwent post-mortem analysis and the most common bacterial organism isolated was found to be *Streptococcus pneumoniae*. Bacteria were also isolated from patients hospitalised with influenza H1N1 and surviving the resulting pneumonia and other complications, which occurred with a frequency of 19% and *Streptococcus pneumoniae* being the main pathogen of co-infection in 54% of cases. In contrast to this review, *Streptococcus pneumoniae* was one of the less frequently isolated organisms from patients with COVID-19 co-infection and this may suggest that specific viral factors play a role in types of bacteria that can colonise and infect injured lung tissue. However, MacIntyre *et al.* (75) also found that *Actinobacter baumanii* was a common bacteria accounting for co-infection in influenza H1N1 virus cases, which was a frequent organism isolated from patients with COVID-19 and thus, suggesting the contrary in that there are likely to be shared viral, bacterial and host factors influencing the occurrence and course of co-infection in viral respiratory disease. The findings of MacIntyre *et al.* (75) have also been supported in a meta-analysis by Klein *et al.* (76) who explored the frequency of co-infections across all types of influenza viruses and found that the rate varied between 2% and 65% and the types of bacteria were comparable to that reported formerly. However, these reviews were both affected by marked inter-study heterogeneity (I2 >90%), which increases concern for biased estimates of co-infection rates across the informing studies and thus, the pooled rates are unreliable. Despite this, the bacteria of co-infections in patients with influenza H1N1 virus were predominantly commensal or acquired organisms with affinity for the upper respiratory tract and thus, the immunosuppressive state of critical illness and/or the insertion of artificial lines through the naso- or oro-pharynx could directly introduce such organisms into the lower respiratory tract to encourage secondary infection(77).Indeed, such factors could also account for the high rate and adverse clinical impact of COVID-19 co-infections reported in this review, although the commonest organisms were different from those of influenza virus and thus, the iatrogenic transference of commensal bacteria may only confer a small contribution to co-infections in COVID-19.

However, the bacteria accounting for COVID-19 co-infections are more comparable to the types of co-infections evident in the previous coronaviruses of MERS and SARS-COV-1. For example, Arabi *et al.* (78)showed that co-infections among a sample of 12 patients with MERS-COV in a Saudi Arabian hospital included the pathogens of influenza B virus and *Streptococcus pneumoniae*, although co-infection only occurred in two patients and the overall sample was poorly representative of the wider MERS-COV population. In another study but one conducted among a cohort of

patients affected by SARS-COV-1, Hwang *et al.* (79)showed that respiratory co-infective pathogens were limited to Aspergillus species and whilst this fungus was identified in this review, the reliability of the findings was also limited by the small sample size. Whilst these studies also showed that MERS-COV and SARS-COV-1 observed high mortality rates, no comparisons were made between co-infected and non-co-infected cases to elicit any differences in outcomes. However, co-infections appear to have been much less common when compared to that reported for the current SARS-COV-2 pandemic. Indeed, in a review of 47 cases of MERS-COV in Saudi Arabia, Assiri *et al.* (28)found that there were no confirmed cases of co-infection with either bacteria, non-coronaviruses or fungi. Whilst this suggests that co-infection in COVID-19 is more common, such inferences cannot be reliably drawn given that the risk of co-infection is markedly complex, receiving influence from host, viral, bacterial, fungal, other pathogen and environmental factors.In this review, gram-negative bacteria were frequent pathogens among co-infected patients with COVID-19 and indeed, these have been previously associated with hospital acquired infections and specifically, hospital-acquired and ventilator-associated pneumonias (80). Hospital-acquired pneumonias tend to be associated with higher morbidity and mortality rates in persons with respiratory disease and distress as a result of the additional inflammatory and infective burden that can overwhelm compensatory responses that attempt to preserve ventilation and gas exchange(81).However, it is important to acknowledge that the detection and confirmation of bacterial organisms can be dependent upon the quality and site of specimens taken, as well as the immunogenic responses of seroconversion, that can lead to delays in antibody production, and bacterial expression factors, which may lead to false-positive results as a result of cross-reactivity (82). The detection of serum immunoglobin M for *Mycoplasma pneumoniae* has been previously shown to have poor specificity for the organism and thus, there is a high risk of over-estimating co-infection rates when relying upon antibody tests(83). This may account for the some of the variance in the detection of bacteria among patients with COVID-19 reported in this review, although as viruses and fungi tend to be isolated using more sensitive and specific techniques, such as polymerase chain reaction, false-positives are unlikely to have been a major issue among the informing studies(84).

In regard to viral co-infections in patients with COVID-19, the most common viruses were the influenza and respiratory syncytial viruses and indeed, given the predominant emergence of SARS-COV-2 during the colder winter months of the year and with peaks of common respiratory viruses occurring over this time, such co-infections are not a surprising finding(85). However, some diligence has to be adopted when interpreting the detection of viruses by either polymerase chain reaction or other tests given that false-positive results can still arise but these are much less frequent when compared to bacterial antibody tests and thus, the findings of this review and other co-infection studies are reliable with moderate to high certainty(86). In addition to the seasonal risks of respiratory co-infection, the reasons for the detection of various respiratory viruses in patients with COVID-19 is not well understood but may be due to the virologic advantage of co-infection where host exposure and responses to different viral antigens and injurious effects may induce a competitive micro

environmental benefit for SARS-COV-2 infection and invasion(87).However, what is more complex to understand is the impact of co-infective competitiveness among respiratory viruses upon disease severity and prognosis in patients with COVID-19 disease. Previous studies have shown that co-infection of the lower respiratory tract between non-coronaviruses can produce a clinical phenotype and outcome similar to that of mono-virus infection, although others have shown that co-infection can impair host immune defences and lead to a more severe clinical course and related morbidity and mortality (88, 89). Indeed, the latter factor is most likely to account for at least some of the greater morbidity and mortality observed in persons with COVID-19. In this review multi-organ dysfunction, ventilator dependence and death were common and reported to occur more rapidly as compared to cases lacking co-infection. In a prior retrospective cohort of patients admitted to a number of intensive care units for the treatment of respiratory distress secondary to viral co-infections due to influenza A and B viruses, Goka *et al.* (90) showed that the mortality rate was significantly higher, than compared to those lacking co-infection. The odds ratio was reported to be 22.0 (95% CI 2.2, 219) and the level of significance was high (p=0.008), although the confidence interval was broad, which reduces certainty in the significance of the effect reported. A similar observation was also found for influenza virus and respiratory syncytial virus co-infection, although the mortality effect was not statistically significant between groups (p>0.05). However, there is little literature to support the clinical impact of co-infection in the context of COVID-19, although one animal study showed that co-infection with SARS-COV-1 and reovirus led to a high and rapid mortality rate in a porcine model but this cannot be generalised to reflect the outcomes occurring in humans or for models infected with SARS-COV-2(91). Another previous study explored the impact of co-infection upon cells and pigs infected with a respiratory syndrome virus and influenza and of interest was that co--infection was not only associated with undesirable effects but the outcomes depended upon which virus initially colonised and infected the models(92). In this review and its informing studies reporting upon COVID-19 co-infections, the initial virus to colonise and infect patients (SARS-COV-2 versus other pathogens) was not and cannot be reliably ascertained using currently employed tests with the exception that there can beconsiderable temporal differences in detection of co-infective pathogens. In theory, the order and temporality in the colonisation and infection of pathogens in patients with COVID-19 could impact the clinical course and outcomes as a result of dynamic host susceptibilities to infection and tissue injury over the course of SARS-COV-2 infection(93).Another factor that may be responsible for variances in the clinical outcomes of persons with COVID-19 co-infection is the inhibitory effect that viruses can impart upon other viruses, which has been previously supported by Shinjoh *et al.* (94) where influenza A virus was found to supress the replication of respiratory syncytial virus *in vitro*. However, little is known about such viral-viral interactions occurring with SARS-COV-2 and thus, this requires exploration in future research as will be discussed in the relevant subsection of this report.

The third pathogen identified from respiratory isolates of patients with COVID-19 in this review were fungi and mostly the fungal organisms of Aspergillus and Candida species, which is partly surprising given that fungal respiratory disease tends to only occur in individuals with chronic immunosuppressed systems, such as due to cystic fibrosis or acquired immunodeficiency syndrome(95).However, there have been a

number of recent reports of invasive fungal infections occurring in patients with COVID-19, particularly invasive aspergillosis, and this may suggest that SARS-COV-2 factors enhance the risk of fungal infection and invasion or that patients with COVID-19 are at risk of significant immunosuppression(96, 97).These reports revealed that the majority of patients developed invasive aspergillosis even in the absence of prior immunodeficiency and they observed moderate to severe acute respiratory distress syndrome that often required last-resort intervention using extracorporeal membrane oxygenation to support gas exchange and prevent death. Indeed, a similar clinical course has also been previously reported for co-infections of influenza virus and Aspergillus species among populations also lacking previous immunosuppression, suggesting that unique viral-fungal interactions may be responsible for rapid immune system dysfunction(98, 99).Notably, the frequency of invasive fungal infections may have been under-reported thus far among the COVID-19 literature given that accurate diagnosis relies upon bronchoscopic aspiration – an investigation that is associated with respiratory aerosols and thus, this is likely to alienate clinicians in performing the test given the risks to themselves and other staff and patients(100). In addition, the management of fungal respiratory disease often relies upon drugs with marked nephrotoxicity and thus, such medicines often have to be discontinued in patients with critical illness due to the frequent onset of acute renal and multi-organ failure(101).Finally, the treatment of patients with non-fungal COVID-19 co-infections could also be compounded by multi-drug resistant bacteria and/or viruses, which were seldomly detected among informing studies of this review and thus, poor response to therapy would have clear implications for prognosis(102).

4.3 Review Strengths

The primary methodological strengths of this review are that is represents the first review to explore the impact of co-infection upon the outcomes of patients with COVID-19 and thus, it has the opportunity to both influence the clinical management and outcomes of COVID-19 disease and areas of priority for ongoing research. In addition, an evidence-based search for relevant literature was performed and appraisal and synthesis of the findings was conducted in accordance with the PRISMA approach, which is known to help account for and reduce bias(35).Moreover, the findings of this review were considered in light of each studies limitations and thus, the confidence and certainty of the findings were reported with sufficient objective as to reduce concern for reporting bias. Furthermore, reporting bias was also avoided by the narrative approach that this study adopted where all the findings pertinent to COVID-19 co-infection among the informing studies were reported descriptively. Finally, the author adopted an objective approach to evaluating and validating the findings of this review by performing an extensive comparison of the results with that of the wider literature – both noting and acknowledging clear differences in the evidence base and using uncertainties to inform recommendations for future COVID-19 research.

4.4Review Limitations

As formerly noted, the findings of this review have provided the first-insight into the influence of co-infections upon outcomes of patients affected by COVID-19, although

certainty in the results must be considered in view of some limitations. Firstly, a search for relevant literature was informed by review recommendations and recall accuracy research but there is a risk that some studies were incidentally missed from this review due to the global extent of COVID-19 and the publication of related literature across numerous mediums. Moreover, as COVID-19 research continues, it is likely that co-infection data will continue to emerge with time and such studies cannot be captured by a point-in-time literature search. These studies may reveal additional insight into the impact of co-infection upon COVID-19 outcomes, which will require an updated review. Secondly, studies were also limited to English language and this may have heightened the risk of incidental study exclusions. Thirdly, the review primarily sought to explore the clinical impact of co-infection upon outcomes, such as morbidity and mortality, but with limited literature directly reporting upon the comparative differences between co-infected and non-co-infected cohorts, the ability to answer the research question was limited. This was also exacerbated by studies who reported upon clinical outcomes that were affected by small sample sizes, which reduces reliability and confidence in the effects reported. Finally, the review author has limited knowledge and expertise within the field of infectious diseases and thus, there is a risk that the discussion section and the recommendations derived are insufficiently informative. However, it is hoped that the extent of literature reading and evaluation offset the former risk and thus, there is confidence that strong and impactful conclusions were generated.

4.4 Implications and Recommendations for Clinical Practice

Overall, the findings of this review have some important implications for clinicians and other health professionals involved in the care of persons with COVID-19 both locally in the UK and worldwide. Firstly, this review revealed that co-infection rates are more common in COVID-19 than previously reported for other epidemics where the mean frequency was 6.7%. Therefore, it is imperative that clinicians remain vigilant in the diagnostic workup of patients with suspected and/or confirmed COVID-19, in order to prevent under-detection and under-treatment of infective pathogens that can readily exacerbate the clinical course and outcomes. As all patients with suspected COVID-19 and those with confirmed COVID-19 but mild disease cannot be tested for various other respiratory pathogens given the considerable demand and cost of performing such tests, it is recommended that persons with clinically significant COVID-19 are tested for an array of bacteria, viruses and fungi that are known to co-exist with SARS-COV-2 as this may markedly alter clinical management. Secondly, it was identified that bacterial co-infections were the most common pathogen isolated from specimens of patients with COVID-19 and as the types of bacteria were not always those typical of those colonising the upper respiratory tract, it is likely that bacterial colonisation may be arising from nosocomial or iatrogenic sources. Therefore, it is imperative that health care workers remain highly compliant with infection prevention and control guidelines, in order to help avoid the transmission of nosocomial bacteria that may ultimately lead to co-infection and worse outcomes. In addition, clinician decisions to insert invasive oropharyngeal and/or nasopharyngeal devices should be considered in light of the risks of iatrogenic related co-infection of the lower respiratory tract, although this is likely to be unavoidable in persons admitted to intensive care where such indwelling devices are fundamental to maintaining

homeostasis of the respiratory and gastrointestinal organ systems.Thirdly, as this review could not elicit any considerable risk factors associated with COVID-19 co-infection from the data of informing studies, recommendations about the management of co-infection risk among patients with suspected and/or confirmed COVID-19 cannot be derived. However, based on consistent observations and previous literature that persons with immunosuppressive states observe the greatest risk of co-infection, it may be appropriate for health workers to implement additional precautions to prevent the transmission of opportunistic hospital pathogens. Finally, given that co-infections appear to be associated with poorer outcomes, including greater morbidity and mortality, clinicians should absorb and utilise the information provided by this review to inform communicative exchanges with patients and their families, in order to provide advanced warning of the risk of poorer outcomes as a means to conveying honesty and appropriate clinical judgement.

4.5 Implications for COVID-19 Guidelines and Policy

At present, various clinical guidelines are available to guide clinical decisions regarding the management and care of persons with COVID-19. In the UK and in regard to co-infection, the National Institute for Health and Care Excellence (NICE) have yet to develop specific guidelines for the purpose of diagnosing and treated co-infective pathogens and this is likely be accounting for regional variances in the detection and reporting of COVID-19 co-infections(103). Therefore, it is strongly recommended that the NICE guidelines for COVID-19 are updated to include additions regarding clinical vigilance of co-infection, the methods used to diagnose co-infective pathogens and the various options to treat co-infective bacteria, viruses and fungi. There has been a publication by NICE regarding the prescription of antibiotics for pneumonia in patients with COVID-19 but the guidance is limited in its scope and specificity, advocating simple microbiological culture, empirical therapy and tailored therapy following the results of culture(104). Thus, this guidance should also be revised to accommodate antibiotic recommendations that are most likely to provide coverage of co-infecting bacteria identified in this review given that the avoidance of empirical therapy may reduce the emergence and spread of multi-drug resistance. Similarly, other guidelines provided by the WHO and CDC are also lacking in specificity and recommendations for co-infections in COVID-19 (105, 106).Specifically, the WHO guidelines recognise that the testing for other respiratory viruses and bacteria should be considered but this is only recommended when there are clinically signs of co-infection – indeed, such signs may be markedly difficult to recognise, particularly in patients with moderate to severe COVID-19 pneumonia, which could readily mask pneumonic contributions by co-infective pathogens. Moreover, the WHO guidelines fail to recognise the need to test for fungal respiratory organisms as a potential co-infective pathogen and thus, there is a risk that morbid and mortal-promoting complications, such as invasive aspergillosis, go undetected. Therefore, the WHO guidelines should be updated to recommend the routine testing of co-infective bacteria, viruses and fungi in patients with clinically significant COVID-19 disease, in order to help improve detection and to inform treatment that

could affect patients outcomes. Poor acknowledge of co-infection and a lack of related recommendations is also present among the CDC guidelines and the majority of guidelines for other countries and thus, the former recommendations should be shared with guideline developers, worldwide, in order to help improve COVID-19 care on the largest possible scale. Such recommendations however, co-exist with considerable implications at the policy level, given that increased proactivity in testing COVID-19 patients for co-infective pathogens will confer additional and substantial healthcare costs and resource utilisation. Thus, to help support the ability of laboratories to analyse specimens for a range of bacteria, viruses and fungal organisms, governmental and organisational bodies should commit additional monetary funds. In addition, it is important that laboratories are provided with sufficient staffing and technological support, in order to ensure specimens can be analysed efficiently and appropriately and thus, patients with co-infections can be treated in a timelier manner, which could help to improve morbidity and mortality.

4.6 Conclusion

In summary, this systematic review responded to a body of emerging literature concerning SARS-COV-2 by exploring the impact of co-infections upon clinical outcomes in patients with COVID-19 – an aim that sought to inform and improve clinical practice, guidelines and policy, in order to help improve patient outcomes on a global scale. A search for relevant literature was undertaken in June 2020 using a number of credible databases and filtering and eligibility processes identified a total of 20 studies for review. These studies were predominantly case reports, case series and cohort studies and given the nature of their design, the risk of bias was generally high due to selection and various other systematic biases. A narrative approach was adopted to explore the evidence of interest and the most pertinent findings were as follows. The mean frequency of co-infection among patients with COVID-19 was found to be 6.7%, the commonest co-infective pathogens included *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, influenza virus A, respiratory syncytial virus, rhinovirus, enterovirus, non-SARS-COV-2 coronaviruses and Candida species and generally, co-infection was associated with greater morbidity and mortality when compared to non-co-infected cohorts. Specifically, morbidity among hospitalised patients comprised a higher occurrence of pneumonia, acute respiratory distress syndrome, respiratory failure, sepsis, acute renal failure, acute cardiac dysfunction, poor weaning from ventilation and ventilator dependence, protracted lengths of hospital and/or intensive care unit stay and a case fatality rate was estimated to be 10%, which is higher than the overall mean mortality rate for COVID-19 (5%). The observation that co-infections in patients with COVID-19 are associated with poorer outcomes is one that appears to be present, but given the limitations of the informing evidence, it cannot be made with high certainty. Despite this, the primary research question (*what is the impact of respiratory co-infections upon morbidity and mortality in persons with SARS-COV-2/COVID-19?*) has been deemed to have been sufficiently addressed given that no other available literature could alter the answer to the question, which are reflected in the implications

and recommendations sections of this review. Responses to the research objectives also helped to address the review question as follows:

- Objective 1: Explore the prevalence and types of SARS-COV-2/COVID-19 co-infections reported across the emerging literature to inform comparisons upon clinical outcomes with non-co-infected cases and to ascertain the pathogens contributing to morbidity and mortality rates [6.7% and *Mycoplasma pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Klebsiella pneumoniae,* influenza virus A, respiratory syncytial virus, rhinovirus, enterovirus, non-SARS-COV-2 coronaviruses and Candida species].
- Objective 2: Evaluate the impact of co-infections upon morbidity and mortality outcomes in the defined patient group by comparing outcomes with non-co-infected cases of COVID-19 [Higher occurrence of pneumonia, acute respiratory distress syndrome, respiratory failure, sepsis, acute renal failure, acute cardiac dysfunction, poor weaning from ventilation and ventilator dependence, protracted lengths of hospital and/or intensive care unit stay and a case fatality rate of 10%]
- Objective 3: Identify the implications of objectives 1 and 2 for current clinical practice and specifically, the management of COVID-19 and infection and prevention control strategies [as detailed in subsections 4.4 and 4.5].
- Objective 4: Determine ongoing knowledge gaps to generate recommendations for future research [see below].

Finally, the exploration and evaluation of literature relevant to co-infection in COVID-19 enabled the author to identify a number of knowledge gaps – information that would be useful to informing and improving COVID-19 management in the future. Firstly, the majority of the literature pertaining to co-infections in patients with COVID-19 have yet to specifically explore the impact of co-infection upon morbidity and mortality outcomes when directly compared to a non-co-infected group and thus, future research should attempt to conduct such studies using a prospective or retrospective observational approach. Secondly, there is an unclear understanding of the effect of the initial infecting pathogen upon the course of COVID-19 disease and as it may be possible that lung parenchymal infection by non-coronaviruses may block the replication of SARS-COV-2, this is an important avenue to explore. Thirdly, there is little understanding of the interactions occurring between co-infective pathogens and between co-infective pathogens and human hosts and thus, research should also prioritise exploring these molecular level factors as this may lead to the identification of novel therapeutic and/or vaccine targets. This is particularly important given that few effective treatment options exist for patients with life-threatening COVID-19 with interventions largely being supportive and doing little to alter prognosis. Finally, research should also aim to address one of the main limitations of the informing literature, small sample sizes, and thereby, conduct a large scale study to derive more reliable data concerning the frequency of co-infections, the types of co-infective pathogens in COVID-19 and the impact of co-infection upon clinical outcomes.

WORKS CITED

Anderson RM, Heesterbeek H, Klinkenberg D, Hollingsworth TD. How will country-based mitigation measures influence the course of the COVID-19 epidemic? The Lancet. 2020;395(10228):931-4.

Burki TK. Coronavirus in China. The Lancet Respiratory Medicine. 2020;8(3):238-46.

Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ. 2020;368(1):1091-8.

Livingston E, Bucher K. Coronavirus Disease 2019 (COVID-19) in Italy. Jama. 2020;1(1):1-8.

Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. J Med Virol. 2020;92(4):401-2.

Lupia T, Scabini S, Mornese Pinna S, Di Perri G, De Rosa FG, Corcione S. 2019 novel coronavirus (2019-nCoV) outbreak: A new challenge. Journal of Global Antimicrobial Resistance. 2020;21(1):22-7.

Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, et al. The Socio-Economic Implications of the Coronavirus and COVID-19 Pandemic: A Review. International journal of surgery. 2020;17(1):1-8.

Rajgor DD, Lee MH, Archuleta S, Bagdasarian N, Quek SC. The many estimates of the COVID-19 case fatality rate. The Lancet Infectious Diseases. 2020;1(1):1-3.

Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. American journal of hematology. 2020;1(2):1-8.

University of Oxford. The economic impact of COVID-19 2020 [Available from: https://www.research.ox.ac.uk/Article/2020-04-07-the-economic-impact-of-covid-19.

WHO. Coronavirus disease (COVID-19) pandemic 2020 [Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.

WHO. Coronavirus disease (COVID-2019) situation reports 2020 [Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.

Worldometer. Coronavirus Cases 2020 [Available from: https://www.worldometers.info/coronavirus/.

Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. Jama. 2020;323(13):1239-42.

Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis. 2020;94(2):91-5.

Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020;395(10229):1054-62.

17. Joseph C, Togawa Y, Shindo N. Bacterial and viral infections associated with influenza. Influenza Other Respir Viruses. 2013;7(2):105-13.

18. Cox MJ, Loman N, Bogaert D, O'Grady J. Co-infections: potentially lethal and unexplored in COVID-19. The Lancet Microbe. 2020;1(1):11-21.

19. Paget C, Trottein F. Mechanisms of Bacterial Superinfection Post-influenza: A Role for Unconventional T Cells. Frontiers in immunology. 2019;10(1):336-46.

20. van der Sluijs KF, van der Poll T, Lutter R, Juffermans NP, Schultz MJ. Bench-to-bedside review: bacterial pneumonia with influenza - pathogenesis and clinical implications. Critical care. 2010;14(2):219-27.

21. CDC. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - United States, May-August 2009. MMWR Morbidity and mortality weekly report. 2009;58(38):1071-4.

22. Du RH, Liu LM, Yin W, Wang W, Guan LL, Yuan ML, et al. Hospitalization and Critical Care of 109 Decedents with COVID-19 Pneumonia in Wuhan, China. Annals of the American Thoracic Society. 2020;1(2):1-10.

23. Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical Features of 69 Cases With Coronavirus Disease 2019 in Wuhan, China. Clinical Infectious Diseases. 2020;2(1):1-10.

24. Zhou Q, Gao Y, Wang X, Liu R, Du P, Wang X, et al. Nosocomial Infections Among Patients with COVID-19, SARS and MERS: A Rapid Review and Meta-Analysis. medRxiv. 2020;1(1):1-8.

25. Póvoa HCC, Chianca GC, Iorio NLPP. COVID-19: An Alert to Ventilator-Associated Bacterial Pneumonia. Infectious Diseases and Therapy. 2020;1(1):1-8.

26. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;63(5):61-e11.

27. Zahariadis G, Gooley TA, Ryall P, Hutchinson C, Latchford MI, Fearon MA, et al. Risk of Ruling out Severe Acute Respiratory Syndrome by Ruling in another Diagnosis: Variable Incidence of Atypical Bacteria Coinfection Based on Diagnostic Assays. Canadian respiratory journal. 2006;13(1):1-10.

28. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. The Lancet: Infectious diseases. 2013;13(9):752-61.

29. Nowak MD, Sordillo EM, Gitman MR, Paniz Mondolfi AE. Co-infection in SARS-CoV-2 infected Patients: Where Are Influenza Virus and Rhinovirus/Enterovirus? J Med Virol. 2020;1(1):1-7.

30. Farrugia P, Petrisor BA, Farrokhyar F, Bhandari M. Research questions, hypotheses and objectives. Canadian Journal of Surgery. 2010;53(4):278-81.

31. Pollock A, Berge E. How to do a systematic review. International Journal of Stroke. 2018;13(2):138-56.

32. Gough D, Thomas J, Oliver S. Clarifying differences between review designs and methods. Syst Rev. 2012;1:1-9.

33. Misra DP, Agarwal V. Systematic Reviews: Challenges for Their Justification, Related Comprehensive Searches, and Implications. Journal of Korean medical science. 2018;33(12):92-8.

34. Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. Health Information and Libraries Journal. 2009;26(1):91-108.

35. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Annals of internal medicine. 2009;151(4):65-94.

36. National Institute for Health Research. PROSPERO 2020 [Available from: https://www.crd.york.ac.uk/PROSPERO/.

37. Grewal A, Kataria H, Dhawan I. Literature search for research planning and identification of research problem. Indian Jounral of Anaesthesia. 2016;60(9):635-9.

38. Morris ZS, Wooding S, Grant J. The answer is 17 years, what is the question: understanding time lags in translational research. Journal of the Royal Society of Medicine. 2011;104(12):510-20.

39. Thakre SB, Thakre SS, Thakre AD. Electronic biomedical literature search for budding researcher. Journal of clinical and diagnostic research. 2013;7(9):2033-7.

40. Bramer WM, Rethlefsen ML, Kleijnen J, Franco OH. Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. Systematic Reviews. 2017;6(1):245-7.

41. Preston L, Carroll C, Gardois P, Paisley S, Kaltenthaler E. Improving search efficiency for systematic reviews of diagnostic test accuracy: an exploratory study to assess the viability of limiting to MEDLINE, EMBASE and reference checking. Syst Rev. 2015;4:82-9.

42. Ecker ED, Skelly AC. Conducting a winning literature search. Evid Based Spine Care J. 2010;1(1):9-14.

43. Aromataris E, Riitano D. Constructing a search strategy and searching for evidence. A guide to the literature search for a systematic review. American Journal of Nursing. 2014;114(5):49-56.

44. JBI. Critical Appraisal Tools 2020 [Available from: http://joannabriggs-webdev.org/research/critical-appraisal-tools.html.

45. Mhaskar R, Emmanuel P, Mishra S, Patel S, Naik E, Kumar A. Critical appraisal skills are essential to informed decision-making. Indian Journal of Sexually Transmitted Diseases. 2009;30(2):112-9.

46. Steckler A, McLeroy KR. The Importance of External Validity. American Journal of Public Health. 2008;98(1):9-10.

47. Mathes T, Klassen P, Pieper D. Frequency of data extraction errors and methods to increase data extraction quality: a methodological review. BMC Medical Research Methodology. 2017;17(1):152-8.

48. Cochrane Collaboration. Data extraction forms 2019 [Available from: https://dplp.cochrane.org/data-extraction-forms.

49. Lisy K, Porritt K. Narrative Synthesis: Considerations and challenges. International journal of evidence-based healthcare. 2016;14(4):201-7.

50. Haidich AB. Meta-analysis in medical research. Hippokratia. 2010;14(1):29-37.

51. Medical Research Council. MRC ethics series: Good research practice: Principles and guidelines 2012 [Available from: https://mrc.ukri.org/publications/browse/good-research-practice-principles-and-guidelines/.

52. Arashiro T, Nakamura S, Asami T, Mikuni H, Fujiwara E, Sakamoto S, et al. SARS-CoV-2 and Legionella co-infection in a person returning from a Nile cruise. Journal of Travel Medicine. 2020;27(3):1-7.

53. Azekawa S, Namkoong H, Mitamura K, Kawaoka Y, Saito F. Co-infection with SARS-CoV-2 and influenza A virus. IDCases. 2020;20(1):1-8.

54. Fan BE, Lim KGE, Chong VCL, Chan SSW, Ong KH, Kuperan P. COVID-19 and mycoplasma pneumoniae coinfection. American journal of hematology. 2020;95(6):723-4.

55. Konala VM, Adapa S, Gayam V, Naramala S, Daggubati SR, Kammari CB, et al. Co-infection with Influenza A and COVID-19. Eur J Case Rep Intern Med. 2020;7(5):1-10.

56. Touzard-Romo F, Tapé C, Lonks JR. Co-infection with SARS-CoV-2 and Human Metapneumovirus. R I Med J. 2020;103(2):75-6.

57. Wu X, Cai Y, Huang X, Yu X, Zhao L, Wang F, et al. Co-infection with SARS-CoV-2 and Influenza A Virus in Patient with Pneumonia, China. Emerg Infect Dis. 2020;26(6):1324-6.

58. Wehl G, Laible M, Rauchenzauner M. Co-infection of SARS CoV-2 and influenza A in a Pediatric Patient in Germany. Klinische Padiatrie. 2020;1(1):1-5.

59. Cuadrado-Payan E, Montagud-Marrahi E, Torres-Elorza M, Bodro M, Blasco M, Poch E, et al. SARS-CoV-2 and influenza virus co-infection. The Lancet. 2020;395(10236):84-9.

60. Stochino C, Villa S, Zucchi P, Parravicini P, Gori A, Raviglione MC. Clinical characteristics of COVID-19 and active tuberculosis co-infection in an Italian reference hospital. The European respiratory journal. 2020;1(1):1-8.

61. Wee LE, Ko KKK, Ho WQ, Kwek GTC, Tan TT, Wijaya L. 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(1):104436-42.

62. Blasco ML, Buesa J, Colomina J, Forner MJ, Galindo MJ, Navarro J, et al. Co-detection of respiratory pathogens in patients hospitalized with Coronavirus viral disease-2019 pneumonia. J Med Virol. 2020;1(1):1-8.

63. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-13.

64. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens. Jama. 2020;323(20):2085-6.

65. Lin D, Liu L, Zhang M, Hu Y, Yang Q, Guo J, et al. Co-infections of SARS-CoV-2 with multiple common respiratory pathogens in infected patients. Sci China Life Sci. 2020;63(4):606-9.

66. Lv Z, Cheng S, Le J, Huang J, Feng L, Zhang B, et al. Clinical characteristics and co-infections of 354 hospitalized patients with COVID-19 in Wuhan, China: a retrospective cohort study. Microbes and infection. 2020;22(4-5):195-9.

67. Ma S, Lai X, Chen Z, Tu S, Qin K. Clinical Characteristics of Critically Ill Patients Co-infected with SARS-CoV-2 and the Influenza Virus in Wuhan, China. Int J Infect Dis. 2020;96(1):683-7.

68. Tadolini M, Codecasa LR, García-García J-M, Blanc F-X, Borisov S, Alffenaar J-W, et al. Active tuberculosis, sequelae and COVID-19 co-infection: first cohort of 49 cases. The European respiratory journal. 2020;1(1):1-8.

69. Wang M, Wu Q, Xu W, Qiao B, Wang J, Zheng H, et al. Clinical diagnosis of 8274 samples with 2019-novel coronavirus in Wuhan 2020 [Available from: https://europepmc.org/article/ppr/ppr112805.

70. Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, et al. Co-infection with respiratory pathogens among COVID-2019 cases. Virus research. 2020;285(1):198005-10.

71. Norvell DC. Study types and bias-Don't judge a study by the abstract's conclusion alone. Evidence-based spine-care journal. 2010;1(2):7-10.

72. Murad MH, Sultan S, Haffar S, Bazerbachi F. Methodological quality and synthesis of case series and case reports. BMJ Evidence-Based Medicine. 2018;23(2):60-5.

73. Tofthagen C. Threats to validity in retrospective studies. J Adv Pract Oncol. 2012;3(3):181-3.

74. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. The Journal of infection. 2020;1(1):1-8.

75. MacIntyre CR, Chughtai AA, Barnes M, Ridda I, Seale H, Toms R, et al. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza a(H1N1)pdm09. BMC infectious diseases. 2018;18(1):637-47.

76. Klein EY, Monteforte B, Gupta A, Jiang W, May L, Hsieh Y-H, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. Influenza and other respiratory viruses. 2016;10(5):394-403.

77. Chertow DS, Memoli MJ. Bacterial coinfection in influenza: a grand rounds review. Jama. 2013;309(3):275-82.

78. Arabi YM, Arifi AA, Balkhy HH, Najm H, Aldawood AS, Ghabashi A, et al. Clinical Course and Outcomes of Critically Ill Patients With Middle East Respiratory Syndrome Coronavirus Infection. Annals of internal medicine. 2014;160(6):389-97.

79. Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, Butany J. Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mod Pathol. 2005;18(1):1-10.

80. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;51(1):81-7.

81. Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and mortality: a systematic review of observational studies. Critical care medicine. 2009;37(10):2709-18.

82. Landry ML. Immunoglobulin M for Acute Infection: True or False? Clinical and Vaccine Immunology. 2016;23(7):540-2.

83. Sobieszczańska BM, Kasprzykowska U, Duda-Madej A, Secewicz A, Marciniak J, Gościniak G. Relevance of serology for Mycoplasma pneumoniae infection among children with persistent cough. Adv Clin Exp Med. 2014;23(2):185-90.

84. Lemmon GH, Gardner SN. Predicting the sensitivity and specificity of published real-time PCR assays. Annals of Clinical Microbiology and Antimicrobials. 2008;7(1):18-24.

85. Shu YL, Fang LQ, de Vlas SJ, Gao Y, Richardus JH, Cao WC. Dual seasonal patterns for influenza, China. Emerg Infect Dis. 2010;16(4):725-6.

86. Cohen AN, Kessel B. False positives in reverse transcription PCR testing for SARS-CoV-2. medRxiv. 2020;1(2):1-8.

87. Pinky L, Dobrovolny HM. Coinfections of the Respiratory Tract: Viral Competition for Resources. PloS one. 2016;11(5):1-9.

88. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response. The Pediatric infectious disease journal. 2005;24(7):605-10.

89. Brand HK, de Groot R, Galama JM, Brouwer ML, Teuwen K, Hermans PW, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. Pediatric pulmonology. 2012;47(4):393-400.

90. Goka E, Vallely P, Mutton K, Klapper P. Influenza A viruses dual and multiple infections with other respiratory viruses and risk of hospitalisation and mortality. Influenza Other Respir Viruses. 2013;7(6):1079-87.

91. Liang L, He C, Lei M, Li S, Hao Y, Zhu H, et al. Pathology of guinea pigs experimentally infected with a novel reovirus and coronavirus isolated from SARS patients. DNA Cell Biol. 2005;24(8):485-90.

92. Dobrescu I, Levast B, Lai K, Delgado-Ortega M, Walker S, Banman S, et al. In vitro and ex vivo analyses of co-infections with swine influenza and porcine reproductive and respiratory syndrome viruses. Vet Microbiol. 2014;169(1-2):18-32.

93. Ami Y, Nagata N, Shirato K, Watanabe R, Iwata N, Nakagaki K, et al. Co-infection of respiratory bacterium with severe acute respiratory syndrome coronavirus induces an exacerbated pneumonia in mice. Microbiol Immunol. 2008;52(2):118-27.

94. Shinjoh M, Omoe K, Saito N, Matsuo N, Nerome K. In vitro growth profiles of respiratory syncytial virus in the presence of influenza virus. Acta Virol. 2000;44(2):91-7.

95. Li Z, Lu G, Meng G. Pathogenic Fungal Infection in the Lung. Frontiers in immunology. 2019;10(1):1524-.

96. Alanio A, Dellière S, Fodil S, Bretagne S, Mégarbane B. Prevalence of putative invasive pulmonary aspergillosis in critically ill patients with COVID-19. The Lancet Respiratory Medicine. 2020;8(6):48-56.

97. Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F, et al. COVID-19 associated pulmonary aspergillosis. Mycoses. 2020;63(6):528-34.

98. Schauwvlieghe A, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. The Lancet Respiratory medicine. 2018;6(10):782-92.

99. Koehler P, Bassetti M, Kochanek M, Shimabukuro-Vornhagen A, Cornely OA. Intensive care management of influenza-associated pulmonary aspergillosis. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(12):1501-9.

100. Wahidi MM, Lamb C, Murgu S, Musani A, Shojaee S, Sachdeva A, et al. American Association for Bronchology and Interventional Pulmonology (AABIP) Statement on the Use of Bronchoscopy and Respiratory Specimen Collection in Patients with Suspected or Confirmed COVID-19 Infection. J Bronchology Interv Pulmonol. 2020;1(1):1-12.

101. Barnett LMA, Cummings BS. Nephrotoxicity and Renal Pathophysiology: A Contemporary Perspective. Toxicological Sciences. 2018;164(2):379-90.

102. Hsu J. How covid-19 is accelerating the threat of antimicrobial resistance. BMJ. 2020;369(1):1983-9.

103. NICE. Coronavirus (COVID-19) 2020 [Available from: https://www.nice.org.uk/covid-19.

104. NICE. COVID-19 rapid guideline: antibiotics for pneumonia in adults in hospital 2020 [Available from: https://www.nice.org.uk/guidance/ng173/chapter/3-Initial-approach-to-antibiotic-treatment-choices.

105. WHO. Clinical management of COVID-19 2020 [Available from: https://www.who.int/publications/i/item/clinical-management-of-covid-19.

106. CDC. Interim Clinical Guidance for Management of Patients with Confirmed Coronavirus Disease (COVID-19) 2020 [Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html.

Appendices

Appendix 1. JBI checklist questions for cohort studies

	Yes	No	Unclear	Not applicable
1. Were the two groups similar and recruited from the same population?	☐	☐	☐	☐
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	☐	☐	☐	☐
3. Was the exposure measured in a valid and reliable way?	☐	☐	☐	☐
4. Were confounding factors identified?	☐	☐	☐	☐
5. Were strategies to deal with confounding factors stated?	☐	☐	☐	☐
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	☐	☐	☐	☐
7. Were the outcomes measured in a valid and reliable way?	☐	☐	☐	☐
8. Was the follow up time reported and sufficient to be long enough for outcomes to occur?	☐	☐	☐	☐
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	☐	☐	☐	☐
10. Were strategies to address incomplete follow up utilized?	☐	☐	☐	☐
11. Was appropriate statistical analysis used?	☐	☐	☐	☐

Appendix 2. JBI checklist questions for case reports (44).

	Yes	No	Unclear	Not applicable
1. Were patient's demographic characteristics clearly described?	☐	☐	☐	☐
2. Was the patient's history clearly described and presented as a timeline?	☐	☐	☐	☐
3. Was the current clinical condition of the patient on presentation clearly described?	☐	☐	☐	☐
4. Were diagnostic tests or assessment methods and the results clearly described?	☐	☐	☐	☐
5. Was the intervention(s) or treatment procedure(s) clearly described?	☐	☐	☐	☐
6. Was the post-intervention clinical condition clearly described?	☐	☐	☐	☐
7. Were adverse events (harms) or unanticipated events identified and described?	☐	☐	☐	☐
8. Does the case report provide takeaway lessons?	☐	☐	☐	☐

Appendix 3. JBI checklist questions for case series (44).

	Yes	No	Unclear	Not applicable
1. Were there clear criteria for inclusion in the case series?	☐	☐	☐	☐
2. Was the condition measured in a standard, reliable way for all participants included in the case series?	☐	☐	☐	☐
3. Were valid methods used for identification of the condition for all participants included in the case series?	☐	☐	☐	☐
4. Did the case series have consecutive inclusion of participants?	☐	☐	☐	☐
5. Did the case series have complete inclusion of participants?	☐	☐	☐	☐
6. Was there clear reporting of the demographics of the participants in the study?	☐	☐	☐	☐
7. Was there clear reporting of clinical information of the participants?	☐	☐	☐	☐
8. Were the outcomes or follow up results of cases clearly reported?	☐	☐	☐	☐
9. Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	☐	☐	☐	☐
10. Was statistical analysis appropriate?	☐	☐	☐	☐