Atypical bacterial co-infections among patients with COVID-19: A study from India

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Abstract
Emerging evidence shows co-infection with atypical bacteria in coronavirus disease 2019 (COVID-19) patients. Respiratory illness caused by atypical bacteria such as Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila may show overlapping manifestations and imaging features with COVID-19 causing clinical and laboratory diagnostic issues. We conducted a prospective study to identify co-infections with SARS-CoV-2 and atypical bacteria in an Indian tertiary hospital. From June 2020 to January 2021, a total of 194 patients with laboratory-confirmed COVID-19 were also tested for atypical bacterial pathogens. For diagnosing M. pneumoniae, a real-time polymerase chain reaction (PCR) assay and serology (IgM ELISA) were performed. C. pneumoniae diagnosis was made based on IgM serology. L. pneumophila diagnosis was based on PCR or urinary antigen testing. Clinical and epidemiological features of SARS-CoV-2 and atypical bacterial-positive and -negative patient groups were compared. Of the 194 patients admitted with COVID-19, 17 (8.8%) were also diagnosed with M. pneumoniae (n = 10) or C. pneumoniae infection (n = 7). Confusion, headache, and bilateral infiltrate were found more frequently in the SARS-CoV-2 and atypical bacteria co-infection group. Patients in the M. pneumoniae or C. pneumoniae co-infection group were more likely to develop ARDS, required ventilatory support, had a longer hospital length of stay, and higher fatality rate compared to patients with only SARS-CoV-2. Our report highlights co-infection with bacteria causing atypical pneumonia should be considered in patients with SARS-CoV-2 depending on the clinical context. Timely identification of co-existing pathogens can provide pathogen-targeted treatment and prevent fatal outcomes of patients infected with SARS-CoV-2 during the current pandemic.

KEYWORDS
atypical pneumonia, co-infections, COVID-19, respiratory pathogens, SARS-CoV-2
1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is identified for the first time at the end of 2019 in Wuhan, China, and is responsible for a contagious respiratory disease known as Coronavirus disease (COVID-19). The virus has spread rapidly in many parts of the world, and as of August 20, 2021, there have been 209.87 million confirmed cases of COVID-19, including 4400 thousand deaths worldwide. In India, 32.35 million confirmed cases and 433 thousand deaths were reported as of August 20, 2021. Clinical presentations of COVID-19 vary from asymptomatic infection to fatal disease with acute respiratory distress syndrome (ARDS) and multiorgan failure.

Bacterial co-infections associated with COVID-19 have been frequently reported; however, their proportions are very low compared to previous influenza pandemics. Lansbury et al. found that 7% of hospitalized patients with COVID-19 had bacterial co-infections, with a higher rate of 14% in intensive care unit (ICU) patients. Co-infection with SARS-CoV-2 and atypical bacteria such as Legionella pneumophila, Mycoplasma pneumoniae, and Chlamydia pneumoniae has been identified in few studies. Due to overlapping clinical presentations and image features, it is difficult to distinguish between SARS-CoV-2 and bacteria causing atypical pneumonia. Besides this, it is unclear whether co-infection with atypical bacteria can cause worse clinical outcomes in COVID-19 patients. Here, we conducted a prospective study to determine the prevalence of co-infection related to atypical bacteria in patients admitted with COVID-19 in an Indian tertiary hospital. We also describe the demographic and clinical features, laboratory parameters, complications, and clinical outcomes of such co-infections.

2 | METHODS

2.1 | Study population

From June 1, 2020 to January 30, 2021, we prospectively enrolled patients with SARS-CoV-2, admitted to the COVID-19 wards and ICU of the All-India Institute of Medical Sciences (AIIMS), New Delhi, India. The Institute Ethics Committee of AIIMS had approved the study protocol. The diagnosis of SARS-CoV-2 was based on either a real-time reverse-transcription polymerase chain reaction (RT-PCR) or a cartridge-based nucleic acid amplification test (CB-NAAT), or a rapid antigen test on combined oropharyngeal/nasal swabs.

2.2 | Data source and collection

Patient data were mainly collected using a standard questionnaire or extracted from electronic medical records. The following data were mainly collected: hospital admission details, demographics (age, gender, and presence or absence of underlying health conditions), clinical and laboratory data, chest X-ray, details of ICU admission, mechanical ventilation, antibiotic treatment, and fatal outcome.

2.3 | Specimen collection and laboratory testing

A total of 194 patients with laboratory-confirmed COVID-19 were also tested for Legionella spp., M. pneumoniae, and C. pneumoniae. We collected a combined oropharyngeal/nasal swab (flocked swabs in viral transport medium; HiViral™) urine, and blood from each patient and transported them to the laboratory, where all the following microbiological investigations were performed: Respiratory PCR for Legionella spp. and M. pneumoniae, immunoglobulin M (IgM) Serology for M. pneumoniae and C. pneumoniae, urinary antigen test for L. pneumophila serogroup 1 (Lp1).

2.4 | Real-time PCR for Legionella spp. and M. pneumoniae

Two respiratory PCR were used during the study period: a real-time PCR targeting the ssrA gene for detecting Legionella spp. including L. pneumophila and another real-time PCR targeting the CARDs toxin gene for diagnosing M. pneumoniae. Briefly, 100 µl of total nucleic acid was extracted from 200 µl of the swab specimens using a QIAamp DNA mini kit (Qiagen). First, 200 µl of lysis buffer (Buffer AL) and 20 µl of proteinase K (20 mg/ml) were added to 200 µl of the respiratory sample, and the mixture was incubated at 56°C for 10 min. Following manual lysis, the samples were placed on the QIAGEN QIAcube instrument for automated nucleic acid extraction using the QIAGEN protocol. The extracted nucleic acids were then tested for Legionella spp. and M. pneumoniae using two previously published real-time PCR assays. Samples with a cycle threshold (Ct) value of <40 were determined as positive. Whenever a positive result was observed, the testing was repeated in triplicates for confirmation. A specimen was considered real-time PCR-positive for Legionella spp. or M. pneumoniae if the sample tested positive in at least two of the three repeats. Each real-time PCR assay is reported to have a specificity of 100% and a sensitivity (limit of detection [LOD]) of 20 fg per reaction (data not shown).

2.5 | Urinary antigen testing (UAT) for L. pneumophila serogroup 1

L. pneumophila antigen in urine was detected using BinaxNOW Legionella urinary antigen ICT kit (Alere), specific for Lp1. The manufacturer’s instructions were followed for performing the UAT. The assay offers a sensitivity of 95% and specificity of 95% for Lp1.

2.6 | Serology for M. pneumoniae and C. pneumoniae

The serologic diagnosis was made based on commercial ELISA kits (NovaLisa, NovaTec Immunodagnostica GmbH). These kits were routinely used to detect serum M. pneumoniae IgM (MYCM0350) and
C. pneumoniae IgM (CHLM0510) in clinical practice at our facility. Briefly, these assays used single 1:101 dilutions of serum in sample buffer and included cut-off calibrators in determining samples as positive or negative. As control of assay performance, a positive and negative control were also included on each plate per assay. The manufacturer’s instructions were followed for performing the assays and interpreting antibody determinations. A value of >11 NTU (Novatex units) obtained in a single determination was considered positive. These tests offer a sensitivity of 94.4% and 90% and specificity of >95% and 99% for M. pneumoniae and C. pneumoniae, respectively.

2.7 | Definitions

The diagnosis of COVID-19 was based on positivity by RT-PCR or CB-NAAT or rapid antigen test on oropharyngeal/nasal swab samples. A patient was considered having co-infection with atypical bacteria if they had a positive result for at least one of the microbiological investigations (Legionella spp. PCR/L. pneumophila UAT or M. pneumoniae PCR/IgM or C. pneumoniae IgM). Pneumonia was defined based on the WHO guidelines as fever, cough, fast breathing, or difficulty in breathing in a patient. The clinical severity of COVID-19 was determined based on the Indian Council of Medical Research (ICMR) criteria (Clinical management protocol: COVID-19, Version 5).

2.8 | Statistical analysis

Categorical variables were expressed as numbers (percentages) and compared using the χ² test or Fisher’s exact test. Continuous variables were shown as median (interquartile ranges) and compared between two independent groups using the t test or Wilcoxon rank-sum test as per the distribution of the data. Statistical significance was defined at a p < 0.05. Statistical software, STATA/SE version 14.2 (StataCorp LP), was used for all the analysis.

3 | RESULTS

Among 194 patients with laboratory-confirmed COVID-19, 17 (8.8%) were co-infected with M. pneumoniae or C. pneumoniae. Co-infection with SARS-CoV-2 and M. pneumoniae was identified in 10 (5.2%) patients. PCR made the diagnosis of M. pneumoniae in three patients and serology (IgM) in the remaining seven. C. pneumoniae was diagnosed based on IgM serology in seven (3.6%) patients with SARS-CoV-2. Simultaneous detection of both M. pneumoniae and C. pneumoniae was not seen in any SARS-CoV-2 positive patients. All the patients (n = 194) were negative for Legionella PCR and UAT. The analysis was performed on the total number of patients who tested positive for M. pneumoniae or C. pneumoniae without distinction between the two bacteria.

3.1 | Demographic and clinical characteristics of patients co-infected with SARS-CoV-2 and M. pneumoniae or C. pneumoniae

Characteristics of COVID-19 patients with and without M. pneumoniae or C. pneumoniae co-infections are shown in the Table. Patients co-infected with SARS-CoV-2 and atypical bacteria did not differ significantly in age and gender from those without atypical pathogens. The median age of COVID-19 patients with M. pneumoniae or C. pneumoniae co-infection (n = 17) was 50 years (range 17–77 years), and 14 (82.4%) were male. Most patients (14, 82.4%) in M. pneumoniae or C. pneumoniae co-infection group had at least one comorbid condition, mainly hypertension (7, 41.2%), diabetes mellitus (5, 35.7%), and renal disease (3, 21.4%). Two (14.2%) patients had a malignancy history, and five (35.7%) had neurological complications. Of these, only neurological complications were significantly more common in patients with SARS-CoV-2 and M. pneumoniae or C. pneumoniae than those with only SARS-CoV-2 (35.7% vs. 3.4%, p = <0.001).

Most common signs in patients with SARS CoV-2 and M. pneumoniae or C. pneumoniae were fever (17, 100%), cough (11, 64.7%), dyspnea (11, 64.7%), confusion (7, 41.2%), and headache (5, 29.4%). It was observed that confusion (41.2% vs. 16.4%, p = 0.012) and headache (29.4% vs. 5.6%, p = 0.005) were significantly higher in M. pneumoniae or C. pneumoniae co-infected group than in only SARS-CoV-2 positive patients. Radiological findings were available only for 11/17 (64.7%) patients. Bilateral infiltrates were reported more frequently in M. pneumoniae or C. pneumoniae co-infection group (90.9% vs. 54.9%, p = 0.025). A higher proportion of patients in the M. pneumoniae or C. pneumoniae co-infection group were reported as having severe COVID-19 pneumonia (64.7% vs. 40.7%, p = 0.335), although the difference was not statistically significant.

With regard to the laboratory findings, a statistically significant intergroup difference was not observed for any parameters. Patients co-infected with atypical bacteria had comparable total leukocyte and platelet counts, C-reactive protein, and procalcitonin values (Table 1).

Of the 17 patients with M. pneumoniae or C. pneumoniae co-infection, 15 (88.2%) patients received antibiotics, and 6 (35.29%) patients received antiviral treatment. Seven (41.1%) patients received antibiotics active against atypical pathogens, including azithromycin (n = 3), doxycycline (n = 3), and levofloxacin (n = 1) with no overlap. Eight patients (47.1%) received agents with broad-spectrum coverage, either amoxicillin/clavulinate combination, or piperacillin/tazobactam combination, or cefoperazone/sulbactam combination.

Patients in the M. pneumoniae or C. pneumoniae co-infection group were more likely to develop ARDS (76.5% vs. 46.9%, p = 0.023) than only SARS-CoV-2 positive patients. Complications, such as pneumonia (82.4% vs. 68.4%, p = 0.281) and shock (47.1% vs. 24.9%, p = 0.052) were more common in the M. pneumoniae or C. pneumoniae co-infection group, although the difference was not statistically significant. Significantly more patients in the M. pneumoniae or C. pneumoniae co-infection group required ventilatory support.
TABLE 1 Baseline characteristics of patients with COVID-19 with and without *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* co-infection

| Characteristics | M. pneumoniae and C. pneumoniae co-infection status | Negative (n = 177) | Positive (n = 17) | p value |
|-----------------|-----------------------------------------------|-------------------|-----------------|-------|
| Age in years median (range) | 50 (15–86) | 50 (17–77) | 0.270 |
| Age group (years) | | | |
| 15–44 | 66 (37.3) | 8 (47.1) | 0.675 |
| 45–64 | 80 (45.2) | 6 (35.3) |
| >65 | 32 (18.1) | 3 (17.6) |
| Male | 125 (70.6) | 14 (82.4) | 0.405 |
| Concurrent conditions (any one) | 127 (71.7) | 14 (82.4) | 0.569 |
| Hypertension | 58 (32.8) | 7 (41.2) | 0.483 |
| Diabetes mellitus | 57 (32.2) | 5 (35.7) | 0.814 |
| Renal disease | 32 (18.1) | 3 (21.4) | 1 |
| Malignancy | 14 (7.9) | 2 (14.2) | 0.637 |
| Neurologic disease | 6 (3.4) | 5 (35.7) | <0.001 |
| Signs and symptoms | | | |
| Fever | 154 (87) | 17 (100) | 0.230 |
| Duration of fever (Median days [range]) | 4 (1–15) | 4 (1–10) | 0.231 |
| Cough | 105 (59.3) | 11 (64.7) | 0.665 |
| Dyspnea | 101 (57.1) | 11 (64.7) | 0.542 |
| Chest pain | 16 (9) | 1 (5.9) | 1 |
| Hypoxia | 8 (4.5) | 1 (5.9) | 0.570 |
| Confusion | 29 (16.4) | 7 (41.2) | 0.012 |
| Headache | 10 (5.6) | 5 (29.4) | 0.005 |
| Myalgia | 21 (11.9) | 3 (17.6) | 0.448 |
| Positive chest radiography findings | 87/102 (85.3) | 11/11 (100) | 0.354 |
| Bilateral infiltrations | 56/102 (54.9) | 10/11 (90.9) | 0.025 |
| Laboratory parameters | | | |
| Abnormal hemoglobin<sup>a</sup> | 136 (76.8) | 12 (70.5) | 0.534 |
| Leukocytosis<sup>b</sup> | 83 (46.9) | 8 (47.1) | 0.994 |
| Lymphopenia<sup>c</sup> | 124 (70.1) | 13 (76.5) | 1 |
| Thrombocytopenia<sup>d</sup> | 65 (36.7) | 8 (47.1) | 0.411 |
| Elevated AST<sup>e</sup> | 100 (56.5) | 11 (64.7) | 0.564 |
| Elevated ALT<sup>f</sup> | 71 (40.1) | 10 (58.8) | 0.157 |
| Elevated C-reactive protein (≥6 mg/dl) | 70 (153 (45.8) | 10/16 (62.5) | 0.202 |
| COVID-19 severity | | | |
| Asymptomatic/mild disease | 68 (38.4) | 4 (23.5) | 0.335 |
| Moderate disease | 37 (20.9) | 2 (11.7) |
| Severe disease | 72 (40.7) | 11 (64.7) |
| Treatments | | | |
| Antivirals | 22/158 (13.92) | 6 (35.2) | 0.022 |
| Antibiotics | | | |
| Combination antibiotics | 72/162 (44.4) | 8 (47.1) | 0.837 |
| Fluoroquinolones | 9/162 (5.6) | 1 (5.9) | 1 |
| Macrolides | 16/162 (9.9) | 3 (17.6) | 0.397 |
| Doxycycline | 49/162 (30.2) | 3 (17.6) | 0.402 |
| Cephalosporins | 29/162 (17.9) | 2 (11.8) | 0.741 |
| Corticosteroids | 35/158 (22.15) | 7 (41.1) | 0.081 |
| In-hospital complications and outcome | | | |
| Required mechanical ventilation | 86 (48.6) | 13 (76.5) | 0.040 |
| Required ICU admission | 124 (70.1) | 15 (88.2) | 0.159 |
| Duration of hospital stay (Median days [range]) | 13 (1–27) | 17 (7–30) | 0.004 |
| Pneumonia | 121 (68.4) | 14 (82.4) | 0.281 |

TABLE 1 (Continued) M. pneumoniae and C. pneumoniae co-infection status

| Characteristics | Negative (n = 177) | Positive (n = 17) | p value |
|-----------------|--------------------|------------------|-------|
| Elevated procalcitonin (>0.1 ng/ml) | | | |
| Abnormal IL-6<sup>g</sup> | 109/134 (81.3% | 14/15 (93.3) | 0.471 |
| Abnormal blood urea nitrogen<sup>h</sup> | 78 (44.1) | 9 (52.9) | 0.508 |
| Abnormal creatinine<sup>i</sup> | 81 (45.8) | 9 (52.9) | 0.571 |
| Abnormal serum ferritin<sup>j</sup> | 91/146 (62.3) | 10/16 (62.5) | 0.989 |
| Total leukocyte count (×10³ cells/µl) | 10.7 (1.1–38.6) | 9.6 (2.9–19.4) | 0.998 |
| Platelet count (×10³ cells/µl) | 175 (22–449) | 154 (29–476) | 0.149 |
| C-reactive protein (mg/dl) | 4.45 (0.021–37) | 7.18 (0.17–25) | 0.322 |
| Procalcitonin (ng/ml) | 0.41 (0.01–100) | 0.55 (0.01–39) | 0.835 |

<sup>a</sup>Hemoglobin<sup>b</sup>Leukocytosis<sup>c</sup>Lymphopenia<sup>d</sup>Thrombocytopenia<sup>e</sup>Elevated AST<sup>f</sup>Elevated ALT<sup>f</sup>Elevated C-reactive protein (≥6 mg/dl)
TABLE 1 (Continued)

| Characteristics | M. pneumoniae and C. pneumoniae co-infection status |  |
|-----------------|----------------------------------------------------|---|
|                 | Negative (n = 177) | Positive (n = 17) | p value |
| ARDS            | 83 (46.9)          | 13 (76.5)         | 0.023   |
| Shock           | 44 (24.9)          | 8 (47.1)          | 0.052   |
| Died            | 58 (32.8)          | 11 (64.7)         | 0.029   |

Abbreviations: ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; ICU, intensive care unit; IL, interleukin.

†Reference range from mild infections affecting the upper respiratory system among all age groups. The clinical presentations of M. pneumoniae range from mild infections affecting the upper respiratory tract to radiologically confirmed pneumonia that needs hospital admission. The IgM antibody of M. pneumoniae was positive for this patient, and sputum RT-PCR tested positive for SARS-CoV-2. The patient was given both antivirals (lopinavir/ritonavir, peramivir, interferon-α2b) and antibiotics (azithromycin and levofloxacin) and subsequently recovered.

Retrospective studies from the United States and Italy have identified co-infection with SARS-CoV-2 and M. pneumoniae based on serologies in 1.7% and 1.1% of patients, respectively. Studies conducted in Spain, UK, and China also showed relatively low percentages of SARS-CoV-2 and M. pneumoniae co-infection (0.97%, 1.49%, and 8.6%, respectively). The proportion of patients co-infected with COVID-19 and M. pneumoniae in our study fall within this range (5.1%). Meanwhile, in a Chinese study involving pediatric COVID-19 patients, co-infection with M. pneumoniae was very high; 47%. These differences in the proportion of co-infection may be attributable to the selection of case-patients (adults or children), the detection method employed (nucleic acid amplification-based or serology-based), and the geographic factors.

A few studies have raised concerns about C. pneumoniae co-infection in patients. An Italian study reported co-infection with C. pneumoniae in 2.7% (5/180) of COVID-19 patients based on serology. Similarly, in a US study, 4.7% (2/42) of the patients with SARS-CoV-2 tested positive for C. pneumoniae also using a respiratory PCR. The detection rate of C. pneumoniae in the present study is in concordance with the previous reports.

Legionella spp. are responsible for Legionnaires’ disease, severe pneumonia in individuals with underlying medical conditions. L. pneumophila and COVID-19 co-infection was not identified in our patient population. However, in the literature, co-infection with SARS-CoV-2 and L. pneumophila had been rarely reported. Arashiro et al. described a patient who returned from a Nile Cruise experienced mild cough, diarrhea, malaise, and ground-glass opacity on chest CT. Both Legionella UAT and SARS-CoV-2 RT-PCR were positive for this patient. He was treated with azithromycin and supportive care but could not be saved.

The majority of the patients with SARS-CoV-2 and atypical bacteria co-infection presented with fever, cough, and dyspnea, and showed bilateral infiltrates, and received ventilatory support. The clinical symptoms of SARS-CoV-2 and bacteria causing atypical pneumonia are similar; besides, viral and bacterial pneumonia may have overlapping imaging findings. Therefore, a differential diagnosis based only on these symptoms may be challenging, and laboratory confirmation is required. Nucleic acid amplification tests such as PCR are the diagnostic method of choice for M. pneumoniae and C. pneumoniae because of their high sensitivity and specificity compared to serology. However, serologic assays for M. pneumoniae

(76.5% vs. 48.6%, p = 0.040) as compared to its counterpart. A slightly high proportion of patients in the atypical bacteria co-infection group required ICU admission (88.2% vs. 70.1%, p = 0.159), but without statistical significance.

The median hospital stay duration was significantly longer in the M. pneumoniae or C. pneumoniae co-infection group compared to that of patients with only SARS-CoV-2 (median length of stay [LOS] 17 days [range 7–30 days] vs. median LOS 13 days [range 1–27 days], p = 0.004). The proportion of fatal cases (64.7% vs. 32.8%, p = 0.029) was significantly higher in the M. pneumoniae or C. pneumoniae co-infection group than patients with only SARS-CoV-2. Patients more likely to have a fatal outcome were those of older age having co-morbid conditions.

4 | DISCUSSION

Current literature shows co-infection with SARS-CoV-2 and other respiratory pathogens, and the data is still evolving. In the present study, we report co-infections due to atypical bacteria in SARS-CoV-2 infected patients. Due to similar clinical signs and symptoms, it is challenging to differentiate between COVID-19 and other types of respiratory infections.

M. pneumoniae commonly causes infections of the respiratory system among all age groups. The clinical presentations of M. pneumoniae range from mild infections affecting the upper respiratory tract to radiologically confirmed pneumonia that needs hospital admission. Co-infection of M. pneumoniae has been identified in viral pneumonia. In the present study, 5.1% of patients with SARS-CoV-2 had M. pneumoniae co-infections diagnosed by PCR or serology. Coexistence of M. pneumoniae and SARS-CoV-2 have been reported in former studies. Ziang et al. described an adult female patient having cough and chest congestion with ground-glass opacities in computed tomography (CT). The IgM antibody of M. pneumoniae was positive for this patient, and sputum RT-PCR tested positive for SARS-CoV-2. The patient was given both antivirals (lopinavir/ritonavir, peramivir, interferon-α2b) and antibiotics (azithromycin and levofloxacin) and subsequently recovered.

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Legionella spp. are responsible for Legionnaires’ disease, severe pneumonia in individuals with underlying medical conditions. L. pneumophila and COVID-19 co-infection was not identified in our patient population. However, in the literature, co-infection with SARS-CoV-2 and L. pneumophila had been rarely reported. Arashiro et al. described a patient who returned from a Nile Cruise experienced mild cough, diarrhea, malaise, and ground-glass opacity on chest CT. Both Legionella UAT and SARS-CoV-2 RT-PCR were positive for this patient. He was treated with azithromycin and supportive care but could not be saved.

The majority of the patients with SARS-CoV-2 and atypical bacteria co-infection presented with fever, cough, and dyspnea, and showed bilateral infiltrates, and received ventilatory support. The clinical symptoms of SARS-CoV-2 and bacteria causing atypical pneumonia are similar; besides, viral and bacterial pneumonia may have overlapping imaging findings. Therefore, a differential diagnosis based only on these symptoms may be challenging, and laboratory confirmation is required. Nucleic acid amplification tests such as PCR are the diagnostic method of choice for M. pneumoniae and C. pneumoniae because of their high sensitivity and specificity compared to serology. However, serologic assays for M. pneumoniae
and C. pneumoniae can still be helpful when molecular assays and culture are not available or adjunct to the PCR.26

Bacterial co-infections in SARS-CoV-2 may play a role in the prognosis of the disease and result in significant morbidity and mortality.5,13 In the present study, there were a significantly higher proportion of patients with M. pneumoniae or C. pneumoniae co-infection need mechanical ventilation support and were likely to develop complications. Similar findings are also reported in a study from Europe.27

Presently, no specific treatment exists for COVID-19, and supportive care is the mainstay. Treatment with hydroxychloroquine, remdesivir, tocilizumab, and lopinavir/ritonavir have been used in certain situations.6,28 The drug of choice for atypical pneumonia include fluoroquinolones, macrolides, and tetracyclines.15,29 In our M. pneumoniae or C. pneumoniae co-infection group, only seven patients received agents with atypical pneumonia coverage (either azithromycin, or fluoroquinolones, or doxycycline). The timely identification of atypical bacteria can influence treatment decisions and improve disease outcomes.

### 4.1 Limitations of the study

Our study has a few limitations. All COVID-19 patients admitted to our facility were not simultaneously tested for atypical pathogens. Therefore, the true incidence of co-infection remains unclear. A lower respiratory tract specimen (e.g., sputum, BAL, endotracheal wash, lung tissue) is the suitable sample for Legionella diagnosis; however, collection of invasive respiratory samples in COVID patients was restricted to prevent aerosol-generating procedures that pose a significant risk to health care staff and patients. We could not use molecular methods to diagnose C. pneumoniae infections, for which we could rely only on serology. Lastly, the use of serology for diagnosis of M. pneumoniae and C. pneumoniae is fraught with problems related to low specificity resulting from cross-reactivity and persistence of antibodies from prior infection. Furthermore, a single IgM is not reliable, but rather needs to be interpreted in conjunction with acute and convalescent IgG measurement, which is often challenging to obtain.

However, this report represents baseline information regarding the clinical features, laboratory results, and outcome of patients co-infected with SARS-CoV-2 and atypical bacteria. Clinicians should consider other respiratory pathogens, including atypical bacteria, during the management of COVID-19 patients. Timely identification of co-existing pathogens can provide targeted treatment and prevent the fatal outcomes of patients during the current pandemic.

### 5 CONCLUSION

Our report highlights co-infection with atypical bacteria should be considered in patients with SARS-CoV-2 depending on the clinical context. Co-infections with other respiratory pathogens during the ongoing pandemic may cause clinical and laboratory diagnostic issues. Besides this, bacterial co-infections may also cause prolonged hospital stay, increased morbidity, and mortality if they remain undiagnosed. Physicians should anticipate bacterial co-infections and exclude other treatable pathogens, including atypical bacteria, during the ongoing pandemic. Similarly, COVID-19 testing should be simultaneously performed even though pathogens other than SARS-CoV-2 are identified. Larger prospective studies are required to shed further light on the true incidence of these co-infections and their impact on the clinical course of COVID-19 patients.

### ACKNOWLEDGMENTS

The authors acknowledge the Indian Council of Medical Research (ICMR), and the All-India Institute of Medical Sciences (AIIMS) for an intramural research grant. We thank all clinicians from COVID wards and ICUs for enrolling patients, all team members of COVID-19 testing facility of AIIMS, New Delhi. We also thank Ms. Aarzoo Sirohi and Mr. Surender Singh for their technical support. This study received special intramural funding from AIIMS, New Delhi (IR COVID-13).

### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

### ETHICS STATEMENT

The Ethical Committee of the AIIMS has provided approval for this study (Ref. No.: IEC-287/17.04.2020, RP-35/2020).

### DATA AVAILABILITY STATEMENT

All the data generated or analyzed during this study are included in this article.

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How to cite this article: Chaudhry R, Sreenath K, Batra P, et al. Atypical bacterial co-infections among patients with COVID-19: A study from India. J Med Virol. 2022;94:303-309. https://doi.org/10.1002/jmv.27324