Emerging Bacterial Pathogens in the COVID-19 Era: Chryseobacterium gleum—A Case in Point

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Abstract

Introduction In the ongoing severe acute respiratory syndrome coronavirus 2 pandemic, a long hospital stay and empirical broad-spectrum antibiotics make the patients prone to acquire nosocomial infections especially with unconventional organisms, and Chryseobacterium gleum is one such rare nosocomial pathogen.

Methods The given study is a case-series-based study conducted from September 2020 to April 2021 in which clinically suspected pneumonia patients who recovered from coronavirus disease 2019 (COVID-19) were included.

Results Seventeen C. gleum isolates were obtained in pure culture from the tracheal aspirates of nine COVID-19 patients (including repeat samples to rule out colonization) within a period of eight months (September 2020–April 2021). Our records showed that there has been an increase in the number of isolates of C. gleum obtained in respiratory samples in 2020. We also did a review of literature of all the cases of C. gleum pneumonia reported till now.

Conclusion To the best of our knowledge, this is the first study reporting the isolation of this rare pathogen from COVID-19 patients with clinical significance in a large cohort of patients. Therefore, it becomes important to consider this pathogen as a significant cause of respiratory infections, especially in patients recovered post COVID-19.

Keywords ► emerging ► bacterial ► COVID-19 ► Chryseobacterium gleum ► pneumonia

Introduction

Chryseobacterium gleum is a nonfermentative, gram-negative bacillus belonging to the family Flavobacterium. It comes under the Centers for Disease Control and Prevention (CDC) group IIb which also comprises Chryseobacterium indologenes and other species within the genus Chryseobacterium.¹ The species in these genera cause healthcare and device-associated infections primarily due to their ability to survive in aqueous environments and by forming biofilms. Majority of the Chryseobacterium species cause infections of the urinary tract and the lower respiratory tract, largely in hospitalized patients with underlying comorbid conditions.²⁻⁵ In the recent times, due to the availability of different diagnostic methods like DNA sequencing and matrix-assisted laser desorption ionization time-of-flight spectrometry (MALDI-TOF MS), it has become easier to differentiate the various species within this genus.

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**Chryseobacterium gleum** Infection in COVID-19 Patients

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*C. gleum* was first identified as a medically relevant pathogen in the SENTRY Antimicrobial Surveillance Program. However, in this study, it was the least frequently isolated species within the genus *Chryseobacterium*, with only two strains identified over the 5-year study period in 16 countries. Though remarkably rare, a growing number of case reports from Europe and Southeast Asia have demonstrated its isolation in respiratory cultures in the recent years. *C. gleum* has been reported to cause a variety of infections ranging from respiratory tract infections, urinary tract infections, pyonephrosis, septicemia, meningitis, wound infections, to peritonitis. The major worrisome issue with this pathogen is its intrinsic resistance against a broad variety of antibiotics including carbapenems, aminoglycosides, and colistin. The problem of multidrug resistant pathogens has led to an increase in the use of these very drugs, thereby selecting out *Chryseobacterium* species in patients who are on prolonged treatment with carbapenems and colistin.

In the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) pandemic, coronavirus disease 2019 (COVID-19)-induced pneumonia has been commonly seen especially in the high-risk groups, with requirement for long hospital stay and empirical treatment especially in the high-risk groups, with requirement for long hospital stay and empirical treatment, with *SARS-CoV2* pandemic, coronavirus disease 2019 (COVID-19)-induced pneumonia has been commonly seen especially in the high-risk groups, with requirement for long hospital stay and empirical treatment in patients who are on prolonged treatment with carbapenems and colistin.

To the best of our knowledge, this is the first case series-based study showing ventilator-associated pneumonia (VAP) due to *C. gleum* in patients recovered post-COVID-19 pneumonia.

**Methodology**

It is a descriptive retrospective study conducted from September 2020 to April 2021 in which clinically suspected pneumonia patients who recovered from COVID-19 pneumonia were included.

**Specimen Processing and Antimicrobial Susceptibility Testing**

During the study period, a total of 40 endotracheal aspirate samples from the clinically suspected pneumonia patients who recovered post-COVID-19 pneumonia were received in the microbiology laboratory and processed for aerobic bacterial culture. Samples were inoculated on 5% sheep blood agar and MacConkey agar followed by an overnight incubation at 37°C. Microbiological reporting of the endotracheal samples was done by the semiquantitative method taking greater than 10^5 colony/mL as significant. To rule out colonization, repeat samples were taken from the same patient. Identification was by MALDI-TOF MS (Vitek MS, Biomerieux Inc., Durham, North Carolina, United States). Antimicrobial susceptibility testing of the isolates was performed on Muller Hinton agar by Kirby Bauer disc diffusion method according to the CLSI 2020 guidelines. Since no standard guidelines for reporting antimicrobial susceptibility testing (AST) for *Chryseobacterium* spp. were available, we performed AST using antimicrobial agents recommended by the SENTRY antimicrobial surveillance program.

**Clinical Correlation**

The cases were followed up to establish clinical correlation. The details of changes in treatment and outcome were noted. Analysis was done using the Microsoft Excel version 16 (Microsoft Corp., Richmond, California, United States). An analysis of frequency of isolation of *C. gleum* in respiratory specimens over the last 5 years, that is, from 2016 to 2020 (prior to the current study period), was also performed.

**Environmental Surveillance**

To trace the source of infection, environmental surveillance was conducted by the hospital infection control team by sampling the patient areas and their adjoining environment (i.e., ventilator ports, beddings, sterile water from the humidifier, oxygen masks, laryngoscope blades, and ventilator tubing). A total of 100 samples were taken for environmental surveillance. These were further inoculated on 5% sheep blood agar and MacConkey agar. Identification was by MALDI-TOF MS (Vitek MS, Biomerieux Inc., Durham, North Carolina, United States).

**Results**

Seventeen *C. gleum* isolates were obtained in pure culture from the tracheal aspirates of nine COVID-19 patients (including repeat samples to rule out colonization) within a period of eight months (September 2020–April 2021). Eight patients were previously positive for COVID-19 and shifted to non-COVID-19 zone of the hospital after getting negative reverse-transcription polymerase chain reaction report for COVID-19 as per the hospital protocol. One patient was detected positive for COVID-19 at the same time when *C. gleum* was isolated from the endotracheal culture. All the patients were initially admitted to the dedicated COVID-19 area of the hospital and had requirement for ventilatory support due to COVID-19-induced pneumonia. The previous cultures of the endotracheal samples of the eight patients sent during their stay in the COVID-19 hospital were bacteriologically sterile. After being shifted to the non-COVID-19 wards, these patients deteriorated clinically (decreased SpO2, increased oxygen requirement, new-onset fever spikes) as well as radiologically (development of new infiltrates on chest X-ray), due to VAP. *C. gleum* was isolated in pure culture from the endotracheal aspirates from all the nine patients. The median time of isolation of *C. gleum* was 15.8 days post-admission and 15.2 days post-mechanical ventilation. Out of the nine patients, four (44.4%) succumbed to the illness. A detailed description of the clinical courses and laboratory parameters is provided in Table 1. An environmental source for this organism could not be identified as *C. gleum* was not isolated from any of the environmental sample cultured. Of this cohort, seven patients had
<table>
<thead>
<tr>
<th>Age in years</th>
<th>Gender</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Case 8</th>
<th>Case 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fever and severe respiratory distress for last 2 days</td>
<td>Fever and shortness of breath for last 5 days</td>
<td>Fever along with chills, myalgia for last 5 days, shortness of breath for 1 day</td>
<td>Fever, cough for 14 days and shortness of breath for 4 days</td>
<td>Dry cough for 2 weeks, fever for 2 days and shortness of breath for 1 day</td>
<td>Abdominal pain and vomiting for last 2 days, altered sensorium, respiratory distress and decreased urine output for last 1 day</td>
<td>Fever and cough along with shortness of breath for 4 days</td>
<td>Fever and cough for last 10 days along with shortness of breath for 3 days</td>
<td>Chest pain, cough associated with few streaks of blood for 3 days, fever, shortness of breath for 2 days</td>
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<td></td>
<td></td>
<td>14 days</td>
<td>16 days</td>
<td>13 days</td>
<td>10 days</td>
<td>10 days</td>
<td>20 days</td>
<td>24 days</td>
<td>24 days</td>
<td>6 days</td>
</tr>
<tr>
<td>Symptoms on presentation</td>
<td></td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis</td>
<td>New onset fever, purulent tracheal secretions, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
</tr>
<tr>
<td>Time period between mechanical ventilation and culture positive for Chryseobacterium gleum</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Clinical picture at the time of isolation of bacteria</td>
<td></td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis</td>
<td>New onset fever, purulent tracheal secretions, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds, worsening gas exchange</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
<td>New onset fever, purulent tracheal secretions, leucocytosis, bronchial breath sounds</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td>Type 2 DM, HTN</td>
<td>Type 2 DM, HTN</td>
<td>Type 2 DM, HTN</td>
<td>Type 2 DM, HTN</td>
<td>None</td>
<td>Type 2 DM</td>
<td>None</td>
<td>Type 2 DM</td>
<td>Type 2 DM</td>
</tr>
<tr>
<td>Empirical antibiotics</td>
<td></td>
<td>Colistin, meropenem</td>
<td>Colistin, meropenem</td>
<td>Colistin, vancomycin</td>
<td>Colistin, meropenem</td>
<td>Meropenem, vancomycin</td>
<td>Colistin, meropenem</td>
<td>Colistin, meropenem</td>
<td>Colistin, meropenem</td>
<td>Colistin, vancomycin</td>
</tr>
<tr>
<td>Radiology</td>
<td></td>
<td>New onset bilateral chest infiltrates-lower lobe of lung</td>
<td>Left lower lobe consolidation, opacity</td>
<td>Bilateral diffuse alveolar opacities</td>
<td>Bilateral dense patchy consolidations, bilateral minimal pleural effusion, bilateral ground glass opacities</td>
<td>Bilateral diffuse peribronchovascular consolidation along with thick-walled cavi ty on right lower lobe region</td>
<td>Bilateral chest infiltrates in the lower lobe of lung</td>
<td>New onset bilateral chest infiltrates, prominent reticular markings</td>
<td>Left lower lobe consolidation, left lower lobe infiltrates</td>
<td>Bilateral chest infiltrates in the lower lobe of lung</td>
</tr>
<tr>
<td>Sensitivity of Chryseobacterium gleum</td>
<td></td>
<td>Sensitive to: cef-tazidime, chloramphenicol, cotrimoxazole, meropenem, minocycline, levofloxacin, piperacillin-tazobactam, ciprofloxacin, tetracycline</td>
<td>Sensitive to: cef-tazidime, chloramphenicol, cotrimoxazole, minocycline, levofloxacin, piperacillin-tazobactam, ciprofloxacin, tetracycline</td>
<td>Sensitive to: cef-tazidime, chloramphenicol, cotrimoxazole, minocycline, levofloxacin, piperacillin-tazobactam, ciprofloxacin, tetracycline</td>
<td>Sensitive to: cef-tazidime, chloramphenicol, cotrimoxazole, minocycline, levofloxacin, piperacillin-tazobactam, ciprofloxacin, tetracycline</td>
<td>Resistant to: Amikacin, meropenem, chloramphenicol,</td>
<td>Sensitive to: cef-tazidime, chloramphenicol, cotrimoxazole, minocycline, levofloxacin, piperacillin-tazobactam, ciprofloxacin, tetracycline</td>
<td>Resistant to: Amikacin,</td>
<td>Sensitive to: chloramphenicol, cotrimoxazole, minocycline, levofloxacin, piperacillin-tazobactam, ciprofloxacin, tetracycline</td>
<td>Resistant to: Amikacin,</td>
</tr>
<tr>
<td>Case</td>
<td>Age in years/Gender</td>
<td>Symptoms on presentation</td>
<td>Blood culture</td>
<td>Neutrophils (41–72%)</td>
<td>Procalcitonin (0.01–0.50 ng/mL)</td>
<td>CRP (0–5 mg/L)</td>
<td>PT/INR (80–100%)</td>
<td>Treatment given</td>
<td>Outcome</td>
<td></td>
</tr>
<tr>
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<td>---------</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70/female</td>
<td>Fever and severe respiratory distress for last 2 days</td>
<td>Sterile</td>
<td>85.9</td>
<td>2.79</td>
<td>73.03</td>
<td>100/0.9</td>
<td>Cotrimoxazole, ceftazidime</td>
<td>Discharged under stable conditions</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>74/male</td>
<td>Fever and shortness of breath for last 5 days</td>
<td>Sterile</td>
<td>89</td>
<td>2.71</td>
<td>184.7</td>
<td>90/1.29</td>
<td>Levoflaxacin</td>
<td>Died (post-COVID-19, septic shock)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50/female</td>
<td>Fever along with chills, myalgia for last 5 days, shortness of breath for 1 day</td>
<td>Sterile</td>
<td>78.2</td>
<td>0.864</td>
<td>270.04</td>
<td>70/1.4</td>
<td>Ceftazidime, piperacillin-tazobactam</td>
<td>Discharged under stable conditions</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28/female</td>
<td>Fever, cough for 14 days and shortness of breath for 4 days</td>
<td>Sterile</td>
<td>7.400</td>
<td>5.3</td>
<td>409</td>
<td>96/1.04</td>
<td>Ceftazidime, piperacillin-tazobactam</td>
<td>Discharged under stable conditions</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>49/female</td>
<td>Dry cough for 2 weeks, fever for 2 days and shortness of breath for 1 day</td>
<td>Sterile</td>
<td>20.000</td>
<td>2.2</td>
<td>333.3</td>
<td>70/0.8</td>
<td>Piperacillin-tazobactam</td>
<td>Died (septic shock, VAP, post-COVID-19 ARDS)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>59/female</td>
<td>Abdominal pain and vomiting for last 2 days, altered sensorium, respiratory distress and decreased urine output for last 1 day</td>
<td>Sterile</td>
<td>20.000</td>
<td>8.1</td>
<td>170.22</td>
<td>96/1.3</td>
<td>Minocycline, piperacillin-tazobactam</td>
<td>Discharged under stable conditions</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>36/male</td>
<td>Fever and cough along with shortness of breath for 4 days</td>
<td>Sterile</td>
<td>13,000</td>
<td>2.2</td>
<td>150.2</td>
<td>78/1.28</td>
<td>Levoflaxacin</td>
<td>Died (post-COVID-19 ARDS, VAP, upper GI bleeding)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>59/female</td>
<td>Fever and cough for last 10 days along with shortness of breath for last 3 days</td>
<td>Sterile</td>
<td>15,000</td>
<td>1.81</td>
<td>24.2</td>
<td>76/1.3</td>
<td>Ceftriaxone, piperacillin-tazobactam</td>
<td>Discharged under stable conditions</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>37/male</td>
<td>Chest pain, cough associated with few streaks of blood for 3 days, fever, shortness of breath for 2 days</td>
<td>Sterile</td>
<td>15,000</td>
<td>29.8</td>
<td>155.55</td>
<td>98/1.4</td>
<td>Levoflaxacin</td>
<td>Died (septic shock, VAP, post-COVID-19 ARDS)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DM, diabetes mellitus; FiO2, fraction of inspired oxygen; GI, gastrointestinal; HTN, hypertension; INR, international normalized ratio; PT, prothrombin time; TLC, total leukocyte count; VAP, ventilator-associated pneumonia.
received colistin and five vancomycin and five meropenem empirically.

Our records showed that there has been an increase in the number of isolates of C. gleum obtained in respiratory samples in 2020 (Fig. 1, after excluding repeat samples from same patients). We also observed that during the study period, out of a total of 10 patients having positive respiratory cultures for C. gleum, nine (90%) had history of COVID-19 infection (Fig. 2).

Discussion and Review of Literature

We found C. gleum to be the causative agent for VAP in nine subjects with COVID-19 disease. Till now there were no reports of the isolation of C. gleum in cases of VAP from patients post-COVID-19 infection. To the best of our knowledge, this is the first study reporting the isolation of this rare pathogen from previously positive COVID-19 patients with clinical significance in a large cohort of patients. Therefore, it becomes important to consider this pathogen as a significant cause of respiratory infections especially in patients recovered post-COVID-19.

Chryseobacterium spp. are emerging gram-negative bacilli belonging to the family of nonfermenters. More than 100 species have been reported in the genus Chryseobacterium but only C. indologenes and C. gleum have been the most isolated species from humans. In the past, these bacteria were mainly isolated from patients with polymicrobial infections that made it difficult to determine their clinical significance. However, in recent times this organism has been isolated in pure cultures from various patient samples, suggesting an emerging role of this organism in causing disease in humans. MALDI-TOF MS has also assisted in the better identification of the various species within the genus Chryseobacterium. In fact, a study by Lo and Chang found MALDI-TOF to be an excellent and cost-effective alternative to 16S rRNA sequencing for its ability to identify C. gleum.

This organism has been mainly associated with hospital-acquired infections (HAIs). Factors like the inherent chlorine resistance of this genus facilitate its persistence within the hospital environment and thereby increasing its likelihood in causing nosocomial infections. Many of the bacteria were mainly isolated from patients with polymicrobial infections that made it difficult to determine their clinical significance. However, in recent times this organism has been isolated in pure cultures from various patient samples, suggesting an emerging role of this organism in causing disease in humans. MALDI-TOF MS has also assisted in the better identification of the various species within the genus Chryseobacterium. In fact, a study by Lo and Chang found MALDI-TOF to be an excellent and cost-effective alternative to 16S rRNA sequencing for its ability to identify C. gleum.

This organism has been mainly associated with hospital-acquired infections (HAIs). Factors like the inherent chlorine resistance of this genus facilitate its persistence within the hospital environment and thereby increasing its likelihood in causing nosocomial infections. Many of the Chryseobacterium spp. especially C. indologenes are well known for their intrinsic resistance to carbapenems and cephalosporins due to the presence of class A and B β-lactamases. A study by Bellais et al reported heterogeneity in metallo β-lactamases in Chryseobacterium species. Chryseobacterium spp. are well known to colonize various medical devices containing fluids such as respirators, humidifiers, and syringes. Many COVID-19 cases require prolonged hospitalization. Along with an increase in need for mechanical ventilation, there is an increased risk of various HAIs due to rare and opportunistic organisms such as C. gleum. During the given period, a total of 18 isolates of C. gleum had been obtained from 10 patients, out of which 17 isolates were from nine COVID-19 positive patients, suggesting a possible association between COVID-19 virus and C. gleum respiratory tract infection. Our systematic review yielded only 18 reports of C. gleum from different clinical specimens like blood, sputum, urine, and pus. In a previously published study from our institute, 19 Chryseobacterium spp. species were isolated from urine samples in patients with urological complaints, of which 15 belonged to Chryseobacterium indologenes and only four to C. gleum. Similarly, in a recent study from North India, 20 isolates of Chryseobacterium spp. were identified over a span of 3 years (2017–2019), where C. indologenes (18/20) was the commonest species followed by C. gleum (2/20), although clinical correlation could not be established for the C. gleum isolates. As far as the role of C. gleum as a causative agent of respiratory infections is concerned, only ten cases have been reported till now. A detailed review of all these cases has been provided in Table 2.

In the present case series, C. gleum was isolated in pure culture from the tracheal aspirates of nine patients and the isolation correlated clinically as well as radiologically. Co-morbid conditions like diabetes mellitus, chronic kidney disease, cardiovascular disease, chronic obstructive pulmonary disease, and malignancies were associated risk factors for acquiring infections with this pathogen. Similar
findings were seen in the present cases where seven patients (77.8%) had underlying comorbidities. Other risk factors associated with the isolation of *C. gleum* as reported in literature\textsuperscript{14,15,26} include exposure to broad-spectrum antibiotics like vancomycin and colistin, prolonged hospitalization, invasive interventions, and medical intensive care unit (ICU) stay (greater than 21 days)\textsuperscript{14,27,29}.

The organisms in the *Chryseobacterium* genus are well known to form biofilms on various medical devices\textsuperscript{15,28} and thus causing device-related HAIs. All the patients in the given study had a history of a prolonged hospital stay including ICU admission, mechanical devices, presence of a central line port, endotracheal tube, and invasive ventilation. In our patients, seven patients had more than 14 days duration of hospital stay before the isolation of *C. gleum* and two of them had more than 21 days of hospitalization. We found *C. gleum* as a causative agent of VAP in nine subjects with severe COVID-19 disease. We also found a high mortality due to *C. gleum*.

*Chryseobacterium gleum* is intrinsically resistant to most antibiotics (carbapenems, colistin) that are used empirically to treat HAIs. Most secondary HAIs in our institute are due to *Acinetobacter baumannii* that is sensitive to carbapenems and colistin. In all our patients, we empirically started colistin on clinical worsening based on our previous

### Table 2: Summary of reported susceptibilities and treatment of *Chryseobacterium gleum* isolates from the respiratory tract to date

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Susceptibility testing criteria</th>
<th>Susceptibility profile</th>
<th>Treatment</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambiase et al\textsuperscript{25}</td>
<td>2007</td>
<td>Italy</td>
<td>NCCLS: not specified</td>
<td>#Resistant: AMK, ATM, CAZ, CTX, FEP, GEN, IPM, MEM, SAM, TZP Susceptible: CIP, LVX, SXT</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Virok et al\textsuperscript{3}</td>
<td>2014</td>
<td>Hungary</td>
<td>EUCAST: <em>Pseudomonas</em> spp.</td>
<td>#Resistant: AMK, DOR, GEN, IPM, MEM, TOB, TZP Susceptible: CAZ, CIP, FEP, LVX</td>
<td>CIP</td>
<td>Responded</td>
</tr>
<tr>
<td>Lo and Chang\textsuperscript{8}</td>
<td>2014</td>
<td>Taiwan</td>
<td>CLSI: other non-Enterobacteriaceae</td>
<td>#Resistant: AMK, AMS, CAZ, CFZ, CRO, CST, FEP, FOX, GEN, IPM, PIP, TZP Susceptible: CIP, MIN, SXT, TGC</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Brkic et al\textsuperscript{9}</td>
<td>2015</td>
<td>Croatia</td>
<td>EUCAST: Gram-negative non-fermentative bacteria</td>
<td>Resistant: CST, DAP, IPM, MEM, VAN Susceptible: CAZ, CIP, FEP, TGC, TZP</td>
<td>TZP</td>
<td>Responded</td>
</tr>
<tr>
<td>Abdalhamid et al\textsuperscript{19}</td>
<td>2016</td>
<td>Saudi Arabia</td>
<td>CLSI: other non-Enterobacteriaceae</td>
<td>Resistant: AMK, CAZ, CIP, CST, FEP, GEN, IPM, MEM, TGC, TZP, VAN Susceptible: LVX, MIN, SXT</td>
<td>LVX</td>
<td>Responded</td>
</tr>
<tr>
<td>Rawat et al\textsuperscript{20}</td>
<td>2017</td>
<td>India</td>
<td>NA</td>
<td>Resistant: NA Susceptible: MIN, SXT, TZP</td>
<td>TZP + SXT</td>
<td>Responded</td>
</tr>
<tr>
<td>Jain et al\textsuperscript{7}</td>
<td>2017</td>
<td>India</td>
<td>CLSI: other non-Enterobacteriaceae</td>
<td>Resistant: AMX, CAZ, CFP, CLI, CRO, CST, CTX, DOX, ERY, FEP, GEN, IPM, MEM, TOB Susceptible: AMK, CIP, DOX, LVX, MIN, SXT, TZP, VAN</td>
<td>LVX</td>
<td>Responded but later on died due to sudden cardiac arrest</td>
</tr>
<tr>
<td>Mirza et al\textsuperscript{21}</td>
<td>2018</td>
<td>Turkey</td>
<td>CLSI: other non-Enterobacteriaceae</td>
<td>Resistant: AMK, GEN, IPM, MEM Susceptible: CAZ, CIP, FEP, LVX, SXT, TZP</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tsouvalas et al\textsuperscript{28}</td>
<td>2020</td>
<td>USA</td>
<td>CLSI: non-fermentative Gram-negative bacilli</td>
<td>Resistant: AMK, ATM, CAZ, CRO, FEP, GEN, IPM, MEM, TOB Susceptible: SXT</td>
<td>SXT</td>
<td>Responded</td>
</tr>
<tr>
<td>Amisha et al\textsuperscript{27}</td>
<td>2021</td>
<td>USA</td>
<td>NA</td>
<td>Resistant: TGC, GEN, TOB Susceptible: AMK, FEP, CAZ, CRO, LVX, MEM, TZP, SXT</td>
<td>SXT, LVX</td>
<td>Died</td>
</tr>
</tbody>
</table>

**Abbreviations:** AMK, amikacin; AMX, amoxicillin; ATM, Aztreonam; CAZ, ceftazidime; CFP, ceferoperazone; CFZ, cefazolin; CIP, ciprofloxacin; CLI, clindamycin; CLSI, Clinical and Laboratory Standards Institute; CRO, ceftriaxone; CST, colistin; CTX, cefotaxime; DAP, daptomycin; DOR, doripenem; DOX, doxycycline; ERY, erythromycin; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; MIN, minocycline; NA, not applicable; NA, not available; NCCLS, National Committee for Clinical Laboratory Standards; PIP, piperacillin; SAM, ampicillin–sulbactam; SXT, trimethoprim–sulfamethoxazole; TGC, tigecycline; TOB, tobramycin; TZP, piperacillin–tazobactam; VAN, vancomycin.

#Multiple strains reported.
antibiogram. We treated C. gleum only after receiving the final culture and sensitivity report. This may have resulted in a delay in initiating the appropriate antibiotics and could explain a high mortality in our series. We have now changed our antibiotic prescription and usually add cotrimoxazole, fluoroquinolones, or ceftazidime in addition to carbapenems or colistin. Centers involved in caring for COVID-19 subjects should investigate all HAs for finding unusual microorganism and modify their antibiotic policy accordingly.

Recent studies have reported increasing incidence of secondary bacterial infections (SBIs) in COVID-19 patients.30–32 The most commonly isolated bacteria are Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas species. In none of these studies Chryseobacterium spp has been isolated as a cause of SBIs. Primarily, the decreased monas species bacterial attachment to host structural cells and proinflammation. Pointed studies are needed to con

underlying comorbid conditions, and mechanical ventilation can predispose the patients to this rare pathogen in causation of pneumonia in a scenario of post-COVID-19 pneumonia.

One important issue in such cases is how to differentiate a true pathogen from a colonizer especially when there is a history of prolonged hospitalization and mechanical ventilator. Isolation of the same organism in pure culture with high bacterial count and in repeat samples along with the clinical picture supports the notion that an isolated microbe is a pathogen and cannot be ignored as a mere contaminant, but no specific guidelines for repeat cultures exist in the literature.7 In our series, the respiratory secretions grew C. gleum on repeat cultures. Also, all subjects had clinical worsening suggesting C. gleum to be responsible for the clinical worsening. Moreover, 89% (8/9) of the patients had prior respiratory cultures where C. gleum was not isolated, thereby ruling out a carrier state. To the best of our knowledge, there is no evidence of a respiratory carrier state of C. gleum documented in the literature.

No standard guidelines are available from either the CLSI (Clinical and Laboratory Standards Institute) or the EUCAST (European Committee on Antimicrobial Susceptibility Testing) for the antimicrobial susceptibility testing of members within the genus Chryseobacterium. Some studies have used Staphylococcus breakpoints for minimal inhibitory concentration interpretation,4 while others have chosen nonfermenting gram-negative bacilli cutoffs to interpret results.17,19 The SENTRY study (1997–2001) estimated the epidemiology and antimicrobial susceptibility pattern of Chryseobacterium infections worldwide, where the most active antimicrobials were the newer quinolones (garenoxacin, gatifloxacin, and levofloxacin), followed by rifampin, trimethoprim–sulfamethoxazole, ciprofloxacin, and piperacillin–tazobactam.6 In the given case series, the isolates were sensitive to most of the antibiotics. Maximum resistance was seen against amikacin and meropenem followed by chloramphenicol. Previous studies have tested the effects of vancomycin and clindamycin on these bacteria as an identification marker and not for treatment purposes.5,6 As in the previous case reports, the organism has consistently shown susceptibility to trimethoprim–sulfamethoxazole, fluoroquinolones such as levofloxacin and ciprofloxacin or piperacillin–tazobactam (or a combination of these agents), these agents are typically used for therapy.5,7,8,19,21,27,37 However, in our patients levofloxacin was used in three patients and most commonly used antibiotic was piperacillin–tazobactam and ceftazidime.

One interesting observation in the present case series was that the outcome when a single antibiotic was given was poor when compared with those who received a combination of agents. Previous studies in non-COVID-19 patients have shown that treatment by a single antimicrobial agent (piperacillin–tazobactam, quinolones or tetracycline) is curative. Whereas as observed in our cases of COVID-19 patients there can be a possibility that in the already compromised lungs due to the virus-mediated direct damage to the lung epithelium, an intense host immune response in the form of an aberrant cytokine storm required an intense regimen to combat the bacterial burden.33,34

The isolates showed different antibiotic sensitivity profiles and C. gleum were not detected in the environmental samples suggesting thereby that the infections did not originate from a point source. Moreover, in all the cases, similar organism was not isolated from blood cultures indicating a localized respiratory infection without dissemination. One case report till now has reported simultaneous isolation of C. gleum from both blood and tracheal aspirate.5 The previous studies have not reported mortality in cases with C. gleum. However, in our case series, 44.4% patients died and the cause of death in all these patients was septic shock following VAP, so this is the first report to our knowledge documenting the mortality associated with this new emerging nosocomial pathogen. Therefore, it becomes important to consider this pathogen as a significant cause of respiratory infections especially in patients recovered post-COVID-19.

Conclusion

Our report is the first to establish the association of C. gleum with COVID-19 infection as a cause of SBI in this group of patients. This study is also the first to establish the mortality associated with this new emerging pathogen. Critically ill patients in ICUs, with mechanical devices, receiving broad-spectrum antibiotics are at risk of developing healthcare-
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associated infections due to this pathogen. Since it is inherently resistant to carbapenems and colistin, its rapid and accurate identification in the laboratory, preferably based on MALDI-TOF MS, is essential for guiding therapy. Moreover, standard in vitro susceptibility methods for this rare organism should be established that can be applied in routine microbiological practices. However, more extensive studies highlighting the exact mechanisms of pathogenesis are required.

Limitations
Molecular techniques could not be performed to say the given case series as an outbreak as we could not retrieve some isolates of C. gleum.

Ethical Approval
The study was approved by the Institute Ethics Committee with reference no. NK/6623/Study/057.

Funding
None.

Conflict of Interest
None declared.

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