

Clinicomicrobiological Profile of Infections by *Achromobacter*: An Emerging Nosocomial Pathogen in Indian Hospitals

Abstract

Background: *Achromobacter* causes opportunistic nosocomial infections in immunocompromised patients with high mortality. It is underreported as it is often misidentified by conventional microbiological methods. **Aims:** The aim of the study is to access the clinicomicrobiological profile and antibiogram of *Achromobacter spp.* from clinical isolates. **Materials and Methods:** It is an observational study done from July 2020 to December 2021 in our hospital. All nonduplicate isolates of *Achromobacter* from blood and respiratory samples were initially identified with VITEK-2 GN card system and further confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Antibiogram and treatment outcomes were also studied. **Results:** *Achromobacter spp.* was isolated from 14 patients. Blood samples yielded most isolates (71.4%; $n = 10$) followed by tracheal aspirate and bronchoalveolar lavage fluid. Bacteremia followed by pneumonia was the most common clinical manifestation of *Achromobacter* infection. All the isolates were identified as *A. xylosoxidans denitrificans* and showed 100% susceptibility to minocycline and piperacillin-tazobactam. Diabetes mellitus and malignancy were the most common underlying condition in these patients. A favorable outcome was seen in 78.6% of the individuals with timely institution of antibiotics and proper diagnosis. **Conclusion:** Infections due to *Achromobacter* are on the rise in developing countries like India. Resistance to many classes of antimicrobials makes its treatment more challenging therefore it should always be guided by antibiograms. The present study highlights the significance of this rare bacterium in patients with malignancies in India and advocates greater vigilance toward appropriate identification of this organism.

Keywords: *Achromobacter*, bacteremia, bronchoalveolar lavage, COVID-19 pneumonia

Introduction

Achromobacter is a rare pathogen that causes opportunistic nosocomial infections in immunocompromised patients, with high mortality.^[1-4] It is underreported as it is often misidentified by conventional microbiological methods.^[5] Its intrinsic and acquired multidrug resistance^[6,7] makes its treatment often difficult and an optimal antimicrobial regimen has not been determined.^[8,9]

Literature from India on this uncommon pathogen is mostly limited to very small series or single case reports and that too in patients of cystic fibrosis (CF). Therefore, we aimed to study the clinicomicrobiological profile and antibiogram of *Achromobacter* species isolated from both pulmonary and extrapulmonary samples of our hospital.

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Materials and Methods

Study design and setting

This study was an observational study, undertaken for 18 months from July 2020 to December 2021 in our university hospital.

Inclusion criteria

All blood and respiratory samples received in the microbiology laboratory for identification, culture, and antibiotic sensitivity during the study were included of which all the clinical isolates identified as *Achromobacter* were studied in detail. Patients' categorization was done according to their clinical conditions like ventilator-associated pneumonia, defined as pneumonia occurring more than 48 h after patients have been intubated and on mechanical ventilation, and bacteremia was diagnosed as per the Pitt bacteremia score. This score is widely used in intensive care settings and ranges from 0 to 14 points, with a score ≥ 4 commonly

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used as an indicator of critical illness and increased mortality.

Sample processing

Blood and respiratory samples, collected from patients, were sent to the microbiology laboratory for identification, culture, and antibiotic sensitivity of the pathogen. For respiratory samples, quantitative culture was performed on sheep blood agar and MacConkey agar with incubation at 37°C. The cutoff point of 10⁵ CFU/mL was considered significant for tracheal aspirate samples to indicate an infection and for bronchoalveolar lavage (BAL) samples, the cut-off was taken as 10⁴ CFU/mL after 48 h of incubation. Blood cultures were performed with the help of automated blood culture systems (Bact/Alert, bioMérieux, France) and were subcultured on sheep blood agar and MacConkey agar after the bottle was flagged positive by this machine.

Initially, the bacterial isolates were identified using the routine Gram staining and biochemical tests used in our laboratory.^[10] The biochemical reactions for this Gram-negative bacillus revealed the results as follows: catalase-positive, oxidase-positive, motile, nonfermenting, indole-negative, triple sugar iron, agar-K/K (alkaline/alkaline) and urease-negative, DNase-negative bacterium.^[11]

With the automated methods, initially, the bacteria was identified with VITEK 2 GN card system (bioMérieux), an automated identification and susceptibility testing system, and finally, its identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). MALDI-TOF is a rapid method for the identification of bacterial isolates to species level in few hours. MALDI-TOF MS uses updated SARAMIS database amended with *Achromobacter* spp. spectra for identification; therefore, the results of this updated MALDI-TOF MS were taken as confirmatory for the identification of bacteria if there is a discordance in results between the two systems.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion and E test method on Mueller–Hinton agar and by VITEK-2 (bioMérieux) system.^[12] The minimum inhibitory concentrations (MICs) of the antibiotics tested were interpreted using the CLSI guidelines for other non-*Enterobacteriaceae* because there are no clear guidelines for sensitivity interpretation of *Achromobacter* isolates.^[13]

Patient follow-up

Demographic details, underlying diseases and other comorbid conditions, type of infection use of invasive procedures, duration of hospital stay, and microbiological identification and antibiotic sensitivity and outcomes were

recorded for all patients. Follow-up visits of the patient were obtained from the outpatient department for the treatment outcomes.

Statistical analysis

Statistical analysis was performed by the Statistical Package for the Social Sciences (IBM-SPSS) software, Version 25. (IBM Corporation, New York, USA.) for descriptive statistics. Categorical data were described using numbers and percentages.

Results

Over a period of 18 months, *Achromobacter* spp. was isolated from 14 patients. The mean age of the patients was 44.3 years (standard deviation = 16.5) and the range varied from 11 to 64 years. There were nine male and five female patients in the study.

Comorbidities were associated with all 14 cases of *Achromobacter* infection. Diabetes mellitus and hypertension were present in 50% ($n = 7$) in 35.7% ($n = 5$) cases, respectively, hematological malignancies in 28.6% ($n = 4$), other malignancies (e.g., breast, pancreas, prostate) in 21.4% ($n = 3$), post-COVID pneumonia and chronic obstructive pulmonary disease (COPD) in 21.4% ($n = 3$), and one case (7.1%) each of renal failure, cerebrovascular accident, systemic lupus erythematosus, liver failure with cirrhosis, and acute pancreatitis [Table 1].

Regarding sample categorization, most isolates were cultured from blood (71.4%; $n = 10$), followed by tracheal aspirate and BAL fluid (28.6%; $n = 4$) [Tables 1]. Subsequently, bacteremia and sepsis were finally diagnosed in 71.4% ($n = 10$) and ventilator-associated pneumonia and lower respiratory tract infection with pneumothorax in 28.6% ($n = 4$). Infection developed in all the patients with underlying hematological malignancies after the initiation of chemotherapy. Nine patients had a central or peripheral line insertion at the time of development of sepsis/bacteremia and five patients required mechanical ventilation due to clinical deterioration in their symptoms. The average duration of hospital stay was 38 days (range: 10–42 days), and the mean time of development of infection after hospitalization was 21 days (range: 10–51 days). Final identification was performed by VITEK-2 GN card system and was confirmed by MALDI-TOF MS. MALDI-TOF MS identified all 14 isolates to subspecies level as *A. xylosoxidans denitrificans* but VITEK 2 identified 12 isolates as *A. xylosoxidans* and for 2 isolates, it gave only identification till genus level as *Achromobacter*. Detailed species identification and scores are listed in Table 2. All 14 isolates identified as *A. xylosoxidans denitrificans* were isolated from ten blood, three BAL, and two tracheal aspirate sample. Detailed sample-wise categorization of *Achromobacter* isolates in positive patients is given in Table 1.

Table 1: Clinical details and outcomes of adult patients (n=14) with *Achromobacter* isolates

Age (years)/sex	Clinical diagnosis	Underlying illness/comorbidities	Sample collected	<i>Achromobacter</i> spp. isolated	Antibiotics administered/changed for <i>Achromobacter</i> infection	Outcome/follow-up
57/male	Sepsis	Carcinoma pancreas, DM	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam, minocycline	Expired
21/female	Sepsis	SLE	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
49/male	Sepsis	ESRD, DM	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
11/male	Sepsis	ALL	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
51/female	Sepsis	Post-COVID-19 pneumonia, DM, HTN	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam, Meropenem	Recovered
52/male	Stroke with intracerebral hemorrhage, VAP	HTN, DM	TA	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam, Minocycline	Recovered
30/male	Sepsis	Acute pancreatitis, HTN	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Expired
22/female	LRTI	ALL	BAL, TA	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
56/male	Pneumothorax, VAP	COPD, DM, HTN	BAL	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam, cotrimoxazole	Left against medical advice
63/male	LRTI	NHL	BAL	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
47/female	Sepsis	AML	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
64/male	Sepsis	Prostate cancer, HTN	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam, minocycline	Recovered
48/female	Sepsis	Breast cancer, DM	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam	Recovered
49/male	Sepsis	Liver failure with cirrhosis, DM	Blood	<i>A. xylosoxidans denitrificans</i>	Piperacillin-tazobactam, minocycline	Expired

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BAL: Bronchoalveolar lavage; COPD: Chronic obstructive pulmonary disease; COVID-19: Coronavirus disease of 2019; DM: Diabetes mellitus; ESRD: End-stage renal disease; HTN: Hypertension; LRTI: Lower respiratory tract infection; NHL: Non-Hodgkin lymphoma; SLE: Systemic lupus erythematosus; VAP: Ventilator-associated pneumonia; *A. xylosoxidans denitrificans*: *Achromobacter xylosoxidans denitrificans*; TA: Tracheal aspirate

Table 2: Comparison of *Achromobacter* spp. identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry mass spectrometry system and the VITEK 2 GN card system

Number of isolates of <i>Achromobacter</i>	MALDI-TOF MS (number of isolates)	Identification score of MALDI TOF MS	VITEK 2 with GN card system (no. of isolates)	Identification score of VITEK 2 system
<i>A. xylosoxidans denitrificans</i> (n=14)	<i>A. xylosoxidans denitrificans</i> (n=14)	100%	1. <i>A. xylosoxidans</i> (n=12) 2. Identified genus only <i>Achromobacter</i> (n=2)	0%

MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry mass spectrometry; GN: Gram negative

All isolates were fully susceptible to piperacillin-tazobactam and minocycline; only one isolate was not susceptible to ticarcillin-clavulanic acid. Resistance to meropenem, cotrimoxazole, levofloxacin, and ceftazidime was exhibited by 14.5%, 28.6%, 35.7%, and 35.7% of the isolates, respectively. Only 3 isolates were susceptible to ciprofloxacin [Figure 1]. All the isolates were sensitive to reserved drug colistin and resistant to amikacin and gentamicin *in vitro*.

Three of the 14 patients died during their hospital stay; the other 11 were discharged in good health with advice for routine follow-up in the outpatient department depending

on the medical condition. One patient was lost to follow-up as he left the hospital against medical advice. Favorable outcome was seen in 78.6% of the individuals with timely institution of antibiotics and proper diagnosis.

Discussion

Achromobacter is a ubiquitous, nonfermenting, Gram-negative bacterium that was originally considered commensals but now is increasingly being recognized as important nosocomial pathogens^[1] causing opportunistic infections in immunocompromised patients. Its infections usually occur in association with immunosuppression,

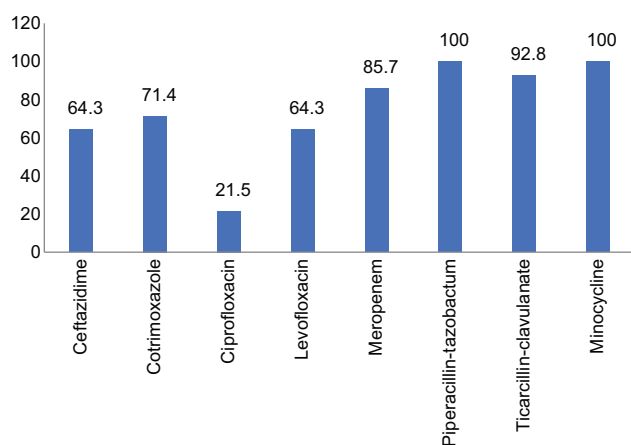


Figure 1: Sensitivity pattern of isolates of *Achromobacter* species (in percentage)

malignancies, acquired immune deficiency syndrome, or organ transplant recipients.^[2,3]

It is a difficult-to-treat organism with a wide antibiotic resistance spectrum and is being increasingly reported worldwide including India.^[1,14,15] *Achromobacter* normally inhabits aquatic sources in the environment and hospitals, as well as the human gut, but it may cause nosocomial and community-acquired infections. Although *Achromobacter* spp. has low virulence, invasive infections can be caused by this bacterium in immunocompromised individuals and neonates showing significant rates of morbidity and mortality.^[16] It was initially isolated from the respiratory tract of people with CF but it can cause a wide range of infections in hosts with other underlying medical conditions. In the present study, bacteremia followed by pneumonia was the most common manifestation of *Achromobacter* infection which is consistent with earlier studies in non-CF patients.^[4] Two subspecies, namely *denitrificans* and *xylosoxidans*, under the species *xylosoxidans* have been noted to cause disease in immunosuppressed populations.^[1] In the present study, the most common predisposing underlying medical condition in patients with *Achromobacter* infections was malignancies, both hematological and solid organ cancers, which emphasizes that bloodstream infection due to multidrug-resistant Gram-negative bacilli is a major cause of morbidity and mortality in these patients.^[16] Although they are studies on this rare pathogen from India, as far as oncology patients are concerned, only few case reports of *A. xylosoxidans* infection which occurred in a cancer patient are reported from India.^[17,18] Hence, our study is the first study on infection caused by *A. xylosoxidans* in patients with an underlying malignancy from our country. The most common comorbidities in our study were diabetes mellitus and hypertension followed by COPD, chronic renal failure, cirrhosis, treatment with high-dose corticosteroids, and use of immunosuppressive drugs which have also been documented in earlier studies.^[19] One

patient had COVID-19 pneumonia who was on steroid and was diabetic which could be cause of immunosuppression leading to infection of this pathogen in this patient.

Recently, there has been an increase in reporting of *Achromobacter* spp. from laboratories in developing countries including India.^[14,15] The main reason behind the identification and reporting of *Achromobacter* spp. is the utilization of automated methods of identification and sensitivity. Its role as a pathogen is underestimated because it is frequently misidentified as other common (i.e., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex, *Acinetobacter* spp.) and rare (i.e., *Pandoraea* spp. and *Ralstonia* spp.) nonfermenting Gram-negative bacilli with conventional methods due to biochemical similarities.^[5,20,21] In our study, all the isolates were identified till subspecies level by MALDI-TOF MS which is also seen in earlier studies.^[22] Hence, MALDI-TOF MS could be considered a rapid and convenient method to sequencing for the identification of such rare nonfermenting bacilli which are often misidentified by conventional methods. The predominance of *A. xylosoxidans* in all types of samples could be because this species is more abundant than other *Achromobacter* in both the natural and hospital environments or there are selective factors that cause its high frequency in clinical samples. Regarding the latter, we suggest that intrinsic resistance to disinfectants particularly quaternary ammonium compounds which have been incriminated in various healthcare-associated infections and pseudobacteremia with *A. xylosoxidans* facilitates its survival in hospital setups.^[23,24] In our study, the average time of development of infection after hospitalization was 21 days (range: 10–51 days). Our findings are consistent with other similar studies by Marion-Sanchez *et al.*, where the mean duration of hospital stay was 23 days (range: 4–94 days).^[4]

In the present study, all the isolates were susceptible to piperacillin-tazobactam and minocycline and most of the patients were successfully treated with them [Table 1]. This was followed by sensitivity to ticarcillin-clavulanic acid, meropenem, cotrimoxazole, levofloxacin, and ceftazidime. All the isolates were sensitive to the reserved drug colistin and resistant to amikacin and gentamicin. *A. xylosoxidans* is characteristically resistant to all aminoglycosides while it expresses variable resistance to trimethoprim-sulfamethoxazole, ciprofloxacin, and ceftazidime.^[16,25,26] Most of the isolates are generally susceptible to carbapenems and antipseudomonal penicillins.^[16,25]

Reported case-fatality rates have varied from 3% for primary or catheter-associated bacteremia to 80% for neonatal infection.^[27] Our study showed favorable outcome in 78.6% of the cases and mortality in the remaining 21.4%. On stratifying the mortality according to the

clinical condition of the patients, most mortality was due to bloodstream infections among the patients.

Conclusion

Infections due to *Achromobacter* are on the rise in developing countries like India. It is often misidentified or faces a delay in identification. Its saprophytic nature further adds to the diagnostic dilemma and often, it is overlooked as a contaminant. Resistance to many classes of antimicrobials, making its treatment more challenging therefore it should always be guided by antibiograms. The present study highlights the significance of this rare bacterium in patients with malignancies in the Indian setting and it advocates greater vigilance toward appropriate identification of this organism.

Ethical statement

The study was approved by institutional ethics committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow (Reference number: PGI/BE/1561/2021).

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Nil.

Conflicts of interest

There are no conflicts of interest.

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