Identification of emerging *Chryseobacterium indologenes* sepsis using automated identification system, Matrix-assisted Laser Desorption Ionization-time-of-flight Mass Spectrometry (MALDI-TOF) and Pulsed-field Gel Electrophoresis (PFGE) typing

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Abstract
Chryseobacterium indologenes is a non-motile, catalase-positive, oxidase-positive, indole-positive, non-glucose-fermenting Gram-negative bacillus that produces yellow coloured colonies on routine culture media. As common environmental saprobe, it is a known colonizer of inanimate objects in the hospital environment. *Chryseobacterium indologenes* is an emerging .pathogen of nosocomial origin in background of increased use of higher antibiotics. In the present study, positive blood cultures bottled from critically ill patients suspected of nosocomial sepsis were inoculated on routine bacteriological media for a period of six months. The identity and antibiotic sensitivity *of Chryseobacterium indologenes* were confirmed by Phoenix automated system® followed by automated MALDI-TOF system and Pulsed Field Gel Electrophoresis (PFGE) typing. All the patients had underlying morbidities (7 out of 7 patients). Phoenix automated system and MALDI-TOF system correctly identified the bacterial isolates. We reiterate the fact that levofloxacin should be the first choice of antibiotic in *Chyrseobacterium indologens* infections followed by cefoperazone + sulbactam. All the isolates were of different clonality as determined by PFGE. To our knowledge, this is the first report on the identification of *Chrysobacterium indologenes* isolates using automated system, MALDI-TOF and PFGE in India. So, in ICUs of tertiary care centers, where higher antibiotics are used, this bacteria can be an important cause of nosocomial bacteremia.

Keywords: *Chryseobacterium indologenes*, sepsis, MALDI-TOF, PFGE, Phoenix automated system.

Introduction
Chryseobacterium indologenes is a rare cause of bacterial infection in immunosuppressed patients and belongs to group Chryseobacteria.[1] Chryseobacteria are a group of nonmotile, catalase-positive, oxidase-positive, indole-positive, non-glucose-fermenting, gram negative bacilli.[2] The genus *Chryseobacterium* includes six species that were previously designated the genus *Flavobacterium*. *Chryseobacterium gleum* and *Chryseobacterium indologenes*, previously known as *Flavobacterium* CDC group IIb, have been clearly differentiated by DNA-DNA homology and eight phenotypic characteristics.[2]

Infections caused by *C. indologenes* are generally associated with indwelling devices and because it is an environmental bacterium and hence, able to cause nosocomial infections.[3] It has been implicated in nosocomial bacteremia, Urinary Tract Infections, pneumonia etc.[3-6] Nearly half of the published research reports refer to nosocomial infections, and a vast majority of patients had underlying immuno-compromised conditions.[4-10]

Studies indicated that the infections caused by *C. indologenes* increased with the increasing use of higher antibiotics like colistin especially in critically ill patients in ICU.[9] However, the treatment against these pathogens is difficult because they are resistant to many antibiotics especially colistin and carbapenems.[6] They are also reported to be sensitive to Levofloxacin.[6,11,12] To date, the clinical significance of *C. indologenes* has not been fully defined. Few cases have been reported about the bacterium in world. Therefore, the present study was undertaken to identify the *C. indologenes* isolates using automated system, MALDI-TOF and PFGE.

Materials and Methods
The present study was conducted in the Microbiology Department, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, a tertiary care institute in the Northern India. Blood samples were collected from ill patients suspected of clinical sepsis within a period of six months (July 2015 to December 2015). Around 10 ml of blood were collected aseptically and put in BD Bactec plus aerobic and anaerobic vial. Afterwards, the bottles were incubated at 37ºC aerobically and anaerobically, respectively for maximum five days. Positive blood culture bottles were inoculated on routine bacteriological media like blood agar and MacConkey agar and incubated overnight. Gram negative, oxidase-positive, non-fermenting, yellow pigment producing colonies were appeared and suspected of *Chryseobacteria.* A characteristic of *Chryseobacteria* was the development of red color upon addition of 10% Potassium Hydroxide solution to the bacterial culture and was due to the production of flexirubin pigment. The identity and

antibiotic sensitivity were confirmed by Phoenix automated identification and sensitivity system®. Further, the isolates were also identified by MALDI-TOF system (VITEK-MS, Biomerieux). The treatment and clinical condition were followed up for each patient. Then, Pulsed Field Gel Electrophoresis (PFGE) was done to type the isolates.

Plugs were prepared as previously described and the genomic DNA was digested with XhoI.[11] The genomic DNA of *Salmonella enterica* serovar Braenderup H9812, digested with XbaI, was used as the molecular weight marker. PFGE was performed using the CHEF-DR II system (BioRad; Hercules, CA, USA). The gel was run at a field strength of 6.0 V/cm, at a reorientation angle of 120°, and from an initial switch time of 0.5 seconds to a final switch time of 26.4 seconds. The result was analyzed by Gel Compar II software (Applied Maths NV, St-Martens-Latem, Belgium).

Results and Discussion

A total of seven isolates of *Chryseobacterium indologenes* were reported from the same ICU in a period of six months. All the isolates were confirmed by Phoenix automated systems and MALDI-TOF (VITEK-MS, BioMerieux). All sepsis developed after 48 hours of admission (range 4- 20 days), and hence, all the cases were of nosocomial infection. All the patients developed sepsis and four patients also developed ventilator associated pneumonia. Moreover, in our study, all patients had underlying morbidities like diabetes mellitus, steroid therapy and prolonged antibiotic therapy (7 out of 7 patients).

With prompt therapy, 5 patients out of seven were recovered from sepsis. 2 of them died of complications due to other conditions. So, we can deduce that the attributable mortality of the bacterial infection is low if properly treated. In our study, 6 out of 7 isolates were sensitive to levofloxacin (86%) followed by Piperacillin + Tazobactam (60%) and cotrimoxazole (60%). Further, the epidemiological relatedness of the 7 isolates was determined by PFGE (Fig. 1). Two isolates (1 and 2) were of similar clonality and rest all were different.

A maximum cases sepsis due to bacterium, *Chrysobacterium indologenes* isolates using automated system, MALDI-TOF and typed by PFGE have been reported from Taiwan, Australia, and Europe.[8,13,14] This is the first time that the bacteria have been studied by automated identification system (Phoenix), MALDI-TOF system and typed by pulsed filed gel electrophoresis in India.

Conclusion

Chryseobacterium indologenes is becoming an important antibiotic resistant nosocomial pathogen especially in ICUs. Though it can cause severe sepsis, the prognosis is good if there is proper culture guided antibiotic therapy. So, this emerging pathogen should be kept in mind in health centers where higher antibiotics are routinely used. Automated identification systems are of great value in diagnosing the pathogen.

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