Necrotizing pneumonia and empyema caused by *Neisseria flavescens* infection

Ling Huang[1,2*], Lan Ma[1,3*], Kun Fan[1,4], Yang Li[1,4], Le Xie[1,4], Wenying Xia[1,4], Bing Gu[1,4], Genyan Liu[1,4]

[1]Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; [2]Department of Laboratory Medicine, the Second Hospital of Nanjing, Nanjing 210003, China; [3]Department of Laboratory Medicine, Gaochun People's Hospital of Nanjing, Nanjing 211300, China; [4]National Key Clinical Department of Laboratory Medicine, Nanjing 210029, China

*These authors contributed equally to this work.

Correspondence to: Genyan Liu. Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University Guangzhou Road, No.300, Nanjing 210029, China. Email: liugenyan@njmu.edu.cn.

Abstract: *Neisseria flavescens* is an uncommon pathogen of human infection, pneumonia and empyema caused by *N. flavescens* is rarely reported. Herein, we report a 56-year-old diabetic patient presenting necrotising pneumonia and empyema due to *N. flavescens* infection. The main clinical manifestation of this patient was high fever, sticky pus and gradually aggravating dyspnea. The chest computed tomography (CT) scan showed there are mass of high density areas around hilus of the left lung, hollow sign with inflammation also appeared. A biopsy specimen was taken from the left principal bronchus by lung puncture biopsy and showed necrosis and inflammation. Microscopic examination of direct smear and culture of sticky pus, much more gram-negative diplococcus was present, pathogen was further identified by Vitek NH card, Vitek MS and confirmed as *N. flavescens* by 16S rRNA gene sequencing finally. Anti-infection therapy following the antimicrobial susceptibility test results was effectively. To our knowledge, this is the first report of pulmonary infection caused by *N. flavescens*.

Keywords: *Neisseria flavescens*; pneumonia; empyema; MALDI-TOF MS; 16S rRNA gene sequencing

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Introduction

Neisseria spp. are part of the commensal flora of mucosal membranes of humans and some animals, and are generally considered non-pathogenic except for *N. gonorrhoea* and *N. meningitidis*. *N. flavescens* often be found in the upper respiratory tract and the oropharynx of humans, and are rarely associated with infectious processes (1). However, when patients in special or immunocompromised conditions, *N. flavescens* can be isolated from blood or cerebrospinal fluid (CSF) occasionally (2-8), but never been isolated from lower respiratory tract.

Herein, we reported a case of a 58-year-old diabetic patient with fatal necrotising pneumonia and empyema due to *N. flavescens* infection. To our knowledge, this is the first report that *N. flavescens* as the pathogen of severe low respiratory tract infection.

Case report

A 58-year-old man was admitted to the hospital because of necrotizing pneumonia and empyema in October 2013. He had experienced nausea, vomiting and little cough ten days before admission, after anti-infection therapy with some cephalosporin in local clinic, the symptoms once getting better, but two days before admission, the patient felt anhelation and dyspnea, then presented to the emergency department of our hospital, non symptomatic remission after dealing with cefodizime and methylprednisolone through intravenous injection temporary, then transferred to the

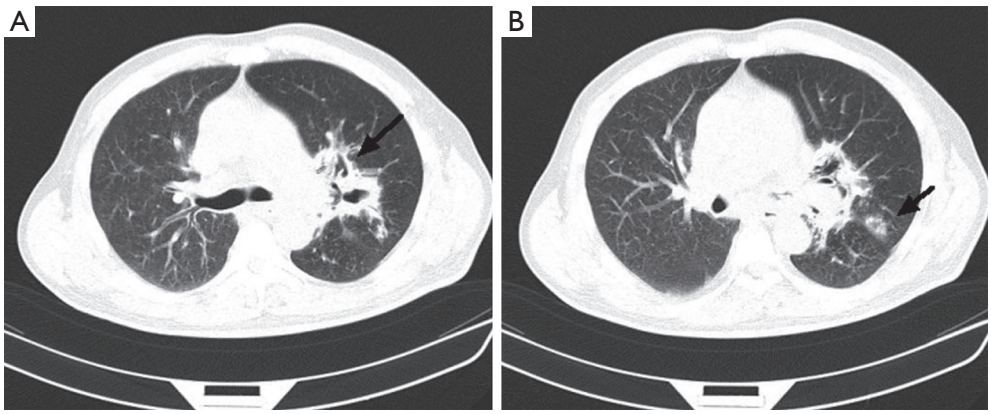

Figure 1 CT scan of the chest. (A) High-density shadow around the hilus of left lung (black arrow); (B) It appears that there is a hollow sign (black arrow) in the peripheral pulmonary. CT, computed tomography.

department of respiration with symptoms of high grade fever (highest temperature is 39.9 ℃/103.82 F), chilling and severe cough with productive of yellow sputum finally.

He has hypertension for four years and controlled well. Four year history of type 2 diabetes and treated with melbine (DMBG) as well as Glipizide, but curative effect is not ideal for fasting blood-glucose more than 10 mmol/L. He also has a smoking history of 20 cigarettes per day for 40 years.

A chest computed tomogram (CT) showed high-density shadow around the hilus of left lung (*Figure 1A* as signed by black arrow), a hollow sign (*Figure 1B* as signed by black arrow) also exists in the left peripheral pulmonary. Initial laboratory tests showed the white blood cell (WBC) count was 36.04×10^9/L (reference level, 4.0×10^9-10.0×10^9/L), the neutrophil cell count and ratio was 33.3×10^9/L (92.4%), the erythrocyte sedimentation rate (ESR) was 115 mm/H, the C-reactive protein (CRP) was 54.1 mg/L (reference level, <5 mg/L).

A transthoracic pulmonary fine-needle aspiration was performed when transferred to the department of respiration. Approximately 2 mL of purulent secretion was obtained and sent for microbiology tests. Direct smear Gram stain was performed and gram-negative diplococci and lots of polymorphonuclear leukocytes can be observed under microscope (*Figure 2A*), acid fast stain was also done and got negative results. The same material was inoculated onto chocolate agar and 5% sheep blood agar (bioMérieux, Shanghai, China). The agar media were incubated at 35 ℃ for 48 h, middle size, bluish grey round opaque colonies were observed. Gram-stain of the pure culture colony was also gram negative cocci. Elementary biochemical properties of this strain were oxidase positive, catalase positive while deoxyribonuclease (DNAse) was negative. The organism was identified with Vitek NH card and Vitek MS successively, but inconsistent results were got, Vitek NH (Ref. V1308 database) identified as *N. flavescens* (99% probability) while Vitek MS (Ref. V2.0 database) identified as *N. subflava* (89.70% probability). Finally, we confirmed this identification as *N. flavescens* (99% probability) by 16S rRNA gene sequencing.

In vitro susceptibility test with agar dilution method was done following the method mentioned in CLSI M45 for *Moraxella catarrhalis*. It is susceptible to penicillin, ampicillin/sulbactam, amikacin, ceftazidime, ciprofloxacin, Trimethoprim-sulfamethoxazole and piperacillin-tazobatam. After one week anti-infection therapy combined piperacillin-tazobatam and Trimethoprim-sulfamethoxazole, the gram negative diplococcic was almost disappeared (*Figure 2B*). But the empyema was not released because of the inflamation and necrosis of cartilagines tracheales (*Figure 3A,B*). Necrosis of cartilagines tracheales lead to tracheal collapse and purulent secretion drainage very uneffective. Finally, the patient was got well after tracheal scaffold implantation and further anti-infective therapy for three weeks.

Discussion

Neisseria is a large genus of commensal bacteria that inhibit mucous membrane surfaces of warm-blooded hosts. There are 11 species that colonize humans include *N. gonorrhoeae*, *N. meningitides*, *N. lactamica*, *N. flavescens*, *N. sicca*, *N. subflava*, *N. mucosa*, *N. cinerea*, *N. elongata*, *N. glycolytica and N. nitroreducens.* Most of these *Neisseria* species are normal inhabitants of the upper respiratory tract and are not considered pathogens (1,9). Up to date, only *N. meningitides*, *N. gonorrhoeae*, *N. mucosa* and *N. sicca* have been reported as causative agents of pneumonia,

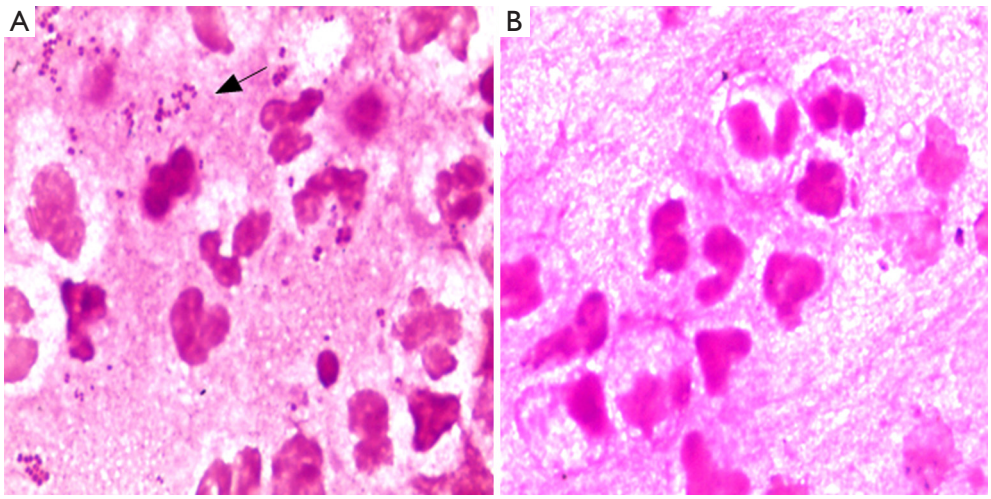

Figure 2 Direct smear Gram stain before and after anti-infection. (A) Direct smear Gram stain of pyogenic fluids before treatment. There are lots of gram-negative diplococcus as well as pyocyte infiltration; (B) Direct smear Gram stain of pyogenic fluids after effective treatment, the diplococcus disappeared. (Gram stain,1,000×).

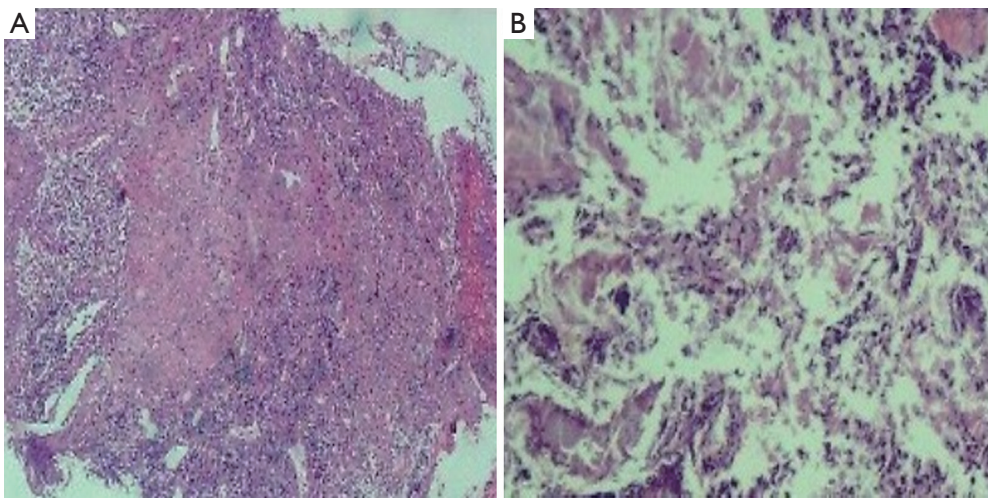

Figure 3 Biospy of principal bronchus mucous membrane. (A) Biospy of distal end of left principal bronchus mucous membrane with deeply acidophilic and fibrinoid necrosis; (B) Biospy of distal end of left principal bronchus cartilage with deeply acidophilic, fibrinoid necrosis and exudation. (H&E stain, 200×).

empyema, bronchopneumonia or bronchiectasis (10-17). Necrotizing pneumonia with empyema caused by *N. flavescens* is the first time reported as we known. Besides as causative agent of pneumonia and empyema *N. flavescens* have else been published as pathogens of septicaemia, meningitis and endocarditis (4-8,18-20).

The clinical symptom and lab tests properties of this case are high fever rate, empyema, elevated WBC, increased CRP value and distinctive imaging changes, all these often lead to a fatal infection as reported infection caused by *N. flavescens* in the other systems (4,6,7,19). We reviewed the literatures and analysed the possible reason may be included the following issues: *N. flavescens* is among the commensal flora of human upper respiratory tract, seldom cause human infection. Most of *N. flavescens* infected patients have severe basic diseases, for example, immunodeficiency and diabetes (2); There are remote causes like dental surgery history, vomiting, chemotherapy and co-infection with HIV or pseudomonas aeruginosa (18,21); Initial experienced clinical application of penicillin and cefixime often failed to cure

Table 1 Supplemental tests which permit differentiation among common gram negative diplococcus (GND)

GND	Oxidase test	Catalase reaction	DNAse test	Nitrate reduction	Acid from				Colistin susceptibility
					G	M	L	S	
N. flavescens	+	Weak	–	–	–	–	–	–	S
N. gonorrhoeae	+	Srong	–	–	+	–	–	–	R
N. meningitides	+	Strong	–	–	+	+	–	–	R
M. catarrhalis	+	Variable	+	+	–	–	–	–	R

Abbreviations: +, most strains positive; –, most strains negative; R, strains grow well on selective medium for *N. gonorrhoeae* and/or show no inhibition around a colistin disk (ten micrograms); acid from G (glucose), M (maltose), L (lactose), S (sucrose).

the *N. flavescens* infection for beta-lactamase producing and *penA* resistant gene expression (5,22-30); severe virulence and inflammatory response caused by lipooligosaccharide of Neisseria lead to septic shock and fibrinoid necrosis and exudation (31). In conclusion, we should pay more attention to human infection caused by *N. flavescens*.

Due to *N. flavescens* may cause severe infection, rapid and accurate identify this organism is more important. As described in this paper, Vitek NH card can be used for accurate identification, but Vitek MS V2.0 database doesn't include *N. flavescens* and should be developed in the future. Among the gram negative diplococcus often cause pulmonary infection, *N. flavescens* can be differentiated from *Moraxella catarrhalis* with DNAse test, differentiated from *N. gonorrhoeae* and *N. meningitides* with rapid acid detection tests and Colistin-susceptible test as summarized in *Table 1*.

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