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Short Communication

The identification, typing, and antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from mink with hemorrhagic pneumonia

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ABSTRACT

The biological characteristics and molecular epidemiology of *Pseudomonas aeruginosa* associated with mink hemorrhagic pneumonia from Shandong province of eastern China were determined in this study. From 2010 to 2011, 30 mink *P. aeruginosa* isolates were identified from lung, fecal and feed samples of clinical cases and subjected to serotyping, antimicrobial susceptibility testing and pulsed-field gel electrophoresis (PFGE) using *Spe*I. The *P. aeruginosa* isolates belonged to four serotypes—21 of type G, four of type I, three of type M, one of type B, and one non-typable strain. The strains were divided into four large groups as determined by PFGE. Isolates from the group 2 were highly homologous and were obtained from the same region as an epidemic. All of the isolates were sensitive to piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, imipenem, amikacin, gentamicin and tobramycin and resistant to ampicillin, cefuroxime and cefuroxime axetil. A high frequency of resistance was found to ampicillin/sulbactam, cefazolin, cefotetan, ceftriaxone, nitrofurantoin, and trimethoprim/sulfamethoxazole (96.7%). Resistance to ticarcillin/clavulanic acid, ciprofloxacin and levofloxacin was less common (13.3%). There was no relationship between antibiotic resistance and serotype distribution of the isolates. The epidemic serotype of *P. aeruginosa* from the mink hemorrhagic pneumonia in Shandong province was type G, which was a clone of commonly found in this province. These findings reveal the genetic similarities and antimicrobial susceptibility profiles of *P. aeruginosa* from clinical cases of mink hemorrhagic pneumonia and will facilitate the prevention and control of the disease in Shandong province of China.

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1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative opportunistic pathogen that commonly infects patients with impaired immune responses as hospital-acquired pneumonia (Sarlangue et al., 2006). In addition to being a human pathogen, *P. aeruginosa* also causes serious harms to dogs, cats and fur-bearing animals such as mink, fox and raccoons (Lin et al., 2012; Hariharan et al., 2006; Shimizu et al., 1974). *P. aeruginosa* has been a major cause of hemorrhagic pneumonia in mink for the last 50 years and the associated mortality is 1–50% (Knox, 1953; Honda et al., 1977). The disease is almost exclusively seasonal (from September to early December) and is characterized by sudden deaths. Moreover the dead minks are typically found with blood around the nostrils and mouth, and severe pathological changes in the lungs (Knox, 1953). *P. aeruginosa* is widespread in mink and fox farm surroundings, contaminating their water, cages, water cups, and feed troughs (Gierløff, 1980). The reason why mink are susceptible to this organism remains unclear.

Three antibiotics – β-lactams, aminoglycosides and fluoroquinolones – are commonly used in the treatment of *P. aeruginosa* infections in humans. However, *P. aeruginosa* resistance to these antibiotics is increasingly reported (Tam et al., 2010). Alarmingly, the isolates of the *P. aeruginosa* carrying bla_{NDM-1} gene was also recently identified (Khajuria et al., 2013). However, there are few studies on antimicrobial resistance in *P. aeruginosa* isolated from animals. We analyzed the susceptibility of mink isolates to fluoroquinolones antibiotics and the associated resistance mechanisms (Gao et al., 2011). It was found that 56.4% of the mink *P. aeruginosa* strains from Rongcheng city in Shandong province were resistant to multiple antibiotics with very high levels of resistance to tetracyclines, fluoroquinolones and cephalosporins. These isolates had limited resistance to spectinomycin. Additionally, these *P. aeruginosa* isolates were generally sensitive to aminoglycosides, such as gentamicin and amikacin and were completely sensitive to imipenem, which has not been used in the veterinary clinics of the study region (personal communication).

In order to effectively treat mink hemorrhagic pneumonia, it is necessary to understand the antimicrobial susceptibility of related pathogens and epidemiological data. In addition to the traditional methods of serotyping, pulsed-field gel electrophoresis (PFGE) is considered as the "gold standard" for *P. aeruginosa* typing (Grundmann et al., 1995; Tenover et al., 1997; Johnson et al., 2007). To facilitate clinical treatment, we collected lung, fecal and feed samples from dead or sick mink with hemorrhagic pneumonia in Shandong province for bacterial isolation, identification, and antimicrobial susceptibility testing. Serotyping and PFGE typing were used to identify epidemic strains and determine the relationship of the isolates obtained from different parts of Shandong province. To our best knowledge, this is the first report describing mink *P. aeruginosa* in China.

2. Materials and methods

2.1. Farm selection and sample collection

Mink farms in Weihai, Linyi, Jiaozhou, and Jinan, Shandong province, China were screened for hemorrhagic pneumonia in the autumn of 2010 and 2011. Fecal samples, feed samples (frozen chicken intestines), and lung samples from dead or sick mink were collected for bacterial isolation.

2.2. Isolation and culture characteristics of P. aeruginosa

Samples were streaked onto nalidixic acid cetrimide medium (NAC Agar, Qingdao Hope Biol-Technology Co., Ltd.) and blood agar (Qingdao Haibo biotech company) and incubated for 18–20 h at 37 °C. A single irregular blue-green colony was picked for Gram staining. Hemolysis was identified by clearing around the colony on blood agar plates.

2.3. Biochemical identification of P. aeruginosa

Isolates were inoculated onto sterile TSA culture medium and incubated at 37 °C for 24 h. A bacterial suspension equivalent to 0.5 McFarland units for each isolate was prepared. The VITEK-2 compact automated microbial identification system (bioMerieux, France Bio Co., Ltd.) was used for physiological and biochemical identification of bacterial isolates.

2.4. O-antigen serotyping

Serotyping was performed using the slide agglutination method and commercially available polyvalent I, II and III group specific antisera against 14 O antigens (Denka Seiken Co., Ltd., Japan). All isolates were incubated on blood agar plates for 18 h at 37 °C. A clean glass slide was prepared with a drop of serum on the sample side and a drop of normal saline on the control side. A typical bacterial colony was suspended in normal saline. One loop of the dense suspension was placed adjacent to each drop on the slide. The slide was tilted back and forth to mix the two drops and observed for signs of agglutination. The negative saline control was performed to identify no spontaneous agglutination. The positive sample demonstrated agglutination within one minute on the antisera side and no agglutination with the negative saline control (Homma, 1982).

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all *P. aeruginosa* isolates was performed using the VITEK-2 compact automated microbial identification system and the results were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute documents M31-A3 (2008) and M100-S21 (2011). The following antimicrobial agents were tested using the VITEK 2 AST-GN13 cards (bioMerieux, France), including β-lactams (ampicillin, ampicillin/sulbactam, piperacillin,