

ERIC-PCR genotyping of *Pseudomonas aeruginosa* isolates from haemorrhagic pneumonia cases in mink

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a significant pathogen of mink and the cause of haemorrhagic pneumonia, an acute fatal disease in farmed mink.

Results: Among 90 *P. aeruginosa* isolates from haemorrhagic pneumonia in mink from 16 farms in Shandong province, China, 43 genotypes were identified by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), with a diversity index of 0.96. The most prevalent ERIC-PCR types were type 18, found in 16 isolates, and type 39, found in 15 isolates. Four serotypes were detected, with serotype G (55.6 per cent) being the most frequent.

Conclusions: These results showed that there was a high degree of clonal diversity among mink *P. aeruginosa* clinical isolates in this study.

INTRODUCTION

Pseudomonas aeruginosa is a significant pathogen of mink and the cause of haemorrhagic pneumonia, an acute fatal disease in farmed mink (Shimizu and others 1974). *P. aeruginosa* was first described as a cause of haemorrhagic pneumonia in mink in 1953 (Knox 1953). The disease is almost always seen in the autumn months and can cause an epizootic on the mink farm with mortalities ranging from 1 to 50 per cent (Knox 1953, Honda and others 1977, Salomonsen and other 2013). *P. aeruginosa* is an opportunistic pathogen and is ubiquitous in the environment on mink and fox farms (Gierløff 1980).

Haemorrhagic pneumonia in mink was first discovered in China in 1983. Since 2000, haemorrhagic pneumonia has occurred in many provinces (Bai and others 2011). Approximately 84 million minks are farmed in Shandong province, and this province has the largest number of mink farms in China (Bai and others 2011). Bai and others (2011) and Qi and others (2014) showed that *P. aeruginosa* is present in most mink farms in

the Shandong province, so it is necessary to understand the epidemiology of haemorrhagic pneumonia in mink in this region.

Genotyping of *P. aeruginosa* isolates by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) has been used to study the epidemiology of *P. aeruginosa* in Australia and Brazil (Stehling and others 2010, Kidd and others 2011). Previous studies in China have characterised *P. aeruginosa* isolates by serotype and pulsed field gel electrophoresis analysis. In this study, *P. aeruginosa* isolates from haemorrhagic pneumonia in mink were characterised by serotyping and ERIC-PCR.

METHODS

Ninety isolates of *P. aeruginosa* were recovered from 16 mink farms in Shandong by the Five-star Animal Health Pharmaceutical Factory of JILIN province during 2011–2012. Isolates were classified as *P. aeruginosa* based on the Microbial Biochemical Identification Tube System and PCR (Song and others 2000). Isolates were serotyped using the slide agglutination method according to the Homma schema (Homma 1976, Long and Gorham 1981) using standard sera.

Bacteria were grown on Luria–Bertani agar plates, and five colonies of each isolate were placed into 5 ml of Luria–Bertani broth and incubated for 12–14 h at 37°C on a rotating shaker. Using the TIANamp Bacteria DNA Kit, total DNA was extracted from a 1 ml suspension containing 10⁸ colony forming units. Isolates were genotyped using ERIC-PCR according to Versalovic and others (1991).

RESULTS

With a diversity index (D) of 0.96, 43 genotypes (1–43) were identified among 90 isolates examined by ERIC-PCR (Fig 1). The most prevalent ERIC-PCR types were type 18, found in 16 isolates, and type 39, found in 15 isolates. The next most frequently

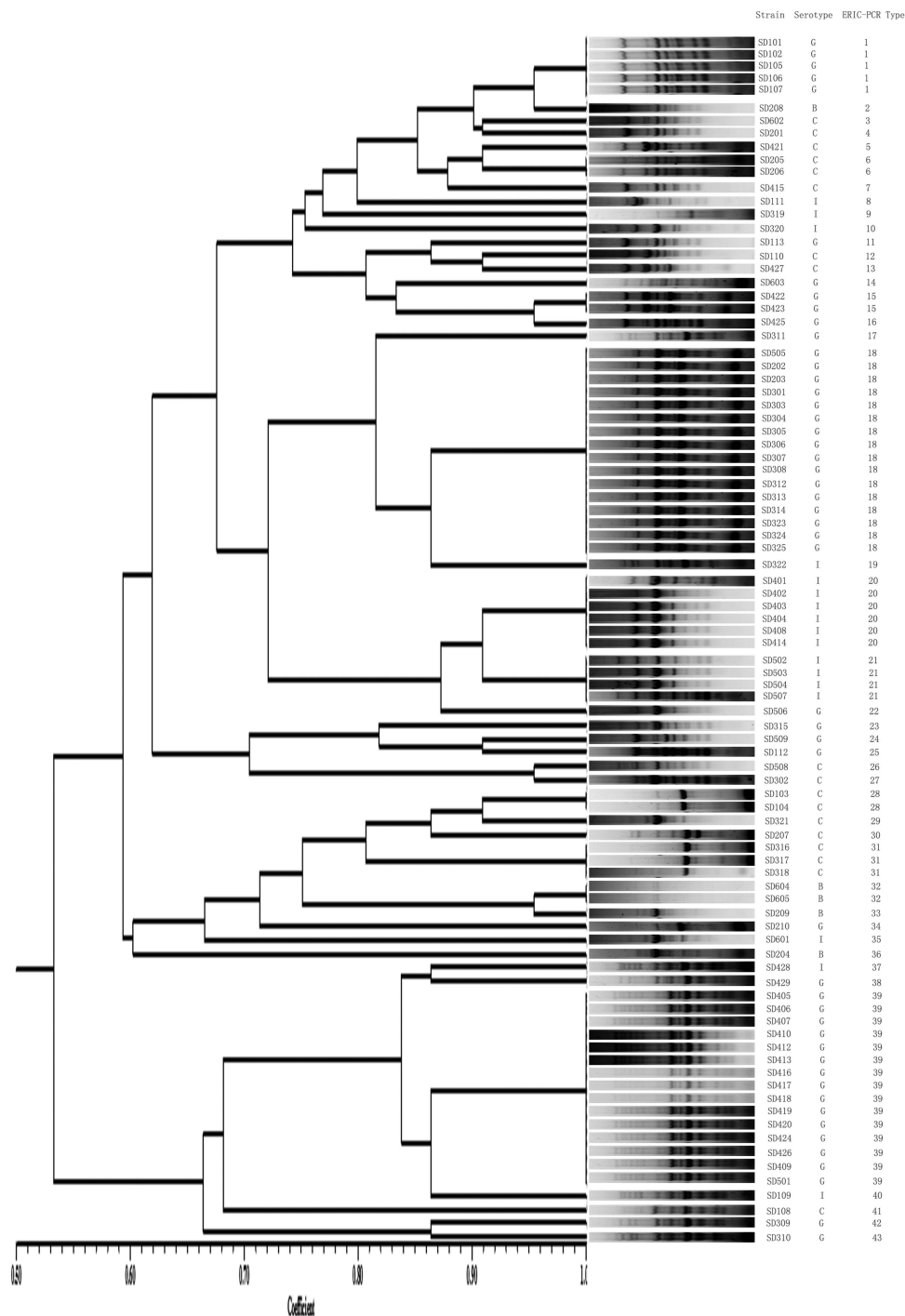


FIG 1: Cluster analysis by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) fingerprinting of 90 *Pseudomonas aeruginosa* isolates from haemorrhagic pneumonia in mink in Shandong province, China. The scale indicates the percentage of genetic similarity. Columns (left to right) indicate list of isolates, serotype and ERIC-PCR type. Isolates were classified into 43 genotypes using 0.96 similarity as the cut-off

identified types were 20 (six isolates), 1 (five isolates) and 21 (four isolates). The remaining genotypes contained 1–3 isolates each.

The 90 isolates of *P. aeruginosa* were classified into four serotypes (G, B, C and I); serotype G was the most frequent (50/90, 55.6 per cent). Serotype C was found in 18 isolates, serotype I was found in 17 isolates and serotype B was found in five isolates.

DISCUSSION

In this study, we found that serotype G strains had the highest genetic diversity and were divided into 15 different genotypes. Diversity was also found within serotype C (13 genotypes), serotype I (eight genotypes) and serotype B (four genotypes). Strain diversity was observed within Shandong province; the highest diversity was identified in Weifang (29 isolates, four serotypes, eight

genotypes). Genotyping (D=0.96) was more discriminatory than serotyping (D=0.66). Serotype G was the dominant serotype among the isolates. This is in accordance with previous observations from China, the USA and Europe (Nordstoga 1968, Long and Gorham 1981, Bai and others 2011, Hammer and others 2003). In this study, on three farms, both the genotype and serotype of the isolates was highly diverse. On all other farms, the isolate serotype was relatively simple while the genotype was complex.

Our results showed that genotyping by ERIC-PCR was more discriminatory than serotyping of *P. aeruginosa* isolates. Hence a combination of genotyping and serotyping is the best way to characterise *P. aeruginosa* isolates.

ERIC-PCR can be used in outbreak investigations and to assist in the control of *P. aeruginosa* in mink farms by vaccination (Tazumi and others 2009, Macedo and others 2011). Worldwide, inactivated vaccines are now used to prevent haemorrhagic pneumonia in minks. The effectiveness of each vaccine is determined by the difference between candidate vaccine strains and epidemic strains. Therefore, knowledge of the genetic diversity of *P. aeruginosa* is of value in understanding vaccine failures and helping to select strains for homotypic vaccines.

Contributors M-mH performed the ERIC-PCR genotyping of *Pseudomonas aeruginosa* isolates and wrote the manuscript. L-zM analysed all of the results. M-mH, X-pL and JZ isolated all of the *P. aeruginosa* strains. M-mH, X-fL and HL identified all of the isolates. All authors reviewed the manuscript and approved submission to the journal.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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