Genetic diversity of different avian strains of Pasteurella multocida recovered during four outbreaks in India

Kumaragurubaran KARThIK*®, Vishwanathan DILLIBABU®, Ramalingam MAHAPRABHU®, Ramasamy BHARATHI®, Kaliyaperumal MANIMARAN®, Kulasekaran SHOBA
Central University Laboratory, Tamil Nadu Veterinary and Animal Sciences University,
Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Received: 09.10.2017 • Accepted/Published Online: 10.02.2018 • Final Version: 09.08.2018

Abstract: Pasteurella multocida causes fowl cholera in avian species and type A is predominantly reported from outbreaks of fowl cholera. Biochemical, serological, and molecular methods are employed for its diagnosis and typing. Utilizing rapid molecular tools like repetitive extragenic palindromic (REP) PCR and enterobacterial repetitive intergenic consensus (ERIC) PCR, P. multocida isolates from four outbreaks (three from Chennai and one from Ahmadabad, India) were characterized and typed to determine their relationships. A total of 36 isolates were recovered from the outbreaks, including one isolate from a parakeet, which was also subjected to characterization by conventional and molecular analysis. All of the isolates were found to be capsular type A based on PCR assay capsular typing. ERIC- and REP-PCR showed differences in the banding patterns among different outbreak isolates, and also among the geographical regions. Differences were also noticed among different host species, as the banding pattern in the ERIC- and REP-PCR differed; the analysis of results also revealed the same. All of the isolates were found to be sensitive to enrofloxacin and ceftiofime among the antibiotics used in the study. It was found that different strains might have been involved in the different outbreaks reported in the study. The results show that molecular typing methods like ERIC- and REP-PCR are useful epidemiological tools for classifying the strains.

Key words: Pasteurella multocida, ERIC-PCR, REP-PCR, fowl cholera, genotyping

1. Introduction
Fowl cholera (FC) is an acute septicemic deadly disease of various poultry species caused by Pasteurella multocida, a gram-negative, nonmotile, nonspore-forming, aerobic, rod-shaped bacterium (1). Pasteurella multocida mainly colonizes the respiratory tract of healthy animals and birds, but can cause disease under stress conditions (2). FC has been reported throughout the world and is known to cause significant mortality, leading to economic losses for poultry farmers (3,4). FC outbreaks have been reported from several parts of India at various times (5). Based on the sugar (dulcitol and sorbitol) fermentation test, P. multocida is divided into 3 subspecies, namely P. multocida subsp. multocida, P. multocida subsp. gallicida, and P. multocida subsp. septica (1). Diagnosis of FC relies on isolation and identification of the causative agent by employing conventional methods, followed by capsular typing using an indirect hemagglutination test and serotyping (6,7). Though 4 serogroups (A, B, D, and F) have been reported from poultry, Pasteurella multocida type A:1 has been implicated in the majority of outbreaks of FC (8).

Conventional diagnosis and phenotypic differentiation tools have long been used to identify FC isolates, but these methods are time-consuming and laborious; hence, molecular assays like polymerase chain reaction (PCR) are employed for accurate diagnosis (9,10). Recent advances in diagnosis have aided in molecular characterization of the isolates using techniques like enterobacterial repetitive intergenic consensus (ERIC) PCR, restriction endonuclease analysis (REA), random amplified polymorphic DNA (RAPD), multilocus sequencing typing (MLST), and repetitive extragenic palindromic (REP) PCR (4,11,12). These methods have higher reproducibility and better discriminatory power; thus, these methods can be employed for epidemiological studies of the isolates (12).

A few studies have reported the isolation of similar strains of P. multocida from the same farm over time, while some studies have reported the isolation of different genotypes during different outbreaks over time from the same farm (13,14). ERIC-PCR and REP-PCR were used in earlier studies to characterize the avian P. multocida isolates recovered from different regions of India to