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Molecular characterization of field isolates of *Pasteurella multocida* from poultry

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Fowl cholera, a contagious, often fatal bacterial disease of domestic and wild avian species caused by *Pasteurella multocida*, is identified on the basis of clinical signs and lesions and microscopic demonstration of *P. multocida*. Isolates of *P. multocida* having both capsular serogroup and somatic serotype in common may be distinguished by PCR and restriction endonuclease analysis (REA) using *Hha*I and *Hpa*II (Wilson *et al.* 1992). The study reports presence of *P. multocida* isolates in poultry by PCR and their molecular characterization using REA.

Swabs (69) from infra-orbital sinus (IOS) of suspected poultry were collected streaked onto brain heart infusion (BHI) agar with 5–10% defibrinated sheep blood and made selective by addition of antimicrobial agents amikacin, bacitracin, potassium tellurite, gentamicin sulphate and clindamycin phosphate for isolation of bacilli. Isolates were characterized on their cultural, morphological and biochemical characteristics as per Quinn *et al.* (1994) and antibiogram carried out by Bauer *et al.* (1966). Antimicrobial discs against cephotaxime, cephalexin, enrofloxacin, gentamycin, erythromycin, pefloxacin, chloramphenicol, ciprofloxacin, oxytetracycline and streptomycin were used. For use in PCR, cell lysates were prepared from infra-orbital sinus swabs and 2–3 days old colonies in 0.1 ml TE buffer and boiling for 10 min. The DNA was stored at –20°C for further use. For restriction analysis, genomic DNA was extracted from the avian *P. multocida* isolates and one standard P52 strain (obtained from IVRI) by phenol chloroform method (Wilson 1987). The integrity of the DNA samples was checked by running them in 0.8% agarose. Concentration and purity of the genomic DNA was determined by optical density (OD) at 260 nm and 280 nm (Sambrook *et al* 1989). PCR was used directly on 19 IOS swab samples and on 5 avian *P. multocida* isolates using genus specific primers as per Townsend *et al.* (1998). PCR products were analysed by agarose gel electrophoresis and visualized under UV transilluminator. Genomic DNA of field isolates and P52 *P. multocida* were subjected to the restriction digestion with *Hpa*II and *Hha*I (Sambrook *et al* 1989). Five mg of genomic DNA was digested, with 30 units of each RE in a 30ml reaction volume consisting of 1X respective dilution buffer, overnight at 37°C in a water-bath. Samples were mixed with 6 ml of loading dye and electrophoresed in 0.8% agarose containing ethidium bromide (0.5 mg/ml) at 30 volts in a 21 cm long gel for approximately 15 h in a horizontal gel electrophoresis unit in 1X TBE buffer. The agarose gels were visualized under UV transilluminator and the band patterns were compared with the standard molecular weight marker (lambda phage DNA digested with *Eco*RI and *Hind* III) run along with the sample.

Samples (69) from suspected poultry birds from 43 different farms in the region of Punjab yielded *P. multocida* in 5 cases. All the isolates were non-haemolytic and produced small, circular glistening and dew drop like colonies on the blood agar. Bacilli were Gram negative, coccobacilli and non motile. All the isolates showed positive reaction for catalase and oxidase, produced indole, reduced nitrates to nitrites, and were negative for Voges Proskauer, methyl red, urease and citrate utilization test. The biochemical results were in accordance with most other workers (Heddleston 1976, Mohan *et al.* 1994). Glucose, mannose, sucrose, galactose, mannose, xylose, sorbitol and mannitol were fermented by all the isolates as observed by Heddleston (1976). All the isolates were sorbitol and xylose positive and negative for trehalose, arabinose and dulcitol, indicating that all these isolates belonged to subspecies *P. multocida* subsp. *multocida* (Quinn *et al.* 1994). These results are in close agreement with those reported by Heddleston *et al.* (1976) and Arora *et al.* (2005). However, Butt *et al.* (2003) have reported non fermentation of lactose, dulcitol, inositol by majority of *P. multocida* isolates but variable fermentation reaction for arabinose and maltose. All the isolates were sensitive to pefloxacin, chloramphenicol, ciprofloxacin and enrofloxacin. Majority of isolates showed sensitivity to cephalexin, cephotaxime, erythromycin and gentamicin and resistance to oxytetracycline and streptomycin. Variable sensitivity to

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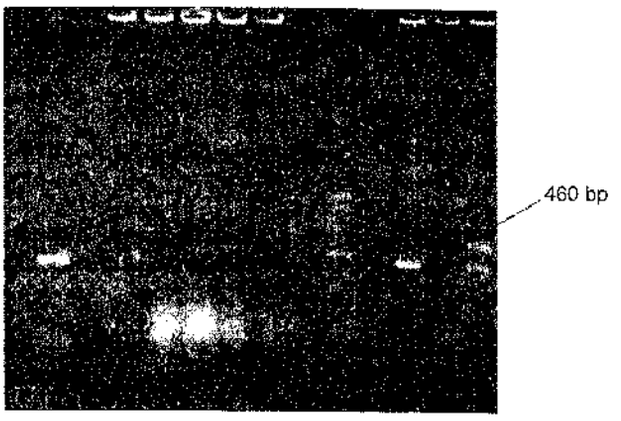

Fig 1. Gel electrophoresis of PCR amplified product of P. multocida from infra orbital sinus (IOS) swabs
• Lane 1–A_{35}(+); • Lane 2–A_{36}(-); • Lane 3–A_{37}(+); • Lane 4–A_{38}(-); • Lane 5–A_{39}(-); • Lane 6–A_{4}0(-); • Lane 7–A_{41}(-); • Lane 8–Negative Control; • Lane 9–Marker; • Lane 10–A_{45}(-); • Lane 11–A_{51}(+); • Lane 12–A_{46}(-); • Lane 13–P52

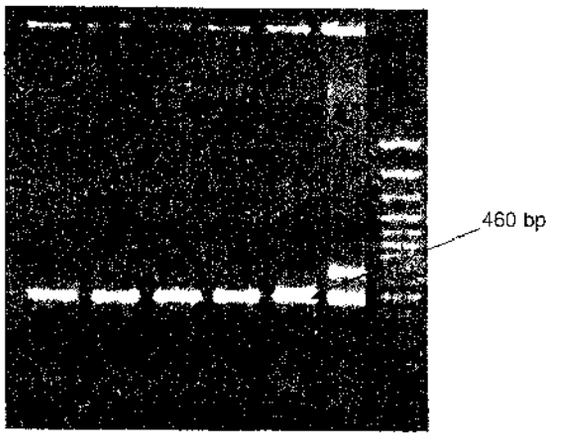

Fig 2. Gel electrophoresis of PCR amplified product of *P. multocida* from cultures of isolates
• Lane 1–1 Isolate; • Lane 2–2 Isolate; • Lane 3–3 Isolate; • Lane 4–4 Isolate; • Lane 5–5 Isolate; • Lane 6–P52 Isolate; • Lane 7–Marker 100 bp DNA Leader

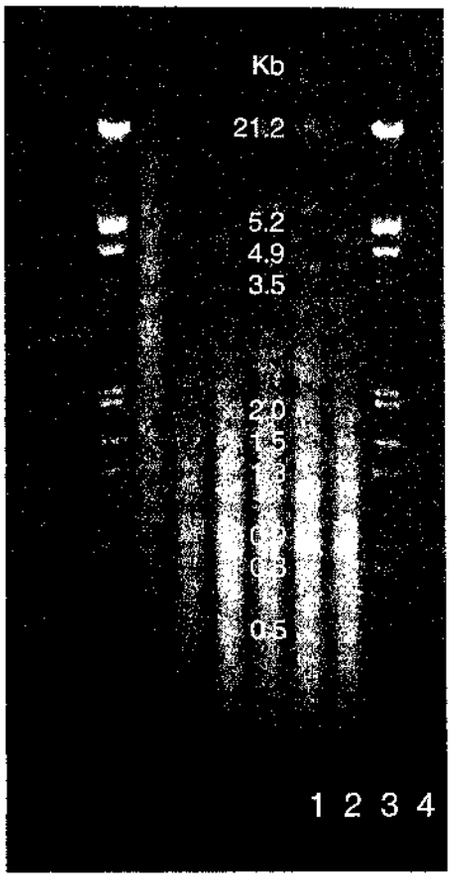

Fig 3. Gel electrophoresis band patterns of *P. multocida* genome digested with restriction endonuclease *Hha*I
• Lane 1–Lambda marker digested with *Eco*RI and *Hind*III; • Lane 2–P52; • Lane 3–1 (A_{15}); • Lane 4–2 (A_{16}); • Lane 5–3 (A_{35}); • Lane 6–4 (A_{37}); • Lane 7–5 (A_{51}); • Lane 8–Lambda marker digested with *Eco*RI and *Hind*III

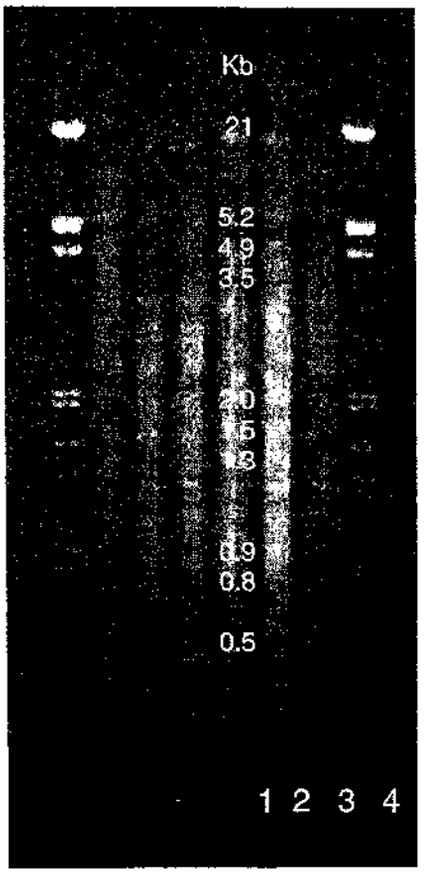

Fig 4. Gel electrophoresis band patterns of *P. multocida* genome digested with restriction endonuclease *Hpa*II
• Lane 1–Lambda marker digested with *Eco*RI and *Hind*III; • Lane 2–P52; • Lane 3–1 (A_{15}); • Lane 4–2 (A_{16}); • Lane 5–3 (A_{35}); • Lane 6–4 (A_{37}); • Lane 7–5 (A_{51}); • Lane 8–Lambda marker digested with *Eco*RI and *Hind*III

different antibiotics have been reported by different workers (Gupta *et al.* 1996, Morishita *et al.* 1996, Rajini *et al.* 1995, Shivachandra *et al.* 2005 and Arora *et al.* 2005). PCR amplified the DNA of all the 5 isolates and 5 IOS swabs giving an amplicon of approx. 460 bp, typical of *P. multocida* (Figs 1, 2). These results are in accordance with the findings of Townsend *et al.* (1998), Lee *et al.* (2000) and Shivachandra *et al.* (2005). The restriction endonuclease analysis (REA) using *Hha*I and *Hpa*II yielded 2 distinct profiles. With *Hha* I (Fig. 3) majority of distinguishing bands were in the range of 3.3 to 22 kb, which depicted 2 banding patterns in 5 avian *P. multocida* isolates. Isolates 1, 2 and 5 showed common profile, which was different from isolates 3 and 4. Upon digestion with *Hpa*II (Fig. 4), majority of distinguishing bands were in the range of 3.6 to 19.3 kb. It also depicted 2 different profiles with isolates—1, 2 and 5 having common banding profile and isolates 3 and 4 showed other common banding profile. Similar patterns of genome fragments were observed by Shivachandra *et al.* (2006). A total of 28 different profiles were recoganized by Wilson *et al.* (1993) when *Hha* I typing was used on 63 avian *P. multocida* isolates. *Hpa* II produced fragments that are distinct and better separated (Wilson *et al.* 1992). The present study also showed that *Hpa* II and *Hha* I can be employed for the genomic differentiation of avian isolates.

SUMMARY

Study reports isolation and molecular characterization of *P. multocida* from poultry by using PCR and REA. Swabs (69) from 43 different poultry farms were processed and 5 cases were positive for *P. multocida*. All the *P. multoicda* isolates were oxidase, catalase positive, non-haemolytc and showed no growth on MLA. Biochemically isolates belonged to *P. multocida* subsp. *multocida*. Isolates were sensitive to enrofloxacin, ciprofloxacin, pefloxacin and chloramphenicol. PCR was positive for 5 infra-orbital sinus swabs. In REA using enzymes *Hha* I and *Hpa* II, the isolates revealed two distinct banding patterns.

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