Molecular characterization of Salmonella enterica serotype Enteritidis isolated from chickens by REP-PCR, BOX A1R and antibiotic resistance

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INTRODUCTION

*Salmonella enteritidis i*s an important pathogen for avian production and public health. In the last 30 years, a dramatic increase in the incidence of Salmonella enteritidis around the world has been described (Liebana *et al.*, 2011). In Peru, this increment is associated to clinical cases, carcasses contamination and antimicrobial resistance in *Salmonella enteritidis* from poultry. Variability studies are important for understand the adaptation and evolution of microorganism include Salmonella. The molecular techniques for typing and characterization of genetic variability in microorganisms are useful to this propose. REP-PCR, BOX A1R have been found to be extremely reliable, reproducible, rapid and highly discriminatory (Albufera *et al.,* 2009). The aim of present report is described the variability of *Salmonella enteritidis* isolates using molecular techniques and antimicrobial profiling.

MATERIALS AND METHODS

Thirty samples of suspect Salmonella clinical cases from broilers during period of January to November 2011 were isolation using Salmonella-Shigella (SS) agar, brilliant green agar, bismuth sulphite agar, and MacConkey agar. Bacterial colonies showing morphological characteristic for Salmonella were then confirmed by biochemical tests. Antibiotic profile for ciprofloxacine, norfloxacine, phosphomicine, enrofloxacine, trimethropim sulfa and oxytetracycline were realized.

Genomic DNA from 30 bacterial colonies was extracted using Wizard Genomic DNA isolation kit (Promega). The genus, specie and serotype identification were confirm to PCR using the protocols described by Soumet *et al.* (1999).
REP-PCR and BOXAIR-PCR was performed using the primers described by Dombek *et al.* (2000) and performed in a Veriti Termocycler (Applied Biosystems) similar to described by Albufera *et al.* (2009). REP-PCR and BOX A1R DNA fingerprints were transform to banding matrix and dendogram using the genetic distance method with GelAnalyzer 2010a and Mega v4.0 softwares.

RESULTS AND DISCUSSION

The thirty *Salmonella enteritidis* strains were identified on differential agar media, by biochemical tests and molecular serogrouping with similar results. Twenty eight of thirty Salmonella enteritidis isolates (93.3%) were resistant to more than one antimicrobial. The most common antibiotic resistance pattern was to oxytetracycline and Trimethropim with 83.3% and 76.6% respectively. Six isolates (20%) showed a multidrug resistance profile to more than 4 antibiotics similar to reported by Zhao et al., (2007). BOX A1R and REP-PCR showed moderate genetic heterogeneity between isolates similar to RFLPs studies described by Zhao et al., (2007), REP-PCR, phage typing and virulence genes described by Dias de Oliveira et al., (2007).

RESULTS AND DISCUSSION

UPGMA analysis showed 4 genetic clusters (Figure 1), however, no relationship was observed between genotypic profile and antimicrobial resistance patterns.

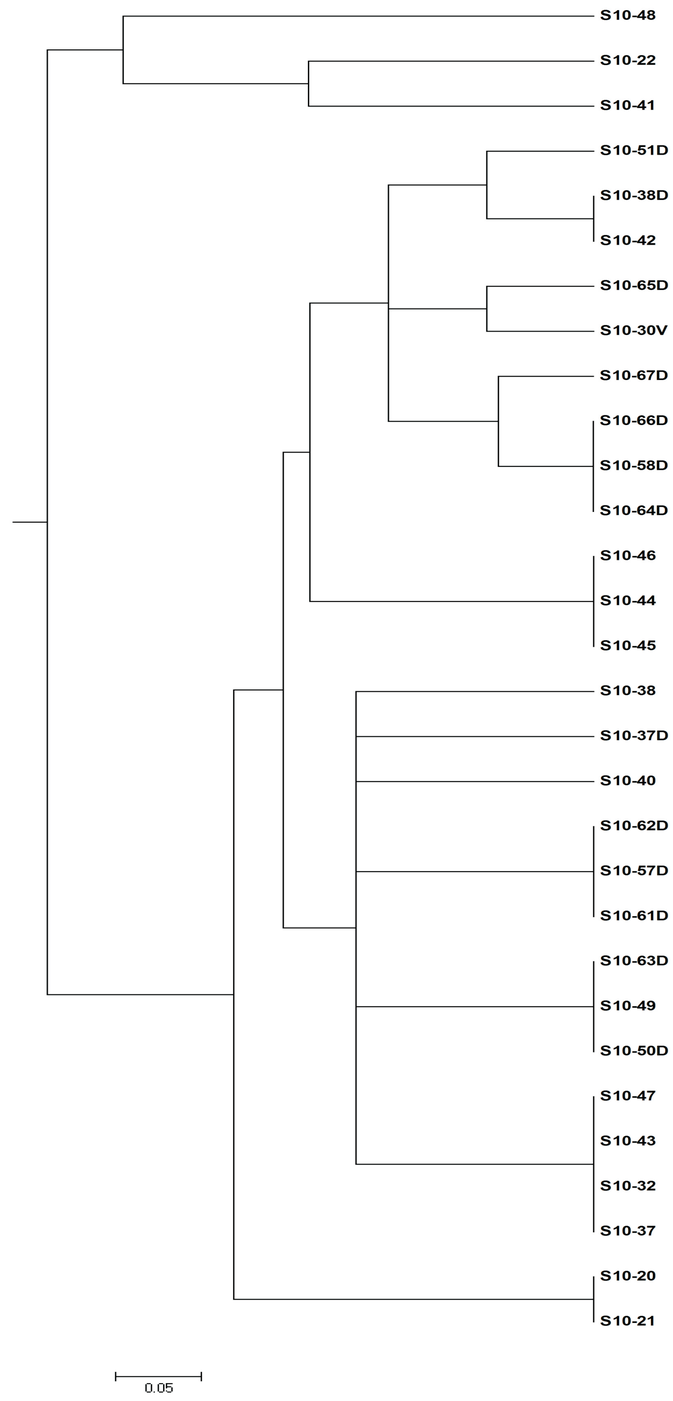

Figure 1. UPGMA dendogram of the cluster analysis based on DNA fingerprinting performed by REP-PCR of S. enteritidis isolates.

Our results suggest than REP-PCR and BOXA1R DNA fingerprint are useful tools for differentiating Salmonella enteritidis strains isolated from poultry. However, the REP-PCR showed a greater discriminatory power to differentiate closely related Salmonella isolates than BOXA1R method. .

CONCLUSION

The results showed high level to antibiotic resistance (93.3%) and moderate genetic heterogenety of Salmonella enterica serovar enteritidis from poultry.

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