Similar genomic pattern to vaccine strain among *Bordetella pertussis* isolates in Iran

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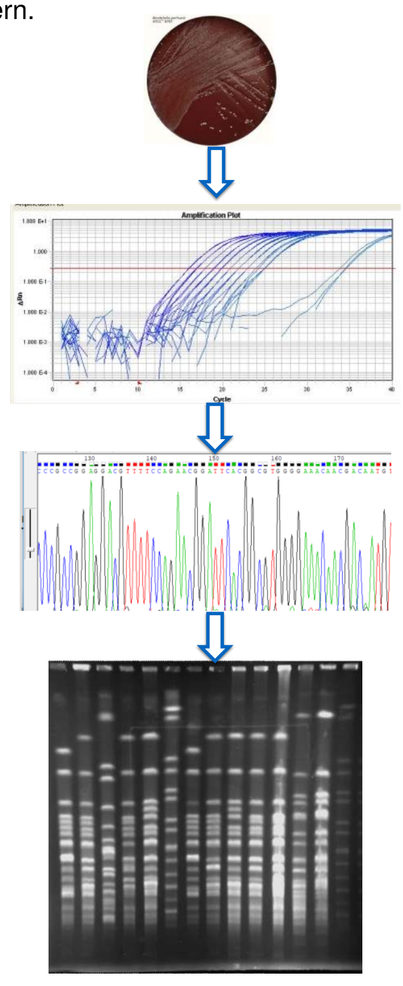
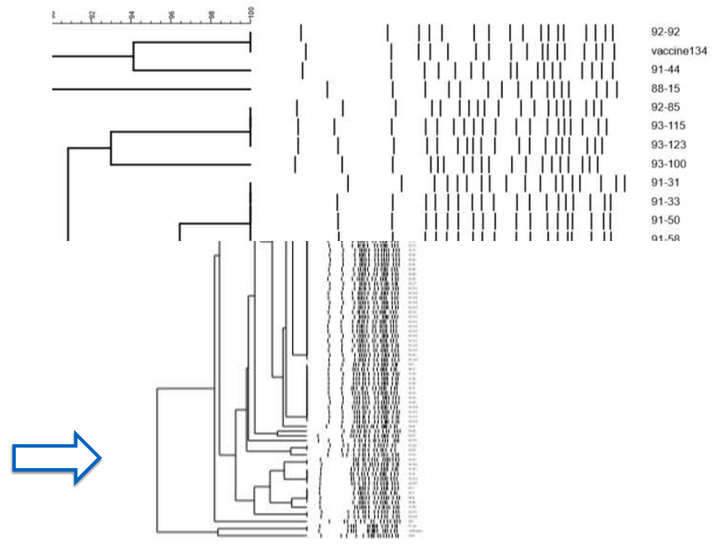
Background:
The problem of whooping cough (pertussis) as a vaccine preventable disease is still unresolved worldwide. The spread of ***Bordetella pertussis*** as the agent of this respiratory illness has been observed globally. Despite a long history of vaccination with high coverage in children, a resurgence of pertussis has been observed in many countries. Adaptation of *B. pertussis* isolates is one of the major reasons for pertussis reemergence. To identify the discrimination between local and vaccine strains, the genomic patterns and allele types of *B. pertussis*, vaccine and circulating strains isolated from clinical specimens in Iran were analyzed in this study.
There are different DNA-based techniques to identify epidemiological characterization and differences between *B. pertussis* strains, such as **pulsed-field gel electrophoresis (PFGE)**. PFGE as a gold standard method is still valuable in epidemiologic studies to compare circulating isolates and vaccine strains.
The aim: This study is the first investigation of the clonal relationship between B. pertussis clinical and vaccine strains. These data will provide good information in terms of allelic variation and dissemination of B. pertussis clinical isolates in Iran. Also, since the vaccination program in Iran has not changed for over 60 years, this study will help shed light on what steps need to be taken to provide more effective vaccines in the future.

Mooi, F.R., 2010. Bordetella pertussis and vaccination: the persistence of a genetically monomorphic pathogen. Infection, Genetics and Evolution 10, 36-49.
Fry, N.K., Neal, S., Harrison, T.G., Miller, E., Matthews, R., George, R.C., 2001. Genotypic variation in the Bordetella pertussis virulence factors pertactin and pertussis toxin in historical and recent clinical isolates in the United Kingdom. Infection and immunity 69, 5520-5528.
Advani, A., Hallander, H.O., Dalby, T., Krogfelt, K.A., Guiso, N., Njamkepo, E., von Könnig, C.H.W., Riffelmann, M., Mooi, F.R., Sandven, P., 2013. Pulsed-field gel electrophoresis analysis of Bordetella pertussis isolates circulating in Europe from 1998 to 2009. Journal of clinical microbiology 51, 422-428.

Methods:
A total of 6490 nasopharyngeal Dacron swab samples were collected from pertussis suspected cases and transported on Regan-Lowe from April 2008 to March 2015 and only one isolate from 2005. All swab samples from different provinces of Iran were transported to the Pertussis Reference Laboratory of Pasteur Institute of Iran. A total of 100 *B. pertussis* strains were isolated from the samples. Biochemical tests, slide agglutination and real-time PCR were done to detect *B. pertussis* strains. DNA extraction was performed using the PCR template preparation kit (Roche Diagnostics, Germany). Real time PCR by targeting *IS481* and *ptxP* were also done to confirm *B. pertussis* using ABI systems (Applied Biosystem). Sequencing of *ptxP*, *ptx and prn* genes was carried out to determine the allele types of these virulence factors. Ultimately, the genomic patterns of *B. pertussis* strains were investigated by PFGE using *Xba*I restriction enzyme. Vaccine strains *B. pertussis* 134 and 509 were also analyzed.
PFGE profiles were analyzed by GelCompar II software version 4 (Applied Maths, Belgium). Salmonella enteric serotype Braenderup strain H9812 was used as size marker. The unweighted pair group method using the arithmetic average (UPGMA) algorithm was used as the clustering method, with 2% band tolerance and 2% optimization settings with the Dice coefficient.

Results:
The results of PFGE showed that considering standard and vaccine strains in analysis of genomic profiles, 24 PFGE patterns were obtained that clustered into 17 PFGE groups. We found only one strain with the genomic pattern similar to vaccine strain *B. pertussis* 134 with the same virulence profile except *ptx*A (*ptxP*1, *ptxA*1 and *prn*1). This strain was isolated in 2014 from a 2 months age infant without any vaccination. However, B. pertussis 509 showed the distinct PFGE pattern.





Conclusions:
Of all 100 studied isolates until 2014, we found only one isolate showing the genomic pattern like vaccine strain. However, the clonal spread with different virulence profile from vaccine strains has been observed among the circulating strains. It may be suggested that strain variation between vaccine and local isolates may have an important effect on pertussis persistence and outbreaks in Iran like other parts of the world.
Strains with a predominant pattern in the dendrogram may have a high potential for expansion from one person to person and the ability to survive host immune system and vaccination. Meanwhile recent vaccine strains in Iran has have a different genetic pattern from predominant circulating strains that may cause low efficiency of pertussis vaccine. Only one strain like old ans standard strain Bp 134.