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Research Article

Molecular analysis of Bordetella bronchiseptica from respiratory-diseased patients.

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Abstract

Background:Bordetellabronchiseptica isa Gram-negative bacterium which known to cause respiratory disease in pet animals, and can colonize the human respiratory tract. Recently, B.bronchiseptica infections arerestricted to humans and increasing worldwide.

Aim: Molecular indication of B.bronchiseptica in respiratory-diseased patientswith sequencing and phylogenetically analyzing close-relationship betweenstudy and global isolates, as well as measuring levels of interleukin-10 in study individuals.

Materials and methods: An overall 150 patients with various clinical signs were subjected to collection of nasal swabs for molecular testing by the conventional PCR assay.Then, some positive B.bronchisepticaisolates were sequenced and analysedphylogeneticallyby the MEGA-11 software to identify its identity to the global NCBI-GenBankB.bronchiseptica isolates / strains.Also, samples of venous blood were obtained from the study population as well as from 30 healthy individuals to estimate serologically the levels of IL-10.

Results: This study revealed that 10.99% of respiratory-diseased patients were positively infected with B.bronchiseptica. Phylogenetically,analyzing of study isolatesdetected the presence of similarity and mutations / changes at ranges of 98.14-99.91% and 0.0004-0.002%, respectively. Phylgenetic tree and multiple sequence alignment demonstrated that identity of study B.bronchiseptica isolates was to Mexican B.bronchiseptica strain (GenBank ID: FJ867619.1). The levels of IL-10 were elevated significantly in respiratory-diseased patients when compared to healthy, but not between the positively and negatively B.bronchiseptica infected patients.

Conclusion: This study implicates molecularly,for the first time in Iraq, B.bronchiseptica in respiratory-diseased patients,close-relationship between the study and global isolates throughout phylogenetic analysis, and the level of IL-10 in respiratory-diseasedand the healthy population.
Furthermore molecular studies are greatly needed to insight the role of B.bronchiseptica in other human infections.

Keywords: Gram-negative bacteria, PCR, IL-10, ELISA, NCBI, Iraq

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1. Introduction

Bordetellabronchiseptica is a small Gram-negative, aerobic, rod-shaped bacterium which belongs to the Alcaligenaceae family in the Burkholderiales Order under the Class of Betaproteobacteria (Kadhim et al., 2025; Mohammad et al., 2025). This bacterial pathogen that first characterized by Bordet and Gengou in 1906, is frequently implicated in infectious respiratory diseases of pet animals, dogs and cats, can cause a highly contagious condition characterized by whooping cough in particularly in children and less severe or chronic infections in adults (Mattoo and Cherry, 2005;Nieves and Heininger, 2016). To establish an infection, adherence of B.bronchisepticato apical surface of respiratory epithelial ciliais crucial as it allows the bacterium to evade mucociliary clearance mechanisms that represent the primary host defense (Brown, 2022). Indeed, the ongoing challengeof B.bronchiseptica infections, particularly the emergence of multidrug resistant strains necessitates a deeper understanding of its antimicrobial susceptibility to guide effective therapeutic interventions (Kadlec and Schwarz, 2018; Yi et al., 2024).

IL-10 is a pleiotropic cytokine primarily recognized for potent immunosuppressive properties, playing a fundamental role in maintaining immune homeostasis and preventing excessive inflammatory responses (Carlini et al., 2023). This anti-inflammatory cytokine is widely produced by various cell types and exerts broad effects on target cells, critically modulating the immune response during diverse physiological and pathological conditions (Kany et al., 2019; Saxton et al., 2023).Dys-regulation in IL-10 signaling, such as loss of function mutations in its receptor, has been directly linked to severe hyper-inflammatory disorders, underscoring its indispensable role in controlling immune-mediated pathologies (Kumaret al., 2019; Aktay-Cetin et al., 2025). The multifaceted nature of IL-10 extends beyond mere immune suppression as it also influences cellular proliferation, differentiation, and tissue repair mechanisms (Steen et al., 2020).

Despite the clear role of B.bronchiseptica in respiratory infections, the diagnostic approaches to identify causative agents have traditionally relied on isolation from carefully collected swabs, recent advancements in molecular diagnostics, quantitative (real-time) and qualitative (conventional) PCR, have demonstrated superior sensitivity for detecting B.bronchiseptica compared to traditional culture methods, even in cases where antimicrobial treatment may have impaired bacterial growth (Calderaro et al., 2022; Jasim andRadhy, 2025; Rose et al.,2025).This includes an in-depth exploration of its taxonomic placement within the genus of Bordetella, its complex array of virulence factors that facilitate colonization and immune evasion, as well as variations in clinical presentation across different hosts (Mattoo and Cherry, 2005; Belcher et al., 2021).

In Iraq, no previous or recent studies have been done to detect the role of B.bronchisepticain respiratory infections of human. Hence, this study aims to molecular indication of B.bronchiseptica in respiratory-diseased patients with sequencing and phylogenetically analyzing close-relationship between study and global isolates, as well as measuring levels of interleukin-10 in study individuals.

2. Materials and methods

Samples

An overall 150 attendingpatients for private clinics at Al-Qadisiyah province (Iraq) during January (2025) to March (2025) with various clinical signs were selected and subjected to direct collection of nasal swabs under aseptic conditions as well as draining of blood with gel tubes. Additionally, blood was drained from a 30 healthy individuals who served as a control group.At laboratory, the swab samples were used to extraction the DNAs, while blood samples were centrifuged at 5000rpm for 5 minutes and the obtained sera were kept frozen at (-20°C)until be used for serological measurement of IL-10.

Moleculartesting

After preparation the study samples at room temperature, DNAs were extracted from the nasal swab samples following the manufacturer instructions of the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). Then, Accupower ® PCR PreMix kit and one set of primers (F: 5'-TCCTGTGCAACTGACGGTAC -'3 and R: 5'-CCCAACATCTCACGACACGA -'3) designed based on NCBI-GenBank isolate (ID: NR_025949.1) targeting the16S rRNA gene, were served for preparingtheMasterMix tubes at a final volume of 20μL. For DNAs amplification, the MasterMix tubeswere transferred to the Thermal Cycler system (BioRad, USA). Electrophoresis of agarose-gel (1.5%) stained with ethidium bromide was done at 100V and 80Am for 90min, and the positive bands were detected at an approximately 622bp using the ultraviolet system.

Phylogenetic analysis

The DNAs of some positive isolates were sequenced, and submitted in NCBI-GenBank database. For phylogeny, MEGA-11 software and NCBI-Multiple Sequence Alignment (MSA) Viewer were served to analyzing the study isolates by ClustralW Alignment, Homology Sequence Identity and Phylogenetic Tree Analysis.

Serological examination

Quantitative sandwich Human Interleukin 10 ELISA kit (catalogue number: SL0967Hu)wasserved in the present study to measurement the levels of IL-10 in respiratory-diseased patients (total number: 150) as well as in healthy individuals (total number: 30). Briefly, the serum samples and kit contents (standard and reagents) were prepared at room temperature, processed, and the optical density (OD) for all tested samples were read by the ELISA reader. Finally, concentration of IL-10 was measured in serum samples based on OD values of both the standards and serum samples as well as the concentrations of standard.

Statistical analysis

One-way ANOVA in the GraphPad Prism software was served for detecting significant differences between the obtained values (mean ± standard error) of study population at $p<0.05$ (Al-Gharban, 2017; Ibraheim et al., 2023).

3. Results

Molecular results

The findings of testing 150 respiratory-diseased patients revealed that 19 (12.67%) of nasal swab samples were positives by PCR assay while 131 (87.33%) samples were negatives (Figure 1).

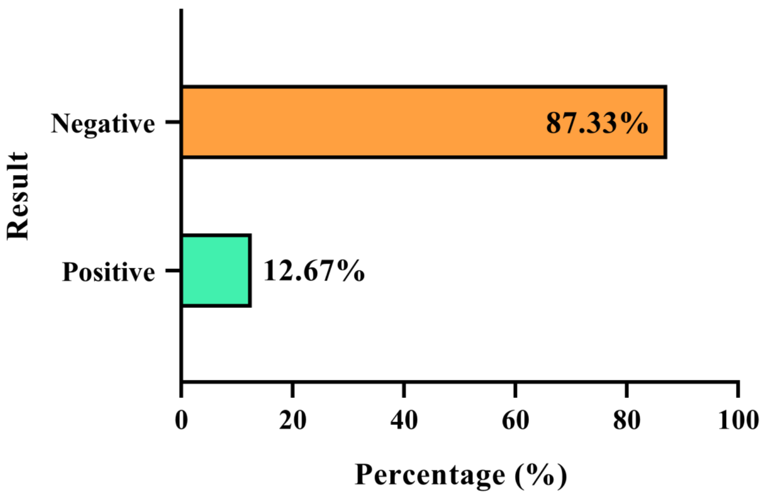

Figure (1): Total molecular results for testing 150 nasal swabs by PCR assay

In the NCBI-GenBank database, submitting of 10 study isolates were done with the respective names started from Bordetellabronchiseptica isolate RAH1 to Bordetellabronchiseptica isolate RAH10. Then, sequence data were subjected to multiple sequence alignment (MAS) by ClustralW alignment in MEGA-11 software as well as by the NCBI-Viewer. Phylogenetic tree analysis and Homology sequence identity detected the presence of similarity and mutations / changes at ranges of 98.91-99.14% and 0.0004-0.002%, respectively with the global NCBI-BLAST B.bronchiseptica strain / isolates. However, marked identity for study B.bronchiseptica isolates was identified for Mexican B.bronchiseptica strain (GenBank ID: FJ867619.1), (Figures 2-4, Table 1).

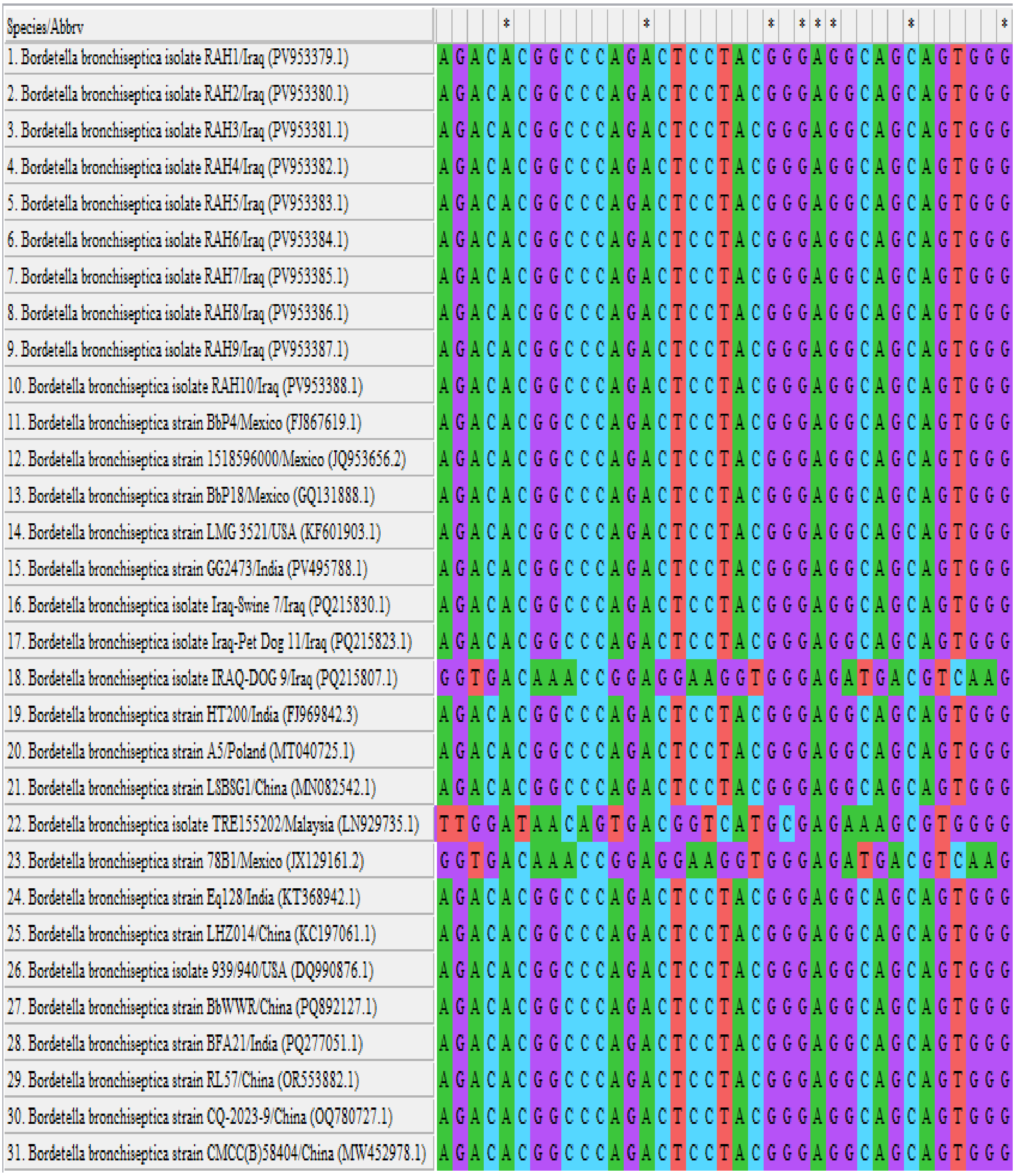

Figure (2): Multiple sequence alignment of local and global B. bronchiseptica isolates / strains by MEGA-11

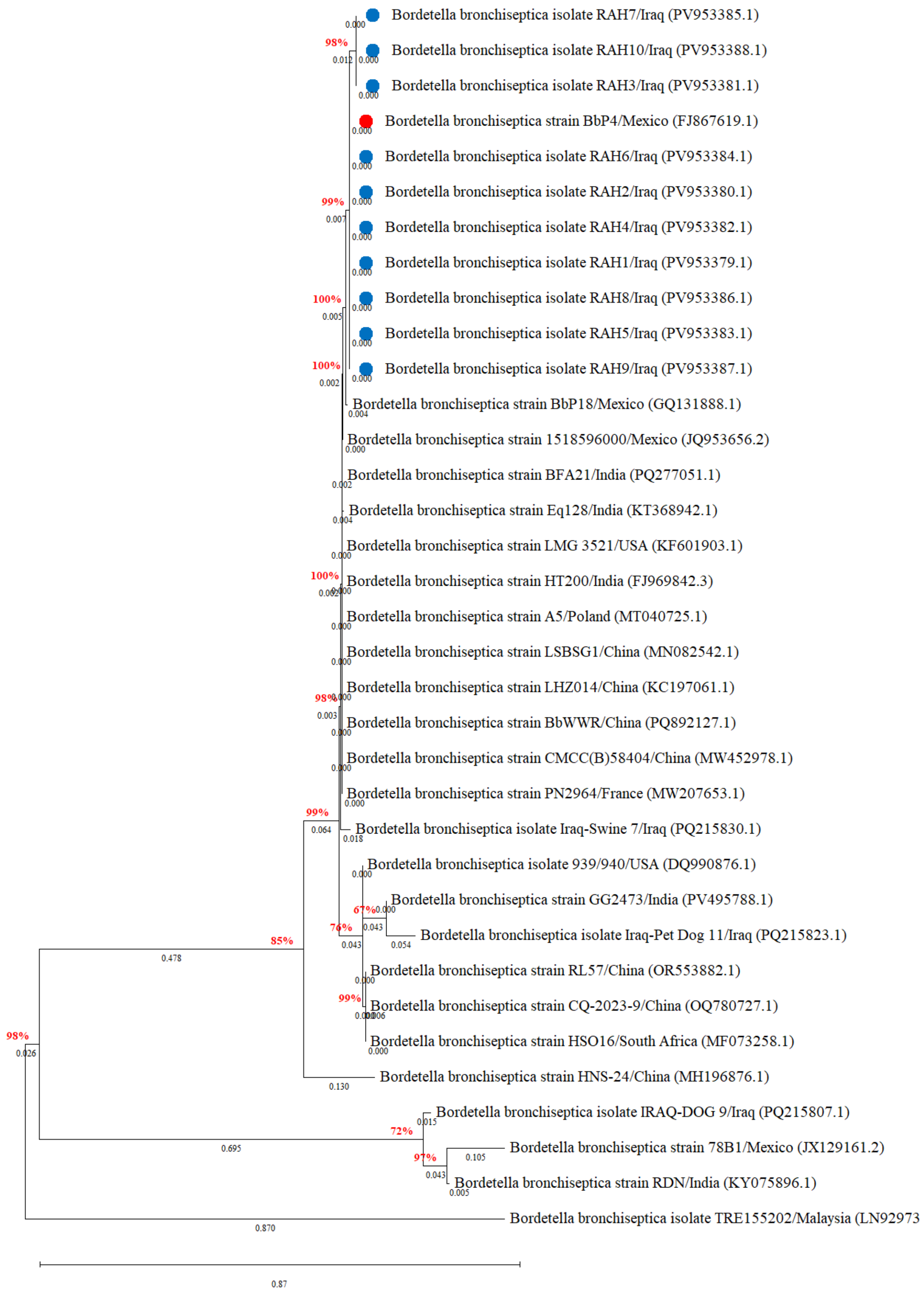

Figure (3): Phylogenetic tree analysis of local and global B. bronchiseptica isolates / strains

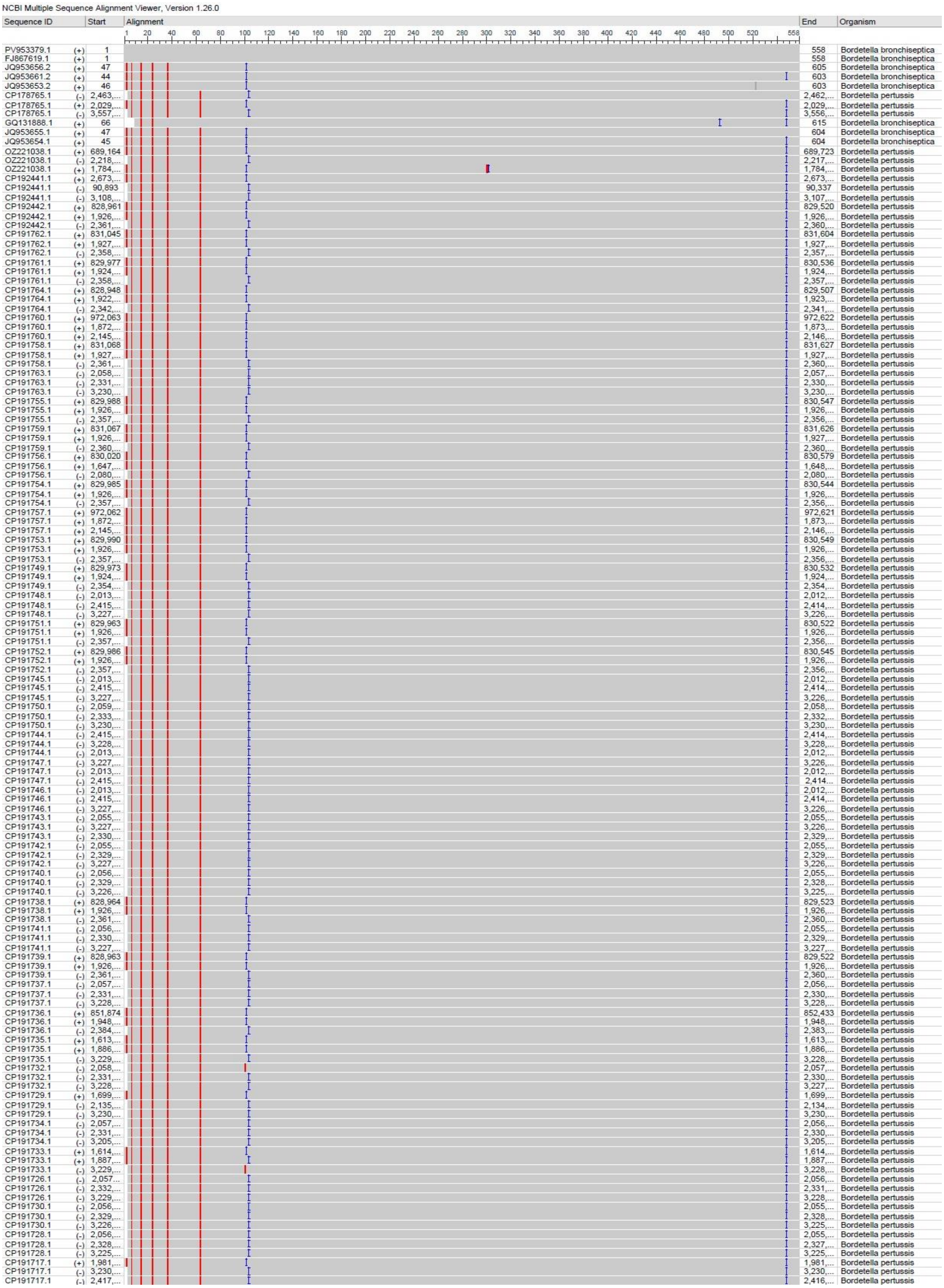

Figure (4): Multiple sequence alignment of local and global B. bronchiseptica isolates / strains by NCBI-Viewer

Table (1): Homology sequence identification for local and NCBI-BLAST B.bronchisepticaisolates / strains

Local isolate		NCBI-BLAST isolate				Identity(%)
Name	Access. No.	Host	Source	Country	Access. No.	
RAH1	PV953379.1	Human	Nasal swab	Mexico	FJ867619.1	98.97
RAH2	PV953380.1	Human	Nasal swab	Mexico	FJ867619.1	98.95
RAH3	PV953381.1	Human	Nasal swab	Mexico	FJ867619.1	99.11
RAH4	PV953382.1	Human	Nasal swab	Mexico	FJ867619.1	99.09
RAH5	PV953383.1	Human	Nasal swab	Mexico	FJ867619.1	99.13
RAH6	PV953384.1	Human	Nasal swab	Mexico	FJ867619.1	99.14
RAH7	PV953385.1	Human	Nasal swab	Mexico	FJ867619.1	99.10
RAH8	PV953386.1	Human	Nasal swab	Mexico	FJ867619.1	99.14
RAH9	PV953387.1	Human	Nasal swab	Mexico	FJ867619.1	99.05
RAH10	PV953388.1	Human	Nasal swab	Mexico	FJ867619.1	99.12

IL-10 levels

The findings of quantitative ELISA among totally 180 study individuals involving 150 respiratory-diseased patients and 30 healthy ones as a control revealed that the values (M ± SE) of respiratory-diseased patients (47.474 ± 2.547pg/ml) were significantly higher ($p<0.0028$; 95%CI: 12.29 to 62) than reported in healthy individuals (14.145 ± 0.888pg/ml), (Figure 5).

In comparison between the positively infected respiratory-diseased patients with B. bronchiseptica(total number: 19) and negativelyinfected respiratory-diseased patients (total number: 131), the concentrations of IL-10 were differed insignificantly ($p<0.0593$; 95%CI: 37.08 to 55.93) between the infected (49.259 ± 3.145pg/ml) and non-infected (45.688 ± 4.059pg/ml) respiratory-diseased patients (Figure 6).

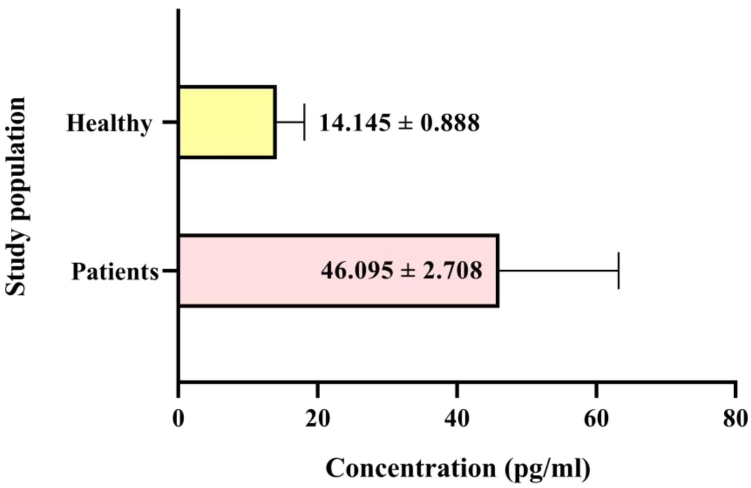

Figure (5): Levels of IL-10 among the study population (patients: 150; healthy: 30)

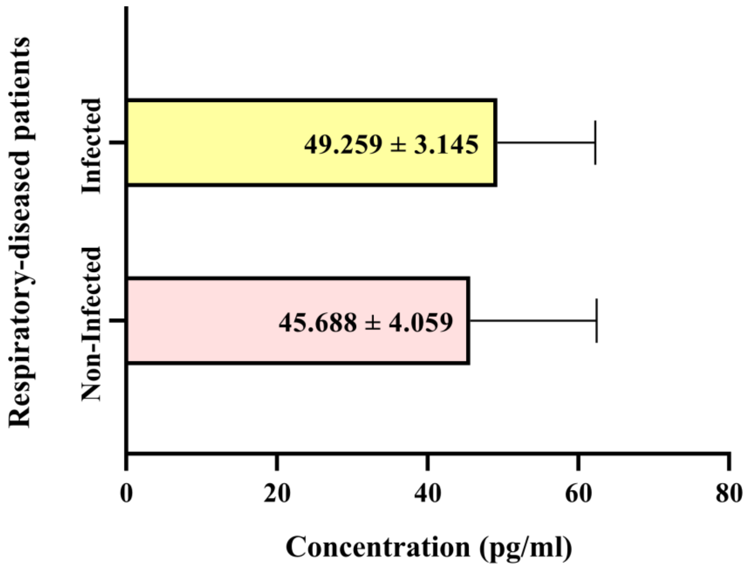

Figure (6): Levels of IL-10 among the respiratory-diseased patients (total number: 150)

4. Discussion

The results of present study indicated molecularly that B. bronchiseptica was found in 12.67% of nasal swab samples of respiratory-diseased patientssuggesting that this pathogen might play a considerable role in complicating of respiratory infections and increasing the antibiotic susceptibility. In earlier evidences, B. bronchiseptica in humanshas been verified in several cases;for example, a 26 years old male was showed the signs of right lower lobe atelectasis and high body temperature after intubation on a respirator in addition to a 54 years old male suffering from a severe sore throat, cough, difficulty swallowing, and difficult breath(Woolfrey and Moody, 1991). Gueirard et al. (1995) identified the presence of microorganismin a study case of woman with bronchopneumonia, with demonstratingthe role of rabbit in transmission of infection. Dworkin et al. (1999) detected by culture an existence of bacterium in persons diseased with HIV infections. Later, García-de-la-Fuente et al. (2015) examinedtwenty twoill individualshaving the pathogen. Woods et al. (2020) reporteda case with a history of chest trauma from pedestrian collision, cough, hemoptysis, and fever with negative screening results for autoimmune diseases and immunodeficiency virus. Then, several researchers have been addressed the specific immunological responses elicited by the bacterium, differentiating between local mucosal immunity and systemic antibody production, and delineated the most effective diagnostic methodologies (Caulfield et al., 2023; Miguelena Chamorro et al., 2023; Parrish andGestal, 2024). Other studies highlighted the importance of understanding the host immune responses to B. bronchiseptica particularly considering how the environmental and co-infection viral factors can compromise host immunity and predispose animals to more severe respiratory diseases (Macpherson et al., 2022; Zafar et al., 2025). However, pathological alterationrelated toB. bronchisepticain animals and humans is restrictedfor inferencewhich collectedthroughoutinfected animalsorhuman autopsies (Kadhimet al., 2025; Malik et al., 2025).

Ourphylogeneticallydat demonstrated the identity of study isolates to the Mexican strain. The 16S rRNAgene is an exclusive and ubiquitous molecular mark with critical roles in identification of bacterial pathogenssuch as B. bronchiseptica(Einarsson and Boutin, 2019), especially when usual culturing techniques are inadequate. It can be discriminated at species level by specific phylogenies in its highly conserved and variable regions so that it is a cornerstone in clinical microbiology in bacterial species identification (Nunes Ramos et al., 2025). The ubiquitous occurrence of this gene in all described species of bacteria and archaea, is relatively uniform distribution with conserved areas to design universal primers, make it an excellent ribotyping tool in prokaryotes (Barreto et al., 2014; Nazir et al., 2019). The full-length 16S rRNA gene with a length of about 1,500 base pairs augments its usefulness by incorporating enough phylogenetic information to aid in high-resolution taxonomic classification that is beyong the reach of shorter sequence fragments (Weinroth et al., 2022). In addition, improved sequencing methodology, including deep sequencing of 16S rRNA gene amplified by universal primers, has transformed research into the nature of diverse microbial communities, including uncultured species (Abellan-Schneyder et al., 2021). This gene stands out as especially useful as its hypervariable domains allow species level differentiation to occur even on closely related bacteria taxa (Bose and Moore, 2023).

In the present study, the findings of IL-10 were showed a significant elevation in values of respiratory-diseased patients compared to healthy; however, insignificant variation was seen between the molecularly infected and non-infected B. bronchiseptica patients. Cytokines like Il-1, IL-6, and IL-10 are diverse group of signaling molecules that play a crucial role in regulating immune responses, inflammation, and cell communication within the body by modulation of antigen presentation by immune cells and the direct suppression of pro-inflammatory cytokine production, thereby preventing excessive immune activation that could lead to tissue damage (Gulati et al., 2016; Kany et al., 2019). Also, cytokines act as messengers, influencing the behavior of various cells including immune cells and coordinating the body's response to threats like infections and tissue damage (Harvanová et al., 2023). The intricate balance maintained by IL-10 is particularly relevant in the context of respiratory diseases where chronic inflammation and immune-dys-regulation contribute significantly to pathogenesis(de AraújoMorais et al., 2021; Junainah et al., 2025). Despite its recognized anti-inflammatory effects, emerging evidence points to complex, sometimes paradoxical roles for IL-10 in promoting fibrosis, particularly in chronic inflammatory settings (Steen et al., 2020). However, the precise role of IL-10 in conditions like lung fibrosis remains complex, given its capacity to down-regulate inflammation and being classified as a Th2 cytokine (Huaux, 2021). Given the role, therapeutic modulation of IL-10 activity represents a promising strategy for a range of immune disorders, necessitating a thorough exploration of its signaling pathways and downstream effectors (Wang et al., 2019; Nie et al., 2025).

5. Conclusion

This study implicates molecularly, for the first time in Iraq, B.bronchiseptica in respiratory-diseased patients, close-relationship between the study and global isolates throughout phylogenetic analysis, and the level of IL-10 in respiratory-diseased and the healthy population.Furthermore molecular studies and genetic surveillance are greatly needed to insight the role of B.bronchiseptica in other human infections, and to adapt active prevention strategies and developing new therapeutic interventions that can effectively target emerging resistant strains and reduce the burden of infection.

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