Clinical and molecular study of upper respiratory tract infections of cats caused by *Bordetella bronchiseptica* in cats at Baghdad city

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ABSTRACT

This study included clinical and molecular study of *Bordetella bronchiseptica* from upper respiratory tract of 150 cats with and without respiratory signs of (60 local breed and 90 different breed) and both sexes (70 male and 80 female) with different ages including 85 cats more than one year and 65 less than one year private veterinary clinics and Baghdad veterinary hospital in Baghdad city at the period from October 2023 to September 2024.Totally, 150 nasal swabs were collected from cats at aseptic condition to carry out for bacteriology isolation by culturing Blood agar and Bordet Gengou agar base for culturing of *B. bronchiseptica*. Then the bacteria were examined by Gram stain and apply different biochemical and antimicrobial study using Vetik analysis of isolated bacteria with confirmative diagnosis by molecular detection of *FIM* gene of *B. bronchiseptica*. The results of clinical study showed that the most frequent signs were fever and dyspnea. The total isolation rate of *B. bronchiseptica* was 4.6%. The DNA of 7 isolates of *B. bronchiseptica* was successfully amplified by using specific primers at 425 bp of *FIM* gene of *B. bronchiseptica.* The isolates of *B. bronchiseptica* showed a high degree of resistance (100%) to Piperacillin, cefotaxime, Aztreonam, Doxycycline, Minocycline and Tetracycline, while these isolates were sensitive to Levofloxacin (85.7%) and Tobramycin (57.1%). The infection rate of *B. bronchiseptica* were increased at age group less than one year and females. No significant variation was observed among the breed of cats, infection rate of *B. bronchiseptica* was increased in Himalaya than other breed. The results revealed to a significant variation of infection rates among the months of study, the highest percentage of infections by *B. bronchiseptica* appeared in December (23%). The results of sequencing of seven isolates of *B. bronchiseptica* showed homology with strains in Gene bank. It had been deposited under accession numbers for *B.bronchiseptica* respectively; CP019934.1, CP049918.1, CP022962.2 LR134480.1, CP020819.1, CP014013.2, CP132332.1, AP014582.1, X74119.1, AY289621.1, and HE965806.1. In conclusion, the results of phylogenetic study revealed that the local isolates *B. bronchiseptica* of variable origins, these confirm their importance as primary pathogens of many diseases due to their widespread in the environment and their ability to infect humans and different animals.

Keywords: *Bordetella bronchiseptica,* Clinical study, *FIM* gene, Cats, Baghdad, Vitek

Introduction

Cats are one of the most common household pets in the world and their potential for transmission of infectious disease is also well with spreading zoonotic agents as toxoplasmosis (Al-Ani *et al.*, 2020; Alkubaisi and Al-Zubaidy, 2023). However, recognition should additionally be given to respiratory infections acquired from pets. The respiratory tract infections in cats are highly contagious and easily speared, making the prevention the first and the most important step in avoiding the risks of further complications associated with the inflection (Weese *et al.*, 2015). Respiratory disorders arise from the interplay of various factors, including the host's

immunological and physiological traits, the etiological agent such as a virus, bacterium, or mycoplasma, and environmental conditions (Hamzah and Ibrahim, 2024; Jbr and Jumaa, 2024). Feline herpesvirus (FHV) and feline calicivirus (FCV) are the most common pathogens causing upper respiratory infection, but *Chlamydia felis*, *Mycoplasma felis* and *Bordetella bronchiseptica* are also reported to be involved (Kennedy *et al.*, 2024; Al-Jumaa *et al.*,2024). Respiratory infections are common in cats, especially in high- density populations such as shelters, breeding catteries, and feral cat colonies. A variety of viruses, bacteria, fungi, and protozoa cause these infections, which negatively impact feline health. While vaccines have greatly reduced the incidence of serious respiratory disease in cats, they have not eliminated the highly contagious pathogens that cause them (Greene, 2012). Symptoms of upper respiratory tract infections include clear or colored discharge from the eyes or nose, coughing, sneezing, swelling of the mucous membranes around the eyes (conjunctivitis, ulcers in the mouth, lethargy, and anorexia. In rare cases, cats may have trouble breathing (Smith, 2005; Lappin *et al.*, 2017). Epistaxis also occur as a signs of respiratory infection (Quimby and Lappin, 2009). Different options are available for diagnosis of respiratory tract infections firstly by the isolation of the causative bacteria by culturing on selective media and biochemical testes, polymerase chain reaction (PCR) (Al-Abedi and Al-Amery, 2021; Mansour and Hasso, 2021), and enzyme-Linked immunosorbent assay (ELISA) as well as the using of X-rays, Computerized Tomography (CT) scan and Magnetic resonance imaging (MRI), (Greene, 2012; Jarad *et al*., 2019; Al-Maliki and Atyia, 2020; Gharban and Yousif, 2020). Respiratory tract infections in cats are complex conditions and caused by different types of viruses and bacteria, and typically initiated by stress-induced immunosuppression within infected pets (Tanaka *et al.*, 2012; Dorn *et al.*, 2017). The most commonly types of bacteria which isolated from upper respiratory tract of cat including: *Enterobacter spp*., *Streptococcus spp., Pseudomonas spp.* and *Serratia* spp. (Lee *et al.*, 2021; Fernández *et al.*, 2023). While the most prevalent bacteria isolated by other researchers from lung washes of cats with bacterial pneumonia was the enteric organisms including: *Escherichia coli* and *Klebsiella spp.* as well as *Pasteurella spp., Staphylococcus spp.* and *Bordetella bronchiseptica* (Dear, 2020). *Bordetella* is a member of the family Alcaligenaceae, classified under the class Betapro-teobacteria and comprises seventeen designated species, which include both animal-associated and environmental bacteria (Ivanov *et al.* 2016). *Bordetella bronchiseptica* is an aerobic, Gram-negative coccobacillus long recognized as a respiratory

pathogen of several animal species. It is occasionally associated with opportunistic infection in people, including those in contact with infected cats (Wernli *et al*., 2011). The field cases were reported with varying signs from upper respiratory tract disease to more severe coughing and bronchopneumonia, and even fatal (Little, 2000). Bordetellosis is especially prevalent in cats in some shelter, pet store, and boarding facilities where large numbers of potentially stressed animals may have been in close contact with one another. Infections with *B. bronchiseptica* frequently occur in concert with respiratory viral and/or Mycoplasma spp. infections. *Bordetella bronchiseptica* can persist in the environment for at least 10 days and is capable of growth in natural water sources, but is susceptible to most disinfectants provided they are used correctly. *Bordetella bronchiseptica* can be isolated from apparently healthy cats and cats with respiratory disease, but has been clearly associated with respiratory disease in cats (Sykes, 2014).

Material and Methods

Animals Study

Of 150 cats with and without respiratory signs of (60 local breed and 90 different breed), both sexes a 70 male and 80 female with different ages including more than one year (85) and less than one year (65) October 2023 to September 2024. Totally, nasal swabs were collected from cats at aseptic condition to carry out for bacteriology according to clinical signs and history.

Bacteriological examination

The nasal swabs were streaked on blood agar (Esmaeel 2009; Saher 2009; Saleh, 2010; Al-Shafee and Abdulwahid, 2024); the suspected *Bordetella colonies* were subculture on selective media Bordet Gengou agar base then incubated aerobically at 37°C for 24-48 hours (Jorgensen *et al*., 2015). The growing colonies were visually inspected to determine their size, shape, and color. The suspected colonies were examined by Stained smears and biochemical tests using VITEK 2 compact system (Markey *et al.*, 2014; Abdullah and Al-Gburi, 2024).

Antimicrobial susceptibility test

Antimicrobial susceptibility test of *B. bronchiseptica* was performed using the vitek-2 system accuracy and fully automated system for Gram negative bacteria were applied according to instructions of the manufacturer. The antibiotics used are Piperacillin / Tazobactam, Amikacin,

Cefazolin, Ceftazidime, Cefepime, Imipenem, Gentamicin, Ciprofloxacin, Levofloxacin, and Tigecycline (Khudhair and AlAubydi, 2023; Al-Sajad and Alsalim, 2024).

PCR assay for detection of FIM gene

Genomic DNA was extracted according to gDNA Bacteria extraction kit.The purity and concentration of extracted DNA were measured using a Nanodrop spectrophotometer (Thermo Fisher, USA), with results displayed on a digital screen. The identity of *B. bronchiseptica* isolates was confirmed using specific primers for the *FIM* gene: F -5'-TGAACAATGGCGTGAAAGC-3' and R -5'-TCGATAGTAGGACGGGAGGA T-3'. The product size was 425 pb, as designed by Xin *et al.* (2008). The process of DNA amplification was executed using a thermal cycler, beginning with an initial denaturation step at 94°C for 5 minutes. This was followed by 40 cycles consisting of denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 40 seconds. The final extension occurred at 72°C for duration of 10 minutes. Agarose gel electrophoresis was employed to load the isolated DNA, a crucial step in the completion of the PCR test. This process was employed to verify the retrieved DNA.

Sequencing

The PCR products for seven samples of each *B. bronchiseptica* were sent to Macrogen-Korea for sequencing. The sequences were analyzed by using the UPGMA method (Sneath and Sokal 1973). The phylogenetic tree was down by evolutionary distances were computed using the Maximum Composite Likelihood method and used substitutions per site as units for sequencing polymorphism. The Evolutionary analyses were conducted in MEGA (Al–Rubaye and Al-doori, 2023; Yousif, 2023; Gharban *et al*., 2023).

Statistical analysis

The statistical Analysis System was conducted using the SPSS program (2020), employing odds ratios and risk factors to analyze and estimate significant differences in all study data (Badawi and Yousif, 2020).

Ethical approved

The ethical and research committee of the College of Veterinary Medicine at the University of Baghdad, Ministry of High Education and Scientific Research, approved this investigation. Iraq

project number: pg. 2237. The herd s´ owners gave their verbal agreement before samples were taken.

Results

Clinical study of cats

The results of clinical signs were showed increasing with fever (36.6%) and dyspnea (30%), followed by coughing (26.6%) and other clinical signs as shown in Table 1.

Clinical signs	No. of infected cat (%)	Odds ratio (CI 95%)
Fever	55 (36.6%)*	3.76 (1.84- 7.66)
Dyspnea	45(30%) *	2.86 (1.39- 5.90)
Coughing	40(26.6%) *	2.35 (1.12-4.90)
Mandibular lymphadenomegaly	32(21.3)	1.77 (0.83-3.78)
Sneezing	30(20%)	1.56 (0.73 - 3.34)
Mucopurulent nasal discharge	29(19.3%)	1.55 (0.73 - 3.34)
Conjunctivitis	20(13.3%)	Non
Gurgling respiratory sound	20(13.3%)	Non

Isolation and identification

Out of 150 nasal swabs, seven swabs were positive to *Bordetella bronchiseptica*, the colonies of isolates appeared on blood agar appear as glistening without heamolysis (Figure 1 A), while culture on Bordet Gengou agar base appear as smooth, opaque, viscid, grayish white (Figure 1 B).

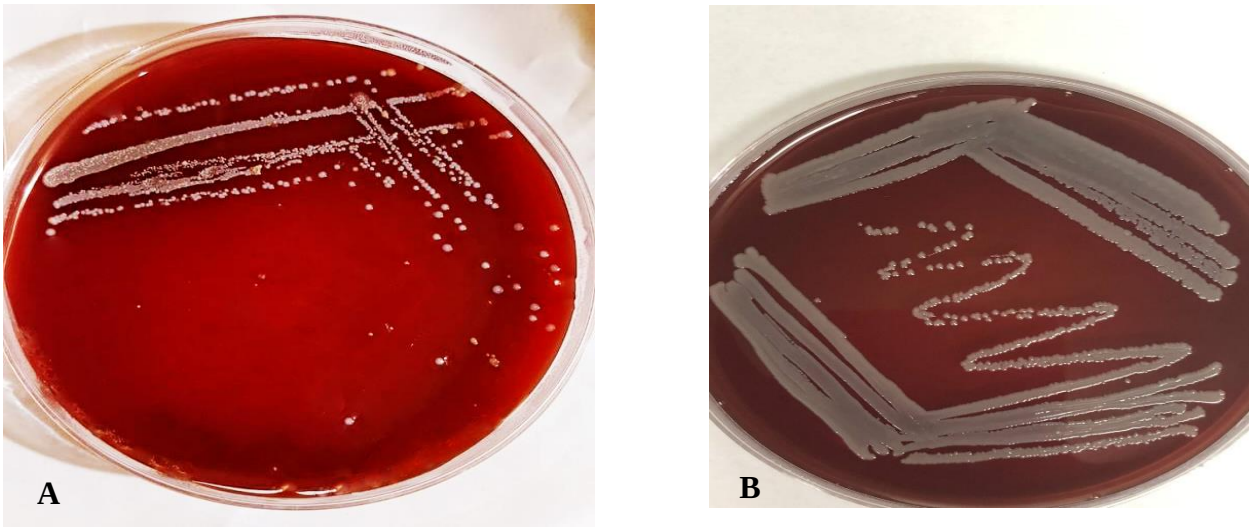

Figure (1): *Bordetella bronchiseptica* colonies. A) On blood agar, B) on Bordet gengou agar base

Antimicrobial susceptibility testing

The isolates of *B. bronchiseptica* were showed a high rate of multidrug resistance with percentage (100%) to: piperacillin, cefotaxime, aztreonam, doxycycline, minocycline and tetracycline. While the isolates were sensitive to levofloxacin (85.7%) and tobramycin (57.1%).

Table 2: Antimicrobial susceptibility test of B. bronchiseptica by vitek-2 system

Antibiotic	Isolation	Sensitive	Intermediate	Resistant
Ampicillin/sulbactam	7	0(0%)	0(0%)	0(0%)
Piperacillin/ sulbactam	7	0(0%)	0(0%)	7(100%)*
Cefoxitin	7	0(0%)	0(0%)	0(0%)
Cefotaxime	7	0(0%)	0(0%)	7(100%)*
Ceftazidime/avibactam	7	0(0%)	0(0%)	0(0%)
Ceftolozana/ tazobactam	7	0(0%)	0(0%)	0(0%)
Aztreonam	7	0(0%)	0(0%)	7(100%)*
Doripenem	7	0(0%)	0(0%)	0(0%)
Meropenem / vaborbactam	7	0(0%)	0(0%)	0(0%)
Tobramycin	7	4(57.1%)	2(28.5%)*	0(0%)
Levofloxacin	7	6(85.7%)*	1(14.2)	0(0%)
Doxycycline	7	0(0%)	0(0%)	7(100%)*
Minocycline	7	0(0%)	0(0%)	7(100%)*
Tetracycline	7	0(0%)	0(0%)	7(100%)*
Tigecycline	7	0(0%)	0(0%)	0(0%)
Chloramphenicol	7	0(0%)	1(14.2%)	5(71.4%)
Colistin	7	0(0%)	0(0%)	0(0%)
		Chi: 1.4; df:1 (P: 0.23) non-significant 4.5 (0.33-60) non-significant	Chi: 0.42; df:1 (P: 0.51) non-significant 2.4 (0.16-34) non-significant	Chi: 0.603; df:1 (P: 0.433) non-significant 2.8 (0.191-40) non-significant

Molecular detection of B. bronchiseptica by using FIM gene

The overall isolation rate for *B. bronchiseptica* was documented as 4.6% (7/150) using conventional PCR method, Figure 2.

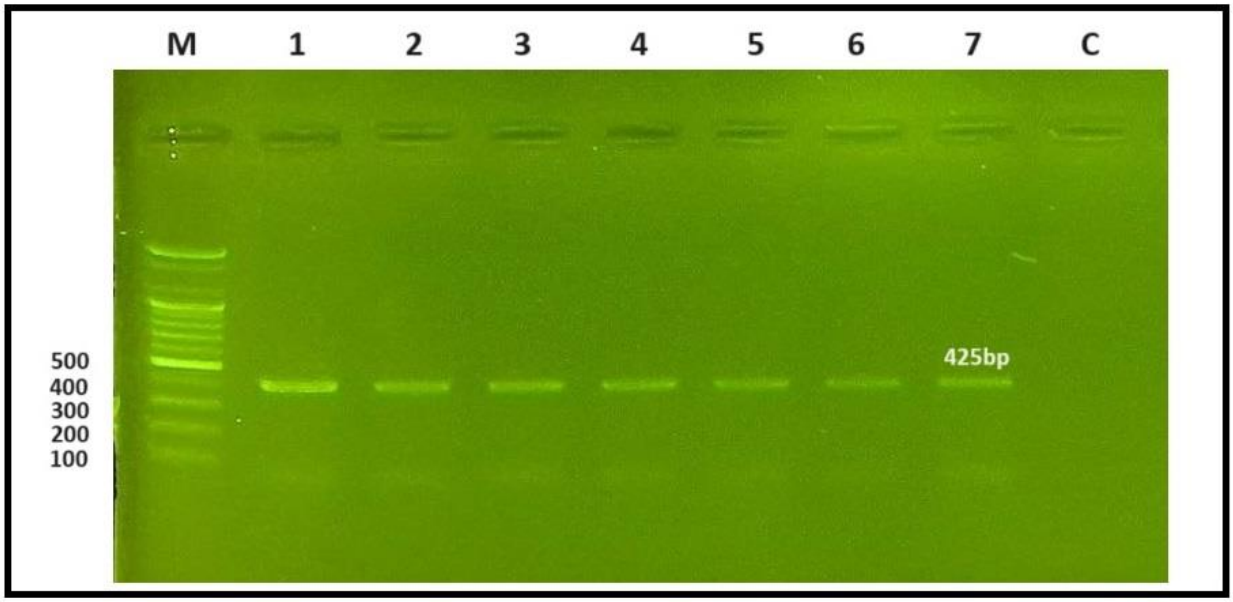

Figure (2): Gel electrophoresis (1.2%) 80 mAm, 100 vol. with red safe stain show the result of amplification of 425 bp of *FIM* gene of *B. bronchiseptica*. M: DNA Laddar; C: Negative results

Sequence of FIM gene

The findings of the current investigation indicated that the genetic sequences of local isolates of *B. bronchiseptica* from nasal swabs of cats originated from several countries, including Japan, the Netherlands, China, the USA, the United Kingdom, Hungary, and South Korea, with a similarity of 99%, Figure 3.

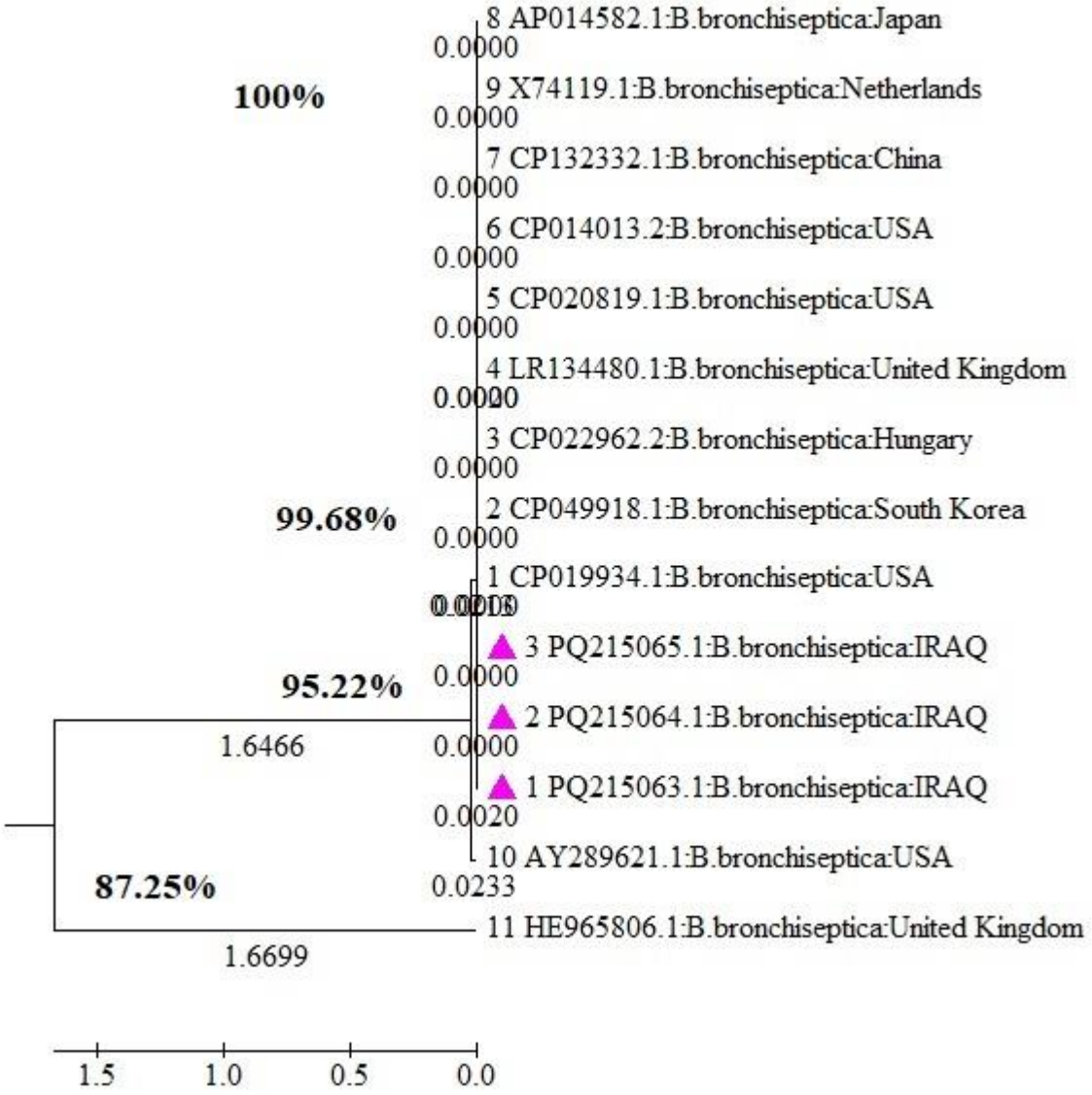

Figure (3): Phylogenetic tree of *Bordetella bronchiseptica*

Prevalence of B. bronchiseptica according to age, sex, breeds and months

Age

The current results revealed that the infection rate of isolation of *B. bronchiseptica* was not significantly affected by the age of the cats, the highest infection rate of isolation of *B. bronchiseptica* was recorded in cats less than one year 5 (7.6%) as shown in Table 3:

Table 3: Infection rates of *B. bronchiseptica* according to age.

Age/year	No. of tested cats	No. of positive cats for *B. bronchiseptica* isolates	Percentage
≤ 1	65	5	7.6%
> 1	85	2	2.3%
Total	150	7	OR (CI 95%) 4.62 (0.88-20.59)

Non-significant

Sex

The present results showed non-significant Variation between the isolation rate of *B. bronchiseptica* according to the sex of rate as showing in Table (4). The current study showed a high infection rate in Female (50%) than in male (4.2%).

Table (4): Infection rates of *B. bronchiseptica* according to sex

Sex	No. of tested cats	No. *B. bronchiseptica* isolates	Percentage
Males	70	3	4.2%
Females	80	4	5%*
Total	150	7	**OR (CI 95%) 1.26 (0.32-4.84)**

*significant

Breeds

The current results revealed non-significant variation among the different breeds of cats, the isolation rate of. *B. bronchiseptica* was increased in stray cats 4 (6.6%) as shown in Table (5)

Table (5). Infection rates of *B. bronchiseptica* according to breed

Breeds of cats	No. of tested cats	No. of cat's positive for *B. bronchiseptica*
Local (stray)	60	4(6.6%)
Himalya	26	2(7.6%) *
Shirazi	24	1(4.1%)
Scottish	20	0(0%)
Chinchilla	20	0(0%)
Total	150	7
OR (CI 95%); 2.08 (0.60-7.16); Non-significant		

Months

The highest percentage of isolation of *B. bronchiseptica* was recorded in December (23%), November (15.3%), October and January at equal rate (7.6%); while negative results were reported in other months of the year. Table (6)

Table (6). Infection rates of *B. bronchiseptica* according to month

Month	No. of tested cats	No. of positive for *B. bronchiseptica*
January	13	1(7.6%)
February	13	0(0%)
March	13	0(0%)
April	12	0(0%)
May	12	0(0%)
June	12	0(0%)
July	12	0(0%)
August	12	0(0%)
September	12	0(0%)
October	13	1(7.6%)
November	13	2(15.3)
December	13	3(23%)*
Total	150	7
OR (CI 95%); 3.43 (1.45-8.11); *Significant		

Discussion

These results are agreement with Garbal *et al.* (2016) who recorded that isolates of *B. bronchiseptica* were identified depending on bacteriology culture and biochemical analysis. Also the current results agreed with Markey *et al.,* (2014). The isolates of *B. bronchiseptica* in the current investigation exhibits colony shape, Gram staining and biochemical reaction that are similar to that recorded in previous research (Milanov *et al.,* 2018); Garbal *et al.,* (2016) and Petrovic *et al.* (2017) were recognized that *B. bronichseptica* the most common respiratory causes in cats which associated with tracheobronchitis, conjunctivitis, rhinitis and pneumoniae. While (Mcmanus *et al.,* 2014) in the Southeast United States observed that *B. bronchiseptica* were more prevalent in both clinically affected and nonclinical cats. In Canada (Gourkow *et*

*al.,*2013) reported that one of the most recently recorded respiratory causes of upper respiratory tract infection is *B. bronchiseptica* which include the clinical signs: sneezing, nasal discharge, ocular discharge, conjunctivitis, coughing, oral ulceration, fever and lethargy, either alone or any combination.

The current results demonstrated that the total isolation rate of *B.bronchiseptica* from nasal swab of cats was (4.6%) using vetik system and conventional polymerase chain reaction , similar rate of isolation (4.8%) were recorded in Eastern Canada (Walter *et al .,* 2020) in a population of shelter cats with respiratory signs. On the other hand, the current results are in disagreement with study results (Lister and Leutenegger, 2015) who reported a high infection rate with *B.bronchiseptica* (44%) by bacterial culture and PCR technique in cats with upper respiratory tract signs . In a study performed by (Lobova *et al.,* 2019) in Czechia detected that *B.bronchiseptica* were confirmed more often from oropharynx than from conjunctival swabs of cats with isolation rate (5.6%). Mavrids *et al.*,(2022) in England found that (39%) of lower respiratory samples were positive for bacterial growth , the isolation rate of *B.bronchiseptica* was (2.3%). The current results demonstrated that the isolates of *B.bronchiseptica* were multidrug resistance (MDRs) resistance (100%) to the following antibiotics type: Piperacillin, Cefotaxime, Aztreonam, Doxycycline, Minocycline and Tetracycline while the isolates were sensitive to Levofloxcin (85.7%) and Tobramycine (57.1%). Litster *et al*. (2012) in America noticed that the oral administration of Amoxicillin clavulanic acid or Doxycycline appeared to be more effective for treatment of cats with upper respiratory signs due to infection with *B.bronchiseptica*. On the same line Mavrides *et al.* (2022) found that the isolates of *B.bronchiseptica* from cats showed a high susceptibility to Amoxicillin-clavulanate. On other hand, (Zhang *et al.,* 2021) in Chine recorded that the *B.bronchiseptica* isolates from pigs with respiratory diseases were resistant to Ampicillin (83.98%), Cefotaxime (30.39%), Chloramphenicol (12.71%), Gentamicin (11.60%), Florfenicol (11.60%), Tetracycline (8.84%), Amoxicillin (8.29%), Tobramycin (6.63%) and Ceftriaxone (4.97%). In Czech Konvalinova *et al.* (2016) mentioned that the isolates of *B.bronchiseptica* from oropharynx swabs of cats were sensitive to Doxycycline, Trimoxazole, Enrofloxacine, Pradofloxacine and Cefovecin. The present results regarding the isolates *B.bronchiseptica* showed a high rate of multidrug resistance may attribute to many factors: *B.bronchiseptica* colonizes the ciliated epithelial cells of upper respiratory tract, the systemic antibiotics may not attain adequate tissue levels at the site of infection , the bronchial-alveolar

blood barrier limits diffusion and only drugs of low molecular weight and high lipophilicity achieve the therapeutic levels (Cohn, 2011). In America, Rodriguez and Berliner (2023) had been conducted to detect *B.bronchiseptica* isolates with identical resistance patterns from lung cultures of cats with respiratory signs, the isolates were resistant to Cefpodoxime, Ceftiofur, Doxycycline and Trimethoprim/sulfate while the isolates demonstrated susceptibility to Imipenem, Chloramphenicol and Fluoroquinolones. The current findings revealed to increase of infection rate with *B.bronchiseptica* at age group less than one year (7.6%), these results were supported with (Rodriguez and Berliner, 2023) in America whom found that *B.bronchiseptica* infection was more prevalent at age groups of cats (2-4 months).The present results were conflict with Lobova *et al.* (2019) in Czechia who recorded a high infection rate with *B.bronchiseptica* in cats at age group more than one year (8.3%) and 1-3 years (4.4%). While (Pasmans *et al.*, 2001) in Belgium noticed that the infection rate in cats younger than 6 months of age was equal to that in older cats. According to the sex, the infection rate of *B.bronchiseptica* in present study was (4.2%) in male and (5%) in females, while (Rodriguez and Berliner 2023) mentioned that the infection rate was increased in males (81.25%) than in females (18.75%). The difference in results can be explained by several reasons related to genetic, immune and environmental physiologyical factors. The number of isolates of *B.bronchiseptica* in present study were increased in local (stray) cats as compared with the other breeds of cats, particularly the stray cats often have high prevalence rates for most infectious agents that are associated with direct contact with other cats as well as they don't subjected to vaccination programs against any pathogens. There are no available studies in the world regarding with the isolation rates of *B.bronchiseptica* in relation to cat breeds. The climatic effect on the rate of infection with *B.bronchiseptica* was obviously observed, a significant high infection rates were recorded in cold months; December, November, October and January, respectively. This reveals that *B.bronchiseptica* is stable and has ability for remaining long period in the environment. Moreover, the reproduction of cats increased actively at cold environment, so that high number of susceptible new born kittens found at this period which leads to increase the rate of infection with bacteria (Greene, 2012). *B.bronchiseptica* is an important pathogenic bacteria in agriculture and veterinary medicine (Mattoo and cherry, 2005). Currently, it is a well-recognized etiological agent in many domestic and wild animal diseases (Petrovic et al., 2017). The current results revealed to a successful amplification of (*FIM*) gene of *B.bronchiseptica* by PCR at 425 bp. The

results agreed with (Xin *et al.*, 2008) in China who detected *B.bronchiseptica* isolates harboring (*FIM*) gene in nasal mucus of rabbits at the same size fragment. In Russia, the researcher (Konyaev, 2020) detected *B.bronchiseptica* with percentage 3.7% as well as the other pathogens (*Chlamydia Felis, Mycoplasma Felis* and *Feline calci virus*) that cause upper urinary tract infection by real time reverse transcription. In another study was done by (Litter and Leutenegger, 2015) to compare various pathogens detection rates using real-time PCR test and bacterial culture in conjunctival, nasal and oropharyngeal swabs from 18 shelter housed cats with clinical upper respiratory signs, the results revealed agreement between PCR results and bacterial culture for *B.bronchiseptica* (75%), also there was combination between the different types of swabs submitted for PCR. On other hand in Czech, Lobova *et al.* (2019) confirmed the presence of FhaB gene of *B.bronchiseptica* using multiplex qPCR, the researchers recorded that *B.bronchiseptica* were only detected in oropharynx of cats with various forms of respiratory diseases.

The phylogenetic tree analysis was based on the partial sequencing of *FIM* gene in the local isolates of *B. bronchiseptica* used for the confirmative genetic detection. Evolutionary analyses were performed in MEGA6. The present results revealed to a clear convergence between the local isolates of *B.bronchiseptica* strains and the world isolated strains, but from different sources. The study showed that there is a close relationship with *B.bronchiseptica* isolated from the homosapines with accession number (CP019934.1, CP020819.1 and AY289621.1) from United Kingdom (Weigand *et al.*, 2017 and Cumming *et al.*, 2003) respectively. On the other hand a close relationship with *B.bronchiseptica* isolated from pigs suffering from rhinitis with accession number (CPO22962.2 and APO14582.1) and from various sources, including Hungary and Japan (Okada *et al.*, 2014; Nicholson *et al.*, 2020), respectively. The local isolates were showed compatibility with strains of *B.bronchiseptica* isolated from dogs suffering from respiratory signs including Kennel cough with accession number (HE965806.1) in United Kingdom (Park *et al.*, 2012).

Conclusion

The current investigation demonstrated the significant effect of *B. bronchiseptica* as the primary causative agent in feline respiratory infections. The PCR method utilizing the *FIM* gene serves as an effective diagnosis for identifying *B. bronchiseptica*.

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