



## Research paper

Molecular epidemiology of *Pasteurella multocida* in cats: First multilocus sequence typing (MLST)-based insights from IranBaharak Mirzavand<sup>a</sup>, Darioush Gharibi<sup>a,\*</sup>, Dolores Cid<sup>b</sup>, Ana Isabel Vela<sup>b</sup>,  
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## ABSTRACT

*Pasteurella multocida* is a zoonotic bacterium commonly found in the oropharyngeal microbiota of cats; despite its clinical relevance, molecular data on feline isolates in Iran remain limited. This study aimed to provide molecular and phenotypic characterization of *P. multocida* isolates obtained from feline sources in Iran. This study characterized 18 *P. multocida* isolates recovered from oral swabs of cats previously sampled in Ahvaz, Iran. After polymerase chain reaction (PCR) confirmation targeting the *kmt1* gene, multilocus sequence typing (MLST) was performed using the Rural Industries Research and Development Corporation (RIRDC) scheme. The MLST analysis assigned the 18 isolates to 14 distinct sequence types (STs). High genetic diversity was observed, with no ST dominating the population. Our results identified several STs, including ST30, ST344, ST487, and ST551, that have been reported worldwide, highlighting their epidemiological relevance. This study provides the first MLST data for feline *P. multocida* in Iran and contributes to global understanding of the species' genetic diversity.

## 1. Introduction

*Pasteurella multocida* is a facultative anaerobic, Gram-negative, opportunistic pathogen relevant to both veterinary and human medicine (Peng et al., 2019; Wilkie et al., 2012). *P. multocida* is the causative organism of several infections and serious diseases in animals, such as septicemia, respiratory disease, and has high zoonotic potential (Hasani et al., 2025). Several companion animals, such as cats and dogs, commonly harbor *P. multocida* as part of their normal nasopharyngeal microbiota and as a potential opportunistic pathogen (Razali et al., 2020; Wilson and Ho, 2013). This bacterium can cause a variety of infections in humans, including rhinitis, pneumonia, and abscess formation, particularly following animal bites (Piorunek et al., 2023).

Although companion animal ownership is common in Iran, reliable national statistics on the total number of pet cats are lacking; the genetic origin and diversity of cat-derived *P. multocida* isolates are still unknown (Yusefi et al., 2019; Ziagham et al., 2024). Multilocus sequence typing (MLST) is a highly discriminatory and reproducible method for analyzing isolates across different hosts and geographical regions (Hotchkiss et al., 2011; Yoshida et al., 2025). This technique has used

effectively for identifying genetic diversity and population structure in *P. multocida*, and is well suited for both epidemiological investigations and evolutionary studies in geographic- and host-specific contexts (Calderón Bernal et al., 2023; Hotchkiss et al., 2011). Here, we report the first MLST-based characterization of feline *P. multocida* isolates from multiple districts within Ahvaz city. The study aimed to assess the genetic diversity of these *P. multocida* isolates and compare them with international *P. multocida* populations, providing insights into their epidemiology and potential zoonotic relevance.

## 2. Materials and methods

## 2.1. Sample collection and bacterial cultivation

The *P. multocida* isolates used in this study originated from a previous investigation (Ziagham et al., 2024), in which oral swabs were collected from 100 cats presented to the Veterinary Teaching Hospital of Shahid Chamran University, located in Ahvaz city, Khuzestan Province, Iran. The sampled population included both privately owned and stray cats referred from various districts across the city. In the previous study, 29

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*P. multocida* isolates were characterized using both phenotypic and molecular methods. For the present work, all 29 isolates were retrieved from  $-80^{\circ}\text{C}$  storage and reactivated on blood agar. Of them, 18 *P. multocida* isolates were successfully revived and were included in the MLST analysis.

## 2.2. *P. multocida* confirmation

Before MLST, the identity of all revived isolates was confirmed by polymerase chain reaction (PCR) targeting the *kmt1* gene, as described previously (Townsend et al., 1998), to ensure the purity and genetic stability of the cultures before shipment for analysis in our previous study (Ziaghani et al., 2024). *P. multocida* reference strain ATCC 43137<sup>T</sup> was included as a positive control.

## 2.3. DNA extraction

Genomic DNA was extracted using the boiling method. Briefly, 2 mL of overnight Brain Heart Infusion (BHI) broth culture (Merck, Germany) was centrifuged at  $12,500 \times g$  for 2 min. Supernatant was thrown away, and 200  $\mu\text{L}$  sterile distilled water was added to the pellet, and recentrifuged at the same settings. The final pellet was resuspended in 100  $\mu\text{L}$  of sterile distilled water, boiled for 10 min, chilled on ice for 5 min, and centrifuged at  $3000 \times g$  for 3 min. Lastly, 50  $\mu\text{L}$  of supernatant containing genomic DNA was transferred to a sterile microtube and stored at  $-20^{\circ}\text{C}$  until utilization.

## 2.4. Multilocus sequence typing

MLST was conducted according to the Rural Industries Research and Development Corporation (RIRDC) scheme originally described previously for *P. multocida* (Subaaharan et al., 2010). The expanded and updated version of the scheme, along with allele definitions and sequence type profiles, was accessed via the PubMLST platform (Jolley et al., 2018).

Seven housekeeping loci (*adh*, *est*, *gdh*, *mdh*, *pgi*, *pmi*, and *zwf*) were amplified using the standardized primer sets available at the PubMLST database. PCR amplification and sequencing were performed according to the protocol provided on the PubMLST website. PCR cycling conditions were as follows: initial denaturation at  $94^{\circ}\text{C}$  for 5 min; 30 cycles of denaturation at  $96^{\circ}\text{C}$  for 10 s, annealing at  $50^{\circ}\text{C}$  for 5 s, and extension at  $60^{\circ}\text{C}$  for 4 min (Jolley et al., 2018). PCR products were sequenced in forward and reverse orientation after decontamination. Sequences were trimmed, aligned, and deposited in the PubMLST database to allocate allele numbers and identify sequence types (STs). Alignments of sequences were conducted using MEGA software, and distributions of STs

were compared with the global database to assess the genetic diversity of feline isolates.

## 2.5. Data analysis

MLST types were compared with global *P. multocida* isolates from various hosts, in particular felines, available in the PubMLST database to evaluate genetic diversity, clonal complex affiliations, and host specificity. Phylogenetic relationships were assessed using BioNumerics (version 7.6) with UPGMA clustering and categorical coefficients.

## 3. Results

All 18 revived isolates yielded the expected 460 bp amplicon with the *kmt1* PCR, confirming their identity as *P. multocida*. MLST was completed for all isolates. Analysis of the seven housekeeping loci revealed 14 distinct STs (Table 1), indicating high genetic diversity within the sample set. ST344, ST365, and ST551 were the most common, accounting for 7 isolates (38.8 %). All other STs were represented by one isolate each.

A minimum spanning tree (MST) constructed from the 18 feline isolates (Fig. 1) showed no dominant lineage and demonstrated multiple single-locus variant (SLV) and double-locus variant (DLV) relationships among STs. A second MST was constructed using the MLST profiles of the 18 feline *P. multocida* isolates from this study, along with 120 global feline isolates retrieved from the PubMLST database (Fig. 2). Moreover, it also been shown that the Iranian feline *P. multocida* isolates were distributed across several clusters rather than forming a single group, indicating a high level of heterogeneity. Three MLST types (ST30, ST344, and ST551) were affiliated with the globally recognized multi-host clonal complexes CC9 and CC13. Of note, ST181 and ST444 were not assigned to any known clonal complex.

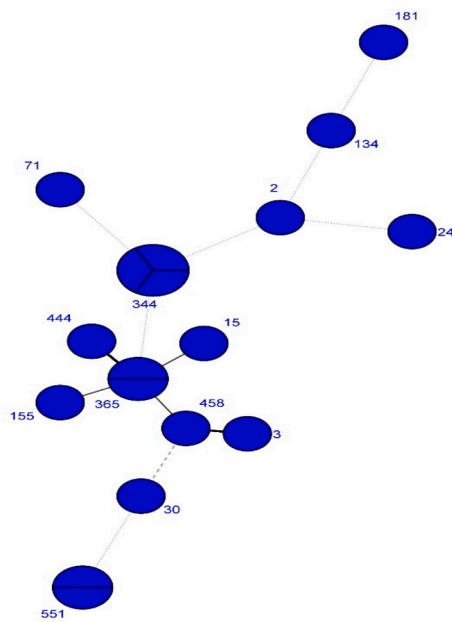
Comparison with the PubMLST database demonstrated that several *P. multocida* STs identified here—such as ST30, ST344, ST487, and ST551—have been previously reported in both cats and other hosts across multiple countries, suggesting broad global distribution (Fig. 3). In contrast, ST181 and ST444 were not assigned to any known clonal complex in PubMLST, indicating possible regional circulation or restricted sampling in other countries.

## 4. Discussion

To the best of our knowledge, this is the first study to perform MLST of *P. multocida* isolates recovered from cats in Iran. Therefore, the present data fills an important gap in the molecular epidemiology of *P. multocida* in Iran. Our observations provide valuable insights into the

**Table 1**  
Allele diversity results in seven housekeeping genes among *P. multocida* isolates.

Isolate	Source	<i>adh</i> <sub>F</sub>	<i>est</i> <sub>F</sub>	<i>pmi</i> <sub>F</sub>	<i>zwf</i> <sub>1F</sub>	<i>mdh</i> <sub>F</sub>	<i>gdh</i> <sub>F</sub>	<i>pgi</i> <sub>F</sub>	ST
1	Cat	1	5	23	1	1	1	11	365
2	Cat	21	5	76	76	5	2	84	344
4	Cat	1	5	10	17	1	1	11	15
5	Cat	4	51	45	18	11	26	2	134
6	Cat	1	18	18	8	12	12	11	30
7	Cat	1	5	10	1	1	6	11	3
9	Cat	1	5	23	1	1	1	11	365
10	Cat	1	5	10	1	1	12	11	458
11	Cat	2	7	16	6	4	5	7	24
12	Cat	4	106	79	8	15	79	81	551
13	Cat	21	24	35	21	7	22	39	71
14	Cat	1	3	23	1	1	12	11	155
15	Cat	2	2	2	2	2	2	2	2
18	Cat	21	5	76	76	5	2	84	344
21	Cat	33	13	50	47	11	3	58	181
22	Cat	1	5	23	1	1	1	78	444
23	Cat	4	106	79	8	15	79	81	551
25	Cat	21	5	76	76	5	2	84	344



**Fig. 1.** Minimum Spanning Tree (MST) based on MLST analysis of feline *P. multocida* in Ahvaz, Iran. Each circle represents a sequence type (ST), and circle size is proportional to the number of isolates. Solid lines indicate single-locus variants (SLVs), and dashed lines indicate double-locus variants (DLVs).

population structure, genetic diversity, and possible evolutionary dynamics of *P. multocida* strains associated with cats. In this study, the results indicate an extremely heterogeneous population structure, with 18 feline *P. multocida* isolates assigned to 14 different STs, among which ST344, ST365, and ST551 were the most commonly identified. This high number of STs observed among *P. multocida* isolates highlights the substantial genetic heterogeneity of feline *P. multocida* in this region. Comparison with global data revealed that several *P. multocida* STs, including ST30, ST344, and ST551, have been previously reported in cats and other hosts worldwide (Allen et al., 2025; Hotchkiss et al., 2011; Petersen et al., 2014; Robinson et al., 2011). These MLST types in this study and their comparison with others (Hotchkiss et al., 2011; Jolley et al., 2018) indicate that feline *P. multocida* isolates in Iran share globally distributed lineages with zoonotic significance.

In this study, the identification of ST30, ST344, and ST551 within CC9 and CC13—widely recognized as multi-host clonal complexes—indicates that feline isolates in Iran share globally distributed lineages with zoonotic significance (Hotchkiss et al., 2011; Zhu et al., 2020). These complexes contain isolates commonly associated with livestock, implying overlapping ecological reservoirs and possible cross-species transmission pathways. This observation reinforces the role of companion animals as intermediate hosts in the broader epidemiological network of *P. multocida*. In contrast, ST181 and ST444 showed no clonal-complex affiliation, which may reflect localized microevolutionary events or insufficient global sampling of feline populations.

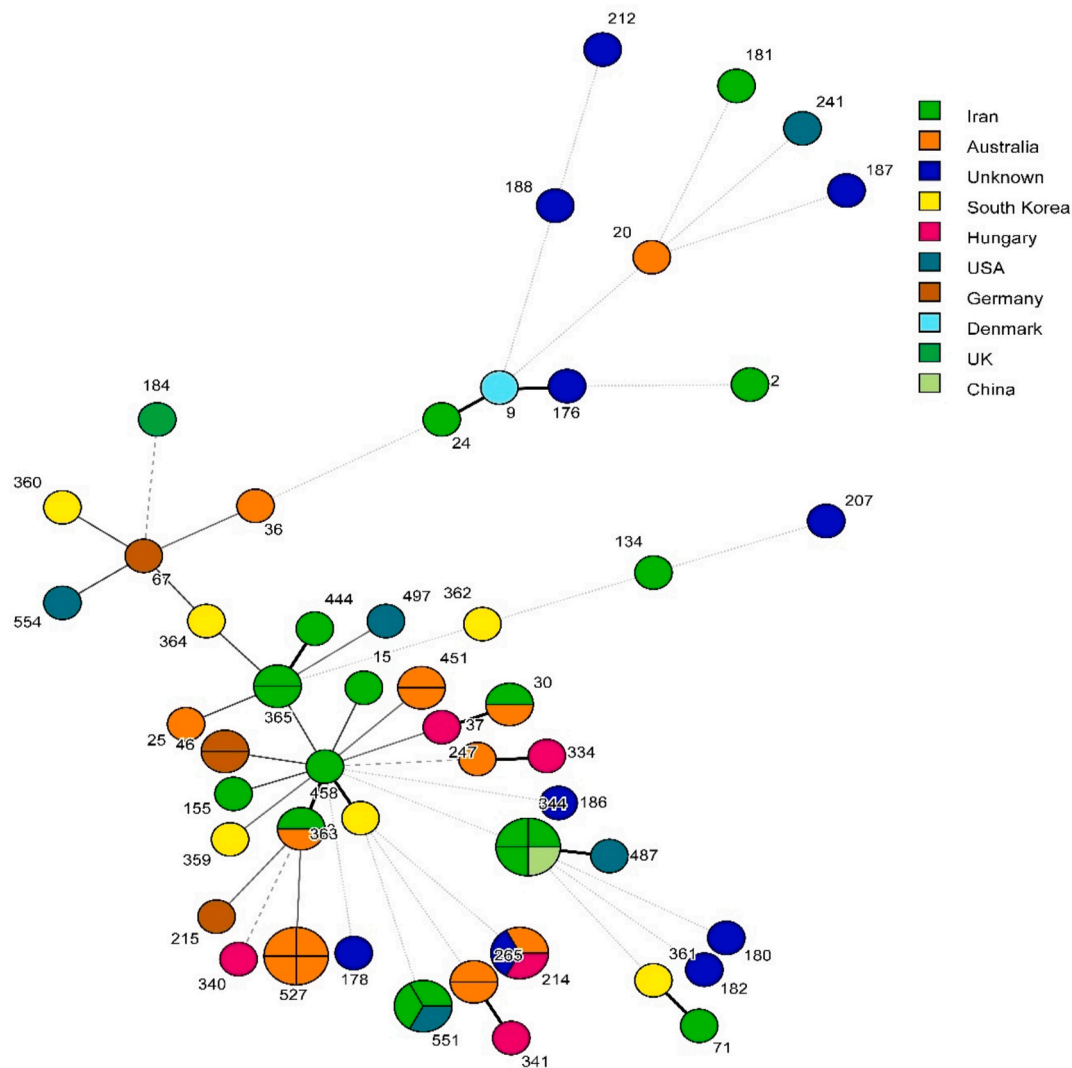
Most molecular work on *P. multocida* in Iran has centered on ruminant and avian disease, where capsular, lipopolysaccharide (LPS), and virulence profiles indicate genotypic diversity across pneumonic pasteurellosis cases in cattle and sheep (Mirhaghoye Jalali et al., 2017; Mombeni et al., 2021; Tahamtan and Mirghafari, 2016). Our feline *P. multocida* MLST dataset complements—and extends—those observations by showing overlap between cat-derived STs and STs reported from livestock/epidemiologically coherent with everyday interfaces between companion animals, peri-urban livestock, and shared environments in Iran. Collectively, these patterns argue for integrated (“One Health”) molecular surveillance that samples cats alongside ruminants, poultry, and, where feasible, human clinical isolates to trace interspecies flow more precisely (Skoufos et al., 2025). The high degree of genetic

diversity observed in *P. multocida* feline isolates is consistent with reports from other geographic regions and animal hosts. For instance, Hotchkiss et al. (Hotchkiss et al., 2011) showed that *P. multocida* strains isolated from cattle and other hosts clustered into multiple MLST types without strict host specificity, suggesting overlapping ecological niches and frequent interspecies transmission. Similarly, Bernal et al. (Calderón Bernal et al., 2023) demonstrated that bovine isolates from both healthy and diseased animals carried diverse genotypes, supporting the concept that *P. multocida* circulates widely across populations with limited host adaptation. Our findings align with these studies, reinforcing the notion that cats may act as incidental carriers of *P. multocida* strains that also circulate in livestock and poultry populations.

Given that Iran harbors substantial populations of both companion animals and food-producing livestock, the detection of overlapping *P. multocida* MLST types across species raises important questions regarding potential epidemiological linkages (Moein Jahromi et al., 2025). Comparison with previous Iranian studies also reveals an important gap. Earlier work has primarily focused on toxigenic and non-toxigenic *P. multocida* strains associated with pneumonic pasteurellosis in cattle and sheep (Mombeni et al., 2021). Our study, therefore, fills a critical void by providing the first feline MLST dataset of cats from Iran, enabling future comparative studies and database enrichment. Although no novel STs were identified, the deposition of these feline genotypes into the PubMLST database will contribute to global efforts to map the population structure of *P. multocida*. There was no clear relationship in this research between sex or age and ST, which implies that these factors may not determine its distribution. Our findings support the view that *P. multocida* is a generalist pathogen with weak host specialization (Smith et al., 2021). Nevertheless, localized microevolution within cats cannot be ruled out, particularly given the high degree of allelic diversity detected.

From a zoonotic perspective, the identification of *P. multocida* STs known to circulate in food animals within feline isolates is significant (Allen et al., 2025). Cats are frequent companions of humans, and bite or scratch injuries represent a well-documented route of *P. multocida* transmission to people, leading to soft tissue infections or even systemic disease (Kim et al., 2014). Some ST types have been isolated from birds, pigs, and humans around the world (Allen et al., 2025; Peng et al., 2018). ST15 was reported from humans in France, and ST71 was isolated from humans in India (Jolley et al., 2018). These findings highlight the zoonotic transmission and emphasize the importance of these ST types in *P. multocida* in domestic animals. Our findings, therefore, underscore the potential public health relevance of cats as reservoirs of diverse *P. multocida* genotypes. The overlap of feline isolates with those from ruminants and poultry may increase the risk of introducing strains with distinct virulence or antimicrobial resistance profiles into human populations.

This study has some limitations that must be noted. To begin with, the relatively small number of isolates will not exactly reflect the full genetic diversity of *P. multocida* that is found among cats in Iran as a whole. Second, the sampling was geographically limited and cannot allow findings to be generalized to the entire country. Third, while MLST was useful in determining the genetic relatedness of the isolates, other molecular technologies like whole-genome sequencing (WGS) or virulence/resistance gene profiling would have given better information about strain history and likely pathogenicity. Also, epidemiological information like clinical context, environmental exposures, and direct comparison with animal- and human-derived isolates was not provided, making it difficult to analyze zoonotic associations. Subsequent studies using larger sample sets, wider geographic ranges, and parallel genomic and epidemiologic investigations will be required to further delineate the population structure, host adaptation, and zoonotic risk of feline *P. multocida* isolates. However, despite these limitations, the present paper addresses three major concerns: (1) Iranian cats carry genetically heterogeneous *P. multocida* isolates, (2) some feline *P. multocida* STs are congruent with those of ruminants and poultry, indicating potential



**Fig. 2.** Minimum Spanning Tree (MST) comparing the 18 feline isolates from this study with 120 global feline *P. multocida* isolates retrieved from the PubMLST database. Circles are colored by the corresponding countries of origin of the strains: blue indicates feline isolates obtained in this study; other circles are isolates obtained from other countries. Each circle is labeled with its corresponding sequence type (ST). When multiple isolates share the same ST, the circle size increases accordingly. Full details of ST–host–country associations are provided in Supplementary Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interspecies transmission, and (3) cats should be considered as potential reservoirs of zoonotic *P. multocida* strains of interest for veterinary and human medicine. Ongoing molecular surveillance, ideally including companion and livestock animals and clinical isolates from humans, will be necessary to have a comprehensive understanding of the epidemiology, host ecology, and zoonotic risk of this pathogen.

## 5. Conclusion

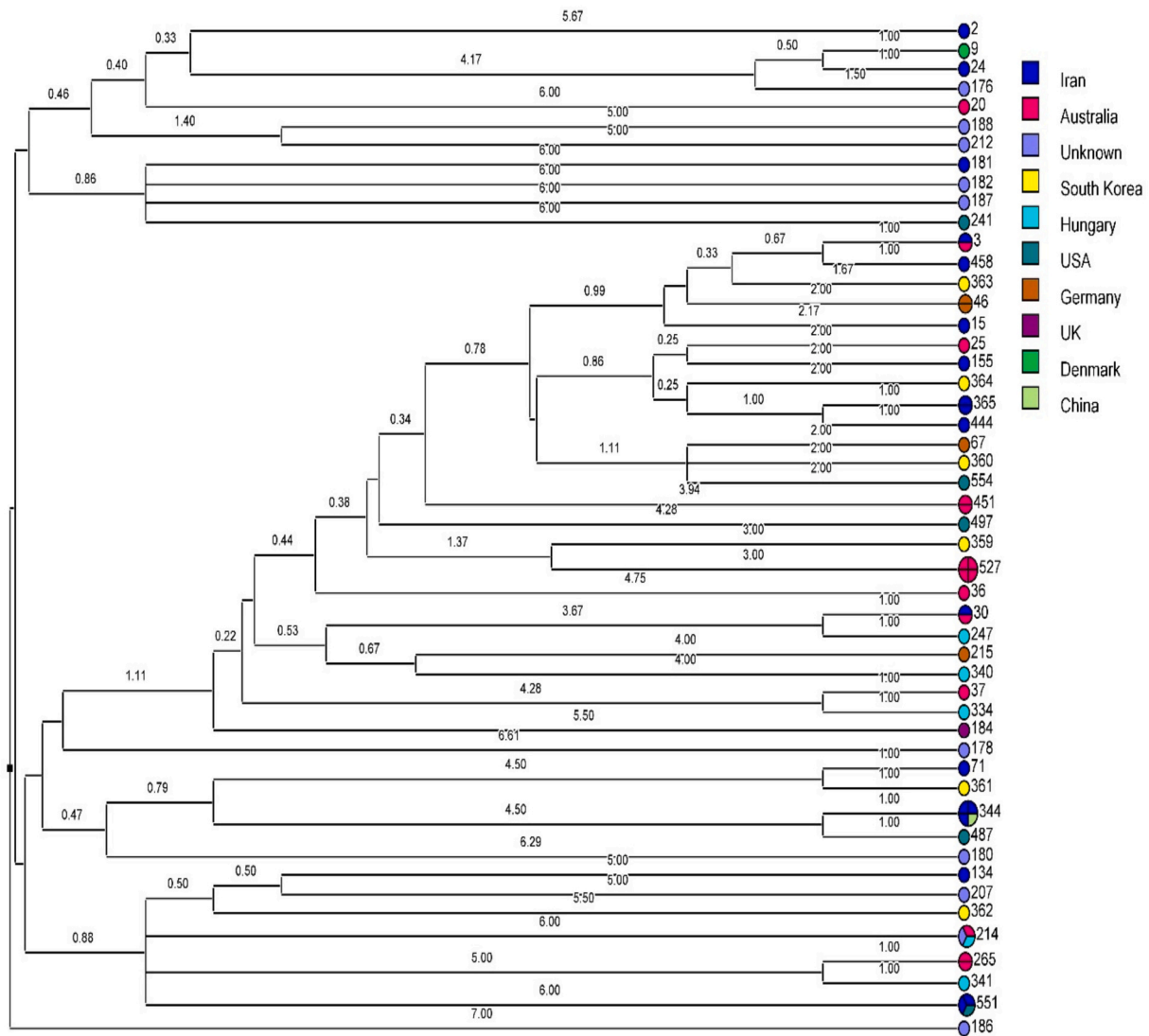
The current study provides the initial assessment using MLST of the nature of the *P. multocida* isolates obtained from felines in Iran. The discovery of 14 sequence types among the 18 isolates indicates a high degree of genetic diversity and serves as evidence that *P. multocida* is a genetically diverse multi-host bacterium. Each of the sequence types found within the current study has been previously identified within felines or other animal hosts found in various geographic locations elsewhere in the world. This suggests that the feline isolates found within Iran represent a global lineage.

The presence of feline isolates belonging to widely recognized multi-host clonal complexes further highlights the epidemiological relevance

of cats within the broader ecology of *P. multocida*. Although the limited sample size and restricted geographic scope preclude broad generalization, these findings establish an essential baseline for future studies and underscore the value of including companion animals in molecular surveillance programs. Expanded investigations incorporating larger sample sizes, wider geographic coverage, and genome-based analyses will be necessary to clarify host associations, transmission pathways, and the zoonotic potential of feline-derived *P. multocida*. Overall, our results provide new details for a more comprehensive understanding of its epidemiology and zoonotic significance.

## CRediT authorship contribution statement

**Baharak Mirzavand:** Writing – original draft, Software, Methodology, Investigation, Data curation. **Dariouosh Gharibi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization. **Dolores Cid:** Writing – review & editing. **Ana Isabel Vela:** Writing – review & editing, Validation. **Mohammad Khosravi:** Visualization, Supervision. **Keyvan Tadayon:** Writing – review & editing, Software.



**Fig. 3.** Dendrogram and cluster analysis of feline *P. multocida* isolates based on multilocus sequence typing (MLST). The dendrogram illustrates the genetic relationships among feline *P. multocida* isolates inferred from concatenated MLST loci using a distance-based clustering method. Branch lengths represent genetic distances between isolates, with numerical values along branches indicating relative divergence. Each terminal node corresponds to an individual isolate, labeled by its isolate number. Colored circles denote the country of origin of each isolate, as indicated in the legend (Iran, Australia, South Korea, Hungary, USA, Germany, UK, Denmark, China, and isolates with unknown origin). Isolates clustering closely together indicate high genetic similarity and potentially shared evolutionary lineages, whereas longer branch separations reflect greater genetic diversity. The analysis demonstrates both geographically mixed clusters and region-associated groupings, highlighting the global genetic heterogeneity of feline *P. multocida* strains.

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### Declaration of competing interest

The authors declare no potential conflict of interest.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2025.105874>.

### Data availability

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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