Investigating the antibiotic resistance pattern of *Corynebacterium pseudotuberculosis* strains isolated from livestock in Mashhad

Amin Molla Ahmadian Kaseb[1,a], Hamid Reza Farzin[2,b, ✉], Arash Chaichi Nosrati[1,c], Majid Jamshidian Mojaver[2,d], Leila Modiri[1,e]

[1] Department of Microbiology, La.C., Islamic Azad University, Lahijan, Iran.

[2] Razi Vaccine and Serum Research Institute, Agriculture Research, Education and Extension Organization (AREEO), Mashhad, Iran.

[a]ORCID: 0009-0006-0937-3673; [b]ORCID: 0000-0002-2961-5477; [c]ORCID: 0000-0001-6168-7525; [d]ORCID: 0000-0002-6334-0622; [e]ORCID: 0000-0002-2133-5796

✉Corresponding author: Hrfarzin@yahoo.com

Abstract

Introduction: *Corynebacterium pseudotuberculosis* is the causative agent of caseous lymphadenitis, one of the common and chronic diseases in sheep and goats. The disease hampers growth, increases carcass condemnation, incurring substantial daily losses for small-holder farms.

Objective: The present study aimed to examine antibiotic resistance patterns, and determine the inhibition coefficient of isolates against commonly used antibiotics in livestock.

Material and Method: In this study, 91 samples were collected from adult sheep that presented with lymph-node swelling and purulent discharge. The material was inoculated into brain-heart infusion (BHI) broth and incubated; cultures were then sub-cultured onto blood agar plates for further growth. Isolates suspected to be *Corynebacterium pseudotuberculosis* were identified and subsequently subjected to antibiotic susceptibility testing, performed by the Kirby–Bauer disk-diffusion method and by determining minimum inhibitory concentrations (MICs).

Result: Antibiotic sensitivity testing showed that the isolates had the lowest resistance to ciprofloxacin and gentamicin antibiotics at 15.3% and 15.8% respectively, and the highest resistance to penicillin and vancomycin antibiotics at 84.42% and 78.39% respectively. Based on MIC results for antibiotics commonly used in livestock treatment, inhibitory effects were between 8 to 64 micrograms per ML. *Corynebacterium pseudotuberculosis* is identified as one of the causative agents of skin abscesses in sheep in the province.

Conclusion: Given the high economic losses caused by this disease, preventive measures such as developing effective vaccination programs and adopting alternative control strategies are necessary. Furthermore, more extensive epidemiological studies are needed to assess the prevalence of caseous lymphadenitis in small ruminants in Iran.

Keywords: Antimicrobial resistance, Caseous lymphadenitis, *Corynebacterium pseudotuberculosis*, Minimum inhibitory concentration, Small ruminants.

1. Introduction

Caseous lymphadenitis (CLA), also known as cheesy gland, is an infectious disease primarily caused by the bacterium *Corynebacterium pseudotuberculosis* [2]. This condition is characterized by the formation of abscesses in the lymph nodes, which can become necrotic and exhibit a cheese-like consistency [3]. While primarily observed in sheep and goats, it can also affect other animals, including cattle, and rarely, humans [5]. Affected animals may present with symptoms such as swollen lymph nodes, weight loss, and general discomfort [4]. Affected lymph nodes may rupture and discharge pus, leading to chronic infections. This disease is chronic and can cause significant economic losses in affected flocks due to reduced production and the costs associated with treatment and management. Transmission occurs through direct contact with infected animals or contaminated environments. Bacteria typically enter the host through skin abrasions or cuts [11, 20]. They can also enter through mucous membranes, particularly in the oral cavity or respiratory tract. Upon entering the lymphatic system, bacteria localize at a site, proliferate, and evade the host's immune response, leading to infection [14]. The host's immune system reacts to the infection, leading to the accumulation of immune cells at the site. This reaction leads to inflammation and the formation of purulent abscesses. Bacteria cause necrosis of tissue within the lymph nodes [12], resulting in the formation of caseous abscesses. These abscesses have a cheese-like consistency due to the accumulation of dead immune cells, bacteria, and necrotic tissue. Common antibiotics used to treat infections caused by *Corynebacterium pseudotuberculosis* include penicillin, tetracyclines, sulfonamides, and macrolides [13]. Resistance can develop to these common classes, making treatment more challenging [25]. Antibiotic resistance in *Corynebacterium pseudotuberculosis* is a growing concern, influenced by factors such as inappropriate antibiotic use and chronic infections [10]. The development of resistant strains complicates treatment and poses economic and public health challenges. Some strains may produce enzymes that inactivate antibiotics [15]. Furthermore, efflux pumps can remove antibiotics from bacterial cells, reducing their effectiveness. Alterations in bacterial structure can also prevent effective antibiotic binding [2]. Contaminated environments and poor hygiene practices can contribute to the spread of resistant strains among animal populations. The presence of antibiotic-resistant strains can complicate therapeutic options and lead to prolonged infections and increased disease incidence in affected animals. Regular monitoring of antibiotic resistance patterns in *Corynebacterium pseudotuberculosis* is essential for effective management and treatment strategies. Implementing responsible antibiotic use practices, including appropriate dosage, duration, and avoiding unnecessary use, can help mitigate the development of resistance [4]. Exploring alternative treatments, such as vaccines or non-antibiotic therapies, may provide additional options for managing infections without contributing to resistance [7].

The aim of this research is to investigate the extent of antibiotic resistance using the Kirby-Bauer method and to determine the minimum inhibitory concentration (MIC) of bacterial growth in isolates of *Corynebacterium pseudotuberculosis* obtained from sheep affected with caseous lymphadenitis in the city of Mashhad.

2. Materials and Methods

2.1. Sample collection

From early spring 2020 to spring 2021, a total of 91 abscess specimens were collected from adult sheep and goats slaughtered at a municipal slaughterhouse in Mashhad, Iran. The samples represented approximately 10% of abscesses showing gross pathological features compatible with caseous lymphadenitis (enlarged lymph nodes with caseous or purulent content). Animals with superficial lymph-node swellings and caseous or purulent lesions were first identified and examined by a veterinarian, and only those with lesions consistent with CL were included in the study.

Under aseptic conditions, abscess material was collected from enlarged superficial lymph nodes (primarily prescapular and prefemoral nodes) using sterile syringes and needles after surface disinfection. Approximately 1 mL of purulent or caseous exudate from each lesion was aspirated and immediately transferred into sterile tubes. Samples were transported to the microbiology laboratory on ice and processed within 4 h of collection.

2.2. Bacteriological isolation and identification

For primary isolation, 200–300 µL of each abscess sample was inoculated into brain–heart infusion (BHI) broth (Merck, Germany) and incubated aerobically at 37 °C for 24 h [6]. After enrichment, a loopful of each culture was streaked onto 5% sheep blood agar (Merck, Germany) and incubated aerobically at 37 °C for 24–48 h. Suspicious colonies, typically small, smooth, white to cream-colored with narrow zones of hemolysis, were selected for further analysis.

In parallel, when solid tissue fragments from lymph nodes were available, approximately 1 cm^3 of tissue was aseptically excised and directly streaked onto blood agar and nutrient agar plates, followed by incubation under aerobic and microaerophilic conditions at 37 °C for 24–48 h [5].

Presumptive *Corynebacterium* colonies were examined by Gram staining and microscopy. Gram-positive, pleomorphic, club-shaped rods consistent with *Corynebacterium* morphology were subjected to routine biochemical tests, including catalase, oxidase, urease, and CAMP tests, according to standard procedures and published protocols [2,5,6]. Isolates showing phenotypic and biochemical characteristics compatible with *Corynebacterium pseudotuberculosis* were selected for antimicrobial susceptibility testing.

This study represents, to the best of our knowledge, one of the first systematic investigations in northeastern Iran to characterize both disk diffusion susceptibility patterns and MIC profiles of *C. pseudotuberculosis* isolates recovered from sheep with caseous lymphadenitis in Mashhad.

2.3. Antimicrobial susceptibility testing (disk diffusion method)

The antimicrobial susceptibility pattern of *C. pseudotuberculosis* isolates was determined by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar supplemented with 5% defibrinated sheep blood (Merck, Germany), following Clinical and Laboratory Standards Institute (CLSI, 2022) recommendations for *Corynebacterium* species. The following antibiotic disks (Merck, Germany) were tested: tetracycline (30 µg), vancomycin (30 µg), ciprofloxacin (5 µg), penicillin (10 units), rifampicin (5 µg), and gentamicin (10 µg).

Pure colonies from 24-h cultures on blood agar were suspended in sterile saline and adjusted to the turbidity of a 0.5 McFarland standard (approximately 1×10^8 CFU/mL). The standardized suspension was used to prepare lawn cultures on Mueller–Hinton agar with 5% defibrinated sheep blood using sterile cotton swabs. After allowing the plates to dry for 10–15 min at room temperature, antibiotic disks were placed on the surface using sterile forceps. The plates were incubated aerobically at 37 °C for 24–48 h.

After incubation, the diameters of the inhibition zones (in mm) around each disk were measured. Results were interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints for *Corynebacterium* spp. Where specific breakpoints for this genus were not available, interpretive criteria for closely related Gram-positive bacteria were used as a reference and clearly indicated in the analysis.

For quality control of the disk diffusion procedure, *Staphylococcus aureus* ATCC 25923 (or PTCC 1112, according to the actual strain used in your lab) was used as a positive control strain in each run [16]. Sterile saline inoculated and cultured under the same conditions served as a negative control to check for contamination. All susceptibility tests were performed in duplicate (on two separate days) to ensure reproducibility, and the mean values were used for analysis.

2.4. Determination of minimum inhibitory concentration (MIC)

MICs of selected antibiotics were determined using the agar dilution method according to CLSI guidelines for *Corynebacterium* spp. The following antibiotics were tested: vancomycin, ciprofloxacin, penicillin, rifampicin, and gentamicin. Antibiotic powders (Sigma-Aldrich, USA) were reconstituted and diluted in sterile physiological saline or appropriate solvent (e.g. ethanol) according to the manufacturer's instructions and CLSI recommendations. The working solutions were added to molten Mueller–Hinton agar supplemented with 10% defibrinated sheep blood to obtain a series of final concentrations.

For each antibiotic, a panel of ten two-fold dilutions was prepared, covering a range that included the CLSI reference concentrations for *Corynebacterium* spp. (e.g. 0.5–64 µg/mL; the exact range for each antibiotic is presented in Table 1). After solidification, agar plates were dried at room temperature before inoculation.

Bacterial suspensions were prepared from fresh 24-h cultures on blood agar and adjusted to a 0.5 McFarland standard. A 1:100 dilution of this suspension (approximately 10^4 CFU/spot) was prepared in sterile saline, and 10 µL of the diluted suspension was spotted onto the surface of

each antibiotic-containing plate using a sterile micropipette or applicator. Plates were incubated aerobically at 37 °C for 24–48 h. The MIC was defined as the lowest antibiotic concentration that completely inhibited visible growth.

Staphylococcus aureus PTCC 1113 was used as a quality control strain to verify the accuracy of MIC determinations. Control plates without antibiotics were included in each batch to ensure viability of the inoculum. All MIC assays were performed in duplicate, and MIC values were recorded as the modal value of the two independent readings. Descriptive statistics (minimum, maximum, and percentage distributions of MIC values) were calculated using Microsoft Excel 2019 (Microsoft Corp., USA) (Table 1).

Table 1. Minimum and maximum concentrations used for antimicrobial susceptibility testing with MIC determination.

Antibiotics	MIC (µg/mL)			Minimum Inhibitory (µg/mL)	Maximum Inhibitory(µg/mL)
	Sensitive µg/mL	Intermediate µg/mL	Resistance µg/mL	µg/mL	µg/mL
Vancomycin	≤4	-	-	0/25	8
Ciprofloxacin	≤1	2	≥4	0/25	8
Penicillin	≤1	2	≥4	0/25	8
Rifampicin	≤1	2	≥4	0/25	8
Gentamycin	≤4	8	≥16	1	32

2.5. Statistical analysis and study novelty

Data from disk diffusion (zone diameters and susceptibility categories) and MIC testing were entered into Microsoft Excel 2019. Categorical data (susceptible, intermediate, resistant) were summarized as frequencies and percentages. Where applicable, differences in resistance rates between antibiotics were evaluated using chi-square (χ^2) tests, with a significance level set at $p < 0.05$.

3. Results

3.1. Isolation and identification of *Corynebacterium pseudotuberculosis*

Out of 91 abscess samples collected from sheep and goats with lesions compatible with caseous lymphadenitis, a total of 10 isolates (11.0%) were identified as *Corynebacterium pseudotuberculosis* based on cultural, microscopic, and biochemical characteristics.

After 24–48 h of aerobic incubation at 37 °C on 5% sheep blood agar, suspected colonies appeared as small, white, dry, matte, convex colonies surrounded by narrow zones of β-hemolysis. Gram staining revealed Gram-positive, pleomorphic, club-shaped rods arranged singly, in palisades, or in "Chinese-letter" formations.

Biochemical testing of the isolates showed the following profile: catalase positive, urease positive, nitrate reduction negative, and CAMP test positive (enhancement of the hemolysis of *Rhodococcus equi*). The reference strain *C. pseudotuberculosis* NCTC 3450 showed an identical biochemical profile (Table 2). Representative growth of *C. pseudotuberculosis* on blood agar after 48 h and the Gram-stained morphology are shown in Figure 1. The CAMP test result with *R. equi* is illustrated in Figure 2, and typical catalase and nitrate test reactions are presented in Figures 3 and 4, respectively.

Figure 1. Growth of *Corynebacterium pseudotuberculosis* on 5% sheep blood agar after 48 h of incubation at 37 °C, showing small, white, dry colonies with narrow β-hemolysis, and Gram-stained smear demonstrating pleomorphic Gram-positive rods.

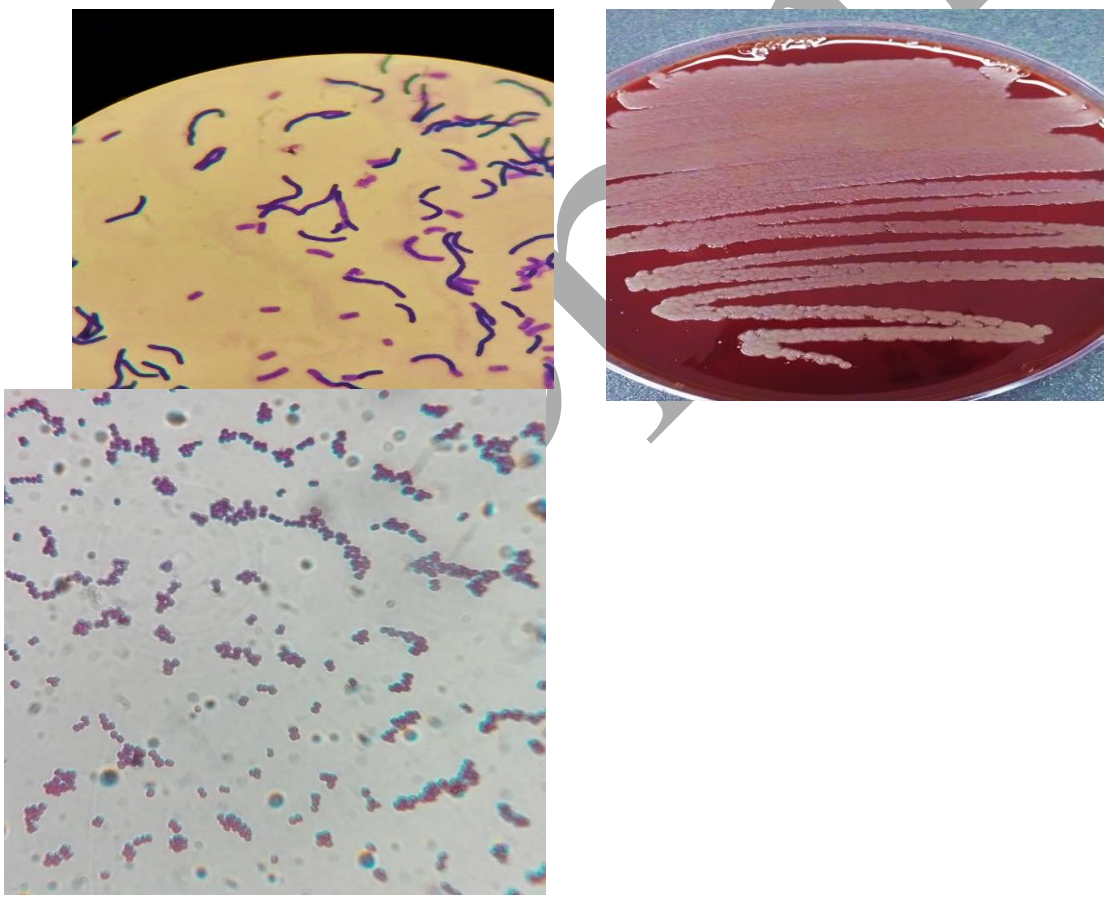
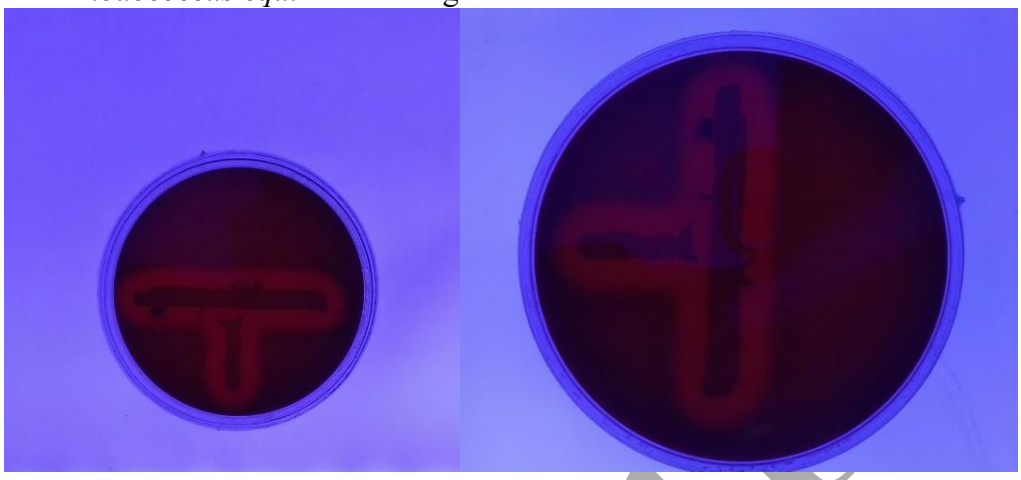


Fig2. Positive CAMP reaction of *Corynebacterium pseudotuberculosis* isolates enhancing the hemolysis of *Rhodococcus equi* on blood agar.



189 **3.3. Biochemical Tests**

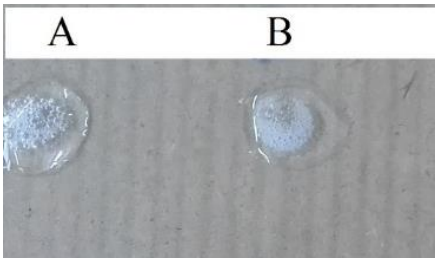
190 Biochemical characteristics of *Corynebacterium pseudotuberculosis* isolates and reference strain
191 NCTC 3450.

192 **Table 2.** Biochemichal tests

Test	Result
Catalase	+
Nitrate	-
Urease	+

195

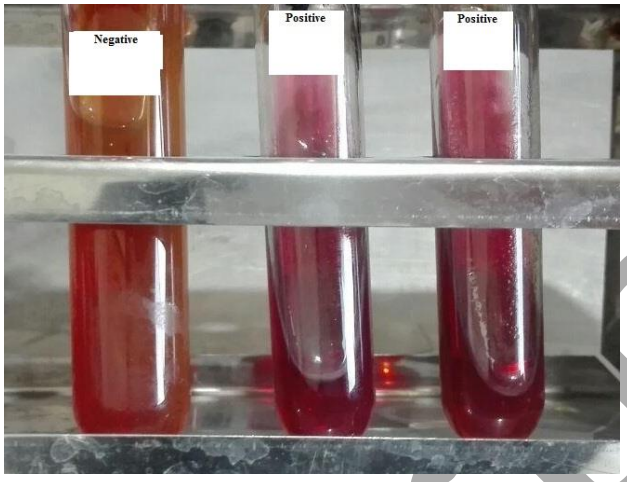
196 **Fig 3.** Catalase test demonstrating bubble formation after addition of 3% hydrogen peroxide to a
197 *C. pseudotuberculosis* colony.



198

199

Fig 4. Nitrate reduction test showing absence of nitrate reduction (negative reaction) in *C. pseudotuberculosis* isolates.



3.2. Antimicrobial susceptibility by disk diffusion

The antimicrobial susceptibility pattern of the *C. pseudotuberculosis* isolates was evaluated using the Kirby–Bauer disk diffusion method. Overall, the isolates showed low susceptibility to most of the tested antibiotics.

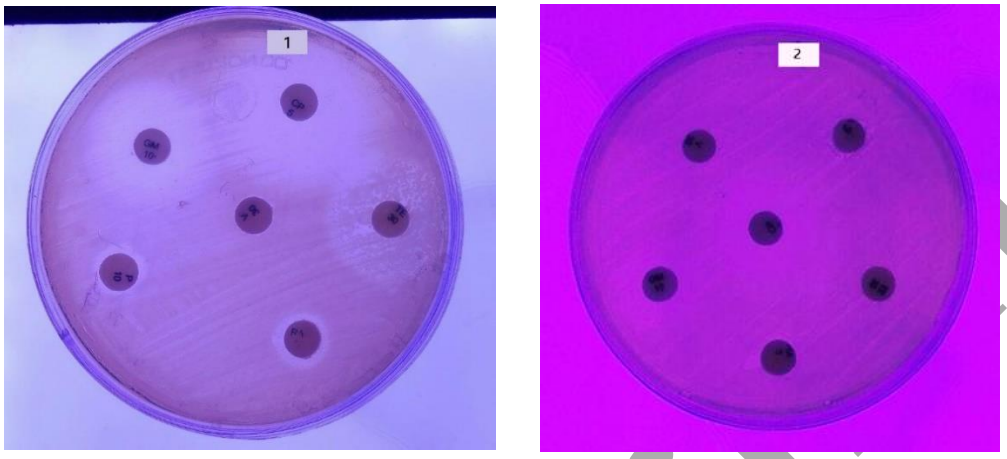
As summarized in Figure 6, the proportions of susceptible isolates to tetracycline, vancomycin, ciprofloxacin, penicillin, rifampicin, and gentamicin were 38%, 22%, 70%, 16%, 22%, and 42%, respectively. Correspondingly, the highest resistance rates were observed for penicillin and vancomycin, while the lowest resistance was recorded for ciprofloxacin and gentamicin, in agreement with the percentages reported in the Abstract.

Figure 5 shows representative Mueller–Hinton agar plates (with 5% sheep blood) with antibiotic disks and bacterial growth, including disks with absent or very small inhibition zones indicating resistance.

Table 3. Mean inhibition zone diameters (mm) for the tested antibiotics.

Antibiotic	Disk content(µg)	Mean zone (mm)	SD (mm)	Range (mm)
Tetracycline	30	18.2	3.5	12-24
Vancomycin	30	13.1	2.8	9-18
Ciprofloxacin	5	22.4	4.1	16-30
Penicillin	10	11.3	2.5	7-16
Rifampicin	5	15.6	3.2	10-21
Gentamicin	10	20.1	3.7	14-26

Figure 5. Representative Mueller–Hinton agar plates (with 5% sheep blood) showing inhibition zones around antibiotic disks used for susceptibility testing of *C. pseudotuberculosis* isolates (Kirby–Bauer method). Some disks show absent or small inhibition zones, indicating resistance.

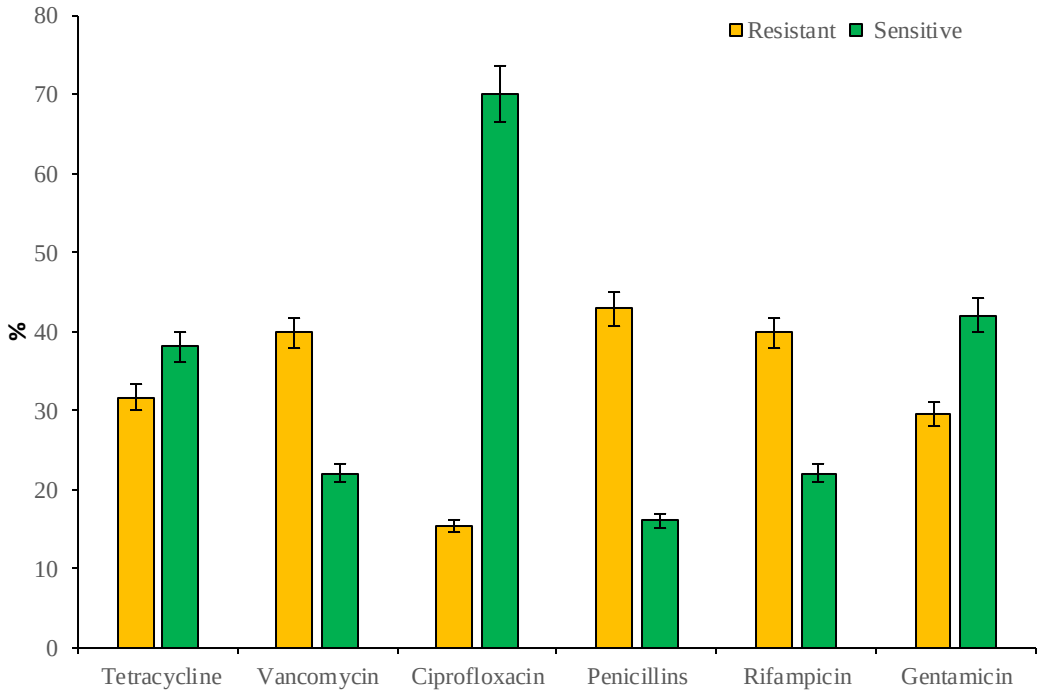


3.3. Minimum inhibitory concentrations (MICs)

The results of MIC testing showed that *Corynebacterium pseudotuberculosis* isolates were inhibited by most antibiotics within a concentration range of 0.25 to 64 µg/mL. In general, ciprofloxacin and gentamicin showed lower MIC values compared with the other antibiotics, whereas penicillin and vancomycin tended to have higher MICs, which is consistent with the higher resistance rates observed in the disk diffusion assay.

As shown in Table 3, inhibition of bacterial growth by tetracycline, vancomycin, ciprofloxacin, penicillin, rifampicin, and gentamicin was achieved at various concentrations within this range, with the majority of isolates being inhibited between 16 and 32 µg/mL for most antibiotics.

Figure 6. Frequency of susceptibility, intermediate response, and resistance of *Corynebacterium pseudotuberculosis* isolates to tetracycline, vancomycin, ciprofloxacin, penicillin, rifampicin, and gentamicin, as determined by disk diffusion testing.



236

237 **3.4. Determination of bacterial growth – MIC**

238 The minimum inhibitory concentrations (MICs) of tetracycline, vancomycin, ciprofloxacin,
239 penicillin, rifampicin, and gentamicin were determined for the 10 *C. pseudotuberculosis* isolates
240 using the agar dilution method. Serial twofold dilutions of each antibiotic were prepared, ranging
241 from 0.25 to 64 µg/mL.

242 Overall, MIC values for the isolates fell within this range, with most isolates being inhibited at
243 concentrations between 16 and 32 µg/mL (Table 3). Ciprofloxacin and gentamicin generally
244 showed lower MIC values, indicating comparatively higher in vitro activity against the isolates,
245 whereas penicillin and vancomycin tended to have higher MICs, consistent with the elevated
246 resistance rates observed in the disk diffusion assay. For most antibiotics, no growth was
247 detected at the highest concentration tested (64 µg/mL), although a subset of isolates required
248 this concentration for penicillin.

249

250

251

252

253

Table 4. MIC levels of *Corynebacterium pseudotuberculosis* isolates against the tested antibiotics.

Antibiotics Concentration	Antibiotics					
	Tetracycline	**Vancomycin**	**ciprofloxacin**	**Penicillin**	**Rifampicin**	**Gentamycin**
<0/25µg/ml	2/27%	4/68%	3/89%	5/4%	13/55%	0%
0/25 µg/ml	34/09%	7/8%	11/86%	2/7%	16/94%	4/16%
0/5 µg/ml	4/5%	12/5%	33/76%	18/91%	15/25%	14/58%
1 µg/ml	31/81%	21/87%	3/89%	8/1%	6/77%	8/33%
2 µg/ml	6/81%	15/62%	5/19%	13/51%	16/94%	10/41%
4 µg/ml	4/5%	14/06%	12/98%	2/7%	3/38%	31/25%
8 µg/ml	11/36%	6/25%	2/59%	27/2%	8/47%	6/25%
16 µg/ml	2/27%	10/93%	11/86%	10/81%	10/16%	16/66%
32 µg/ml	2/27%	4/68%	14/28%	10/81%	8/47%	8/33%
64 µg/ml	0%	0%	0%	0%	0%	0%

4. Discussion

Caseous lymphadenitis (CLA) is a chronic suppurative disease of small ruminants caused by *Corynebacterium pseudotuberculosis* and characterized by abscess formation in superficial and internal lymph nodes. In the present study, *C. pseudotuberculosis* was isolated from a subset of abscess lesions in slaughtered sheep and goats in Mashhad, confirming its role as one of the etiological agents of skin and lymph node abscesses in livestock in this region. Although the isolation rate was relatively low, it should be considered that abscesses in small ruminants can have multiple causes, and not all caseous lesions are necessarily due to *C. pseudotuberculosis*. Differences in sampling strategies, lesion selection, and diagnostic methods may partly explain the variation in isolation frequencies between studies.

The most important finding of this work is the detection of a concerning pattern of antimicrobial resistance among the *C. pseudotuberculosis* isolates. In our study, the lowest resistance rates were recorded for ciprofloxacin and gentamicin, whereas the highest resistance was observed for penicillin and vancomycin. This pattern is broadly consistent with several previous reports on *C. pseudotuberculosis* in small ruminants. For example, Rizk et al[1]. examined antibiotic resistance to penicillin, ciprofloxacin, neomycin, erythromycin, streptomycin, methicillin and novobiocin in isolates from sheep and goats with CL and found the highest sensitivity to ciprofloxacin and the highest resistance to penicillin, similar to our results. Mohamadzadeh et al [4]. also reported resistance to doxycycline, tetracycline and ceftriaxone in isolates from sheep with CL in Binalud, Iran, indicating that resistance to several commonly used drugs is already established in this region.

Other studies have reported different resistance profiles, which likely reflect regional variation in antimicrobial usage patterns and selection pressure. Li et al [2]. found that most resistance in *C. pseudotuberculosis* isolates from goats in southwestern China was associated with nitrofurantoin and furazolidone, whereas all isolates were susceptible to vancomycin, norfloxacin, cefadroxil, clarithromycin and cefepime. In contrast, Tawab et al [3]. reported high resistance rates to co-amoxiclav (76.9%), enrofloxacin (57.7%), clindamycin (53.8%), amoxicillin (53.7%) and

ciprofloxacin (23.1%) in isolates from sheep, and Damaty et al [5]. showed that 100% of isolates from sheep and goats with CL in Egypt were resistant to bacitracin and florfenicol, whereas none were resistant to norfloxacin, and the highest resistance was observed to penicillin and erythromycin. Taken together, these studies highlight that antimicrobial resistance in *C. pseudotuberculosis* is highly variable and influenced by local antimicrobial use, but resistance to penicillin and some other first-line agents is common and increasing.

The MIC results in our study, which showed inhibition of most isolates at concentrations between 8 and 64 µg/mL for the panel of tested antibiotics, are in agreement with the disk-diffusion findings. Lower MICs for ciprofloxacin and gentamicin suggest better in vitro activity, whereas higher MICs for penicillin and vancomycin are consistent with reduced susceptibility. These observations are comparable to reports of multidrug-resistant phenotypes in other *Corynebacterium* species isolated from human infections, where unpredictable and sometimes extensive resistance patterns complicate therapy and necessitate routine susceptibility testing [16,18,21,22]. Although our study did not investigate resistance genes, previous work has demonstrated that corynebacteria may harbor multiple mechanisms of resistance, including antibiotic-inactivating enzymes, efflux pumps and target modifications, which together can give rise to multidrug-resistant (MDR) phenotypes.

Despite the in vitro susceptibility of some isolates to several antibiotics, CL remains a major problem in sheep and goat flocks. This apparent discrepancy is largely due to the pathophysiology of the disease: the thick fibrous capsule surrounding the abscesses markedly limits antibiotic penetration in vivo, and bacteria can persist within these encapsulated lesions even when MIC values suggest susceptibility. As a result, antibiotic treatment of CL is often unsatisfactory, with high rates of relapse and persistence of infection at flock level. Therefore, antimicrobial therapy should not be relied upon as the sole control measure and, whenever used, should be guided by susceptibility testing to avoid further selection of resistant strains.

From a herd health and public health perspective, the presence of antibiotic-resistant *C. pseudotuberculosis* is of concern. Persistent infection in flocks contributes to ongoing economic losses through decreased productivity, carcass trimming or condemnation, and increased treatment and management costs. Moreover, considering *C. pseudotuberculosis* as a potential zoonotic agent, the circulation of resistant strains in animal populations could also pose risks to humans who are in close contact with infected animals or contaminated materials.

Effective control of CL in endemic areas such as Iran requires a multifaceted approach. The most reliable flock-level control measure remains the identification and removal (culling or segregation) of clinically affected animals, particularly those with recurrent abscesses. Preventive measures should include educating farmers to minimize skin trauma during handling and shearing, using properly maintained and disinfected equipment, and controlling external parasites that may facilitate bacterial entry through skin lesions. In the longer term, research on immunostimulatory compounds and vaccine development is essential to provide more effective preventive tools [13,15,17]. In addition, regular surveillance studies at regional and national levels are needed to better estimate the prevalence of CL and to monitor trends in antimicrobial resistance, which will help in updating treatment guidelines and designing rational antimicrobial use policies in small ruminants.

This study has some limitations, including the relatively small number of isolates and the restriction to a single slaughterhouse and geographical area, which may limit the generalizability of the findings. Nevertheless, it provides useful baseline information on the presence of *C. pseudotuberculosis* in abscess lesions in Mashhad and on the current resistance patterns of isolates from this region. Future work should include larger sample sizes, multiple regions, and molecular characterization of isolates and resistance genes.

5. Conclusion

In the present study, the antibiotic resistance pattern of *Corynebacterium pseudotuberculosis* isolated from abscesses of sheep and goats in Mashhad was evaluated. The isolates showed the lowest resistance to ciprofloxacin and gentamicin (15.3% and 15.88%, respectively) and the highest resistance to penicillin and vancomycin (42.84% and 39.78%, respectively). These findings confirm the presence of *C. pseudotuberculosis* as one of the causative agents of skin abscesses and caseous lymphadenitis in small ruminants in this province and indicate a substantial risk of therapeutic failure when infections are treated empirically with traditional first-line antibiotics.

Given the high economic losses associated with CL, essential precautionary measures are required, including the development and implementation of effective vaccination programs, removal or segregation of clinically infected animals, improvement of biosecurity and hygiene practices, and strict adherence to prudent antibiotic use. Larger-scale epidemiological studies are needed to assess the prevalence of CL at both flock and animal levels and to obtain quantitative data on its economic impact in small ruminant production systems in Iran.

Acknowledgment

We would like to express our sincere gratitude to Ms. Amiri for her valuable assistance throughout the research process. Her contributions were instrumental in the successful completion of this study. We also extend our appreciation to the staff of the Razi Institute in Mashhad for their cooperation and support in this research project. Their collaboration greatly facilitated our work and enhanced the quality of our findings. Their combined efforts have significantly contributed to the success of this research endeavor.

Ethics

Not Applicable

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests statement

The authors declare that they have no conflict of interest.

Authors' contribution

AMAK Conducted data collection and drafted the manuscript. HRF Conceptualized and designed the initial study. ACHN and LM Provided consultation and guidance in drafting the manuscript. MJ Performed data analysis and interpretation of results. All authors reviewed and approved the final version of the manuscript.

Funding

No funding was obtained for this study.

Data Availability

The datasets generated and analysed during the present study are not publicly accessible. They can be obtained only in coordination with the corresponding author, who will provide them upon reasonable request.

AI usage statement

"The authors used ChatGPT (OpenAI, GPT-4, March 2024 release) solely to assist with reference formatting and minor English-language editing. The tool was not employed for data generation, analysis, interpretation or scientific content creation, and all AI-suggested changes were reviewed and approved by the authors.

Refrences:

1. Abebe D, Sisay Tessema T, Osman SA, et al. Determination of *Corynebacterium pseudotuberculosis* prevalence and antimicrobial susceptibility pattern of isolates from

lymph nodes of sheep and goats at an organic export abattoir, Modjo, Ethiopia. Lett Appl Microbiol. 2015;61(5):469-476. doi: 10.1111/lam.12482.

2. Alibi S, Ferjani A, Boukadida J, Cano ME, Fernández-Martínez M, Martínez-Martínez L, Navas J. Occurrence of *Corynebacterium striatum* as an emerging antibiotic-resistant nosocomial pathogen in a Tunisian hospital. Sci Rep. 2018;8(1):9704. doi: 10.1038/s41598-018-27843-2.
3. Clarke JR, Rahman D, Saul Z. A case of recurrent *Corynebacterium jeikeium* endocarditis: unanswered questions for the treatment of chronic endovascular infections. J Infect. 2019;18:e00610. doi: 10.1016/j.jinf.2019.04.008.
4. Dalal A, Urban C, Segal-Maurer S. Endocarditis due to *Corynebacterium amycolatum*. J Med Microbiol. 2018;57(10):1299-1302. doi: 10.1099/jmm.027836-0.
5. Damaty HM, El-Demerdash AS, Abd El-Aziz NK, Yousef SG, Hefny AA, Abo Remela EM, Shaker A, Elsohaby I. Molecular Characterization and Antimicrobial Susceptibilities of *Corynebacterium pseudotuberculosis* Isolated from Caseous Lymphadenitis of Smallholder Sheep and Goats. Animals. 2023;13(2):2337. doi: 10.3390/ani13020237.
6. Dragomirescu CC, Lixandru BE, Coldea IL, Corneli OL, Pana M, Palade AM, Cristea VC, Suciu G. Antimicrobial Susceptibility Testing for *Corynebacterium* Species Isolated from Clinical Samples in Romania. J Antibiot. 2020;9(1):31. doi: 10.1093/jac/dkz452.
7. Dragomirescu CC, Lixandru BE, Coldea IL, Palade AM, Baltoiu M, Dinu S, Popa MI. Comparative analysis of different phenotypic and molecular methods used for the taxonomic identification of *Corynebacterium* spp. isolated from clinical samples in Romania. Biotechnol Lett. 2017;29(12):12926-12933. doi: 10.1007/s10529-017-2446-9.
8. El Tawab A, El-Hofy AA, Hassan FI, El-khayat NI, Rizk ME. Protective and immunogenic characters of inactivated oil adjuvant *Brucella melitensis* vaccine versus Rev-1 vaccine. Biosci Res. 2018;16:2272-2281.
9. Elkayam N, Urazov A, Tuneev K, Chapnick E. *Corynebacterium striatum* bacteremia associated with cellulitis in a patient with cirrhosis. IDCases. 2019;17:e00575. doi: 10.1016/j.idcr.2019.05.002.
10. FitzGerald R, Prosser A, Perera DN. Non-toxigenic penicillin-resistant cutaneous *C. diphtheriae* infection: a case report and review of the literature. J Infect Public Health. 2018;11(1):98-100. doi: 10.1016/j.jiph.2017.11.016.

11. Hah WO, Werth BJ, Butler-Wu SM, Rakita RM. Multidrug-resistant *Corynebacterium striatum* associated with increased use of parenteral antimicrobial drugs. Emerg Infect Dis. 2018;24(11):1908-1910. doi: 10.3201/eid2411.180278.
12. Li H, Yang H, Zhou Z, Li X, Yi W, Xu Y, Wang Z, Hu S. Isolation antibiotics resistance, virulence traits phylogenetic analysis of *Corynebacterium pseudotuberculosis* from goats in southwestern China. J Small Rum Res. 2018;168:69-75. doi: 10.1016/j.jsmr.2018.07.004.
13. Magdy Selim A, Atwa SM, El Gedawy AA, Younis EE. Epidemiological, bacteriological and molecular studies on caseous lymphadenitis in sheep of Dakahlia, Egypt. Anim Biotechnol. 2022;33(5):1655-1660. doi: 10.1080/10495352.2021.1964462.
14. Minkin R, Shapiro JM. *Corynebacterium afermentans* lung abscess and empyema in a patient with human immunodeficiency virus infection. South Med J. 2017;110(4):395-398. doi: 10.1097/SMJ.0000000000000420.
15. Mohamadzadeh R, Farzin HR, Jamshidian Mojaver M. Antibiotic resistance phenotype study in *Corynebacterium pseudotuberculosis* isolated from sheep with cheese lymphadenitis in Binalood city of Mashhad. New Findings Vet Microbiol. 2020;3(1):88-95.
16. Ramos JN, Souza C, Faria YV, da Silva EC, Veras JF, Baio P, Mattos-Guaraldi L. Bloodstream and catheter-related infections due to different clones of multidrug-resistant and biofilm producer *Corynebacterium striatum*. BMC Infect Dis. 2019;19(1):1-11. doi: 10.1186/s12879-019-4617-9.
17. Rizk AM, El-Tawab A, Awad A, Afifi SE, Mohamed SR. *Corynebacterium pseudotuberculosis* infection in small ruminant and molecular study of virulence and resistance genes in Beni-Suef Governorate. Benha Vet Med J. 2019;37(1):122-127.
18. Rudresh SM, Ravi G, Alex AM, Mamatha K, Sunitha L, Ramya KT. Non diphtheritic *Corynebacteria*: an emerging nosocomial pathogen in skin and soft tissue infection. J Clin Diagn Res. 2015;9(12):DC19.
19. Suciu G, Suciu V, Martian A, Craciunescu R, Vulpe A, Marcu I, Fratu O. Big data, internet of things and cloud convergence–an architecture for secure e-health applications. J Med Syst. 2015;39(1):1-8.

20. Syed MA, Ashcherkin N, Sundhu M, Hakam L, Gul S. Recurrent bacteremia with *Corynebacterium striatum* after prosthetic valve replacement a case report. Cureus. 2019;11(5):e4670.
21. Yoon S, Kim H, Lee Y, Kim S. Bacteremia caused by *Corynebacterium amycolatum* with a novel mutation in gyrA gene that confers high-level quinolone resistance. Korean J Lab Med. 2011;31(1):47.
22. Zasada A, Mosiej E. Contemporary microbiology and identification of *Corynebacteria* spp causing infections in human. Lett Appl Microbiol. 2018;66(6):472-483.