Molecular Identification and Antibiotic Resistance of *Moraxella osloensis* Isolated from Ocular Infections in Cattle

Uçan, N.,[1,a] Nuhay, Ç.,[2,b] Hassan, F.,[1,3,c] and Türkyılmaz, S.[4,d*]

[1] Aydın Adnan Menderes University, Institute of Health Sciences, Aydın, Türkiye.

[2] Bornova Veterinary Control Institute, Bacteriology Laboratory, 35010, Bornova, İzmir, Türkiye

[3] Department of Microbiology and Immunology, Faculty of Pharmacy, Modern University for Technology and Information, Cairo, Egypt.

[4] Department of Microbiology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydın, Türkiye.

ORCIDs: [a]: 0000-0002-1758-6744, [b]: 0000-0002-1475-3041, [c]: 0000-0002-6588-3866, [d]: 0000-0002-1363-4534

* **Corresponding author:** Prof. Dr. Süheyla Türkyılmaz Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Işıklı, Aydın, Türkiye. Phone: +90 256 220 60 00/6168; E-mail: sturkyilmaz @adu.edu.tr

ABSTRACT

This study was aimed to identify aerobic bacterial agents isolated from conjunctival swab samples of cattle showing clinical signs of infectious bovine keratoconjunctivitis (IBK) using conventional and genotypic methods, and to determine the antimicrobial resistance profile of a *Moraxella osloensis* isolate, a species rarely associated with ocular infections in cattle. A total of 62 conjunctival swab samples were collected from 62 cattle with clinically suspected IBK from six farms between June 2024 and May 2025. Phenotypic identification of the isolates was performed using conventional bacteriological methods, while species-level identification was achieved by sequence analysis of the 16S rRNA gene region. Isolates suspected of belonging to the genus *Moraxella* were additionally evaluated using an automated identification system (VITEK® 2). Antimicrobial susceptibility testing was performed using the disk diffusion method with eight antibiotics representing seven different antimicrobial classes. Bacterial growth was detected in 44 (71%) of the 62 conjunctival swab samples, including 32 Gram-positive and 12 Gram-negative isolates. BLASTN analysis of the 16S rRNA gene partial sequence (1091 bp) of one Gram-negative isolate revealed a high degree of sequence similarity to *M. osloensis*. The closest match was obtained with the *M. osloensis* strain A1920 reference sequence, showing 98.2% sequence identity and 99% coverage (GenBank: NR_104936.1; E=0.0). Antimicrobial susceptibility testing demonstrated that the isolate was susceptible only to cefotaxime and imipenem, while exhibiting resistance to six other antimicrobial classes, indicating a multidrug-resistant (MDR) phenotype. In conclusion, the findings suggest that *M. osloensis* may be involved in the aetiology of ocular infections in cattle and that such isolates may harbor a high level of antimicrobial resistance, potentially limiting treatment options. Based on the available literature, this study represents the first report of a multidrug-resistant *M. osloensis* isolate recovered from bovine ocular infections in Türkiye, highlighting its potential relevance within the One Health framework.

Keywords: Infectious Bovine Keratoconjunctivitis; *Moraxella* spp.; Antibiotic Resistance; Phenotypic Identification; 16S rRNA; Cattle; One Health.

INTRODUCTION

Keratoconjunctivitis is an ocular disease of farm animals in which numerous factors play a role, including allergic reactions, viral and bacterial infections, and parasitic agents, and its clinical course and aetiology can vary (1). Although systemic diseases in cattle can lead to similar clinical findings, the primary etiologic agent of infectious bovine keratoconjunctivitis (IBK) is considered to be *Moraxella bovis* due to its

high degree of contagiousness and the serious negative effects it causes on animal welfare and productivity (2).

The onset and severity of the disease depend on the complex interactions between host factors, environmental conditions, and the virulence characteristics of the causative microorganism, and disruption of corneal surface integrity is considered an important predisposing factor for the development of infection (2,3).

However, the aetiology of ocular infections is not limited to *Moraxella* species alone. In microbiological examinations of conjunctival swabs, numerous bacterial species, considered as primary agents as well as opportunistic pathogens, can be isolated. While *M. bovis* is the most commonly reported species within the genus *Moraxella*, in recent years other species such as *Moraxella bovoculi* and *Moraxella ovis* have also been shown to be associated with outbreaks (4). In addition, bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Escherichia coli* and *Trueperella pyogenes* are among the other agents that can be isolated in bovine ocular infections (5). Less commonly, atypical microorganisms such as *Pseudomonas stutzeri* (6) and *Mycoplasma bovoculi* (7) have also been associated with keratoconjunctivitis cases. *M. osloensis* is a Gram-negative coccobacillus found mostly as a commensal in the upper respiratory tract and skin mucosa of humans, but it can cause serious clinical conditions such as fatal infections (8), neonatal ophthalmia (9), pneumonia (10), and systemic infections (11), especially in immunocompromised patients.

Recent studies have considered *M. osloensis* and *M. nonliquefaciens* as important *Moraxella* species associated with eye infections in humans (12). The fact that this bacterium, normally considered to have low pathogenicity, can exhibit a significant infectious potential under suitable conditions suggesting that it should also be considered as an opportunistic agent in terms of animal health (8,11,12).

Successful control of ocular infections depends on the accurate and precise identification of the causative agent. However, the large similarity of morphological and biochemical characteristics among *Moraxella* species means that phenotypic methods are insufficient, especially in distinguishing rare or atypical species other than *M. bovis* (2,12). This situation can lead to overlooking the pathogenic roles of species such as *M. osloensis*, also known as a member of the commensal flora (12). Considering the dynamic and multifactorial nature of the etiology of infectious keratoconjunctivitis in cattle, the use of molecular validation methods such as 16S rRNA gene sequencing is important for the accurate identification of such microorganisms (2). Its association with serious ocular infections in humans is considered an important finding supporting the potential pathogenicity of *M. osloensis* (9,12).

In this study, the aim was to identify aerobic bacterial agents obtained from conjunctival swab samples of cattle showing signs of infectious keratoconjunctivitis using conventional and genotypic methods, and to determine the antimicrobial resistance profile of *M. osloensis* isolate, which is rarely associated with ocular infections in cattle.

MATERIAL AND METHODS

Materials

The material for this study consisted of clinical samples collected during field studies conducted in six different cattle farms in İzmir and Aydın provinces between June 2024 and May 2025. Unilateral conjunctival swab samples were taken from a total of 62 cattle exhibiting ocular signs such as excessive lacrimation, photophobia, mucopurulent discharge, conjunctivitis, corneal opacity, and in advanced cases vision loss during clinical examination. This study was designed as a descriptive cross-sectional study aiming to identify rarely reported ocular pathogens; therefore, no sample size calculation was performed beforehand. During the sampling process, animals that had not received systemic or topical antibacterial treatment in the last 15 days were selected.

Before sampling, the animals were appropriately restrained, and the eye area was gently cleaned with sterile gauze moistened with 0.9% NaCl. Next, the lower eyelid was carefully pulled outwards to expose the conjunctival fornix, and swab samples were taken with sterile swab sticks (13). The collected samples were appropriately labelled to ensure proper identification and traceability, and placed in sterile transport tubes to minimize the risk of contamination. All samples were kept under cold chain conditions (4-8°C) and delivered to the laboratory within 24 hours at the latest and subjected to microbiological examination.

Ethical Approval

The research protocol was found ethically appropriate by the Aydın Adnan Menderes University Animal

Table 1. Antibiotics used in the study, evaluation criteria and result (EUCAST, 2024).

Antibiotic Group	Antimicrobial Agent/Abbreviation Disk	Content (µg)	Zone Diameter Limit Values (mm)		Result
			S ≥	R <	
Penicillin	Amoxicillin-clavulanic acid (AUG)	2-1	19	19	R
Cephalosporin	Rcefotaximee (CTX)	5	20	17	**S**
	Ceftriaxone (CRO)	30	24	21	R
Carbapenem	Imipenem (IPM)	10	29	29	S
Fluoroquinolone	Nalidixic Acid (NA)	30	23	23	R
Macrolide	Erythromycin (E)	15	23	23	R
Tetracycline	Tetracycline (TE)	30	26	26	R
Folate Pathway Inhibitor	**Trimethoprim-Sulfamethoxazole (STX)**	**1.25-23.75**	**18**	**15**	**R**

R: Resistant, **S:** Susceptible

Experiments Local Ethics Committee with decision number 64583101/2024/70 dated 06.08.2024.

Bacterial Isolation and Phenotypic Identification

Conjunctival swab samples were inoculated into Brain Heart Infusion Broth (BHIB) (HiMedia Laboratories, Mumbai, India) for pre-enrichment and incubated under aerobic conditions at 37°C for 18 hours. Next, a loopful of the suspension was taken and plated onto Blood Agar (KA; Oxoid, UK) containing 7% sheep blood, along with MacConkey agar, Bile Esculin agar, and Sabouraud Dextrose agar (all from HiMedia Laboratories, Mumbai, India). The plates were incubated at 37°C for 24-48 hours (4). At the end of incubation, the growing colonies were evaluated, and their phenotypic isolation was performed using classical conventional methods. Gram staining was applied to the colonies to determine their microscopic morphology, and basic biochemical tests (oxidase, catalase, motility, hemolysis, lactose fermentation, urease, indole, growth on TSI agar, etc.) were performed (14).

As a result of the preliminary evaluation, the isolate was suspected to belong to the genus *Moraxella* and was further analysed using an automated identification system (VITEK® 2, BioMérieux, France; GN ID cards). The results were interpreted hypothetically, not definitively, at the species level. Due to the high phenotypic similarity between *Moraxella* species, phenotypic identification was performed first and supported by molecular confirmation.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of the suspected *M. osloensis* isolate was performed on blood Mueller–Hinton agar (Oxoid, Basingstoke, UK) using the Kirby–Bauer disk diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023) (15). The plates were incubated at 37°C for 18-24 hours in a laboratory incubator (Nüve, Ankara, Türkiye), and the diameters of the inhibition zones were measured using a digital caliper (Mitutoyo Corporation, Kawasaki, Japan).The antimicrobial panel was designed to reflect agents commonly used in the treatment of bovine ocular infections in routine veterinary practice. Resistance to these drugs is clinically relevant, as it may limit empirical therapy and increase reliance on last-resort antimicrobials. Since the European Committee for Antimicrobial Susceptibility Testing (EUCAST) does not provide specific breakpoints for *M. osloensis*, the *Moraxella catarrhalis* criteria were applied in interpreting the results, as recommended for closely related species (16) (Table 1). The *Escherichia coli* ATCC 25922 strain was used for quality control in the tests. The resistance of isolates to at least three or more antimicrobial classes has been accepted as the criterion for multiple antibiotic resistance (MDR) (17).

Genotypic Identification

DNA Isolation

Genomic DNA isolation from all bacterial isolates obtained in the study was performed using a commercially

Tablo 2. Bacterial genera and isolate numbers that could be identified by phenotypic methods.

Bacterial Genus (n)	Gram Staining	Oxidase	Catalase	Motility	Hemolysis	Lactose	Urea	Indole	TSI	Isolate number (n=44)
Enterococcus spp.	P, coccus	N	N	N	N	D	N	ND	ND	5
Staphylococcus spp.	P, coccus	N	P	N	Non-hemolytic/β	D	D	ND	ND	8
Aerococcus spp.	P, coccus	P	N	N	α hemolysis	P	N	ND	ND	2
Micrococcus spp.	P, coccus	N	P	N	α hemolysis	N	P	ND	ND	2
Corynebacterium spp.	P, pleomorphic	N	P	N	D	D	N	ND	ND	7
Bacillus spp.	P, rod	P	P	P	D	N	D	ND	ND	8
Acinetobacter spp.	N, cocobacillus	N	P	N	N	N	N	N	K/K	3
Pseudomonas spp.	N, rod	P	P	P	N	N	N	N	K/K	5
Stenotrophomonas spp.	N, rod	N	P	P	N	N	D	N	K/K	2
Moraxella spp.	N, cocobacillus	P	P	N	N	N	N	N	K/K	2

P: Positive, **N**: Negative, **D**: Interspecies variable, **ND**: Test not applied, **K/K**: No fermentation, gas–, H_2S–

available DNA extraction kit (InstaGene™ Matrix, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The integrity of the isolated DNA samples was evaluated by 1% agarose gel electrophoresis, and the integrity of the amplifiable genomic DNA was confirmed (18). Purity and concentration measurements were performed with a spectrophotometer (NanoDrop™ Maestro; MaestroNano, Taipei, Taiwan), and samples with an OD260/OD280 ratio in the range of 1.8-2.0 were considered pure. Lower values (<1.8) indicate protein contamination, whereas higher values (>2.0) indicate RNA contamination (19).

Primers

Universal primers 27F (5'-AGAGTTTGATCMTGG CTC AG-3') and 1492R (5'-TACGGYTACCTTGTT ACGACTT-3') were used for amplification of the 16S rRNA gene region of the growing isolates (20).

PCR and Evaluation

PCR reactions were performed in a total volume of 35 µL using a commercial master mix (Solis BioDyne, Tartu, Estonia) (5× FIREPol®, Catalog No: 04-11-00125; containing 12.5 mM $MgCl_2$). Each tube contained 7 µL of 5× master mix, 0.2-0.6 µL of primers at a concentration of 10 pM each, 3 µL of genomic DNA (5-50 ηg/µL), and nuclease-free distilled water for the remaining volume. PCR cycle protocol: Initial denaturation at 95°C for 5 minutes; followed by denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 90 seconds for 30 cycles; finally, final extension at 72°C for 10 minutes.

Amplification products were electrophoresed on a 1% agarose gel at 90 volt for 45 minutes, and the clarity and size of the bands were evaluated. Samples showing no smear formation, primer dimer, or non-specific bands, and exhibiting a single band profile of approximately 1467 bp were considered suitable for sequence analysis (20).

Sequence Analysis

PCR products were purified using the MAGBIO HighPrep™ PCR Clean-up System (AC-60005) kit (MagBio Genomics Inc., Gaithersburg, MD, USA) according to the manufacturer's protocol. Purified DNA samples were analyzed using forward Sanger sequencing with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems (Thermo Fisher Scientific, Foster City, CA, USA) on an Applied Biosystems ABI 3730XL (Thermo Fisher Scientific, Waltham, MA, USA) instrument. Forward sequencing provided high-quality reads covering the variable regions necessary for species-level identification. The resulting sequences were subjected to quality control processes, and the results obtained in FASTA format were compared with reference nucleotide sequences in the NCBI database using the BLAST algorithm. Species-level molecular identification was performed according to the reference sequences showing the highest similarity rate (% identity) (20,21). In this study, 16S rRNA gene sequencing was considered sufficient for reliable taxonomic identification at the genus and, where

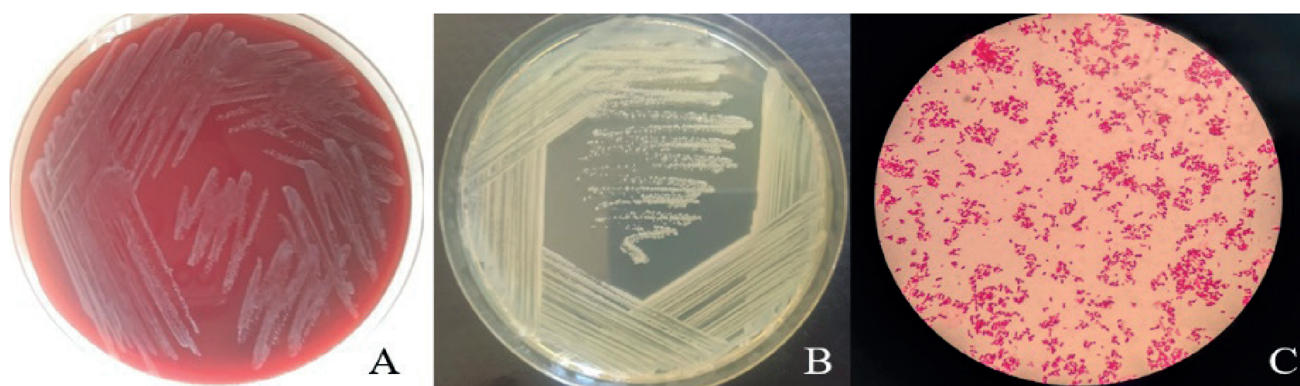

Figure 1. *Moraxella* spp. **A.** Appearance in KA: small, gray-white, smooth-bordered, non-hemolytic colonies, **B.** Appearance in MHA: opaque, smooth-bordered colonies, **C.** B. Gram staining: Bacteria with Gram-negative coccobacil morphology.

applicable, species level. Species-level assignments were based on the closest BLAST match, and similarity percentages are provided to indicate the strength of taxonomic resolution. For isolates showing lower similarity values, identification reflects the closest phylogenetic affiliation.

RESULTS

Bacterial Isolation and Identification

Bacterial growth was detected in 44 samples (71%) of the conjunctival swabs taken from 62 cattle showing clinical keratoconjunctivitis findings from six farms in İzmir and Aydın. From each conjunctival swab sample showing growth, an isolate with the dominant morphology was selected, and a total of 44 isolates were transferred to further identification. All isolates were initially identified at the genus level using conventional phenotypic methods. Species-level identification of all isolates was subsequently performed by 16S rRNA gene sequencing.

In this study, a total of 10 different bacterial genera were identified using phenotypic methods. Six of these genera were Gram-positive (*Enterococcus* spp., *Staphylococcus* spp., *Aerococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp.) and four were Gram-negative (*Acinetobacter* spp., *Pseudomonas* spp., *Stenotrophomonas* spp., *Moraxella* spp.).

During phenotypic identification, one isolate was observed to have a Gram-negative coccobacillus morphology (Figure 1). Biochemical tests showed positive results for oxidase and catalase, while motility, lactose fermentation, hemolysis, urea, and indole tests were negative.

Identification using the VITEK® 2 GN identification system indicated that the isolate belonged to the *Moraxella* group with a probability score of 95%; however, definitive species-level identification could not be achieved. Although the biochemical profile was compatible with *Moraxella lacunata*, *M. nonliquefaciens*, and *M. osloensis*, the system reported the result only as "*Moraxella* group". Evaluation based on parameters such as alanine-phenylalanine-proline arylamidase (APPA), arginine dihydrolase (ADO), L-pyrrolydonyl arylamidase (PyrA), indole production (Indole-α-Lactose reaction) (IARL), D-cellobiose fermentation (dCEL), β-galactosidase (BGAL), and γ-glutamyl transferase (GGT) revealed that the isolate exhibited a biochemical pattern specific to the *Moraxella* genus.

Antibiotic Susceptibility of *M. osloensis* Isolate

In disk diffusion tests, *M. osloensis* isolate was evaluated with eight antibiotics belonging to seven antimicrobial families. The isolate was found to be susceptible only to rcefotaxime and imipenem; it was resistant to amoxicillin-clavulanic acid, ceftriaxone, nalidixic acid, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole. These findings reveal that the isolate is susceptible to one antibiotic each from the cephalosporin and carbapenem groups, and resistant to the other six antimicrobial families, exhibiting multidrug resistance (MDR) (Table 1).

Molecular Identification

To support bacterial identification in all isolates in the study, the 16S rRNA gene region was amplified by PCR. As a result of the reaction performed using universal primers, a specific product of approximately 1467 bp in length was obtained for each isolate (Figure 2).

Sequence analysis identified a total of 12 Gram-negative

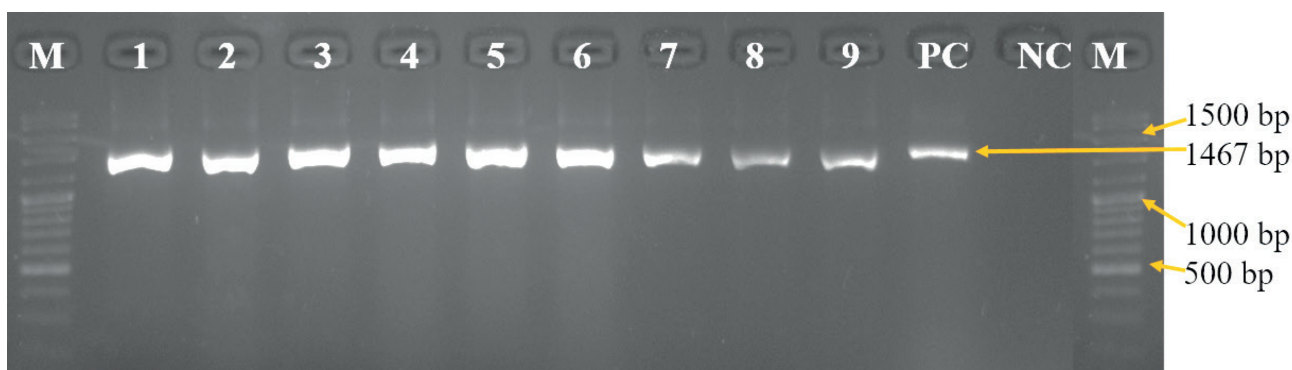

Figure 2. Agarose gel view of PCR products. Field isolates 1-9. **PC**: Positive Control (*E. coli* ATCC 25922), **NC:** Negative Control (DNA-free master mix), **M**: 100 bp DNA Ladder.

bacterial species. Isolates included *Acinetobacter lwoffii* (99.0%), *Pantoea agglomerans* (95.1%), *Brevundimonas pondensis* (99.5%), *Stenotrophomonas chelatiphaga* (82.9%), *Chryseobacterium cheononjinense* (87.6%), *Stutzerimonas nitrilitolerans* (87.5%), *Achromobacter mucicolens* (98.9%), *Massilia oculi* (98.9%), *Pseudomonas koreensis* (98.7%), *Stenotrophomonas lactitubi* (99.5%), *Hafnia paralvei* (98.5%), and *Moraxella osloensis* (98.2%). The percentages shown in parentheses represent the sequence similarity (identity percentage) obtained by comparing the 16S rRNA gene sequences of the isolates with reference sequences in the NCBI GenBank database. These values indicate the degree of nucleotide identity between the isolate sequence and its closest match in the database.

Sequence analysis also revealed a total of 32 Gram-positive bacterial species. Among these isolates were members of the Enterococcaceae and Lactobacillaceae families, such as *Enterococcus villorum* (99.7%), *Enterococcus hirae* (99.7%), *Vagococcus bubulae* (98.9%), *Aerococcus viridans* (99.1%), *Granulicatella elegans* (97.5%), and *Lactobacillus acidophilus* (95.2%). *Staphylococcus chromogenes* (99.1%), *Staphylococcus xylosus* (99.7%), *Staphylococcus saprophyticus* subsp. *saprophyticus* (98.3%), *Staphylococcus sciuri* (98.0%), *Staphylococcus arlettae* (99.0%), *Staphylococcus hominis* (94.3%), *Staphylococcus succinus* (98.2%) and Staphylococcaceae members such as *Mammaliicoccus sciuri* (99.1%) were included. Percentages indicate sequence similarity with GenBank reference sequences, as described above.

In addition, members of Micrococcaceae and Microbacteriaceae such as *Micrococcus luteus* (99.1%), *Kocuria kristinae* (95.1%), *Microbacterium ginsengiterrae* (98.9%) and *Arthrobacter gandavensis* (99.2%); Corynebacteriaceae species such as *Corynebacterium coyleae* (89.0%), *C. macginleyi* (95.1%), *C. afermentans* (97.0%), *C. minutissimum* (95.1%) and *C. jeikeium* (95.4%) were detected. Isolates belonging to the genus Bacillus, namely *B. haynesii* (97.6%), *B. pumilus* (93.4%), *B. licheniformis* (99.0% and 94.1%), *B. circulans* (88.5%), and *B. cereus/thuringiensis/mycoides* (94.1%), were also identified. In addition, species such as *Alloiococcus otitidis* (98.1% and 98.2%), *Aneurinibacillus thermoaerophilus* (88.1%), and *Lactobacillus acidophilus* (95.2%) were determined.

These findings demonstrate that Gram-negative and Gram-positive isolates obtained from conjunctival swab samples exhibit a wide taxonomic diversity and that 16S rRNA gene sequencing provides reliable taxonomic identification at the genus and, where applicable, species level. Species-level identification was based on the closest BLAST match, and similarity percentages are provided to indicate the strength of taxonomic resolution. For isolates showing lower similarity values, taxonomic assignment reflects the closest phylogenetic affiliation based on 16S rRNA gene sequence comparison.

Results of 16S rRNA Sequence Analysis of *M. osloensis* Isolate via GenBank

BLASTN analysis of the 16S rRNA gene partial sequence (1091 bp) of one Gram-negative isolate revealed a high degree of sequence similarity to M. osloensis. The 16S rRNA sequence of the isolated *M. osloensis* sample was compared with the reference sequence (Accession No: NR_104936.1) in the GenBank database using BLAST analysis. The analysis showed that the sequence had a 98.2% similarity to the reference; the maximum score was 1895, the total score was 1895, the E value was 0, and the reference sequence length

was 1522 base pairs. These data indicate that the isolate has a high degree of sequential similarity to the reference sequence and that species-level identification is reliable. Although whole-genome sequencing provides higher taxonomic resolution, previous studies have demonstrated that 16S rRNA gene sequence similarity values above 98% are sufficient for reliable species-level identification within the genus Moraxella, particularly when supported by concordant phenotypic characteristics (22,23).

Fungal Culture

Conjunctival swab samples were incubated in SDA medium (HiMedia Laboratories Pvt. Ltd., Mumbai, India), but no fungal growth was detected in any of them.

DISCUSSION

The aim of this study was to isolate and accurately identify bacteria that might play a role in the aetiology of ocular infections in cattle. In this context, the phenotypic characteristics of bacterial isolates obtained from conjunctival swab samples were evaluated using classical microbiological methods, followed by 16S rRNA gene sequencing and BLAST analysis for molecular confirmation. Although whole genome sequencing offers higher taxonomic resolution, the use of 16S rRNA gene sequencing was considered appropriate in this study, as the primary objective was to support phenotypic identification and achieve reliable taxonomic assignment rather than to perform comprehensive genomic characterization. Furthermore, the antimicrobial susceptibility profile of one *M. osloensis* isolate was interpreted. The study highlights the limitations of phenotypic methods in identifying causative agents of IBKs, while emphasizing the importance of molecular approaches and antimicrobial susceptibility testing in disease control and the development of treatment strategies. This is consistent with studies previously reported in Türkiye and other countries (1,2,12).

In the study, bacterial growth was detected in 44 out of 62 samples (71%); although Gram-positive bacteria were numerically dominant, a remarkable diversity was also observed in the Gram-negative spectrum. In particular, the isolation of environmentally opportunistic bacteria such as *Acinetobacter lwoffii, Pantoea agglomerans, Massilia oculi, Stutzerimonas* spp., and *Hafnia paralvei* suggests that the bovine eye may be a suitable entry point for environmental microorganisms. This explains how the disruption of corneal integrity and the weakening of local defense mechanisms allow opportunistic pathogens to colonize the eye (3,5,13).

The differences between phenotypic identification and 16S rRNA sequence analysis constituted one of the most important findings of the study. While conventional biochemical tests can only identify a limited number of species at the genus level, molecular analyses revealed a significant diversity of species in both Gram-positive and Gram-negative groups. Diagnostic discrepancies observed particularly in the *Enterococcus* and *Corynebacterium* groups demonstrated the inadequacy of classical methods in complex microbial ecosystems such as the cow's eye. The frequent misidentification or incomplete identification of *Vagococcus bubulae*, *Granulicatella elegans, Arthrobacter gandavensis*, and *Microbacterium* species in biochemical tests were concrete examples of this.

One of the most striking findings of the study was the revelation that two isolates, phenotypically assessed as "*Moraxella* spp.", belong to different genera as a result of 16S rRNA sequence analysis. The identification of one of these isolates as *M. osloensis* and the other as *Chryseobacterium cheononjinense* demonstrates that Gram-negative, oxidase-positive, and non-motile bacteria can be grouped as "*Moraxella*-like" under routine laboratory conditions. In particular, the fact that *M. bovis* and *M. bovoculi* are considered primary pathogens in bovine ocular infections can create a diagnostic bias for these isolates with phenotypic similarity (4,12).

Although *M. osloensis* is mostly found as a commensal in humans, it is a species associated with serious ocular infections such as endophthalmitis and neonatal ophthalmitis (9,12). The presence of *M. osloensis* isolated from cattle in this study suggests that this species may also be an opportunistic ocular pathogen in animals. According to the literature review, data on *Moraxella* species isolated from eye infections in cattle in Türkiye are quite limited (2,6).

The fact that the isolated *M. osloensis* strain was found to be susceptible only to rcefotaxime and imipenem, showed resistance to other antibiotic groups, and was evaluated as multi-antibiotic resistant is clinically significant. The detection of resistance to antibiotics such as penicillin derivatives, macrolides, and florfenicol, which are frequently used in the treatment of IBK, shows that empirical treatment approaches may not always be sufficient (12). However, since only one *M. osloensis* isolate was obtained in the study, Although only a single M. osloensis isolate was recovered, its phenotypic

and molecular characterization provides valuable preliminary evidence that warrants further investigation in larger epidemiological studies. It is clear that these findings should not be generalized at the species level but should be evaluated on a case-by-case basis.

The study revealed that distinguishing *Moraxella* species isolated from cattle at the species level using phenotypic and biochemical methods is difficult, and misidentifications can occur between closely related species. Indeed, in a recent study, some strains isolated from cattle with ocular lesions and evaluated as *M. osloensis* using routine diagnostic methods were shown to be genomically different species (*Moraxella oculi* sp. nov.) as a result of advanced molecular analyses. These findings show that identifications based solely on phenotypic approaches may not be reliable, especially in atypical or rare *Moraxella* isolates; and support the necessity for using molecular validation methods for accurate species-level identification (4,12).

The bacterial isolates obtained in this study were collected from conjunctival swab samples taken under sterile conditions from cattle exhibiting clinical signs of infectious keratoconjunctivitis. It should be emphasized that the isolation of these microorganisms does not imply definitive pathogenicity, but rather indicates their potential involvement as opportunistic or secondary agents in the context of compromised ocular defences. Therefore, these isolates are unlikely to represent incidental environmental contamination. However, it is thought that not all isolates are considered primary pathogens, and that environmentally derived and opportunistic species may play a role as secondary infections or accompanying agents. The main aim of the study was not to directly prove pathogenicity, but to accurately and comprehensively reveal the existing microbial diversity.

In this study, the VITEK® 2 system was used as a supporting diagnostic tool. While the system provides reliable results for the species included in the database, it may show limitations in environmentally related and rare species. Therefore, the obtained results were evaluated by comparing them with 16S rRNA sequencing findings, and the final species identifications were based on molecular data.

The association of *M. osloensis* with serious ocular and systemic infections in humans suggests that animal-derived isolates may have potential zoonotic significance and act as reservoirs of resistance. The detection of multidrug-resistant opportunistic bacteria in food-producing animals is of particular concern, as it may contribute to the circulation of antimicrobial resistance across animal, human, and environmental interfaces. The multi-antibiotic resistance profile identified in this study is indicative of potential contributions to the circulation of antimicrobial resistance in animal-human-environment interactions and should be considered a significant risk factor for clinical treatment.

In conclusion, BLASTN analysis of the 16S rRNA gene partial sequence (1091 bp) of one Gram-negative isolate revealed a high degree of sequence similarity to M. osloensis. This study revealed that environmental and atypical bacteria should not be overlooked in the etiology of bovine ocular infections, that conventional diagnostic methods can mask microbial diversity, and that 16S rRNA sequence analysis represents a widely accepted and reliable tool for accurate species identification (20,21). The findings highlight the importance of integrating molecular-based approaches into routine diagnosis to better understand the epidemiology of bovine keratoconjunctivitis and develop effective treatment strategies. Future studies using whole genome sequencing or multilocus sequence analysis may further clarify the taxonomic position and virulence potential of atypical *Moraxella* isolates.

Originality Statement

This article, in whole or in part, has not been submitted for publication elsewhere.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study.

Author Contributions

NU, FH, ST: Conceptualization, methodology development, validation, data analysis, research, original draft writing and editing.

Ç. N.: Validation with the VITEK® 2 system.

All authors have read the final version of the article, agreed on its content, and approved its publication.

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