# Characterization and antimicrobial resistance of *Moraxella ovis* isolates from clinical cases of contagious ovine keratoconjunctivitis in Mexico

## *Caracterización y resistencia antimicrobiana de aislamientos de Moraxella ovis de casos clínicos de queratoconjuntivitis contagiosa ovina en México*

## *Caracterização e resistência antimicrobiana de Moraxella ovis isolados de casos clínicos de ceratoconjuntivite contagiosa ovina no México*

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**Abstract**

**Background:** Contagious ovine keratoconjunctivitis (OKC) causes blindness in sheep and goats and it is associated with a set of bacterial genera of which some species show antimicrobial resistance. **Objective:** To identify phenotypic-genotypic relationship of antimicrobial resistance from *Moraxella* spp. isolates obtained from clinical cases of contagious ovine keratoconjunctivitis (OKC) in Mexico. **Methods:** A total of 209 samples were obtained from clinical cases of OKC in sheep and 60 *Moraxella ovis* isolates were identified by bacteriological techniques and amplification of *16s rRNA* and *rtxA* genes by PCR. All isolates were evaluated in terms of antimicrobial resistance by the disk diffusion susceptibility test and amplification of resistance genes by PCR. **Results:** We found 14 *Moraxella ovis* isolates with antimicrobial resistance (AMR) and five multiresistant (MDR). The *sul1, sul*2, *tetB, qnrA, qnrB,* $Bla_{TEM}$ genes of antimicrobial resistance were amplified, while gene *floR* was not amplified. **Conclusion:** This is the first isolation report of *Moraxella ovis* from ocular lesions in sheep in the State of Mexico, with six antimicrobial resistance genes identified. Results suggest that *Moraxella ovis* plays an important role in the course of the disease and provides a panorama for molecular epidemiological surveillance and bacterial resistance.

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**Keywords**: *antimicrobial resistance; contagious ovine keratoconjunctivitis; epidemiological surveillance; goat; Moraxella spp.; multiresistance; resistance genes; sheep.*

**Resumen**

**Antecedentes:** La queratoconjuntivitis contagiosa ovina (OKC) causa ceguera temporal o permanente en ovinos y caprinos y está asociada a un conjunto de géneros bacterianos, algunos de los cuales muestran resistencia antimicrobiana. **Objetivo:** Identificar la relación fenotípica-genotípica de la resistencia antimicrobiana de aislamientos *Moraxella ovis* obtenidos de casos clínicos de queratoconjuntivitis contagiosa ovina (OKC) en México. **Métodos:** Se obtuvieron un total de 209 muestras de casos clínicos de OKC en ovinos y se identificaron 60 aislamientos de *Moraxella ovis* por técnicas bacteriológicas y amplificación de genes 16s rRNA y *rtxA* por PCR. En todos los aislamientos se evaluó resistencia antimicrobiana mediante prueba de susceptibilidad de difusión en disco y amplificación de genes de resistencia por PCR. **Resultados:** Se encontraron 14 aislamientos de *Moraxella ovis* con resistencia antimicrobiana (AMR) y cinco multirresistentes (MDR). Los genes de resistencia antimicrobiana *sul1, sul*2*, tetB, qnrA, qnrB, Bla*$_{\text{TEM}}$ fueron amplificados, mientras que el gen floR no fue amplificado. **Conclusión:** Este es el primer reporte de aislamiento de *M. ovis* en lesiones oculares en ovinos en el Estado de México, con seis genes de resistencia antimicrobiana identificados. Nuestros resultados sugieren que *Moraxella ovis* juega un papel importante en el curso de la enfermedad y brinda un panorama para su vigilancia epidemiológica molecular y resistencia bacteriana.

**Palabras clave:** *cabra; genes de resistencia; Moraxella spp.; multirresistencia; oveja; queratoconjuntivitis contagiosa ovina; resistencia antimicrobiana; vigilancia epidemiológica.*

**Resumo**

**Antecedentes:** A ceratoconjuntivite contagiosa ovina (OKC) é uma doença infeccioso contagioso que causa cegueira temporária ou permanente em ovinos e caprinos, esta doença está associada a um conjunto de gêneros bacterianos dos quais alguns deles relataram resistência antimicrobiana. **Objetivo:** O objetivo deste estudo foi identificar a relação fenotípica-genotípica da resistência antimicrobiana de *Moraxella* spp. isolados obtidos de casos clínicos de ceratoconjuntivite contagiosa ovina (OKC) no estado do México. **Métodos:** Um total de 209 amostras foram obtidas de casos clínicos de OKC em ovinos.e obtidos e 60 isolados de *Moraxella ovis* foram identificados por técnicas bacteriológicas e amplificação dos genes *16s rRNA* e *rtxA* por PCR. Todos os isolados foram avaliados quanto à resistência antimicrobiana pelo método de teste de suscetibilidade à difusão em disco e pela amplificação de genes de resistência por PCR respectivamente. **Resultados:** Determinamos 14 isolados de *Moraxella ovis* com resistência antimicrobiana (AMR) e cinco multirresistentes (MDR) e amplificou os genes de resistência antimicrobiana *sul1, sul*2*, tetB, qnrA, qnrB, Bla*$_{\text{TEM}}$ e não amplificou o gene floR. **Conclusão:** É o primeiro relato de isolamento de *Moraxella*. ovis em lesões oculares em ovinos no Estado do México e a identificação de seis genes de resistência antimicrobiana. Sugere-se que *Moraxella ovis*. desempenha um papel importante no curso da doença e fornece um panorama de interesse em vigilância epidemiológica molecular e resistência bacteriana.

**Palavras-chave:** *cabra; genes de resistência; Moraxella spp.; multirresistência; ovelha; ceratoconjuntivite contagiosa ovina; resistência antimicrobiana; vigilância epidemiológica.*

## Introduction

Contagious ovine keratoconjunctivitis (OKC) is a disease that causes temporary or permanent blindness in sheep and goats. Its clinical signs range from conjunctivitis to corneal ulcers. Several bacterial pathogens have been isolated from OKC, such as *Moraxella, Mycoplasma*, and *Chlamydia* (Akerstedt and Hofshagen, 2004; Gupta *et al.*, 2014; Jelocnik *et al.*, 2019). However, three species of the genus *Moraxella* have been associated with infectious keratoconjunctivitis in large and small ruminants, such as *Mor. ovis* (Dagnall, 1994a), *Mor. bovis* (Karthik *et al.*, 2017) and *Mor. bovoculi* (Farias *et al.*, 2015). The differential biochemical tests of *Moraxella* species are phenylalaninedeaminase and gelatinase tests, although they may be inconsistent. Molecular tests are based on the amplification of *16s*-23S *rRNA* genes ISR (Shen *et al.*, 2011; O'Connor *et al.*, 2012; Sosa and Zunino, 2013) and the *rxtA* gene that encodes cytotoxin A (Farias *et al.*, 2015).

Keratoconjunctivitis treatment is based on systemic or local antimicrobials such as tetracyclines, sulfonamides, chloramphenicol, and tulathromycin (Alexander, 2010; Maboni *et al.*, 2015), although several *Moraxella* species have shown resistance to those antimicrobials (Catry *et al.*, 2007; Loy and Brodersen, 2014; Maboni *et al.*, 2015). Antimicrobial resistance is associated with the presence of genes within the bacterial genome such as tet and otr that confer resistance to tetracyclines (Martí *et al.*, 2006; Mosquito *et al.*, 2011) *sul* and *dfr* that confer resistance to sulfonamides and trimethoprim (Kerrn *et al.*, 2002; Ho *et al.*, 2009), *cat, cmlA*, and *floR* that confer resistance to phenicols (Schwarz *et al.*, 2004; Chiu *et al.*, 2006), *mph* and *msr* that confer resistance to macrolides and lincosamides (Lüthje and Schwarz, 2006), $Bla_{TEM}$, $Bla_{Rob}$ and $Bla_{CARB}$ that confer resistance to betalactamases (Bush and Jacoby, 2010; Dallenne *et al.*, 2010) and *qnr, gyr*, and *par* that confer resistance to quinolones (Cattoir *et al.*, 2007; Jacoby *et al.*, 2008). Genes such as *tetH, sul2, floR,* $bla_{Rob-1}$, *srt*, (3´)-*Ic, mph* and *msr*, have been described in the genome within a pathogenicity island in *Mor. bovoculi* (Dickey *et al.*, 2016) although no other studies have been reported. Hence, the aim of the present study was to isolate *Moraxella* spp. from ocular lesions in sheep in the State of Mexico (Mexico) and phenotypic-genotypic characterization of the antimicrobial resistance to understand the pathogenesis of these microorganisms in the disease.

## Materials and Methods

The experimental protocol was approved by the Review Commission of the Internal Committee for the Care of Laboratory Animals - Teaching, Research, Service and Production of Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma (State of Mexico, Mexico) (CICUAL-DISP FMVZ).

### *Bacteriological sampling and isolation*

Sample size was calculated considering the clinical cases of OKC through the finite population sampling formula (Jaramillo, 2009). A pilot sampling was used for estimating the *p* indicator, obtaining a prevalence of 0.23 (107/454), and estimating 209 samples. Samples (n=209) were collected from a total of 845 clinically healthy sheep with ocular lesions (e.g. ulcers, blindness) suggestive of OKC by general physical examination and ophthalmological tests in 15 farms from six municipalities of the State of Mexico between February and June 2017. Specimens were obtained by conjunctival swabs in the lesion area without touching the palpebral edge. The samples were placed in Stuart transport medium (STM, Cat. 1058-A, Dibico. Cuautitlán Izcalli, Mexico), maintained in refrigeration at 4 °C, and processed before 24 h (Akerstedt and Hofshagen, 2004).

Regarding sample isolation, inoculations were performed on 5% ovine blood agar plates (ABS, BLL, Cat BD211037. Becton-Dickinson. CDMX, Mexico) and incubated in aerobic conditions at 37 °C for 24-48 h. Colony growth criteria and classical biochemical tests described in the literature were used for identification of *Moraxella* species involved in keratoconjunctivitis (Angelos *et al.*, 2007; Angelos and Ball, 2007; Shen *et al.*, 2011).

*Genotypic identification through the 16s rRNA and rtxA genes of Moraxella* spp.

For DNA extraction, a heated colony was used in a total volume of 100 µl of sterile distilled water heated to 95 ºC for 10 min followed by centrifugation of the cell suspension for 5 min at 9279 G (Eppendorf® Microcentrifuge 5415, Merck KGaA, Darmstadt, Germany) and subsequent DNA collection (Dallenne *et al.*, 2010).

Genes *16s rRNA* and *rtxA* were amplified by a multiplex PCR with a final reaction volume of 25 µl for each one containing: 12.5 µl Master Mix (Gotaq Green Master mix, Cat M7122. Promega Corporation, WI, USA), 1 µl of each primer (*Ovi16S1* F/*Ovis1849* R, *Bviv16S1* F/*Bovi1541* R and *Bovo1915* R) for the *16s rRNA* gene and for the *rtxA* gene (*MbxA* F/MbxA R, *MbvA* F and *MovA* R), 4 µl of bacterial DNA and 7.5 µl nucleases free water (Nuclease-Free Water Cat. P1195. Promega Corporation, WI, USA). The DNA sequences and PCR product sizes are described in Table 1.

The PCR protocol regarding *16s rRNA* gene was as follows: initial denaturation for 5 min at 95 °C followed by 35 cycles: denaturation for 40 s at 95 ºC, alignment for 40 s at 55 ºC, extension for 1 min at 72 ºC; and a final extension for 7 min at 72 °C. For gene *rtxA* 35: cycles denaturation for 50 s at 95 °C, alignment for 50 s at 65 ºC, extension for 1 min at 72 ºC, and a final extension for 4 min at 72 °C carried out in a ThermoCycler (MultiGeneTM Mini, TC 020-24, Labnet International Inc. CA, USA). All the amplification products were identified through horizontal electrophoresis in 1% agarose gels stained with 0.5 µg/mL of ethidium bromide and visualized with a UV transilluminator (Mini-Bis 16mm, DNr Bio-Imaging Systems. Neve Yamin Israel; Shen *et al.*, 2011; Farias *et al.*, 2015). A *Staphylococcus aureus* ATCC 25923 strain was used as negative control.

*Antimicrobial susceptibility tests*

Susceptibility tests were carried out using the disk diffusion method on Mueller Hinton agar (AMH, BD Bioxon. Becton-Dickinson. CDMX, Mexico) supplemented to 5% with ovine blood defibrinated according to the guidelines of the Institute of Clinical and Laboratory standards (CLSI, 2016). The bacterial solution turbidity suspended Mueller-Hinton was adjusted (MH, BBL TM. Becton-Dickinson. CDMX, Mexico) at a scale of 0.5 Mc Farland equivalent to a concentration of 1-2 x$10^8$ CFU/mL, the following antimicrobials were used; ampicillin (10 µg), carbencillin (100 µg), cephalothin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), nitrofurantoin (30 µg) netilmicin (30 µg), gentamicin (10 µg), amikacin (30 µg), sulfamethoxazole/trimethoprim (25 µg), norfloxacin (10 µg), tetracycline (30 µg) and nalidixic acid (30 µg) (BBL ™ Sensi-Disc™.

**Table 1.** Primer designing for *Moraxella* spp. identification using PCR.

| Gene | Species | Primers | Sequences 5'- 3' | Frag-ment size | Reference |
|---|---|---|---|---|---|
| rtxA | *Mor. bovis* | MbxAF | GCA AAA CTG GCA ATG ACG A | 943 bp | Farias *et al.* (2015) |
| | | MbxAR | GTG CCA TTG ACC CAA CTA GC | | |
| | *Mor. bovoculi* | MbvAF | AAT GCT GGT GCT GGT AAC GA | 990 bp | |
| | *Mor. ovis* | MovAR | TGG TTG CAG GGT ATT GGA GC | | |
| 16s rRNA | *Mor. ovis* | Ovi16S1F | GAA CGA TGA GTA TCC AGC TTG CT | 1849 bp | Shen *et al.* (2011) |
| | | Ovis1849R | CTC TTT ACT TTG GTT AAT TAT TTT GTT GGA | | |
| | *Mor. bovoculi* | Bovo1915R | TGT ATT GGG TAC AAT CAC CAT GG | 1859 bp | |
| | | Bviv16S1F | GAA CGA TGA CTA TCT AGC TTG CTA GAT ATG | | |
| | *Mor. bovis* | Bovi1541R | AGC TAT AGA CCC AAT TTA ACT TAC GCT ACT | 1541 bp | |

Becton-Dickinson. CDMX, Mexico). The plates were incubated at 37 °C for 18-24 h. Regarding interpretation of the results, the following profiles were established: sensitive (S), intermediate (I), and resistant (R). An *Escherichia coli* ATCC 25922 strain was used as control. As no standardized criteria for the interpretation of sensitivity exist for *Moraxella* spp., breakpoints established for Gram-negative pathogens related with cattle respiratory disease were used (Maboni *et al.*, 2015) (e.g. critical breakpoints against respiratory pathogens of *Pasteurella multocida, Moraxella catarrhalis, Mannheimia haemolytica, Pseudomona aeruginosa* and *Haemophilus somnus*) (CLSI, 2013; 2016).

*Antimicrobial resistance genes*

Polymerase chain reaction (PCR) was used to identify antimicrobial resistance genes. Seven primers were used. Sequences and amplification products sizes are described in Table 2. Detection of genes *sul1* and *sul2* (sulfonamides), $Bla_{TEM}$ (β-Lactams), *tetB* (tetracyclines), *floR* (florfenicol/chloramphenicol), as well as a multiplex PCR for *qnrA* and *qnrB* genes (quinolones) followed the same reaction conditions previously published (Kerrn *et al.*, 2002; Chiu *et al.*, 2006; Martí *et al.*, 2006; Cattoir *et al.*, 2007; Dallenne *et al.*, 2010). Positive controls used were as follows: *Escherichia coli* ATCC 25922 and other *E. coli* isolates from sewage characterized as phenotypically resistant to AM (ampicillin), CB (carbencillin), CF (cephalothin), CL (chloramphenicol), NA (nalidixic acid), TE (tetracycline), NET (netilmicin), NF nitrofurantoin, CPF (ciprofloxacin) and genotypically possessing *qnrA+, qnrB+* and $Bla_{TEM}$+ genes (Talavera-González *et al.*, 2021).

## Results

A total of 209/861 examined sheep (24.27% prevalence) in six municipalities of the State of Mexico showed lesions compatible with OKC. With respect to the total number of the studied samples (209), we identified 60 isolates of *Moraxella* spp. by biochemical tests. Through the 16s rRNA gene, 54 strains of *Mor. ovis* were correctly identified from the 60 isolates (90%). The 1541 and 1959 pb amplicons corresponding to *Mor. bovis* and *Mor. bovoculi* not amplified by any isolate, respectively. For the *rtxA* gene, 57 strains of *Mor. ovis* were identified of the 60 isolates (95%) (Table 3) and no isolates amplified a band of 943 pb corresponding to *Mor. bovis*.

**Table 2.** Antimicrobial resistance used for the identification of *Moraxella* spp.

| Resistance | Primers | Sequences 5´-3´ | Fragment size | Reference |
|---|---|---|---|---|
| Sulfonamides | *sul1 F* | CGG CGT GGG CTA CCT GAA CG | 433 bp | Kerrn *et al.* (2002) |
| | *sul1 R* | GCC GAT CGC GTG AAG TTC CG | | |
| | *sul2 F* | GCG CTC AAG GCA GAT GGC ATT | 293 bp | Kerrn *et al.* (2002) |
| | *sul2 R* | GCG TTT GAT ACC GGC ACC CGT | | |
| Tetracyclines | *tet B F* | TTG GTT AGG GGC AAG TTT TG | 650 bp | Martí *et al.* (2006) |
| | *tet B R* | GTA ATG GGC CAA TAA CAC CG | | |
| Quinolones | *qnrA F* | AGA GGA TTT CTC ACG CCA GG | 580 bp | Cattoir *et al.* (2007) |
| | *qnrA R* | TGC CAG GCA CAG ATC TTG AC | | |
| | *qnrB F* | GGM ATH GAA ATT CGC CAC TG | 264 pb | Cattoir *et al.* (2007) |
| | *qnrB R* | TTT GCY GYY CGC CAG TCG AA | | |
| β-lactamases | *MultiTSO-T BlaTEM F* | CAT TTC CGT GTC GCC CTT ATT C | 800 bp | Dallenne *et al.* (2010) |
| | *MultiTSO-T BlaTEM R* | CGT TCA TCC ATA GTT GCC TGA C | | |
| Florfenicol/ Chloramphenicol | *floR F* | CTT TGG CTA TAC TGG CGA TG | 266 bp | Chiu *et al.* (2006) |
| | *floR R* | GAT CAT TAC AAG CGC GAC AG | | |

Y=T o C; R=A or G, S=G or C, D=A or G or T, H=A or C or T; M=A o C.

*Antimicrobial susceptibility tests*

The 60 isolates of *Mor. ovis* were sensitive to gentamicin and norfloxacin 100% (60/60). Antimicrobial resistance of 18.3% (11/60) to nalidixic acid was observed, 11.6% (7/60) to nitrofurantoin, 10.0% (6/60) to ampicillin, 6.6% (4/60) to chloramphenicol, 3.3% (2/60) to cephalothin, 3.3% (2/60) to tetracycline, and 1.6% (1/60) to cefotaxime, netilmicin, amikacin and sulfamethoxazole/trimetropim (Table 4).

**Table 3.** *Moraxella* spp. isolates obtained from clinical ovine keratoconjunctivitis cases in sheep in the State of Mexico.

| UPP | Municipality | Animals | Ocular injures* | | | | Samples | Bacteriology | Molecular | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | KC | K | U | | Isolates | *16S rRNA* 1949 pb | *RtxA* 990 pb |
| | | | | | | | | *Mor. ovis* | *Mor. ovis* | *Mor. ovis* |
| | | | 51 | 14 | 2 | - | 67 | 29 | 26 | 26 |
| 4 | Toluca | 189 | 48 | 7 | 1 | - | 56 | 5 | 3 | 5 |
| 3 | Xonacatlán | 117 | 23 | - | - | - | 23 | 4 | 4 | 4 |
| 3 | Lerma | 80 | 20 | 10 | 1 | - | 31 | 10 | 10 | 10 |
| 1 | Capulhuac | 43 | 15 | - | - | - | 15 | 4 | 4 | 4 |
| 1 | Calimaya | 150 | 8 | 8 | - | 1 | 17 | 8 | 7 | 8 |
| 15 | Total | 861 | 16 | 39 | 4 | 1 | 209* | 60 | 54 | 57 |
| | | | | | Prevalence | | 24.27% | 28.70% | 90% | 95% |

*Ocular lesions correspond to the number of animals sampled. UPP: Number of Production Units; C: Conjunctivitis; KC: Keratoconjunctivitis; K: Keratitis; U: Corneal ulcers.

**Table 4.** Susceptibility test of *Moraxella* spp. causing ovine keratoconjunctivitis.

| Antimicrobial | *Mor. ovis* (n= 60) (#) prevalence | | | Zone diameter |
|---|---|---|---|---|
| | Resistance | Intermediate | Susceptible | Interpretive criteria (nearest whole mm) |
| NA | 11(18.3) | 8(13.3) | 41(68.3) | ≤22-≥28 |
| NF | 7(11.6) | 1(1.6) | 52(86.6) | ≤14-≥17 |
| AM | 6(10.0) | 7(11.6) | 47(78.3) | ≤13-≥27 |
| CL | 4(6.6) | 2(3.3) | 54(90.0) | ≤25-≥29 |
| CF | 2(3.3) | 3(5.0) | 55(91.6) | ≤16-≥20 |
| TE | 2(3.3) | 1(1.6) | 57(95.0) | ≤24-≥29 |
| CFX | 1(1.6) | 1(1.6) | 58(96.6) | ≤10-≥13 |
| SXT | 1(1.6) | 6(10) | 53(88.3) | ≥26 |
| NET | 1(1.6) | - | 59(98.3) | ≤12-15 |
| AK | 1(1.6) | - | 59(98.3) | ≤14-17 |
| CP | . | 2(3.3) | 58(96.6) | ≥21 |
| CB | - | 2(3.3) | 58(96.6) | ≤18-≥25 |
| GE | - | - | 60(100) | ≤12-≥15 |
| NOF | - | - | 60(100) | ≤12-≥17 |

#: Isolates; (#): Percentage. AM: Ampicillin; CB: Carbenicillin; CF: Cephalothin; CFX: Cefotaxime; CP: Ciprofloxacin; CL: Chloramphenicol; NF: Nitrofurantoin; NET: Netilmicin; GE: Gentamicin; AK: Amikacin; STX: Sulfamethoxazole/trimethoprim; NOF: Norfloxacin; TE: Tetracycline; NA: Nalidixic acid (CLSI 2013; 2016).

A total of 68.33% (41/60) isolates showed sensitivity to all the antimicrobials used, and 31.66% (19/60) were resistant to one or more antimicrobials. Furthermore, 23.33% (14/60) isolates were resistant to one or two antimicrobials and 8.33% (5/60) were multi-resistant strains (Table 4).

*Phenotypic and genotypic profiles*

*Mor. ovis* presented 36.66% (22/60) and 33.33% (20/60) amplification of *sul1* and *sul*2 gene, respectively. Isolates of *Mor. ovis* 46.66% (28/60) amplified gene $Bla_{TEM}$. The gene that confers resistance to tetracyclines, 8.33% (5/60) of *Mor. ovis* isolates amplified a 650 pb band corresponding to *tetB*. *Mor. ovis* isolates (23/60) 38.33% amplified *qnrA* gene and (22/60) 36.66% *qnrB* gene (Table 5).

## Discussion

An OKC prevalence of 24.27% (209/861) was obtained. This was lower than reported in the United Kingdom (Dagnall, 1994a; 1994b) 72.38% (97/134). Severity of eye lesions was observed, which could be related to the period taken from the samples to the raising systems and predisposing factors. An increase in OKC cases was reported in Norway during the autumn and winter seasons due to animal management: in summer sheep graze without human intervention, while they are confined in barns during the winter (Akerstedt and Hofshagen, 2004). In Mexico, sheep breeding systems vary from intensive, semi-intensive to extensive, mixed, and grazing. In the present study, sampling was carried out in the spring-winter period, which is characterized by the presence of flies, dust, direct sunlight as well as other factors involved in the evolution of the disease (Egwu *et al.*, 1989). Dagnall (1994b) reported a prevalence of 28.86% (28/97) for *Mor. ovis* from ovine isolates. Similarly, Akerstedt and Hofshagen, (2004) obtained a prevalence of 28.23% (24/85) in sheep herds with the disease. Similar data were obtained in this work: 27.75% (58/209) of *Mor. ovis* isolates from sheep with OKC.

The first isolation of *Mor. bovoculi* was reported in the United States (Angelos *et al.*, 2007) and later in countries such as Uruguay (Sosa and Zunino, 2013), Argentina, Norway, and Brazil (Libardoni *et al.*, 2012; Farias *et al.*, 2015). The first report of *Mor. bovoculi* in sheep was made by Farias *et al.* (2015) in Brazil. Karthik *et al.* (2017) were the first researchers who identified *Mor. bovis* from ocular injuries in sheep in India. In our study it was not possible to isolate *Mor. bovis* and *Mor. bovoculi* from ocular injuries in sheep.

Shen *et al.* (2011) amplified the 16s *rRNA* gene in 89.5% (51/57) of the isolates, identifying *Mor. bovoculi* (44/51) and *Mor. bovis* (7/51). In this study 90.0% (54/60) of the isolates were amplified and correctly identified as *Mor. ovis*.

**Table 5.** Phenotypic and genotypic profile of antimicrobial resistance in *Moraxella*.

| Phenotypical resistance | Resistance genes | *Mor. Ovis* (n= 60) | | | Total | Prevalence of re-sistance genes (%) |
|---|---|---|---|---|---|---|
| | | Resistance | Intermediate | Susceptible | | |
| AM | $Bla_{TEM}$ | 5(6) | 6(7) | 17(48) | 28(60) | 46.66 |
| NA | *qnrA* | 8(11) | 1(8) | 14(41) | 23(60) | 38.33 |
| | *qnrB* | 8(11) | 2(8) | 12(41) | 22(60) | 36.66 |
| STX | *sul1* | 1(1) | 1(1) | 20(58) | 22(60) | 36.66 |
| | *sul2* | 1(1) | 1(1) | 18(58) | 20(60) | 33.33 |
| TE | *tetB* | 1(2) | 0(1) | 4(57) | 5(60) | 8.33 |
| CL | *floR* | 0(4) | 0(2) | 0(54) | 0(60) | 0.00 |

#: Gene amplification; #: All isolates. AM: Ampicillin; TE: Tetracycline; CL: Chloramphenicol; STX: Sulfamethoxazole/trimethoprim; NA: Nalidixic acid.

Farias *et al.* (2015) amplified the gene *rtxA* in 100% (33/33) of isolates from bovine and ovine with keratoconjunctivitis, identifying *Mor. bovis* (15/33), *Mor. bovoculi* (11/33) and *Mor. ovis* (7/33). In the present study, we amplified the *rtxA* gene 95% (57/60) of all isolates identifying *Mor. ovis*. Regarding the six *Mor. ovis* isolates that did not amplify the 16s rRNA and the three *Mor. ovis* isolates that did not show amplification for *rtxA* gene, they possibly could be related to the variations in the reading frame of the sequences by deletions or absence of repeated sequence regions surrounding the RTX operon which has been reported in non-hemolytic *Mor. bovis* strains (Angelos *et al.*, 2003). In recent studies, Dickey *et al.* (2018) detected a recombination in the nucleotide sequences within the non-coding regions in the *rRNA* and *RTX* genes in strains of *Mor. bovoculi.*

The first study of antimicrobial sensitivity *in vitro Mor. ovis* strains performed by Elad *et al.* (1988) reported resistance to penicillin, ampicillin, streptomycin and neomycin. Another study, conducted by Catry *et al.* (2007), reported strains resistant to erythromycin. The most recent work by Maboni *et al.* (2015) showed strains resistant to oxytetracycline and penicillin. All the mentioned researchers described *Mor. ovis* strains sensitive to gentamicin, chloramphenicol, florfenicol, and sulfonamides. In the present study we reported strains of *Mor. ovis* susceptible to gentamicin, as well as resistant strains to ampicillin, chloramphenicol, tetracycline, and sulfamethoxazole/trimethoprim.

Oxytetracycline is usually the first choice of treatment for keratoconjunctivitis (Alexander, 2010); however, *Moraxella* spp. showed resistance over time to this antimicrobial (Maboni *et al.*, 2015). Likewise, florfenicol was reported as an effective therapeutic option in keratoconjunctivitis (Gokce *et al.*, 2002; Angelos *et al.*, 2011). The use of antimicrobials is essential for controlling OKC by *Mor. ovis* to avoid exacerbation of lesions associated with other bacterial infections (Dagnall, 1994b).

To the best of our knowledge, this is the first study to amplify the antimicrobial resistance genes *sul1, sul2, tetB, qnrA qnrB y* $Bla_{TEM}$ in *Mor. ovis*. A study associated with antimicrobial resistance determinants (ARD) in *Mor. bovoculi* was performed by Dickey *et al.* (2016); they described 10 ARD located on a genomic island greater than 27 kb in the sequences of *Mor. bovoculi* and Mb58069 isolates that were resistant to florfenicol, oxytetracycline, sulfonamides, and showed intermediate resistance to macrolides.

A similar study, conducted by Roberts *et al.* (1991), described tetracycline-resistant strains of *Mor. catarrhalis* that carry the *tetB* gene on its chromosome. The *tetB* gene has the widest range of Gram-negative bacteria, such as *E. coli* (Medina *et al.*, 2011; Mirzaagha *et al.*, 2011), *Acinetobacter baumannii* (Martí *et al.*, 2006), *Actinobacillus actinomycetemcomitans* (Roe *et al.*, 1995), *Haemophilus influenzae* (Robert and Smith, 1980), and *Treponema denticolaum* (Roberts, 1996).

Bacteria carry resistance genes *sul1, sul*2, *sul3,* and $Bla_{TEM}$ such as *E. coli* (Kerrn *et al.*, 2002; Infante *et al.*, 2005; Ho *et al.*, 2009; Medina *et al.*, 2011; Gnida *et al.*, 2014; Memariani *et al.*, 2015), *Klebsiella pneumoniae, Pseudomona aeruginosa* (Peymani *et al.*, 2017), *Proteus mirabilis* (Feizabadi *et al.*, 2010; Gong *et al.*, 2018), *Salmonella* spp. (Maka *et al.*, 2015), *Stenotrophomonas maltophilia* (Hu *et al.*, 2011). Bacteria carriers of genes *qnrA* and *qnrB* are *K. pneumoniae* (Rodríguez-Martínez *et al.*, 2003), *E coli* (Wang *et al.*, 2003; Jiang *et al.*, 2008; Aguilar-Montes de Oca *et al.*, 2015), *P. aeruginosa*, *Enterobacter cloacae* (Wu *et al.*, 2007), *Actinobacter baumanii* (Touati *et al.*, 2008), *Salmonella enterica* (Murray *et al.*, 2008), *Enterobacter aerogenes, Citrobacter freundii* (Park *et al.*, 2007), *Kluyvera* (Kraychete *et al.*, 2016), among others.

*Mor. ovis* was identified in the present study by using *16s rRNA* and *RtxA* genes with PCR, confirming PCR as the most sensitive test for diagnosing bacterial agents involved in keratoconjunctivitis. It will be possible to establish new criteria for choosing antimicrobials based

on the phenotypic and genotypic characteristics of antimicrobial resistance, which will also allow for molecular epidemiology surveillance of antimicrobial resistance genes in bacterial populations of *Moraxella* spp.

## Declarations

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### *Conflicts of interest*

The authors declare they have no conflicts of interest regarding the work presented in this report.

### *Author contributions*

Acosta-Dibarrat J, Talavera Rojas M, Soriano Vargas E, and Ortiz Arana G designed the experiment. Acosta-Dibarrat J and Ortiz Arana G administered the project. Ortiz Arana G, Palomares-Resendiz EG, Salgado-Miranda C, and Enriquez-Gomez E worked on the aspects involved in the methodology. Ortiz Arana G and Acosta Dibarrat J wrote and prepared the manuscript. All authors provided critical feedback during writing and editing.

### *Use of artificial intelligence (AI)*

No AI or AI-assisted technologies were used during the preparation of this work.

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