

## Biofilm formation and drug resistance determinants of *Moraxella catarrhalis*, *Moraxella osloensis* and *Moraxella lacunata* from clinical samples in Iraq

Manal Hadi Ghaffoori Kanaan <sup>1</sup>, Hiba Riyadh Al-abodi <sup>2</sup>, Sura Saad Abdullah <sup>3</sup>, Mojtaba Memariani <sup>4</sup>, Maryam Kohansal <sup>5</sup>, Abdolmajid Ghasemian <sup>5,\*</sup>

<sup>1</sup>Department of Nursing, Technical Institute of Suwaria, Middle Technical University, Baghdad, Iraq; <sup>2</sup>Department of Environment, College of Science, University of Al-Qadisiyah, Iraq; <sup>3</sup>College of Pharmacy, Ajou University, Suwon 16499, Republic of Korea; <sup>4</sup>Skin Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; <sup>5</sup>Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran

\*Corresponding author: Abdolmajid Ghasemian, Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran, E-mail: majidghasemian86@gmail.com

DOI: 10.22034/HBB.2024.05

Received: September 13, 2023; Accepted: February 3, 2024

### ABSTRACT

In this study, fifty *Moraxella* species were isolated. The antibiotic susceptibility,  $\beta$ -lactamases and minimum inhibitory and bactericidal concentrations were evaluated. The rate of ESBLs among *M. catarrhalis*, *M. osloensis* and *M. lacunata* included 47 % (n=11), 36.84 % (n=7) and 37.5 % (n=3), respectively. The strong biofilm formation was observed among 52 %, 63.5 % and 62.5 % of *M. catarrhalis*, *M. osloensis* and *M. lacunata*, respectively. The rate of the *bro1*, *bro2* and *bla*<sub>CTX-M1</sub> genes in *M. catarrhalis* included 23/23 (100 %), 12/23 (52.17 %) and 5/23 (21.8 %), respectively. Moreover, 14/19 (73.68 %), 8/19 (42.10 %) and 2/19 (10.52 %) of *M. osloensis*, respectively contained these genes. The *bro1* gene was detected in all the *M. lacunata* isolates. *Moraxella* spp highly produced BRO  $\beta$ -lactamases and ESBLs, and deciphered strong biofilm formation.

**Keywords:** *Moraxella* spp, clinical isolates, biofilms, drug resistance

### INTRODUCTION

*Moraxella* species are large Gram-negative, obligate aerobic, non-motile,

boxcar-shaped diplobacilli normally residing within the human upper respiratory tract and are occasionally recovered from the skin and urogenital tract

### *Ghasemian et al.*

[1-2]. Over the past decades, they have emerged as eminent opportunistic human pathogens which mostly cause complications among immunodeficient individuals. The genus encompasses 16 species, but only *M. catarrhalis* is a strict human pathogen. Nowadays, *M. catarrhalis* is regarded as the third most common etiological agent for otitis media and acute sinusitis in children. Along with *Haemophilus influenzae*, *M. catarrhalis* is responsible for a wide spectrum of community acquired respiratory infections such as pneumonia and exacerbations of chronic bronchitis, particularly in elderly individuals with underlying conditions [3-5].

Species other than *M. catarrhalis* are unusual pathogens in humans. *M. osloensis* is a commensal microorganism in the genital tract that may be misdiagnosed with *Neisseria gonorrhoea*. The bacterium has been associated with meningitis [6], endocarditis [7], septic arthritis, osteomyelitis, and catheter-related infections [8]. Most of the infections have been occurred in immunocompromised individuals such as patients with hematological malignancies or transplant recipients. *M. lacunata* is also a commensal inhabitant of the conjunctiva and upper

### *Biofilm formation of Moraxella catarrhalis*

respiratory tract of humans. It can cause a variety of ocular infections including conjunctivitis, keratitis, endocarditis, and otolaryngitis. Cases of ocular infections have been reported from different countries such as Japan, United States and Pakistan. Although keratitis caused by *Moraxella* spp. can be efficiently treated by ophthalmic topical antibiotics, persistent and chronic infections may lead to hypopyon formation and endophthalmitis [9-10]. The *Moraxella* genus is primarily diagnosed via brick shaped formation (diplobacilli). *M. lacunata* is a commensal bacterium with low virulence rate; however, immunodeficient, vulnerable and those patients with underlying diseases are at highest risk of infection [10]. The bacterium was recently isolated from a six months immunodeficient child who received eculizumab [5]. These pathogens have tropism to eye infections with an increasing trend in recent years [11-13]. Several clinical infections such as conjunctivitis, blepharitis, upper respiratory tract, keratitis and systemic infections have been reported from *M. osloensis* [13]. *Moraxella*-associated conjunctivitis caused by *M. osloensis* and *M. nonliquefaciens* persists for weeks, manifested as a follicular conjunctivitis,

*Ghasemian et al.*

and may be misdiagnosed with that caused by *Chlamydia* spp [9].

The surge in antibiotic resistance among respiratory pathogens has evolved gradually as a global issue. A close observation of shifting trends in antimicrobial resistance directly influences the selection of antimicrobial agents to eradicate a particular pathogen. Beta-lactamase-positive strains of *M. catarrhalis* were discovered for the first time in 1977 in Sweden [14]. Over the years, these strains have been reported from multiple countries at different rates exceeding 90.0 % [15]. Unfortunately, increasing resistance towards third-generation cephalosporins, macrolides, and quinolones were also observed in different countries [14-17]. Additionally, antibiotic susceptibility testing for *Moraxella* spp. is not routinely conducted, and almost all the resistance data comes from large cities, with only few studies performed in rural areas. To our knowledge, scarce data on frequencies of antibiotic resistance among *Moraxella* spp. in Iraq are available.

A Biofilm is defined as a multi-cellular bacterial community encompassed in an extracellular polymeric substance matrix. It enhances microbial adhesion, persistence,

***Biofilm formation of Moraxella catarrhalis***

survival, and resistance to antimicrobials by forming physical barrier shielding embedded bacterial species [18]. Biofilm formation capacity has been linked to the colonizing ability of the *Moraxella* species. Until now, most of studies have been focused on *M. catarrhalis* ability to develop biofilm *in vitro*, singly [18] or in combination with other otopathogens [19-21].

In the present study, the antibiotic resistance patterns,  $\beta$ -lactamases and biofilm formation as well as frequencies of those genes encoding common  $\beta$ -lactamases were assessed among *Moraxella* spp. isolated from clinical specimens in Baghdad, Iraq.

## **MATERIALS AND METHODS**

### ***Sampling and identification of bacterial strains***

A total of 260 patients (114 males and 146 females) were enrolled in the present study during Jan 2020-2021 from A and B hospitals. These patients suffered from respiratory infections (including pneumonia, acute rhinosinusitis, and otitis), keratitis, and septicemia. Specimens were collected from respiratory tract (n=189), cornea (n=51) and blood (n=20).

### *Ghasemian et al.*

The inclusion criteria included all the patients with clinical signs and exclusion criteria included consumption of antibiotics during two weeks ago.

Briefly, the samples were cultured onto the sheep blood agar (non-hemolytic, dry, opaque, grey to white and smooth) and chocolate agar media and incubated at 37 °C overnight [3]. Afterwards, suspected colonies were further subjected to routine microbiological and biochemical tests such as API 20NE system (bioMérieux, Marcy l'Etoile, France), Gram staining, oxidase, catalase, DNase and tributyrin tests, reduction of nitrate to nitrite, and carbohydrate fermentation (Merk, Germany). Molecular identification of isolates was also implemented using Polymerase Chain Reaction (PCR). Specific primers included McF: 5'CAGGCCTAACACATGCAAGTC-3', McR: 5'GGGCGGAGTGTACAAGGC3', with 1360 bp product, MoF: 5'AAATGCGAGAACGCAGGTTG3', MoR: 5'CCTTTCGGACTATTGGCGGT3' (CCUG 350 reference genome, 101bp fragment), MIF: 5'CGTGTTGACCGTTTTGACTTT3' and MIR: 3'CATAGATTAGGTTACCGCTGACG5'

### *Biofilm formation of Moraxella catarrhalis*

. In a total volume of 25 µL, Mater Mix (18 µL), deionized H<sub>2</sub>O (3 µL), DNA template (10 µg, 2 µL) and each primer (1.0 µM, 1 µL) were mixed. Detailed PCR reaction conditions have been described by [22-23].

### *Antibiotic susceptibility testing*

The antibiotic susceptibility of isolates was performed on Mueller-Hinton agar medium containing 5% sheep blood by disk diffusion method as previously described [22]. The following antibiotics (MAST, UK) were tested: trimethoprim-sulfamethoxazole (1.25-23.75 µg), tetracycline (30 µg), ampicillin (10 µg), erythromycin (15 µg), cefotaxime (5 µg), cefepime (30 µg), amoxicillin-clavulanic acid (AMC) (2 µg), and ciprofloxacin (5 µg). The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values were determined against tetracycline, erythromycin and cefepime using agar dilution method. Nitrocefin test was also used for β-lactamase production. This test was implemented using 20 µL of each nitrocefin and bacterial solution (0.1 M phosphate buffer, pH 7.0). Color change from yellow to red within 1-2 min indicated a positive test [4]. In addition, the production of Extended-Spectrum β-Lactamase (ESBL) enzymes was evaluated

### *Ghasemian et al.*

using synergy tests (co-amoxiclav plus ceftazidime) as previously described. The both disks were placed near each other onto the MHA medium and an increased growth inhibitory zone between them outlined ESBL production [24]. BRO beta-lactamases were also screened using PCR amplification of BROF: 5'TRGTGAAGTGATTTTKRRMTTG3' and BROR: 5'GCAATTTATTA ACTGGATGTA3' primers yielding 165 (*bro1* gene) and 144 bp (*bro2*) fragments [25].

### ***Minimum inhibitory concentration***

The minimum inhibitory concentration (MIC) of ampicillin, cefotaxime and ceftazidime antibiotics was determined using microdilution broth method. The range of dilutions included 0.5-512 µg/mL. The test was followed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [5].

### ***Biofilm formation assay***

The biofilm formation was assessed using microtiter tissue plate assay, as described earlier [18,20]. Briefly, in 96-well plates, the trypticase soy broth (200 µL) and bacterial suspension equal to the 0.5 Mac Farland turbidity (150 µL) were inoculated in 96-well microplates and incubated

### ***Biofilm formation of *Moraxella catarrhalis****

overnight. The wells were washed by sterile water and subsequently fixed with methanol. The crystal violet dye (0.1%) was added to wells and left for 15min at room temperature. The washing was repeated and the ethanol was added to the wells. Finally, the absorbance rate was measured using an ELISA reader at 590 nm. Those wells contained the only medium without bacterial inoculations were considered as negative control. The biofilm formation levels were measured using the following formula: strongly biofilm ( $OD > 4 \times OD_c$ ), moderate biofilms ( $2 \times OD_c < OD \leq 4 \times OD_c$ ), weak biofilms ( $OD_c < OD \leq 2 \times OD_c$ ), and non-biofilm producer ( $OD \leq OD_c$ ).  $OD_c$  stands for the control absorbance rate [20,24,26-27].

### ***Cellular cytotoxicity assay***

HaCaT (human keratinocytes) cells ( $10^5$  cells/mL) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) added with 10 % fetal bovine serum, penicillin (100 Units/mL), and streptomycin (100 µg/mL), and incubated at 37 °C and 5 % CO<sub>2</sub> in a humidified incubator. Briefly, bacterial suspensions equal to the Half Mac Farland were prepared and inoculated into the fresh medium containing normal cells. Finally, cellular morphology changes were

*Ghasemian et al.*

observed as the result of possible cytotoxic factors produced by bacterial strains [6].

### ***Detection of $\beta$ -lactamase genes***

The DNA was extracted using boiling method. The quality and quantity of DNA was evaluated using NanoDrop device and agarose gel electrophoresis and stored at -20°C. The PCR was employed for the amplification of *bro1*, *bro2*, *bla<sub>CTX-M1</sub>*, *bla<sub>TEM1</sub>*, *bla<sub>SHV</sub>*, *bla<sub>AMPC</sub>*, *bla<sub>NDM</sub>*, *bla<sub>KPC</sub>*, *oxa-23-like*, *oxa-24-like*, *oxa-51-like*, *oxa-58-like*, *bla<sub>OXA-48-like</sub>* genes. The sequences of primers and amplification conditions have been described previously [22,24,28].

The reaction conditions for the genes have been depicted in table 1. In a total volume of 50  $\mu$ L, the PCR master mix (30  $\mu$ L), Taq DNA polymerase (4  $\mu$ L, 1 U, Fermentas-Lithuania), template DNA (10  $\mu$ g, 4  $\mu$ L), ddH<sub>2</sub>O (10  $\mu$ L) and 1  $\mu$ M (1  $\mu$ L) of each primer was added. The amplified products sizes included *bla<sub>CTX-M1</sub>* (550 bp), *bla<sub>SHV</sub>* (1016 bp), *bla<sub>TEM1</sub>* (980 bp), *bla<sub>AmpC</sub>* (440 bp), *bla<sub>IMP1</sub>* (182 bp), *bla<sub>KPC</sub>* (282 bp), *bla<sub>NDM1</sub>* (1015 bp), *bla<sub>OXA-48-like</sub>* (484 bp). The positive and negative control included DNA of strains carrying the genes and distilled H<sub>2</sub>O, respectively.

## ***Biofilm formation of *Moraxella catarrhalis****

### ***Analysis of data***

The data was analyzed using the IBM SPSS software version 21. The analysis of variance (ANOVA) and t-test were used at a significance level of 0.05.

## **RESULTS**

### ***Isolation of *Moraxella* spp from various specimens***

Out of 260 samples, 50 *Moraxella* spp were identified including 23 *M. catarrhalis*, 19 *M. osloensis* and 8 *M. lacunata*. These strains were recovered from respiratory tract (n=32), conjunctivitis (n=14), keratoconjunctivitis (n=3) and blood (n=1) samples, as shown in Table 2.

### ***Antibiotic Susceptibilities of *Moraxella* spp***

Out of 23 *M. catarrhalis* isolates, high resistance rates were observed for ampicillin (78 %, n=18) and cefotaxime (69 %, n=16), followed by trimethoprim-sulfamethoxazole (n=12, 52 %), cefepime (n=10, 43.5 %), AMC (n=8, 34 %), ciprofloxacin (n=10, 43.5%), erythromycin (n=9, 39.2 %) and tetracycline (n=8, 34 %). Moreover, *M. osloensis* resistance pattern included 37 % (n=7), 16 % (n=3), 31.6 % (n=6), 63.15 % (n=12), 10.52 % (n=2), 16

**Ghasemian et al.**

% (n=3), 0 % and 10.52 % (n=2), respectively. The resistance rate of *M. lacunata* to these disks respectively included 25 % (n=2), 0 % (n=0), 50 %

**Biofilm formation of *Moraxella catarrhalis***

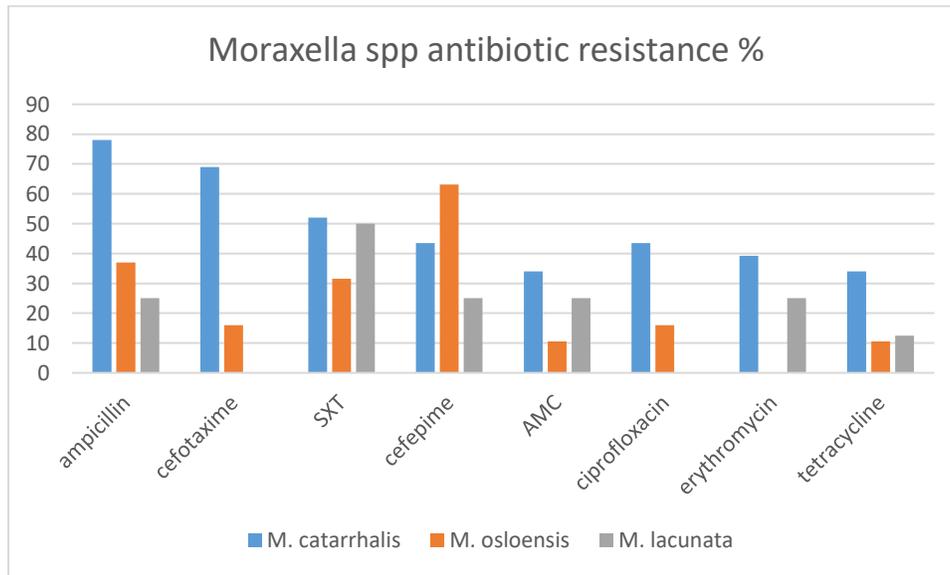
(n=4), 25 % (n=2), 25 % (n=2), 0 %, 25 % (n=2) and 12.5 % (n=1) (Figure 1).

**Table 1.** PCR reaction conditions in this study

Antibiotic resistance genes	Annealing T (°C)	One cycle: 94°C ----- 6 min. 33 cycle: 94°C ----- 70 s Annealing 72°C ----- 90 s One cycle: 72°C ----- 8 min
<i>bla</i> <sub>CTX-M1</sub>	59	
<i>bla</i> <sub>SHV</sub>	56	
<i>bla</i> <sub>TEM1</sub>	61	
<i>bla</i> <sub>AmpC</sub>	61	
<i>bla</i> <sub>KPC</sub>	64	One cycle: 95°C ----- 4 min. 30 cycles: 94°C ----- 45 s Annealing 72°C ----- 40 s One cycle: 72°C ----- 5min
<i>bla</i> <sub>NDM1</sub>	61	
<i>bla</i> <sub>OXA-48-like</sub>	65	
<i>bla</i> <sub>IMP1</sub>	62	

**Table 2.** Distribution of *Moraxella* spp. based on gender and source of samples; comparison between males and females (p=0.004), among various clinical specimens (p=0.002) and association of previous hospitalization (p<0.001) and antibiotic use (p<0.0001)

Demographic data/Species	<i>M. catarrhalis</i> n=23 (%)	<i>M. osloensis</i> n=19 (%)	<i>M. lacunata</i> n=8 (%)	p value
Males	11 (47)	8 (42)	3 (37.5)	
Females	12 (52)	11 (58)	5 (62.5)	
Respiratory	18 (78)	3 (15.78)	4 (50)	
Conjunctivitis	2 (8)	12 (63)	4 (50)	
Kerato-conjunctivitis	0.0	4 (21)	0.0	
Blood	3 (13)	0.0	0.0	
Previous hospitalization	18 (78)	14 (73.7)	8 (100)	<0.001
Previous antibiotic use	19 (82.6)	16 (84)	8 (100)	<0.0001



**Figure 1.** The antibiotic susceptibility pattern of *Moraxella* spp, AMC: amoxicillin-clavulanic acid, SXT: trimethoprim-sulfamethoxazole, X axis: bacterial isolates and antibiotics, Y axis: percentage of resistance.

### ***Ghasemian et al.***

The frequencies of phenotypic ESBL production among *M. catarrhalis*, *M. osloensis*, and *M. lacunata* were 47% (n=11), 36.84 % (n=7) and 37.5 % (n=3), respectively. As for MIC and MBC, *M. catarrhalis* had MICs of 0.5-8 µg/mL against cefepime, 0.25-4 µg/mL for erythromycin and 0.5-4 µg/mL for tetracycline. These values were similar for *M. osloensis* isolates.

### ***Minimum inhibitory concentration***

*M. catarrhalis* minimum inhibitory concentration of ampicillin, cefotaxime, ceftazidime, cefepime, erythromycin and tetracycline respectively ranged 0.5-8 µg/mL, 1-8 µg/mL and 0.5-8 µg/mL, 0.5-8 µg/mL, 0.25-4 µg/mL and 0.5-4 µg/mL, respectively.

### ***Biofilm production***

Among 23 *M. catarrhalis*, 52 % (n=12) and 48 % (n=11) were strong and moderate biofilm producers, respectively. As for *M. osloensis*, 63.15 % (n=12) were strong biofilm producers, while 21 % (n=4) produced moderate biofilms and only 3 (16 %) strains were weak biofilm producers. Concerning *M. lacunata*, strong and moderate biofilm production were observed among 62.5 % (n=5) and 37.5 % (n=3) of strains, respectively.

### ***Biofilm formation of Moraxella catarrhalis***

The association of strong biofilm formation and ESBLs or carbapenemases has been represented in Table 3. In this respect, there was a significant association between ESBL or carbapenemase production and strong biofilm formation among *M. catarrhalis*, as is the case for *M. osloensis* and *M. lacunata*.

### ***Cell cytotoxicity experiment***

In HaCaT human cell line, 14 *M. catarrhalis*, 9 *M. osloensis* and 3 *M. lacunata* isolates at 0.5 Mac Farland concentration caused the deformation and toxicity against cells. A concentration of 10<sup>6</sup> CFU/mL of bacterial strains was used. *M. catarrhalis* strains caused the mean death of 64 % 89 % of cells after 24 h and 48 h, respectively. *M. osloensis* caused the mean death of 56 % and 79 % of cells, after 24 h and 48 h, respectively. In addition, *M. lacunata* caused the death rate of 49 % and 68 % of cells, respectively.

### ***Detection of β-lactamase genes***

BRO β-lactamases were also found in all the *M. catarrhalis*, and *M. lacunata* and 36.84% (n=7) *M. osloensis*. The rate of the *bro1*, *bro2* and *bla<sub>CTX-M1</sub>* genes in *M. catarrhalis* included 23/23 (100 %), 12/23 (52.17 %) and 5/23 (21.8 %), respectively. Moreover, 14/19 (73.68 %), 8/19 (42.10

%) and 2/19 (10.52 %) of *M. osloensis* respectively contained these genes. The *bro1* gene was detected in all the *M. lacunata* isolates. The isolates carried

none of the other  $\beta$ -lactamase genes (Table 4).

**Table 3.** The association of ESBL production and strong biofilm formation in *Moraxella* spp

Isolates	Strong biofilm	ESBLs	<i>p</i> value
<i>M. catarrhalis</i>	52% (n=12)	47% (n=11)	<0.0001
<i>M. osloensis</i>	63.15% (n=12)	36.84% (n=7)	<0.001
<i>M. lacunata</i>	62.5% (n=5)	37.5% (n=3)	0.136

ESBLs: extended-spectrum  $\beta$ -lactamases

**Table 4.** The existence of  $\beta$ -lactamase genes among *Moraxella* spp

Species	<i>bro1</i>	<i>bro2</i>	<i>bro1+bro2</i>	<i>bla</i> <sub>CTXM-1</sub>	MDR phenotype
<i>M. catarrhalis</i>	23/23	12/23	12/23	5/23	9/23
<i>M. osloensis</i>	14/19	8/19	2/19	1/19	2/19
<i>M. lacunata</i>	8/8	1/8	0	0	1/8

MDR: multidrug-resistant

**DISCUSSION**

Given that the prevalence of *Moraxella* species has increased globally over the past decades, we have begun to pay attention to these opportunistic pathogens as significant causes of various infections [29-30]. In

view of the lack of data on *Moraxella* frequencies in Iraq, the present study can be considered a novel study in the country. In our survey, *M. catarrhalis* constitute almost half (%) of the isolated strains, while *M. lacunata* has the least prevalence. As expected, the majority of *M catarrhalis*

### *Ghasemian et al.*

strains (18 out of 23) were isolated from respiratory infections. These results are in agreement with those of previous studies from Pakistan [14], Iran [14], Egypt [31], and China [32]. In the current investigation, *M. osloensis* was the second most prevalent species. *Moraxella* infections, particularly those related to *M. osloensis*, of the eye are scarcely described in the literature [9,33-34]. It has been shown that *M. nonliquefaciens* has been frequently isolated from keratitis and endophthalmitis, whereas *M. osloensis* is implicated in conjunctivitis [9]. Concerning *M. lacunata*, it was the least common species in our study. It has been isolated from ocular infections [35], osteomyelitis [12], endocarditis [36] and septicemia [37].

In our survey, there was no significant difference between males and females but significant higher rate of isolates were from upper respiratory tract. Noticeably, previous hospitalization and antibiotic use were significant risk factors of isolation of drug-resistant strains. *Moraxella* spp cause various opportunistic infections such as Keratitis among immunocompromised patients [10,34,42].

Before 1970s, *Moraxella* was susceptible to beta-lactam antibiotics. However, the

### *Biofilm formation of Moraxella catarrhalis*

pathogen became increasingly resistant towards penicillins and cephalosporins. Across Australia, Europe, and the United States, over 90 % of the strains produce beta-lactamase [14-15,39]. In the present study, more than half of the strains were resistant to ceftazidime (a third generation cephalosporin), which is higher than those reported in other countries such as Pakistan [14] China [15], Taiwan [17], India [39], and Iran [16]. We also noticed that the majority of cefotaxime-resistant strains were ESBL producers. Accordingly, it is clear that antibiotics such as penicillin, ampicillin, cephalosporins, and other beta-lactams can no longer be used as a reliable drug for eradication of *Moraxella* spp infections. Noticeably, high rates of resistance towards tetracycline was also observed in our study, which was higher than those reported from Iran [16], Taiwan [17], and China [15]. This may be due to presence of efflux pumps including AcrAB-OprM, which can lead to MDR phenotype [16,40]. Alarmingly, development of *Moraxella* spp isolates resistance to quinolones and macrolides is a concern [41]. *M. catarrhalis* MIC ranges of 0.5-8 µg/mL, 1-8 µg/mL and 0.5-8 µg/mL, 0.5-8 µg/mL, 0.25-4 µg/mL and 0.5-4 µg/mL was determined against

### *Ghasemian et al.*

ampicillin, cefotaxime, ceftazidime, cefepime, erythromycin and tetracycline respectively. The MIC and MBC values have not been determined in previous studies among *Moraxella* spp, but the species carried macrolide resistance genes [42,43].

BRO  $\beta$ -lactamases were also found in all the *M. catarrhalis*, and *M. lacunata* and 36.84 % (n=7) *M. osloensis*. The rate of the *bro1*, *bro2* and *bla*<sub>CTX-M1</sub> genes in *M. catarrhalis* included 23/23 (100 %), 12/23 (52.17 %) and 5/23 (21.8 %), respectively. Moreover, 14/19 (73.68 %), 8/19 (42.10 %) and 2/19 (10.52 %) of *M. osloensis* respectively contained these genes. The *bro1* gene was detected in all the *M. lacunata* isolates. The isolates carried none of the other  $\beta$ -lactamase genes.

The strong biofilm formation was observed among 52 %, 63.5 % and 62.5 % of *M. catarrhalis*, *M. osloensis* and *M. lacunata*, respectively which were significant associated to the ESBL production. In other words, these isolates had significantly higher ability for biofilm formation. Biofilm formation has not been evaluated in previous studies, but several virulence factors of *M. catarrhalis* such as outer membrane proteins (USPA1, USPA2H and

### *Biofilm formation of Moraxella catarrhalis*

USPA2V) causing immune evasion and serum resistance, MID Hag causing haemagglutination and IgD binding, CopB participating in iron acquisition has been mentioned [21-24]. We observed that *Moraxella* spp had the ability of deformation and exerting cell cytotoxicity against HCaT human normal cells.

Major limitations of this study included lack of genetic evaluation of virulence factors, gene expression and *in vivo* characterization of *Moraxella* spp pathogenicity mechanisms.

### CONCLUSION

In this study, 23 *M. catarrhalis*, 19 *M. osloensis* and eight *M. lacunata* isolates were identified among clinical samples from hospitalized patients. Isolates were mostly resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline and cefotaxime disks. Majority of isolates carried BRO  $\beta$ -lactamases. ESBLs production was occurred in lower than half of them. The strong biofilm formation was observed in higher than half of *Moraxella* spp. *Moraxella* spp had the ability of deformation and exerting cell cytotoxicity against human normal cells. Future studies will be helpful towards

*Ghasemian et al.*

genetic evaluation of various virulence determinants of *Moraxella* spp.

## REFERENCES

- [1]. Jafari-Sales, Abolfazl, Hossein Soleimani, and Leila Moradi. Antibiotic resistance pattern in *Klebsiella pneumoniae* strains isolated from children with urinary tract infections from Tabriz hospitals. *Health Biotechnol Biopharm.* 2020; 4(1): 38-45.
- [2]. Rezaie, Niloofar, *et al.* Isolation of MDR *Providencia stuartii* from tracheal aspirates of two hospitalized patients. *Health Biotechnol Biopharm.* 2022; 6(3): 73-79.
- [3]. Hays JP. *Moraxella catarrhalis*: A mini review. *J Pediatr Infect Dis.* 2009; 4(03): 211–20.
- [4]. de Vries SPW, Bootsma HJ, Hays JP, Hermans PWM. Molecular aspects of *Moraxella catarrhalis* pathogenesis. *Microbiol Mol Biol Rev.* 2009; 73(3): 389–406.
- [5]. Bicoll, Paige S., *et al.* Eculizumab-associated *Moraxella lacunata* Bacteremia and systemic inflammatory response syndrome in

*Biofilm formation of Moraxella catarrhalis*

a toddler with atypical hemolytic Uremic syndrome. *Clin Med Insights Pediatr.* 2021; 15: 1179556521992367.

- [6]. Roh, Kyoung Ho, *et al.* Three cases of *Moraxella osloensis* meningitis: a difficult experience in species identification and determination of clinical significance. *J Korean Med Sci.* 2010; 25(3): 501–504.
- [7]. Gagnard, Jean-Charles, *et al.* *Moraxella osloensis*, an emerging pathogen of endocarditis in immunocompromised patients? *Swiss Med Wkly.* 2015; 145(3738).
- [8]. Gagnard, Jean-Charles, *et al.* An unusual osteomyelitis caused by *Moraxella osloensis*: A case report. *Int J Surg Case Rep.* 2017;41: 146–49.
- [9]. LaCroce, Samantha J., *et al.* *Moraxella nonliquefaciens* and *M. osloensis* are important *Moraxella* species that cause ocular infections. *Microorganisms.* 2019; 7(6): 163.
- [10]. Papakonstantinou, Panteleimon E., *et al.* *Moraxella keratitis*: epidemiology and outcomes. *Eur J Clin Microbiol Infect Dis.* 2020;

- 39(12): 2317–25.
- [11]. Sawada, Nami, *et al.* Moraxella lacunata infection accompanied by acute glomerulonephritis. *Open Med.* 2020; 15(1): 962–67.
- [12]. Mehmeti, Fortesa, *et al.* Moraxella lacunata subacute osteomyelitis in a child. *J Pediatric Infect Dis Soc.* 2021; 10(2):161–63.
- [13]. Walker DK, Altamimi S, Henneberry J. Moraxella lacunata Bacteremia and Sepsis associated with Ecthyma Gangrenosum: A case report and review of the literature. *Infect Dis Clin Pract.* 2017;25(3): 131–33.
- [14]. Shaikh, Safia Bader Uddin, *et al.* Prevalence and resistance pattern of Moraxella catarrhalis in community-acquired lower respiratory tract infections. *Infect Drug Resist.* 2015; 8: 263.
- [15]. Shi, Wei, *et al.*  $\beta$ -Lactamase production and antibiotic susceptibility pattern of Moraxella catarrhalis isolates collected from two county hospitals in China. *BMC Microbiol.* 2018; 18(1): 1–6.
- [16]. Mohammad Shafiei P, *et al.*

- Investigating the antibiotic resistance prevalence and phenotypic and genotypic evaluation of AcrAB-OprM efflux pump in multidrug-resistant in clinical isolates of Moraxella catarrhalis in Kazerun City, Iran. *Iran J Med Microbiol.* 2020; 14(5): 388–407.
- [17]. Hsu, Shih-Fen, *et al.* Antimicrobial resistance of Moraxella catarrhalis isolates in Taiwan. *J Microbiol Immunol Infect.* 2012; 45(2):134–40.
- [18]. Bair, Kirsten L., and Anthony A. Moraxella catarrhalis promotes stable polymicrobial biofilms with the major otopathogens. *Front Microbiol.* 2020;10:3006.
- [19]. Tan, Aimee, *et al.* Moraxella catarrhalis NucM is an entry nuclease involved in extracellular DNA and RNA degradation, cell competence and biofilm scaffolding. *Sci Rep.* 2019; 9(1): 1–14.
- [20]. Bedenić B, Meštrović T. Mechanisms of Resistance in gram-negative urinary pathogens: From country-specific molecular insights to global clinical relevance. *Diagnostics.* 2021; 11(5): 800.

- [21]. Matejka, Karen M., *et al.* Antibiotic susceptibility of *Moraxella catarrhalis* biofilms in a continuous flow model. *Diagn Microbiol Infect Dis.* 2012; 74(4): 394–98.
- [22]. Million, Matthieu, *et al.* Metagenomic analysis of microdissected valvular tissue for etiological diagnosis of blood culture–negative endocarditis. *Clin Infect Dis.* 2020; 70(11): 2405–12.
- [23]. Gadsby NJ, *et al.* Development of two real-time multiplex PCR assays for the detection and quantification of eight key bacterial pathogens in lower respiratory tract infections. *Clin Microbiol Infect.* 2015; 21(8): 788.
- [24]. Ghasemian A, *et al.* Expression of adhesin genes and biofilm formation among *Klebsiella oxytoca* clinical isolates from patients with antibiotic-associated haemorrhagic colitis. *J Med Microbiol.* 2019; 68(7): 978–85.
- [25]. Khan MA, *et al.*  $\beta$ -lactamase and antibiotic resistances in a global cross-sectional study of *Moraxella catarrhalis* from children and adults.

- J Antimicrob Chemother.* 2010; 65(1): 91–97.
- [26]. Alebel M, *et al.* Extended-spectrum  $\beta$ -Lactamase and Carbapenemase producing gram-negative Bacilli infections among patients in intensive care units of Felegehiwot Referral hospital: A prospective cross-sectional study. *Infect Drug Resist.* 2021; 14: 391.
- [27]. Alkhudhairy, Miaad K., *et al.* Comparison of adhesin genes expression among *Klebsiella oxytoca* ESBL-non-producers in planktonic and biofilm mode of growth, and imipenem sublethal exposure. *Microb Pathog.* 2019; 134: 103558.
- [28]. Kanaan, Manal Hadi Ghaffoori, *et al.* Drug resistance and virulence traits of *Acinetobacter baumannii* from Turkey and chicken raw meat. *Comp Immunol Microbiol Infect Dis.* 2020;70:101451.
- [29]. AkhoundiMeybodi, Zohreh, *et al.* Comparison of surgical site infection using active and passive surveillance methods in two months. *Health Biotechnol Biopharma,* 2022; 6(2): 52-63.
- [30]. Roitman, Karina L., *et al.* Study of the genetic diversity of *Moraxella* spp. isolates

**Ghaseemian et al.**

obtained from corneal abscesses. *Revista Argentina de Microbiología*. 2023; 55(1): 20-24.

[31]. Kanaan, Manal Hadi Ghaffoori, et al. Significance of *Moraxella catarrhalis* as a causative organism of lower respiratory tract infections. *Egypt J Chest Dis Tuberc*. 2017; 66(3): 459–64.

[32]. Liu Y-Li, et al. *Moraxella catarrhalis* macrolide-resistant isolates are highly concentrated in two MLST clonal complexes-CCN10 and CC363. *Front Microbiol*. 2017; 8: 201.

[33]. Inoue H, et al. Clinical characteristics and bacteriological profile of *Moraxella keratitis*. *Cornea*. 2015; 34(9):1105–1109.

[34]. Tobimatsu, Yui, et al. Clinical characteristics of 17 patients with *Moraxella keratitis*. In: *Seminars in Ophthalmol* 2018. 726–32.

[35]. Bharathi, M. Jayahar, et al. Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. *Indian J Ophthalmol*. 2010; 58(6): 497.

**Biofilm formation of *Moraxella catarrhalis***

[36]. Noman, Fatima, et al. “*Moraxella lacunata*” endocarditis treated with penicillin. *J Pak Med Assoc*. 2008;58(6):337.

[37]. Nakayama, Asami, et al. *Moraxella lacunata* infection associated with septicemia, endocarditis, and bilateral septic arthritis in a patient undergoing hemodialysis: a case report and review of the literature. *J Infect Chemother*. 2014; 20(1): 61–64.

[38]. Enright MC, McKenzie H. *Moraxella* (Branhamella) *catarrhalis*-clinical and molecular aspects of a rediscovered pathogen. *J Med Microbiol*. 1997; 46(5): 360–71.

[39]. Raveendran S, et al. *Moraxella catarrhalis*: a cause of concern with emerging resistance and presence of bro beta-lactamase gene—report from a tertiary care hospital in south India. *Int J Microbiol*. 2020; 2020.

[40]. Spaniol V, et al. *Moraxella catarrhalis* AcrAB-OprM efflux pump contributes to antimicrobial resistance and is enhanced during cold shock response. *Antimicrob Agents Chemother*. 2015; 59(4): 1886–94.

***Ghasemian et al.***

- [41]. Lee, Harim, et al. Antimicrobial activity of doripenem and other carbapenems against gram-negative pathogens from Korea. *Microb Drug Resist.* 2011; 17(1): 37–45.
- [42]. Uzuner, Erez, et al. "The role of MiRNA in cancer: pathogenesis, diagnosis, and treatment." *miRNomics: MicroRNA Biol Comput Anal* (2022): 375.
- [43]. Hill DJ, Virji M. A novel cell-binding mechanism of *Moraxella catarrhalis* ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. *Mol Microbiol.* 2003; 48(1): 117–29.
- [44]. Sosa, V. and P. Zunino, Molecular and phenotypic analysis of *Moraxella* spp.

***Biofilm formation of Moraxella catarrhalis***

- associated with infectious bovine keratoconjunctivitis in Uruguay. *Vet J,* 2012. 193(2): 595-97
- [45]. Vergis, J., *et al.*, A comparative study for detection of extended spectrum  $\beta$ -lactamase (ESBL) production by Enterococcal *Escherichia coli* (EAEC) strains using double disc, nitrocefin and PCR assays. *J Microbiol Methods,* 2018. 151: 57-61
- [46]. Clinical and L.S. Institute, *CLSI supplement M100.* 2020.
- [47]. Tao, R., *et al.*, Connexin26 Modulates Radiation-Induced Skin Damage by Regulating Chemokine CCL27 through MAPK Signaling. *Radiation Res,* 2023. 200(3): 281-88.