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First data on antimicrobial susceptibility patterns of *Moraxella catarrhalis* isolates in Lebanon

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Abstract

Background: *Moraxella catarrhalis* is an important bacterial pathogen. However, no data regarding this human pathogenare currently available in Lebanon. This study aimed to determine for the first time the antimicrobial susceptibility profiles of *M. catarrhalis* isolates in Lebanon.

Methods: A total of 34 *M. catarrhalis* strains were isolated from clinical specimensduring the period from November 2010 to March 2019. Bacterial identification was performed using MALDI-TOF MS. Antibiotic susceptibility of allisolates was interpreted according to EUCAST recommendations.

Results: A total of 34 non-duplicated *M. catarrhalis* strains were isolated from patients referred to Nini Hospital in Tripoli, Lebanon. Regarding antibiotic susceptibility rates, the percent susceptibility is 100% to the majority of antibiotics, except ampicillin (7.4%), trimethoprim-sulfamethoxazole (85.3%), nalidixic acid (85.3%), and ciprofloxacin (97.1%).

Conclusion: To our knowledge, this study is the first investigation regarding the antimicrobial susceptibility patterns of *M. catarrhalis* isolates in Lebanon. In addition to the high level of resistance to ampicillin, our findings showed the emergence of resistance to trimethoprim-sulfamethoxazole, nalidixic acid and ciprofloxacin. Even if this study provides useful information to develop effective empirical treatment, we recommend the implementation of reliable diagnostic tools to guide appropria tetreatment.

Keywords

Moraxella catarrhalis; Antimicrobial Resistance; Epidemiology; Lebanon.

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Introduction

Moraxella catarrhalis is an aerobic Gram-negative diplococcus, formerly known as *Branhamella catarrhalis*, that resides exclusively in humans, and commensally colonizes the mucosal surface of the upper respiratory tract, and occasionally the conjunctiva and genital tract [1]. The highest prevalence of colonization was detected among infants and children, which decreased in healthy adults [2]. The role of this bacterium as a disease-causing organism has long been questioned. *M. catarrhalis* was recognized to cause occasionally infections such as acute otitis media, sinusitis, acute bronchitis, pneumonia, and exacerbations of chronic obstructive pulmonary disease, and rarely bacteremia, meningitis, septic arthritis, osteomyelitis, endocarditis, and pericarditis, especially in immunocompromised persons [3]. In fact, the absence of vaccines for prevention and the low number of active antibiotics for treatment of *M. catarrhalis* infections, have considered this bacterium as an important human pathogen [4]. In addition, two major resistance mechanisms have been described in *M. catarrhalis:* the inactivation of antimicrobials by enzymes such as β-lactamases and the decrease in permeability of bacterial cell wall (reducing in the number of porins and/or enhancement of the active efflux system) [1]. The beta-lactamase-producing *M. catarrhalis* was firstly reported in 1976. Today, two distinct BRO-type β-lactamase enzymes (BRO-1 and BRO-2), have been reported worldwide [5]. Regrettably, BRO-positive *M. catarrhalis* strains have increased rapidly in recent years, and are now accounting for more than 90% globally [2]. The evidence of continuing misuse of antibiotics associated with a low level of antibiotic awareness is of global concern [6]. In Lebanon, as other developing countries, national investigations showed an increase in the levels of antimicrobial resistance in clinical and non-clinical settings [7-11]. For instance, a recent nationwide study conducted in 13 different hospitals located in different Lebanese governorates showed a trend of increasing antimicrobial resistance [12]. Even if the epidemiology of antimicrobial resistance is widely studied in Lebanon, there is a lack of studies on antimicrobial resistance in *M. catarrhalis* isolates in this country. Therefore, we decided to assess for the first time the antimicrobial resistance patterns in *M. catarrhalis* strains isolated in North Lebanon.

Material and Methods

This study was conducted in the clinical microbiology laboratory of Nini Hospital during the period from November 2010 to March 2019. The isolation of *M. catarrhalis* strains was performed according to standard protocols proposed by the Référentielen Microbiologie Médicale (REMIC) using a calibrated loop (10 µl) and a blood agar (Bio-Rad®, France) after an incubation for 18 to 24 hours at 35°C in air enriched with 5% CO_2. Bacterial identification was carriedout through the use of API-NH (bioMérieux, Marcy l'Etoile, France). All isolates were transferred to the Laboratoire Microbiologie Santé et Environnement (LMSE) at the Lebanese University for identification confirmation or exclusion using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) (bioMérieux, Marcy l'Etoile, France). The antibiotic susceptibility testing was performed for *M. catarrhalis* isolates by the disk diffusion method on Mueller Hinton supplemented with 5% blood and 20 mg/l β-NAD (MH-F) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The antibiotics tested were ampicillin (AMP; 2 µg), amoxicillin - clavulanic acid (AMC; 2-1 µg), cefotaxime (CTX; 5 µg), cefixime (CFM; 5 µg), tetracycline (TET; 30 µg), minocycline (MNO; 30 µg), erythromycin (E; 15 µg), chloramphenicol (C; 30 µg), nalidixic acid (NA; 30 µg), ciprofloxacin (CIP; 5 µg) and trimethoprim-sulfamethoxazole (SXT; 1.25-23.75 µg).

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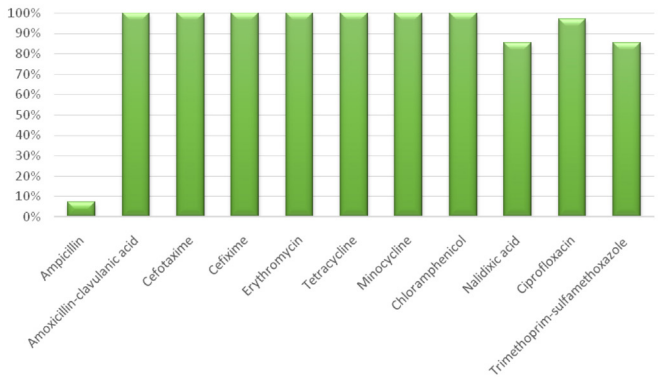
Results

This study was conducted in the North governorate of Lebanon. A total of 34 non-duplicated *M. catarrhalis* isolates were recovered from patients referred to Nini Hospital. Overall, *M. catarrhalis* strains were isolated from nose (n=19), ear (n=7), sputum (n=5), blood (n=1), eye (n=1), and throat (n=1) of 34 patients (23 malesand 11 females, ranging in age from 2 weeks to 77 years, with a mean age of 11.9 years) presenting respiratory like symptoms. Regarding antibiotic susceptibility rates, the percent-susceptibility is 100% to the majority of antibiotics, such as amoxicillin-clavulanic acid, cefotaxime, cefixime,tetracycline, minocycline, erythromycin, and chloramphenicol **(Table 1)**. Higher antibioticresistance rates were observed to ampicillin (92.6%), trimethoprim-sulfamethoxazole (14.7%), nalidixic acid (14.7%), and ciprofloxacin (2.9%) **(Figure 1)**.

Figure 1: Antibiotic susceptibility rates of *Moraxella catarrhalis* isolates.



Discussion

The global increase in the prevalence of *M. catarrhalis* in recent years has drawn the attention to the clinical importance of this bacterium, particularly as an important cause of respiratory tract infections [13]. The present surveillance study in North Lebanon aimed to evaluate the susceptibility patterns of *M. catarrhalis* clinical isolates to several commonly used antibiotic agents in the hopes of helping physicians and infectious diseases specialists in developing effective empirical antibiotic treatment for *M. catarrhalis* infections. These bacteria are almost universally capable of producing of one of two chromosomally encoded BRO β-lactamases, with previous investigations showing production in more than 90% of clinical isolates [2, 14-16]. As expected, the present finding (92.6% of isolates are ampicillin resistant) is compatible with previous investigations **(Table 2)**. This dramatic increase in the prevalence of ampicillin resistant *M. catarrhalis* strains could be regarded as the fastest dissemination of β-lactamase genes within the genus. The BRO genes appear to be chromosomally located but are readily transferred by conjugation with in the *Moraxella* species. Besides the high level of resistance to penicillin, the combination of penicillin with a β-lactamase inhibitor used as a treatment of *M. catarrhalis* infections is still active against 100% of our isolates in almost all countries. However, unfortunately, recent studies found the emergence of *M. catarrhalis* strains resis-

Table 1. Distribution of *Moraxella catarrhalis* isolates according to clinical specimens, and their antibiotic resistance patterns.

		Antibiotic agents (% of resistance)										
Specimen	**N**	**AMP**	**AMC**	**CTX**	**CFM**	**E**	**TET**	**MNO**	**C**	**NA**	**CIP**	**SXT**
Nose	19	84.2	0	0	0	0	0	0	0	15.8	0	15.8
Ear	7	100	0	0	0	0	0	0	0	14.3	0	28.6
Sputum	5	100	0	0	0	0	0	0	0	20	20	0
Blood	1	100	0	0	0	0	0	0	0	0	0	0
Eye	1	100	0	0	0	0	0	0	0	0	0	0
Throat	1	100	0	0	0	0	0	0	0	0	0	0

Ampicillin (AMP); Amoxicillin - clavulanic acid (AMC);Cefotaxime (CTX); Cefixime (CFM); Erythromycin (E); etracycline (TET); Minocycline (MNO); Chloramphenicol (C); Nalidixic acid (NA); Ciprofloxacin (CIP); Trimethoprim-sulfamethoxazole (SXT).

Table 2. Worldwide available data regarding the percentage of resistance of *Moraxella catarrhalis* isolates to major antibiotics.

		Antibiotic agents							
Country	**Year**	**AMP**	**AMC**	**CTX**	**E**	**TET**	**CIP**	**SXT**	**Ref.**
Australia	2010	63.6	0	0	0	1.1	0	13.8	22
United States	2012	96.4	0	0.2	0.5	0.2	0	2.5	33
United States	2009	95.2	4.8	3.2	ND	ND	ND	ND	17
Canada	2014	ND	0	0	ND	0	0	0	18
Canada	2000	ND	0	0	ND	0.7	0	15.7	23
Portugal	2001	81.6	0	0	ND	0	0	ND	34
Greece	2014	47.8	0	0	ND	1.5	0	28.4	24
Ethiopia	2018	94.6	ND	ND	86.6	37.8	16.2	78.4	20
Tunisia	2008	95	0	0	3.8	1.25	0	12.5	21
Iran	2012	0	0	0	0	ND	0	100	25
Taiwan	2012	97.8	0	0	ND	19.8	0	18.5	2
China	2018	74.2	0	0	70.8	6.8	3.4	28.1	19
Thailand	2016	97	0	0	ND	ND	ND	ND	16
Pakistan	2015	63	4	0	59.1	ND	59	59	13
Lebanon	2019	92.6	0	0	0	0	2.9	14.7	This study

Ampicillin (AMP); Amoxicillin - clavulanic acid (AMC); Cefotaxime (CTX); Erythromycin (E); Tetracycline (TET); Ciprofloxacin (CIP); Trimethoprim-sulfamethoxazole (SXT); Not determined (ND).

tant to amoxicillin-clavulanic acid and third generation cephalosporins [17].

Other antimicrobial agents, such as tetracycline, erythromycin, ciprofloxacin, and trimethoprim-sulfamethoxazole have also been empirically used to treat *M. catarrhalis* infections worldwide with apparent success. All clinical isolates collected in this study showed 100% susceptibility to tetracycline and erythromycin which is consistent with the most current international research [18]. Nevertheless, the antimicrobial resistance threat must keep the clinical community vigilant. Regrettably, tetracycline resistance has emerged in numerous developing and developed countries. The higher levels of resistance were reported in China [19], Taiwan [2] and Ethiopia [20]. Furthermore, several studies realized in developing countries indicated unexpected levels of antimicrobial resistance to erythromycin in *M. catarrhalis* isolates, with 59.1% in Pakistan [13], 70.8% in China [19], and 86.6% in Ethiopia [20].

On the other hand, our findings showed the emergence of resistance to trimethoprim-sulfamethoxazole (14.7%), and ciprofloxacin (2.9%). Various previous studies conducted globally reported the spread of resistance to trime thoprim-sulfamethoxazole among *M. catarrhalis* isolates, such as 12.5% in Tunisia [21], 13.8% in Australia [22], 15.7% in Canada [23], 18.5% in Taiwan [2], 28.1% in China [19], 28.4% in Greece [24], 59% in Pakistan [13], 78.4% in Ethiopia [20], and 100% in Iran [25]. Based on the aforementioned data, the spread of cotrimoxazole resistance among *M. catarrhalis* isolates in developing countries is shocking, and reveals a prominent threat jeopardizing the clinical efficacy of this important antibiotic. Moreover, the ciprofloxacin resistancewas detected for the first time among

M. catarrhalis isolates in Lebanon, with a higher rate than that reported in the majority of countries. Surprisingly, two studies conducted in Ethiopia and Pakistan showed high level of resistance to ciprofloxacin, with 16.2% and 59% respectively [13, 20].

Indeed, our generated data are in accordance with recent national studies reporting an increased level of antimicrobial resistance among infectious bacterial agents, a striking low level of antibiotic awareness among Lebanese population, a spread of counterfeit medicines, and a misuse of antibiotics in Lebanon [6-11, 26-32]. Even if this study provides useful information for physicians to develop effective empirical antibiotic treatment for *M. catarrhalis* infections, we advise to pay attention to this prominent issue and we recommend the implementation of reliable identification and antibiotic susceptibility testing in routine laboratory diagnostics to guide appropriate treatment.

In summary, to our knowledge, this study is the first investigation regarding the antimicrobial susceptibility patterns of *M. catarrhalis* isolates in Lebanon. Overall, our isolates showed 100% susceptibility to the majority of tested antibiotics, except ampicillin, trimethoprim-sulfamethoxazole, nalidixic acid and ciprofloxacin. Due to the limited number of isolates analyzed in this investigations, the epidemiologic significance of these results remains to be confirmed. Therefore, additional long-term surveillance studies including a large number of isolates are required to assess the antimicrobial resistance of this important human pathogen.

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