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Original Article

Study of Antimicrobial susceptibility test for *Moraxella catarrhalis* in Hospitalized Patients of respiratory tract Infection

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ABSTRACT

Moraxella catarrhalis, a gram-negative diplococcui, an opportunistic pathogen that infects the human respiratory system. In th present study 20 isolates were collected from 405 individual clinical samples. Based on the sample source, the common sources of *M. catarrhalis* isolates were sputum 10 (50%), followed by throat swabs 8 (40%) and bronchial wash 2 (10%). Based on the culture morphology ("Hockey puck" sign), biochemical characteristics and with the API-NH, out of 354 (87.5%) positive bacterial growths, 20 (5%) isolates were identified as *M. catarrhalis.* In relation to biofilm development, the present study revealed that 5% of isolates were weak biofilm formers and 95% non-biofilm in formers in qualitative method (tube method) .According to CLSI, The most effective antibiotics against *M. catarrhalis* were Amoxicillin-clavulanate and Trimethoprim-sulfamethoxazole the resistance rate was 2 (10%) and 1 (5%) respectively. The aim of the study is to investigate antibiotic resistance in *M. catarrhalis* in hospitalized respiratory tract infection patients.

Keywords: *Moraxella catarrhalis,* Hockey puck, Penicillin resistance, Beta-Lactamase

Introduction

Moraxella catarrhalis (*M. catarrhalis*), which belongs to the family Moraxellaceae, is a gram-negative diplococci, facultative, aerobic, and commensal of the nasopharynx [1]. It is an opportunistic pathogen that infects the human respiratory system; immunocompromised patients, children, and the elderly are particularly susceptible to infection with this pathogen [2]. Healthy people become susceptible to *M. catarrhalis* colonization due to a number of characteristics, including age, family size, socioeconomic level, immunization status, and seasonal change [3]. The detection rate of *M. catarrhalis* in respiratory tract patients has increased to 30% during the last few years, making the bacteria the third most common causative pathogen associated with community-acquired respiratory tract infections (CARTIs) and chronic obstructive pulmonary disease (COPD) in adults, as well as the second most common cause of otitis media (OM) in children and maxillary sinusitis in infants and children [4, 5].

The increase in the detection rate of *M. catarrhalis* in clinical samples was related to the generation of new strains of bacteria that have several virulence factors; many of them that are raised through the plasma membrane are either released outside the cell or generalized to the outer membrane proteins (OMPs) [6]. The new bacterial strains become resistant to antibiotics such as the beta-lactam group and penicillin through the formation of β-lactamases [7]. The virulence factor of *M. catarrhalis* that increases the severity of disease in infected people is biofilm formation. The bacteria within the biofilm are protected from the host immune system by a polymeric matrix

that serves as a barrier. Also, their ability to express different genes increases their resistance to the effects of antibiotics [8]. *M. catarrhalis* develops polymicrobial biofilms with Haemophilus influenzae and Streptococcus pneumonia during respiratory tract infections, which will contribute to the failure of antibiotic action, especially if mixed with bacteria that produce ß-lactamase, such as *M. catarrhalis* [9]. Biofilm formation has an important function in the colonization and persistence of mixed bacterial pathogens in respiratory tract infections [10]. This study is to isolate *M. catarrhalis* from throat swabs, bronchial wash, and sputum of patients with respiratory tract infection, and confirm by API-NH-2, as well as to study the antibiotic resistance of *M. catarrhalis* in hospitalized respiratory tract infection patients.

Methods

Patients and sample collection

The study was carried out in Azadi Teaching Hospital, Pediatric Hospital, Kirkuk General Hospital, and Women and Children's Hospital in Kirkuk city during the period from October 2023 to October 2024. Different clinical samples (sputum, bronchial wash, and throat swabs) were obtained from 405 hospitalized patients. The samples were directly inoculated in brain heart infusion broth (BHIB), which was used as a transport medium, and incubated overnight at 37 ˚C to undergo initial cultivation.

Isolation and identification

Blood agar and chocolate agar were used to culture all the samples and incubated at 37˚C for 24–48 h in a candle jar containing 3–5% carbon dioxide. Gram stain, the colony hockey puck sign and the catalase and oxidase production tests, as well as API-NH, were used for confirmatory identification of the bacteria.

Antibiotic susceptibility

The disc diffusion susceptibility test, referred to as the Kirby-Bauer method, was used. Bacterial growth (1–2 × 10^8 CFU/ml) was cultured on chocolate agar, then incubated for 24 hrs at 37˚C in a candle jar containing 3–5% carbon dioxide. The zone of inhibition was measured with a graduated ruler. The results were classified as sensitive, moderate, or resistant after comparison with the Clinical Laboratory Standards Institute (CLSI) [11]. The biofilm was estimated by the tube method. In this technique, each isolate was cultured overnight at 37˚C in BHIB in a glass tube [12].

Table 1. Antibiotic discs used in the current study and their inhibition zone.

NO.	Antibiotic	Abbreviation	Concentration	Diameter of inhibition zone		
				Sensitive	Intermediate	Resistant`
1	Amoxicillin / clavulanic	AMC	20/10	≥ 24	-	≤ 23
2	Trimethoprim-Sulfamethaxole	COT	1.25/23.75	≥ 13	11-12	≤ 10
3	Tetracycline	TE	30	≥ 29	25-28	≤ 24
4	Clarithromycin	E	15	≥ 24	-	-
5	Azithromycin	AZM	15	≥ 26	-	-

Ethical approval

Official approval was obtained from the Kirkuk Health Directorate (Approval number: 7563) in 2023/9/10.

Results and Discussion

Moraxella catarrhalis isolates from various clinical samples

The frequency of M. catarrhalis isolates was high in the sputum, followed by throat swabs and bronchial wash with percentages of 50%, 40% and 10% respectively as s% respectively. This investigation showed that M. catarrhalis was the sole bacterial isolate found in sputum samples which agrees with astudy [13].

Table 2. Culture results for different clinical samples.

Bacteria / Samples	*M.catarrhalis*	Percentage	Other bacteria	Percentage	No growth	Percentage	Total
Sputum	10	50%	30	9%	40	78%	80
Throat swab	8	40%	299	90%	8	16%	10
Bronchial wash	2	10%	5	1%	3	6%	315
Total	20	100%	334	100%	51	100%	405

Among four hundred and five clinical respiratory tract specimens, only twenty isolates were M. catarrhalis, which means 5% of people were infected with this microbe. When compared to other studies, this result is similar to the isolation rate for M. catarrhalis reported in studies by [14, 15], which recorded 9.8% and 5%, respectively. In contrast, [16] showed that the isolation rate is approximately 3.6% of patients. The current result was in line with that of [17] in India, which reported that a high percentage of M. catarrhalis samples were obtained from sputum samples. In contrast, another study conducted in Nepal by [18] recorded that M. catarrhalis was found in 6.90% of sputum cultures from 405 clinical specimens. Only 354 specimens (87.4%) had a positive culture, while 51 specimens (12.6%) of the current results were negative, which is in line with a study conducted by [19] in Iraq, which showed 6.9% (no bacterial growth) from 204 clinical specimens. These samples may have been exposed to antibiotics, or there may be other causal agents present that require special techniques to detect, including viruses and fungi.

M. catarrhalis identification and isolation

A gram-negative, non-spore-producing, non-motile diplococcus was identified using a Gram stain (Figure 1-A). Colonies were spherical, convex, non-hemolytic, and grayish-white on blood agar (Figure 1-C) and chocolate agar (Figure 1-B); however, they did not develop on MacConkey agar. One of the main distinctions between Neisseria and Moraxella species is the presence of a non-sugar fermenter on Kligler iron agar (Figure 1-F). Because the isolate contained cytochrome C oxidase in the electron transport system, it was oxidase-positive (Figure1-E) and catalase-positive (Figure1-D). API-NH biochemical assays confirmed the identification (Figure 1-G).

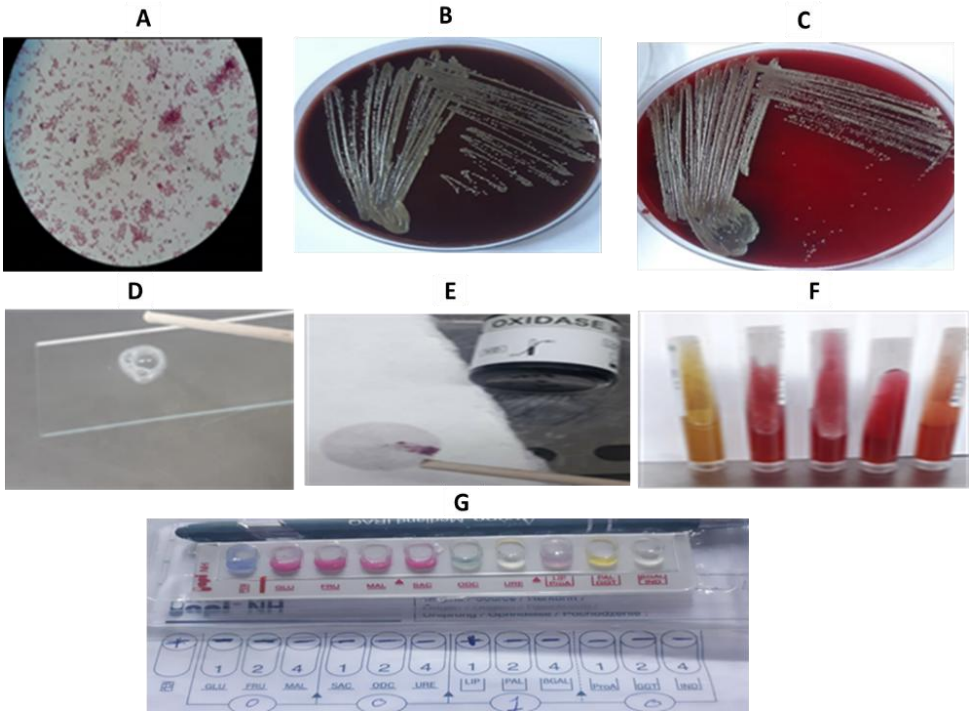

Figure 1. Identification and isolation of M. catarrhalis A. Gram Stain; B. Chocolate agar; C. Blood agar; D. catalase test; E. oxidase test; F. sugar test; G. API-NH

Antimicrobial susceptibility

Table 3 displays the pattern of drug resistance recorded of *M. catarrhalis* isolates. According to the 2023 CLSI standard, among the 20 bacteria identified from clinical samples, the isolates exhibited varying degrees of resistance to the antibiotics examined. Tetracycline

8 (40%) had the highest rate of resistance, followed by Azithromycin 7 (35%) and Clarithromycin 4 (20%), while the most effective antibiotics against *M. catarrhalis* were Amoxicillin-clavulanate 2 (10%) and Trimethoprim-sulfamethoxazole 1 (5%).

Table 3. Antibiotic susceptibility test of *M. catarrhalis*

Antibiotic	Sensitive	Percentage	Intermedate	Percentage	Resistant	Percentage
Tetracycline	10	50%	2	10%	8	40%
Azithromycin	13	65%	-	-	7	35%
Clarithromycin	16	80%	-	-	4	20%
Amoxicillin-clavulanat	18	90%	-	-	2	10%
Trimethoprim-sulfamethoxazole	18	90%	1	5%	1	5%

Macrolides (Azithromycin and Clarithromycin) are protein synthesis inhibitors. In the present study, *M. catarrhalis* isolates showed the highest antibiotic resistance, i.e., 35% and 20%, respectively. These resistance rate results agree with [20, 17], which reported that *M. catarrhalis* showed high resistance to macrolide antibiotics when isolated from clinical specimens. In contrast, another investigation conducted in northern Iran recorded complete susceptibility to Azithromycin and Clarithromycin [11, 21]. Antibiotic resistance to tetracycline in our study was 40%. This resistance rate agrees with another investigation in Iraq [22], which showed high resistance to tetracycline (73.8%). Other studies of isolated clinical specimens showed 70% resistance to tetracycline [11]. The current results are not compatible with [21, 22], which reported only 3.1% resistance to tetracycline.

Amoxicillin/clavulanate resistance was recorded at 10%. This result agrees with [23], which reported that bacterial resistance to augmentin (amoxicillin-clavulanate) increased by 19.23% between 2002 and 2004, compared to 0.25% resistance during that time. Our result disagrees with [24, 17], which found 100% susceptibility. In the current research, antibiotic resistance to trimethoprim-sulfamethoxazole was 5%; this disagrees with [4] and shows relatively little efficacy against *M. catarrhalis*. Furthermore, in our study, no *M. catarrhalis* strain was resistant to all antibiotics, which yields results similar to a study done in Iran [11]. Additionally, study [25], conducted in Iraq during Umrah and Hajj seasons, found that most *M. catarrhalis* isolates from pilgrims and tourists at Umrah were sensitive to every antibiotic used in the study.

It is evident from a comparison of this study with other research on the frequency of antibiotic-resistant strains of *M. catarrhalis* that drugs like tetracycline are no longer effective against *M. catarrhalis* because of persistent resistance reports. The excessive use of antibiotics, as well as geographical and cultural factors including the abuse of medicines and the easy availability of antibiotics all contribute to the rise in antibiotic resistance observed in recent years [11] which agrees with a study done on secondary bacterial infection in COVID-19 pneumonia patients in Iraq [26], were Tetracycline resistance was seen in every bacterial isolate.

Biofilm production

A total of (20) *M. catarrhalis* isolates were tested. Also, one isolate (5%) was a weak biofilm producer while 19 isolates (95%) are non-biofilm producers (figure 2) in tube method.

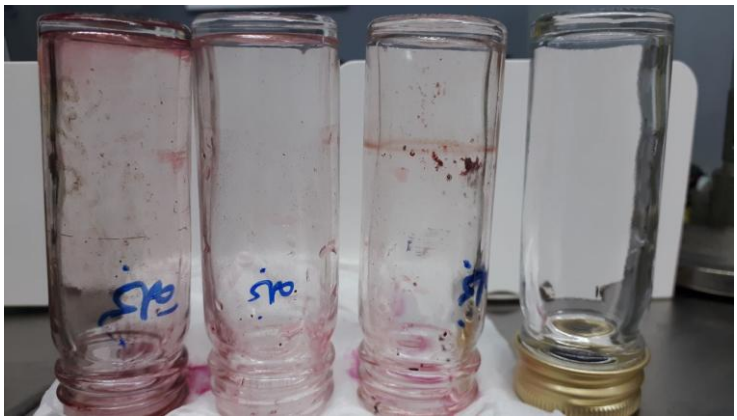

Figure 2. Tube method biofilm formation

The results of this study agree with those recorded by [27], which show that the ability of *M. catarrhalis* strains to create biofilms varies greatly, with most strains producing biofilms but exhibiting very poor biofilm development under various in vitro conditions. This is because some bacterial genes may be associated with environmental factors or host stress, causing pathogenic strains of the bacteria to

overexpress genes, such as those responsible for biofilm formation, in harsh environments to protect themselves from the immune system and from other bacterial invasions, as well as to preserve nutrients [28]. However, in vitro, such as on blood agar, the bacteria are in a limited environment where many important nutrients are present. The biofilms produced by *M. catarrhalis* undergo genetic transformation utilizing cloned portions of DNA. *M. catarrhalis* is more likely to be observed in polymicrobial infections, such as acute otitis media illnesses, than in infections involving a single species, as previously indicated in [29], and agree with a study [30] which found that isolates of biofilm-forming bacteria were more resistant to antibiotics than non-biofilm-forming isolates.

Conclusion

Compared to other clinical specimens, sputum samples had a greater prevalence of *M. catarrhalis* isolates. Antibiotics such as trimethoprim-sulfamethoxazole and amoxicillin-clavulanate are suitable for treating *M. catarrhalis* infection

Conflict of Interest

The authors declare that there is no competing of interests.

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