The Antimicrobial Susceptibility of *Moraxella* Species Other Than *Moraxella Catarrhalis*

Moraxella Catarrhalis Dışı *Moraxella* Türlerinin Antibiyotik Duyarlılığı

Latife İŞERİ,[a]
Teoman APAN,[a]
Esra ŞAHİN[b]

[a]Department of Medical Microbiology, Kırıkkale University Faculty of Medicine, Kırıkkale
[b]Microbiology Laboratory, Niğde Dr. Doğan Baran Hospital for Women's and Children' s Diseases, Niğde

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Yazışma Adresi/*Correspondence:*
Latife İŞERİ
Kırıkkale University Faculty of Medicine, Department of Medical Microbiology, Kırıkkale, TÜRKİYE/TURKEY
liseri2000@yhoo.com

ABSTRACT Objective: Although *Moraxella* species are members of the normal respiratory flora, some species, including *M. catarrhalis*, *M. osloensis*, *M. nonliquefaciens*, and *M. lacunata*, can cause serious infections in humans. The purpose of this study was to investigate the antimicrobial susceptibility of *Moraxella* species other than *M. catarrhalis*. **Material and Methods:** The study included 17 *M. osloensis*, 18 *M. lincolnii*, and three *M. nonliquefacie*, isolated from 100 nasopharyngeal samples. The isolates were identified by conventional methods. Identification to the species level was performed by a RapID NF Plus identification kit (Remel, USA). The antimicrobial susceptibility of the isolates was examined for ampicillin, amoxicillin/clavulanic acid, gentamicin, ceftazidime, ciprofloxacin, erythromycin and trimethoprim-sulfamethoxazole using the disk diffusion method. The results were evaluated according to guideline published by the Clinical and Laboratory Standards Institute. β-Lactamase production was tested using nitrocefin discs. **Results:** The resistance rates of isolates were between 3% and 8% for trimethoprim-sulfamethoxazole, amoxicillin/clavulanic acid. Inhibition zone diameters for ampicillin of 41% of M. osloensis, 33% of *M. nonliquefaciens*, and 11% of *M. lincolnii* isolates were relatively small (≤15 mm). Five *M. osloensis* (29%) and one *M. nonliquefaciens*, isolates were beta lactase positive. **Conclusion:** *Moraxella* species other than *M. catarrhalis* can cause serious infections in humans. Therefore, we suggest that *Moraxella* species other than *M. catarrhalis* should be included in surveillance studies.

Key Words: Moraxella; drug resistance

ÖZET Amaç: *Moraxella* türleri normal solunum florasının üyesi olmasına rağmen, *M. catarrhalis*, *M. osloensis*, *M. nonliquefaciens*, ve *M. lacunata* gibi bazı türler insanda ciddi enfeksiyonlara neden olabilir. Bu nedenle, bu çalışmanın amacı, *M. catarrhalis* dışı türlerin antibiyotik duyarlılıklarını araştırmaktı. **Gereç ve Yöntemler:** Çalışma 100 nazofarengial örneklerden izole edilen 17 *M. osloensis*, 18 *M. lincolnii*, ve 3 *M. nonliquefaciens*, izolatını içermektedir. *Moraxella* türleri geleneksel yöntemlerle tanımlandı. Tür seviyesinde tanımlama RapID NF Plus kit (Remel, USA) ile yapıldı. İzolatların, ampisilin, amoksisilin-klavunik asit, gentamisin, seftazidim, ciprofloksasin, eritromisin ve trimetoprim-sulfametoksazol'e duyarlılıkları disk difüzyon yöntemiyle incelendi. Sonuçlar klinik laboratuvar standartları enstitüsünün kurallarına göre değerlendirildi. Beta laktamaz üretimi nitrosefin diskleri kullanılarak test edildi. **Bulgular:** İzolatların amoksisilin-klavunik asit ve trimetoprim-sulfametoksazole direnç oranları sırasıyla, %3 ve %8 olarak bulundu. *M. osloensis* suşlarının %41'i, *M. nonliquefaciens*, izolatlarının %33'ü *M. lincolnii* izolatlarının %11'inin inhibisyon zonları ampisilin için göreceli olarak düşüktü (≤15 mm). Beş *M. osloensis* (29%) ve bir *M. nonliquefaciens*, izolatı beta laktamaz pozitifti. **Sonuç:** *M. catarhalis* dışı *Moraxella* türleri de, insanda ciddi enfeksiyonlara neden olabilir. Bu nedenle biz survelans çalışmalarına, *M. catarrhalis* dışı *Moraxella* türlerin dahil edilmesini öneriyoruz.

Anahtar Kelimeler: Moraksella; ilaç direnci

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Moraxella species are members of the nasopharyngeal flora. In most cases, these organisms are carried without causing clinical symptoms. However, when the living conditions of individuals altered, they may invade adjacent sites and/or invade the bloodstream, causing disease. *Moraxella catarrhalis* is a known pathogen of the respiratory tract, ears, eyes, and even joints of humans; however, other *Moraxella* species, especially *M. osloensis*, are increasingly being reported as the cause of infections in immunocompromised adults and healthy children and elderly people.[1-3] Han et al. isolated *M. osloensis* from the blood and catheter samples of ten cancer patients over a period of only 18 months. In addition, *M. nonliquefaciens,* has been isolated from patients with infections such as peritonitis and endophthalmitis.[4-7]

For this reason, we investigated the antibiotic susceptibilities of nasopharyngeal *Moraxella* species other than *M. catarrhalis*.

MATERIAL AND METHODS

One hundred nasopharyngeal samples collected from healthy individuals were inoculated onto tryptic soy agar plates (Becton Dickinson, France SA) containing 5% sheep blood and incubated at 37°C in a 5% CO_2 atmosphere for 24 h. Informed consent was obtained from all patients for being included in the study. The two or three of small and pinpoint colonies from every plate were tested for catalase activity, oxidase activity and sugar fermentations. Nonfermentative, catalase and oxidase positive colonies were accepted as *Moraxella*. The identification at species level was performed by a RapID NF Plus identification kit (Remel, USA). *Moraxella* species other than *M. catarrhalis* were included to the study. The antimicrobial susceptibility of the isolates was examined for ampicillin (10 μg) (AMP), amoxicillin/clavulanic acid (20/10 μg) (AMC), gentamicin (10 μg) (GN), ceftazidime (30 μg) (CAZ), ciprofloxacin (5 μg) (CIP), erythromycin (15 μg) (ERY) and trimethoprim-sulfamethoxazole (1.25/23.75 μg) (SXT) using the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).[8] As there are no antimicrobial performance standards for *Moraxella* species other than *M. catarrhalis*, the results were evaluated according to guideline given for *M. catarrhalis* by CLSI.[8] All isolates were tested for β-lactamase production using nitrocefin disks (NCF) as recommended by manufacturer (Sigma Aldrich, Germany).

RESULTS

Thirty-eight *Moraxella* isolates were cultured from the 100 nasopharyngeal samples. The species distribution of the 38 isolates was: *M. lincolnii* (18 isolates), *M. osloensis* (17 isolates), and *M. nonliquefaciens,* (three isolates).

Inhibition zone diameters, representing the level of antibiotic sensitivity (S) of each of the isolates, were evaluated to determine the antibiotic resistance profiles of the isolates. The susceptibility breakpoint of *M. catarrhalis* for SXT is ≥13 mm according to the CLSI.

Ninety-seven percent of the isolates were sensitive to SXT (Table 1). The sensitivity rate for AMC was 92% (35/38) on the basis of the CLSI recommendations (S ≥24 mm) (Table 1).

Pre-determined disk diffusion breakpoints were not available for CAZ, GN, CIP or AMP according to the recommendations of CLSI. In this study, we determined that most (92%) of the isolates had inhibition zone diameters of ≥30 mm for CAZ. However, one multidrug-resistant *M. osloensis* isolate showed a zone of inhibition for CAZ of only 16 mm. As with CAZ, 95% (36/38) of isolates had inhibition zone diameters >20 mm for GN. Despite this, two isolates showed significantly smaller

TABLE 1: Susceptibilities of the isolates to three antibiotics.

Strains (n)	NCF n (%)	SXT n (%)	AMC n (%)	ERY n (%)
M. osloensis (17)	5 (29)	16 (94)	15 (88)	17 (100)
M. lincolnii (18)	0	18 (100)	17 (94)	18 (100)
M. nonliquefaciens (3)	1 (33)	3 (100)	3 (100)	3 (100)
Total (38)	6 (16)	37 (97)	35 (92)	38 (100)

zones of inhibition (diameters=15 mm) for GN than the other isolates. The inhibition zone diameters for CİP of 36 isolates were >25 mm. One *M. osloensis* and one *M. lincolnii* isolate had 15 mm and 20 mm sensitivity zone diameters, respectively. In contrast, the sensitivity zone diameters for ampicillin were relatively small. Fifty-eight percent (22/38) of isolates had a zone diameter ≥20 mm, while 41% (7/17) of *M. osloensis* isolates, 33% (1/3) of *M. nonliquefaciens,* isolates, and 11% (2/18) of *M. lincolnii* isolates had diameters ≤15 mm. The five *M. osloensis* (29%) and one *M. nonliquefaciens,* isolates were beta lactase positive with nitrocefin test. The sixteen percent of the all strains produced beta-lactamase.

DISCUSSION

M. osloensis and *M. nonliquefaciens,* are susceptible to antibiotics and are rarely infectious.[9,10] However, there is no large-scale antimicrobial sensitivity data for *M. osloensis*, *M. nonliquefaciens*, and *M. lincolnii* strains isolated from the nasopharyngeal region of humans, and only a few isolated case reports exist on this subject.[1-7] The isolates used in the current study were directly isolated from nasopharyngeal samples, and included 17 *M. osloensis*, 18 *M. lincolnii*, and three *M. nonliquefaciens,* isolates. In general, the isolates were highly susceptible to SXT and AMC (97% and 97% respectively).

In the current study, 35 (92%) isolates showed a inhibition zone diameter ≥30 mm for CAZ. Thirty-six (95%) of isolates had inhibition zone diameters >20 mm and >25 mm for GN and CIP, respectively. We could not evaluate about sensitivity of strains to these drugs, because sensitivity breakpoints for *Moraxella* have not been reported for CAZ, GN,CIP in guideline of CLSI.

Most *M. catarrhalis* strains produce β-lactamases and are therefore resistant to penicillin. As a result, ampicillin breakpoints have not been provided for *M. catarrhalis* in the guideline of CLSI. Other than *M. catarrhalis*, *Moraxella* species are generally considered to be sensitive to penicillin.[9,10] However, penicillin-resistant and β-lactamase secreting *M. osloensis* isolates have been reported.[11-15]

In this study, sensitivity zone diameters for *M. osloensis* (41%) isolates were relatively small following disk diffusion assays using ampicillin (Table 1), and β-lactamase secretion rate of *M. osloensis* isolates was found high (29%).

Although *M. lincolnii* is not considered a pathogen, *M. catarrhalis, M. osloensis*, and *M. nonliquefaciens,* can cause serious infections in children and in adults with low immunity.[1-7] Therefore, we suggest that *Moraxella* species other than *M. catarrhalis* should been taken into account when carrying out surveillance studies of *Moraxella* infections, and more research should be conducted to assess antimicrobial resistance of these species.

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