DOI:10.25092/baunfbed.826100 *J. BAUN Inst. Sci. Technol., 23(1), 22-29, (2021)*

Antibiotic susceptibilities of Gram-negative bacteria isolated from contact lens storage cases of asymptomatic wearers

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Geliş Tarihi (Received Date): 25.02.2020
Kabul Tarihi (Accepted Date): 04.09.2020

Abstract

The aim of this study was to determine the in vitro antibiotic susceptibilities of various bacteria isolated from contact lens storage cases of asymptomatic wearers. For this purpose, twenty-two bacteria isolates were subjected to in vitro antibiotic susceptibility using Kirby-Bauer disc diffusion method. Four ATCC strains of different genus of bacteria were used as control. The results obtained from the current study showed that gentamicin was the most effective antibiotic against all bacteria tested. In addition, all bacteria tested in this study were resistant to ampicillin, methicillin, penicillin G and vancomycin. According to the results continuous monitoring of antibiotic susceptibilities of bacteria isolated from contact lens storage cases of asymptomatic wearers are needed.

Keywords: *Antibiotic susceptibility, bacterial contamination, contact lens storage cases.*

Asemptomatik kullanıcıların kontakt lens saklama kaplarından izole edilen Gram negatif bakterilerin antibiyotik duyarlılıkları

Öz

Bu çalışmanın amacı, asemptomatik kullanıcıların kontakt lens saklama kaplarından izole edilen çeşitli bakterilerin in vitro antibiyotik duyarlılıklarını belirlemektir. Bu amaçla yirmi iki bakteri izolatının, Kirby-Bauer disk difüzyon yöntemi kullanılarak in vitro antibiyotik duyarlılıkları araştırılmıştır. Kontrol olarak farklı cinslere ait dört ATCC bakteri suşu kullanılmıştır. Bu çalışmadan elde edilen sonuçlar, test edilen tüm bakterilere karşı gentamisinin en etkili antibiyotik olduğunu göstermiştir. Ek olarak, bu çalışmada test edilen tüm bakterilerin ampisilin, metisilin, penisilin G ve vankomisine karşı dirençli oldukları tespit edilmiştir. Bu sonuçlara göre asemptomatik

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kullanıcıların kontakt lens saklama kaplarından izole edilen bakterilerin antibiyotik duyarlılıklarının sürekli izlenmesi gerektiği sonucununa varılmıştır.

Anahtar kelimeler: *Antibiyotik duyarlılığı, bakteriyel kontaminasyon, kontakt lens saklama kapları.*

1. Introduction

Since 125 million people around the world wear contact lenses, it is important to minimize risk factors, especially corneal disorders [1]. Bacterial keratitis is one of the most common infections due to the use of contact lenses in the world. In a study carried in 2008 in Elazığ, contact lens wear was reported as the risk factor of 3.1% of the bacterial keratitis infections [2]. On the other hand, in Sivas, of the 500 patients admitted to the hospital due to various complaints in 2012 September-November, 133 (26.6%) were diagnosed as contact lens-related infection [3]. This situation shows that the rate of contact lens-related ocular infections was multiplied by 8.58 in 4 years. Therefore, contact lens wear may be considered as an important risk factor for eye infections in Turkey. In addition, the fact the patients in Sivas were at 18-30 age, the use of contact lenses in Turkey shows how much popular among the young population [3].

Misuse of contact lenses and inappropriate hygiene practices are the main risk factors of such eye infections among contact lens wearers. Inadequate or improper disinfection during contact lens wear may result in the contamination of contact lens storage cases. On the other hand, bacterial contamination has been reported in previous studies, even in the storage cases of asymptomatic contact lens wearers having no eye disease. However, the contaminant bacteria have the potential to cause infection in contact lens wearers [4-7]. The microorganism spectrum of bacterial keratitis varies according to geographical and seasonal characteristics. In addition, keratitis profile differs between the people inhabiting in rural or urban areas, western and developing countries [8]. *Pseudomonas aeruginosa* is the common causative agent identified in most cases in bacterial keratitis associated with contact lens wear [9]. However, *Serratia marcescens* was isolated as common as *Pseudomonas aeruginosa* in keratitis associated with contact lens wear in a study conducted in Florida [10].

Failure to initiate prompt and appropriate treatment may cause blindness [11]. The most appropriate way in the selection of antibiotics for the treatment of the disease is the identification of the causative bacteria. However, treatment with broad-spectrum antibiotics can be initiated often for the treatment of bacterial keratitis before the identification of the pathogen and performing antibiotic susceptibility tests [12]. This situation may cause bacteria to develop resistance against the antibiotics used [13, 14]. Therefore, susceptibility testing should be performed periodically to determine the resistance trends as suggested by many researchers [15, 16]. In the current study, in vitro antibiotic susceptibilities of various Gram-negative bacteria previously isolated from contact lens storage cases of asymptomatic wearers [17] were determined.

2. Materials and methods

In our previous study [17], contact lens storage case samples were collected from contact lens wearers' and screened for the presence of Gram-negative rod bacteria. Briefly, the sediment obtained from the contact lens storage case content was inoculated onto MacConkey agar and cetrimide-fucidin-cephalosporin supplemented *Pseudomonas* agar base plates for the isolation of both lactose fermenting and nonlactose fermenting Gram-negative bacilli, and *Pseudomonas* bacteria, respectively. All isolated strains were then identified using API 20E and API 20NE systems. Up to date, these strains were stored at −86 °C in a freezing medium.

In the present study, twenty-two of the previously isolated bacteria strains belonging to *Alcaligenes*, *Burkholderia*, *Klebsiella*, *Ochrobactrum*, *Pseudomonas* and *Serratia* genera were subjected to in vitro antibiotic susceptibility testing using Kirby-Bauer disc diffusion method [18]. For this purpose, frozen stocks of bacteria were re-grown again on Mueller-Hinton agar plates at 37°C. After 24 h, all isolates were cloned by successive sub-culturing, and passaged in 5 mL Mueller-Hinton broth for a re-growth at 37°C for 24h. Then, the bacterial suspensions were adjusted to McFarland 0.5 standard and aliquots (100 µL) were spread onto Mueller–Hinton agar (pH 7.2-7.4) plates in duplicate, and then, the standard antibiotic discs (Oxoid) were placed onto the plates and incubated at 37°C for 24 h. The list of the standard antibiotic discs is given in Table 1. *Escherichia coli* ATCC 8739, *Serratia marcescens* ATCC 13880, *Staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 9027 were used as control organisms. In addition, discs containing 0.9% NaCl were used as negative control. After incubation, the inhibition zone diameter around each disc was measured using a ruler, and isolates were classified as susceptible or resistant according to Clinical and Laboratory Standards Institute (CLSI) performance standards [19].

Table 1. Antibiotic discs used during this study.

Name of antibiotic	Amount of antibiotic per disc
Ampicillin	10 µg
Cefotaxime	30 µg
Ceftriaxone	30 µg
Erythromycin	15 µg
Gentamicin	10 µg
Methicillin	10 µg
Penicillin G	10 units
Polymyxin B	300 units
Streptomycin	10 µg
Vancomycin	30 µg

3. Results and discussion

The zone diameters of the bacteria growth found by Kirby-Bauer disc diffusion method using ampicillin, cefotaxime, ceftriaxone, erythromycin, gentamicin, methicillin, penicillin G, polymyxin B, streptomycin, vancomycin antibiotic discs were evaluated according to CLSI performance standards and the susceptibilities of each antibiotic (Table 2) were determined. Control results were recorded successfully.

Table 2. Antibiotic susceptibilities of the bacteria isolates.

Bacteria (n)	Antibiotic Susceptibilities (%)									
	Amp	Cn	Cro	Ctx	E	Met	P	PB	S	Va
Alcaligenes xylosoxidans (4)	0/4 (0)	1/4 (25)	0/3* (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)
Burkholderia cepacia (5)	0/5 (0)	0/5 (0)	0/5 (0)	1/5 (25)	1/5 (25)	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)
Klebsiella ornithinolytica (1)	0/1 (0)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Klebsiella oxytoca (1)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Klebsiella pneumoniae (1)	0/1 (0)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Ochrobactrum anthropi (2)	0/2 (0)	2/2 (100)	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)
Pseudomonas aeruginosa (1)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
Pseudomonas fluorescens (1)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Serratia liquefaciens (2)	0/2 (0)	1/2 (50)	1/2 (50)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)
Serratia marcescens (4)	0/4 (0)	4/4 (100)	1/4 (25)	2/4 (50)	0/4 (0)	0/4 (0)	0/4 (0)	0/4 (0)	1/4 (25)	0/4 (0)
Total Susceptibility	0/22	12/22	4/21	4/22	2/22	0/22	0/22	1/22	2/22	0/22
Susceptibility (%)	0	54.55	19.05	18.18	9.09	0	0	4.55	9.09	0

n: number of the isolates; Amp: Ampicillin; Cn: Gentamicin; Cro: Ceftriaxone; Ctx: Cefotaxime; E: Erythromycin; Met: Methicillin; P: Penicillin G; PB: Polymyxin B; S: Streptomycin; Va: Vancomycin; *one isolate of *Alcaligenes xylosoxidans* was not tested due to technical reasons.

The results showed that gentamicin was the most effective antibiotic against all bacteria tested. In addition, all bacteria tested in the study were resistant to ampicillin, methicillin, penicillin G and vancomycin (Table 2).

According to the susceptibility results, one strain (25%) of *Alcaligenes xylosoxidans* was sensitive to gentamicin. While one strain (25%) of *Burkholderia cepacia* was sensitive to cefotaxime, other one (25%) was sensitive to erythromycin. All (100%) strains belonging to *Klebsiella* genus were sensitive to gentamicin, and *Klebsiella ornithinolytica* and *Klebsiella pneumoniae* strains were also sensitive to ceftriaxone. While two strains (100%) of *Ochrobactrum anthropi* were sensitive to gentamicin, one strain (50%) was also sensitive to erythromycin. One (100%) *Pseudomonas aeruginosa* strain was sensitive to polymyxin B, on the other hand one (100%) *Pseudomonas fluorescens* strain was sensitive to gentamicin. One (50%) strain of *Serratia liquefaciens* was sensitive to gentamicin, cefotaxime, ceftriaxone and streptomycin. All strains (100%) of *Serratia marcescens* were sensitive to gentamicin, one (25%) was sensitive to ceftriaxone, two (25%) were sensitive to cefotaxime and, one (25%) was sensitive to streptomycin (Table 2).

The choice of antibiotics for the treatment of infections caused by bacteria is very important. For this reason, in order to determine the most appropriate treatment

method, it is necessary first to identify the bacterium that is the causative agent of the infection and then to determine the susceptibilities of the antibiotic of this bacterium. Since the antibiotic susceptibilities of bacteria may vary, antibiotic susceptibility testing has been a trend subject that has been investigated by researchers continuously from the past to the present [20]. For these reasons, in the current study in vitro antibiotic susceptibilities of various Gram-negative bacteria previously isolated from contact lens storage cases of asymptomatic wearers [17] were investigated by Kirby-Bauer disc diffusion method.

The current antimicrobial susceptibility testing methods are manual methods that include disc diffusion and gradient diffusion methods, and broth microdilution or rapid automated instrument methods that use commercially marketed materials and equipments. The advantages of disc diffusion method include simplicity requiring no special equipment, providing categorical results that are easily interpreted by all researchers, and flexibility in selecting discs for testing. In addition, this well standardized method is the lowest cost test among all current sensitivity tests. On the other hand, this method is not suitable for testing most of the fastidious or slow-growing bacteria. Other disadvantages of the disc diffusion method include the lack of automation, risk for contamination, and that is a time-consuming method [21, 22].

According to the obtained results from this study, *Pseudomonas aeruginosa*, the most common bacteria isolated from the keratitis infections, was resistant against all antibiotics excluding polymyxin B. However, it was reported in a study [23] that all *Pseudomonas aeruginosa* strains isolated from patients diagnosed with bacterial conjunctivitis were susceptible to gentamicin. Al-Zahrani [24] isolated *Pseudomonas aeruginosa* strains from lenses and eyes of contact lens and non-contact lens wearers and reported that 4.17% were resistant and 4.17% were sensitive to cefotaxime, 20.83% and 12.5% of strains were resistant and sensitive to ceftriaxone, respectively, and all were sensitive to gentamicin. The *Pseudomonas aeruginosa* strain tested in this study was resistant to gentamicin, unlike these reported studies. Similar to this study, Hedayati et al. [25] reported that all *Pseudomonas aeruginosa* strains isolated from corneal ulcers in patients with microbial keratitis were resistant to penicillin and gentamycin. When the results of these studies are compared with the current study, it is seen that *Pseudomonas aeruginosa* strains show different sensitivity to the same antibiotic. Thus, monitoring the antibiotic resistance patterns esp. for *Pseudomonas aeruginosa* strains is essential. In addition, in the light of these studies, the *Pseudomonas aeruginosa* strain tested in this study may be considered as an opportunistic pathogen that may lead to eye infections.

The strains of *Serratia marcescens* tested in our study, other common bacteria isolated from the keratitis infections, showed different sensitivities to the antibiotics. While all strains were sensitive to gentamicin, one (25%) was sensitive to ceftriaxone, two (25%) were sensitive to cefotaxime and, one (25%) was sensitive to streptomycin. Similar to this study, Yoon et al. [26] reported that all *Serratia marcescens* strains isolated from bacterial keratitis cases were susceptible to gentamicin and cefotaxime. According to these results, it may be suggested that the susceptibilities of *Serratia marcescens* strains to the same antibiotic do not vary much.

Although the most effective antibiotic in this study was found as gentamicin, it was noteworthy that the percentage of sensitivity was around 50%. In addition, all bacteria

were resistant to 4 of the 10 antibiotics tested, including ampicillin, methicillin, penicillin G, and vancomycin. This may be related to the fact that these bacteria were isolated from contact lens storage cases, a suitable environment for many bacteria to form biofilm. It was demonstrated by using microscopy techniques that bacteria entered in contact lens storage cases are found in biofilm instead of being planktonic [27]. And, it is well known that biofilm-associated bacteria are more resistant to antibiotics and biocides. Szczotka-Flynn et al. [28] reported that *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Staphlylococcus aureus* formed biofilms on contact lenses, and biofilm-associated bacteria were more resistant than planktonic cells to several soft contact lens care products.

4. Conclusion

The findings of this study re-emphasized the importance of continuous monitoring of the antibiotic resistance testing among the bacteria that are the potentially causative agents of bacterial keratitis in contact lens wearers. In the current study, gentamicin was found to be the most effective antibiotic against all bacteria tested. On the other hand, all the bacteria tested in this study were resistant to ampicillin, methicillin, penicillin G and vancomycin.

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