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Isolation and Resistance Pattern of Bacteria from Infected Corneal Ulcer Patients in Human

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Abstract

A corneal ulcer is an infectious or more severely infective disease of the cornea, which affects the epithelial surface of the corneal stroma after trachoma. Corneal ulcers are the world's second most important cause of blindness. This research was performed to determine the microbiological composition of corneal ulcers and the resistant pattern of bacteria collected from the patients of corneal ulcers from the department of ophthalmology at Rajshahi medical college hospital. The isolated bacteria were gram-negative. In *Klebsiella* selective agar, it showed a purple-magenta-colored colony, as well as in ESBL selective agar; the isolated bacteria showed a purple or blue-colored colony. Thus the isolate has been identified as *Klebsiella pneumoniae* by selective agar test. Eight different antibiotics were used to evaluate the isolate for antibiotic sensitivity using the disc diffusion method. The isolated bacteria showed resistance to three of the antibiotics, namely ampicillin, vancomycin, and bacitracin, and showed susceptibility to five such as gentamicin, amikacin, chloramphenicol, imipenem, levofloxacin. The isolated bacteria exhibited 37.5% resistance and 62.5% sensitivity against those eight antibiotics. According to the findings of this study, *Klebsiella pneumoniae* can be detected in corneal ulcers, and mentioned antibiotics can be used to treat these bacteria.

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Introduction

A corneal ulcer is one of the leading causes of visual incapacity and blindness in middle-income countries, including Bangladesh (Whitcher *et al.*, 2001). According to the World Health Organization, there are 45 million blind people in the world. India accounts for 5.4 million of the world's blind people. It's worth noting that corneal illnesses, such as corneal ulcers are a leading cause of visual loss. By 2020, India's corneal impairment is expected to reach 10.6 million people (Verma *et al.*, 2011). Corneal ulcers are a leading cause of blindness all over the world. Corneal ulcers cause about 10% of blindness incidents (Abubakar *et al.*, 2018). Various microorganisms such as bacteria, fungi, viruses, parasites, and polymicrobial infection can cause a corneal ulcer. Bacteria and fungi are the most prevalent causative microorganisms for corneal infection, depending on geographical and temporal variations. While viral and acanthamoeba keratitis is less common in developing countries, they are significant causes of corneal blindness (Ting *et al.*, 2021). It is a sight-threatening disorder that affects both men and women of all ages all over the world (Bhadange *et al.*, 2013). The corneal epithelial tissue layer provides an efficient shield against most microorganisms, so bacterial keratitis is rarely found in the regular eye (Palioura *et al.*, 2016). In the United States alone, around 930,000 cases receive external medical treatment, and 58,000 patients seek the emergency department (Collier *et al.*, 2014). The economic burden associated with this disease is undefined in developed countries but assumed to be devastating (Cao *et al.*, 2014). In different geographical locations, the epidemiology of corneal ulcers differs significantly, with the larger percentage of corneal ulcers from bacteria recorded from Australia, North America, Singapore, the Netherlands (Shah *et al.*, 2011). Different microbial agents may cause corneal ulceration. Although any organism can infiltrate the corneal stroma if the corneal protective mechanisms such as blinking, tear dynamics, and epithelial integrity are compromised, the microbial causes of suppurative corneal ulcers vary greatly across the globe (Leck *et al.*, 2002). Nowadays, *K. pneumoniae* is deliberated the most important source of hospital-acquired pneumonia in the United States, with 3% to 8% of all nosocomial bacterial infections accounted for by the organism (Jondle *et al.*, 2018). In Bangladesh, 33.55 % of all incidents of unilateral bl inding related to corneal ulcer complications have bee n reported (Zare *et al.*, 2019). Ulcers of the cornea are usually associated with several predisposing influences. Among the significant predisposing classes, trauma, chronic ocular surface disease, contact lens use, corneal surgery, ocular anesthetic disorder, diabetes mellitus, vitamin deficiency, and immune deficiencies are factors linked to the corneal ulcer (Tanure *et al.*, 2000).

Materials and methods

Sample collection

The sample was collected from the corneal ulcer patient from the department of ophthalmology at Rajshahi medical college hospital, Rajshahi, Bangladesh. The sample was collected from 60 years old male patient. Using a sterile swab, corneal scraping was collected by an ophthalmologist with the support of a slit lamp microscope.

Isolation, pure culture and morphological characterization of bacteria

The pure bacterial strain was isolated from a mixed culture of microorganisms using spreading and streaking procedures (Rahman *et al.*, 2021). The bacterial strain was isolated using the LB agar medium, which was incubated at 37 °C for 16 hours (Sanders, 2012). Then blood agar media were prepared (for 100ml blood agar media, 4g of blood agar base were added in 100ml distilled water by autoclaving at 121°C for 20 minutes. When the media were cooled at 55°C then added 5% sheep blood). The blood agar plates were prepared by pouring the media into the Petri dish. After solidification of blood agar plates, we used the spread plate procedure on the blood agar plate (Buck and Cleverdon, 1960). The inoculated blood agar medium was incubated aerobically at 37°C temperature. The bacterial culture media were checked for growth after 24 hours (Katara *et al.*, 2013). Bacterial isolate was formed in a blood

agar plate at 37 °C temperature. Colony morphology, growth patterns size, color, and shape have been reported after overnight incubation. The isolated bacteria were tested in MacConky agar, Hichrome ESBL, and *klebsiella* selective agar for the presence of the pathogen.

Hemolysis activity tests were performed. When bacteria was growing on blood agar, bacteria were classified according to their ability to cause hemolysis. Hemolysis was detected on a blood agar plate by streaking (Kato *et al*., 2017). The isolated bacteria were showed gamma hemolysis on blood agar.

Antibiotic sensitivity test

The disc diffusion method was used to investigate antibiotic sensitivity patterns in isolated bacteria (Bauer *et al*., 1966). In this analysis, 8 different antibiotics were tested to analyze the pattern of sensitivity to the isolated bacteria. For the antibiotic sensitivity test, isolated bacteria were incubated overnight in LB agar media in a shaker incubator with 120 rpm at 37°C for 24 h. 100 µl of bacterial culture was distributed on the LB agar plate with the help of a micropipette. For the distribution of the bacterial culture on the plates, a disinfected glass rod was used. Different commercially accessible antibiotic discs such as ampicillin (10mcg), amikacin (30mcg), bacitracin (10mcg), chloramphenicol (30mcg), gentamicin (120mcg), imipenem (10mcg), levofloxacin (5mcg), vancomycin (30mcg). Using sterile forceps, antibiotic discs were inserted into the LB agar plates and quietly pushed to achieve a good connection with the nutrient medium. Following that, the plates were incubated for 24 hours at 37°C. The inhibitory zones of the plates were calculated with the help of an mm scale followed by the technique mentioned previously (Bauer *et al*., 1966).Table 1 showed the antibiotic sensitivity test of the isolated bacteria.To measure the inhibiton zone we confirmed whether the bacteria are sensitive or resistance.

Results

Isolation of the bacterial strains

Within 24 h incubation, bacterial colonies appeared. Well-separated bacterial Colonies were picked up for further analyses. Pure culture of the bacteria appeared after repetitive cultivation in the plate of blood agar. It showed in Fig. 1A. In the gram staining test, the bacteria appeared a pink color, so it was gram-negative bacteria.

Table 1. Based on the inhibition zone, antibiotic sensitivity patterns of isolated bacterial strain.

Antibiotics	Required MIC	Calculated MIC
Ampicillin (10mcg)	17 mm-24 mm	6mm (Resistant)
Amikacin (30mcg)	13 mm-22 mm	20 mm (sensitive)
Bacitracin (10mcg)	8 mm	6mm (Resistant)
Chloramphenicol (10mcg)	18 mm-30 mm	22mm (sensitive)
Gentamicin (120mcg)	10 mm	21 mm (sensitive)
Imipenem(10mcg)	>23 mm	23 mm (sensitive)
Levofloxacin (5mcg)	>17 mm	17 mm (sensitive)
Vancomycin (30mcg)	17 mm-21 mm	5mm (Resistant)

Note: Resistant (R) 15 mm; Intermediate Resistant (I) =10–15 mm; Susceptible (S) >15 mm (Bauer *et al*., 1966).

Identification of the bacteria

The morphological characteristics, selective agar test of the isolated bacteria were examined, and based on the color change of the media, it was confirmed. The bacterial isolates were gram-negative, large in size, mucoid in shape, grayish-white in color, and growth patterns showed non-hemolytic in blood agar. In Klebsiella selective agar test, the isolated bacteria appeared as a purple-magenta-colored colony, thereby helping in the easy recognition of the organisms. So, the species was *Klebsiella pneumonia*. It showed in Fig. 2A. For further confirmation, the isolated bacteria were grown on ESBL selective agar and they appeared as purple or blue colored colonies.

Fig. 1. Isolate bacterial colonies (A).

It showed in Fig. 2B and the macConkey agar test showed lactose fermenting gram-negative bacteria in Fig. 2C. The isolated bacteria were showed gamma hemolysis because of no hemolysis of RBC or no change of the medium in the adjacent colonies. It is shown in Fig. 2D.

Antibiotic sensitivity test

The isolate was showed different patterns of antibiotic susceptibility to eight commercial antibiotics. The isolate was exhibited resistance to ampicillin, vancomycin, and bacitracin; moreover, the isolate was showed sensitivity to gentamicin, amikacin, chloramphenicol, imipenem, and levofloxacin. Among them, imipenem showed the highest MIC value of 23mm. Figure 3 showed eight antibiotics against isolated bacteria.

Discussion

Bacterial keratitis is a serious eye infection that may cause serious vision loss (McDonnell *et al.*, 1992). In patients with infectious keratitis, prompt care with effective antimicrobial eye drops is important to prevent complications. The seriousness of the corneal infection typically depends on the natural state of the cornea and the infectivity of the pathogenic bacteria (Vajpayee *et al.*, 2000). Antibiotic resistance has recently emerged as a major infection problem. Infectious keratitis has become more common in ophthalmology over the last decade, owing in part to an increase in contact lens wearers and immune-compromised patients (Toriyama *et al.*, 2018). The goal of this study was identification and resistance patterns of bacteria collected from the corneal ulcer patients, Rajshahi medical college hospital, Rajshahi, Bangladesh.

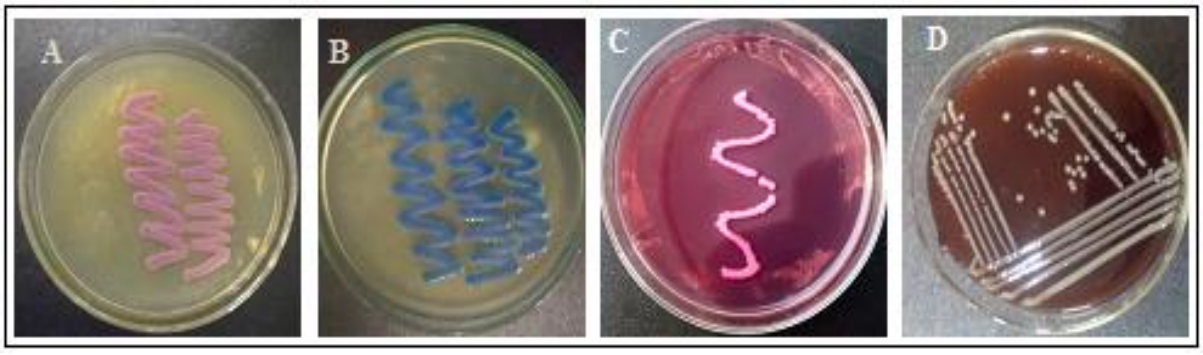

Fig. 2. The klebsiella selective agar test (A) ESBL selective agar test (B) MacConkey agar test (C) Hemolysis activity test (D).

According to the methods suggested by the International Organization for Standardization, the isolation of the proposed bacteria was guided (International Organisation for Standardisation, 2002). The isolated bacteria were gram-negative, large in size, mucoid in shape, grayish-white in color, and growth patterns showed non-hemolytic in blood agar related results were described by Lenchenko (Lenchenko *et al.*, 2020).

In Klebsiella selective agar test, the isolated bacteria were shown a purple-magenta colored colony which was an easy detection method of *Klebsiella pneumonia* and similar result found by Bergey ("Bergey's Manual® Syst. Bacteriol.," 2005) as well as in ESBL selective agar test the isolated bacteria

showed blue colored colony which also indicating the bacteria was *Klebsiella pneumonia* species and related results were described by Flonta (Flonta & Almas, 2011). In macConkey agar, test results were shown that it was lactose fermenting gram-negative pathogenic bacteria, a similar result found by Bruce *et al.* (Bruce *et al.*, 1981).

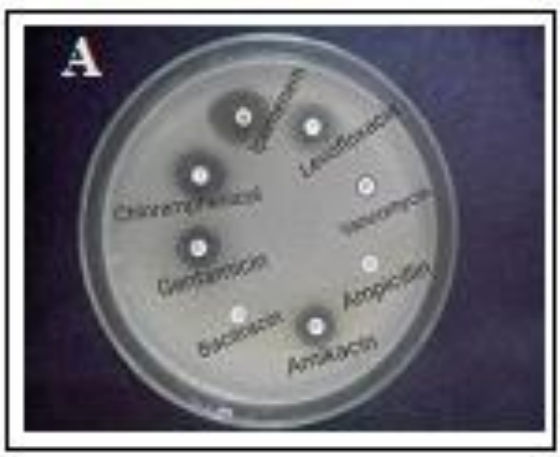

Fig. 3. Antibiotic sensitivity test (A).

Although our identified bacteria was very rare in normal corneal ulcer cases, Kaliamurthy found related results in the case of bacteria isolated from corneal ulcers (Kaliamurthy *et al.*, 2013). In the antibiotic sensitivity test; the isolated bacteria showed that it was sensitive to imipenem, levofloxacin, chloramphenicol, amikacin, gentamicin, and resistant to vancomycin, bacitracin, ampicillin. The isolated bacteria exhibited 37.5% resistance and 62.5% sensitivity against those commercial antibiotics (Jain & Kamble, 2017). Several types of research showed that neomycin, levofloxacin, and tobramycin could be effective against corneal ulcers, which justify our finding (Amatya *et al.*, 2012). The information gathered in this study about etiological agents and antibiograms can assist ophthalmologists in prescribing empirical antimicrobial therapy and devising strategies for proper case management, particularly in areas where laboratory facilities are restricted. This study's antibiotic susceptibility test of the bacterial isolate showed susceptibility to the majority of widely used antibiotics. Most of the isolates were found to be susceptible to chloramphenicol, and levofloxacin commonly used active ophthalmic antibiotics, which was other similar findings of our study (Jain and Kamble, 2017).

Conclusion

In this experiment, the bacterial isolate was magnificently isolated and characterized from the human corneal ulcer patient samples and identified as *Klebsiella pneumonia*.

This bacterial isolate was susceptible to gentamicin, amikacin, chloramphenicol, imipenem, and levofloxacin. Consequently, these antibiotics could be used to control these bacteria in the case they are found to be harmful to humans. More examination work is required to find out the additional effect of these bacterial isolates associated with the human corneal ulcer sample.

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