



Antibiotic Resistance Problems in Pathogenic Bacteria Associated With Cases of Corneal Ulcers

KEYWORDS

Antibiotic Resistance, Corneal Ulcer, Contact Lens

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ABSTRACT Corneal Ulcer means loss of corneal substances as a result of infection and formation of raw, excavated area." Corneal Ulcers can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites and sometimes it is allergic in nature or it can be due to endogenous infections. Bacterial keratitis is serious ocular infectious disease that can lead to significant vision loss. Any infectious process in the cornea producing a keratitis, mild or severe, requires prompt and vigorous treatment with an effective antimicrobial agents to minimize corneal scarring and vision loss. The bacteria are isolated from Corneal Ulcers and to determine the efficiency of empirical antibiotic therapy as the initial treatment for Corneal Ulcer.

INTRODUCTION

Number of blind people in the world is 45 million. Out of which 5.4 million blind people are in our country. Corneal Ulcer is a major cause of blindness throughout the world. About 10% cases of blindness are due to Corneal Ulcer. (Ninama et al., 2011).

Cornea is a clear transparent front part of the eye with a smooth shining surface. That covers Iris, Pupil and anterior chamber. The cornea with the anterior chamber and lens reflects light with the cornea accounting for approximately two-third of the eye's total optical power."Corneal Ulcer means loss of corneal substances as a result of infection and formation of raw, excavated area." (Chatterjee 1988).

Corneal Ulcers can be caused by exogenous infections i.e. by viruses, bacteria, fungi or parasites and sometimes it is allergic in nature or it can be due to endogenous infections. The term keratitis (Corneal Ulcer) had been introduced by "James Wardop" in 1869 in his essay on morbid anatomy of human eye. (Ninama et al., 2011; Chatterjee 1988).

Almost any organism can invade the corneal stroma if the normal corneal defence mechanisms, i.e., lids, tear film and corneal epithelium are compromised. (Garg et al., 1999).

Eighty percent of bacterial corneal ulcers are caused by *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas* species. The ability of an organism to adhere to the edge or base of epithelial defect signatures its pathogenicity. Certain bacterial toxins and enzymes help in the digestion and degradation of the corneal matrix. Bacterial keratitis is an acute or chronic, transient or recurrent infection of the cornea with varying predilection for anatomical and topographical parts of the cornea like marginal or central. It is a potentially sight-threatening corneal infection in humans that is generally found in eyes with predisposing elements, the most common of which is contact lens wear. The epidemiological data reveals the universal occurrence of this disease. With advances in the understanding of its pathogenesis, laboratory investigations and the availability of fourth generation antibiotics, the overall visual outcome in bacterial keratitis has improved with time. Particular attention should be given to this condition as it can progress very rapidly with complete corneal destruction occurring within 24–48 hours. Early diagnosis, which is primarily clinical and substantiated largely by microbiological data, and prompt treatment are needed to

minimize the possibility of permanent vision loss and reduce structural damage to the cornea. (Abdullah et al., 2009).

Bacterial keratitis is serious ocular infectious disease that can lead to significant vision loss. Any infectious process in the cornea producing a keratitis, mild or severe, requires prompt and vigorous treatment with an effective antimicrobial agents to minimize corneal scarring and vision loss. The goal of this study is to isolate the pathogenic bacteria from Corneal Ulcers and to determine the efficiency of empirical antibiotic therapy as the initial treatment for Corneal Ulcer.

MATERIALS AND METHODS

In assessment to isolate and identify the pathogenic bacteria from Corneal Ulcer and study their susceptibility and resistance pattern with various antibiotics, present work was under taken.

Collection of samples: A total of 100 samples were collected during period of June 2013 to March 2014 from ophthalmology hospital, government hospital and clinical laboratories.

Enrichment of samples: Samples were collected in sterile container containing 0.5ml of Brain Heart Infusion Broth (BHI) as enrichment culture medium that supports the growth of bacteria and then transferred immediately to laboratory for further processing. (Kaye et al., 2003)

Isolation and identification of pathogenic bacteria: After incubation loopful of each enriched culture was streaked on CLED agar and Nutrient agar plates were incubated at 37°C for 24 hours. Colonies with different morphological characters and Gram's characters were selected and inoculated on respective selective media viz. Blood agar, Mannitol salt agar, Cetrimide agar, *Pseudomonas* isolation agar (Hi-media), EMB (Eosin Methylene Blue) agar, CLED (Cystine-Lactose-Electrolyte-Deficient) agar, MacConkey agar. All the plates were incubated at 37°C for 24 hours.

All the suspicious screened colonies of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* were then analyzed for their biochemical character viz. Carbohydrate fermentation, IMViC, Enzymes etc. by inoculating into respective media. Further their identification was confirmed by Morphological, Biochemical and Cultural characteristics.

Antibiotic resistance pattern: After identification the isolates were subjected to antibiotic resistance and sensitivity pattern of pathogenic bacteria will be carried out by using disc diffusion technique. (Bauer et al., 1966)

The Antibiotics were used: Moxifloxacin (0.5%), Ofloxacin (0.3%), Tobramycin (1.33%), Cephazolin (5%), Vancomycin (30mcg), Chloramphenicol (30 mcg), Imipenem (10mcg), Gentamicin (10 mcg), Ciprofloxacin (10 mcg), Ceftazidime (30mcg). Antibiotic disc were placed on a lawn culture of the isolate under test on Mueller Hinton Agar (MHA).

RESULTS AND DISCUSSION

Table 1 : Frequency distribution of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolation from clinical samples

Sr. No.	Name of Organism	No. of Isolates	No. of Isolates (%)
1.	<i>Staphylococcus aureus</i>	31	45.59
2.	<i>Pseudomonas aeruginosa</i>	22	32.35
3.	<i>Klebsiella pneumoniae</i>	15	22.06

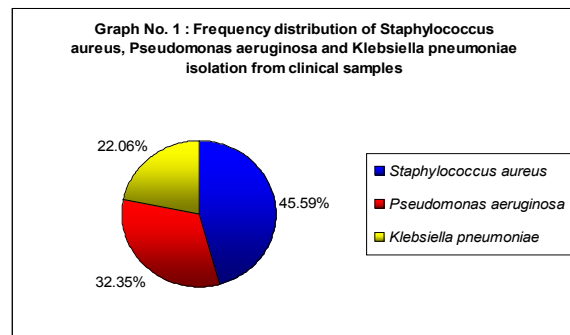
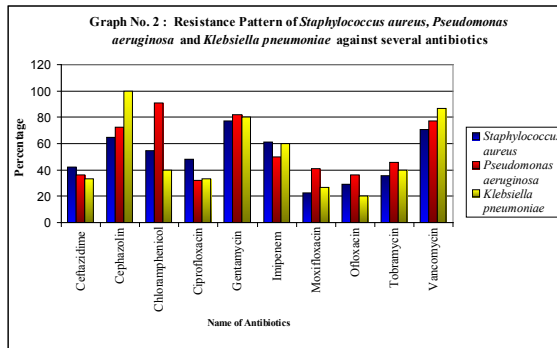


Table 2: Resistance Pattern of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* against several antibiotics

Sr. No.	Antibiotics	No. of Resistance in Percentage		
		<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>
1	Ceftazidime	41.93	36.36	33.33
2	Cephazolin	64.51	72.72	100
3	Chloramphenicol	54.83	90.90	40.00
4	Ciprofloxacin	48.38	31.81	33.33
5	Gentamycin	77.41	81.81	80.00
6	Imipenem	61.29	50.00	60.00
7	Moxifloxacin	22.58	40.90	26.66
8	Ofloxacin	29.03	36.36	20.00
9	Tobramycin	35.48	45.45	40.00
10	Vancomycin	70.96	77.27	86.66



In present study 100 samples were collected during period of June 2013 to March 2014. The patients were of both sex and age groups varying from 20 to 70 years. Out of 100 samples, bacteria were isolated from 37 samples. A total of 68 isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* isolated from 37 samples. Among 68 isolates 31 were *Staphylococcus aureus*, 22 were *Pseudomonas aeruginosa*, 15 were *Klebsiella pneumoniae*. (See Table & Graph No.1)

The organisms were identified based on the colony morphology and biochemical reaction. *S. aureus* isolates are confirmed based on yellowish colony coloration and pigmentation on Mannitol salt agar and golden yellow colonies on Milk agar. *P. aeruginosa* isolates are confirmed based on colony coloration or pigmentation i.e. blue-green colony due to pyocyanin pigment and yellow-green colony due to fluorescent pigmentation or also known as pyoverdine on selective media i.e. Cetrinide agar and *Pseudomonas* isolation agar. *K. pneumoniae* isolates are confirmed based on pale yellowish mucoid colonies on CLED agar and pink mucoid colonies on MacConkey agar.

The sensitivity and resistance pattern of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* against several antibiotics were observed by disc diffusion method on Mueller Hinton Agar (MHA- Hi-media) such as Moxifloxacin, Ofloxacin, Tobramycin, Cephazolin, Vancomycin, Chloramphenicol, Imipenem, Gentamicin, Ciprofloxacin, Ceftazidime. *Staphylococcus aureus* showed 22% to 78% resistance to these antibiotics. *Pseudomonas aeruginosa* showed 31% to 91% resistance to these antibiotics. *Klebsiella pneumoniae* showed 20% to 100% resistance to these antibiotics. The Moxifloxacin was 78% sensitive to *S. aureus* and Ciprofloxacin was 69% sensitive to *P. aeruginosa* and Ofloxacin was 80% sensitive to *K. pneumoniae*. (See Table & Graph No.2)

The ability of an organism to adhere to the edge or base of an epithelial defect signature its pathogenicity. Membrane appendages such as fibrillae in Gram-positive organisms, fimbriae and glycocalyx in Gram-negative bacteria help these organisms adhere to damaged epithelial cells and stroma. The adhering quality of *Pseudomonas aeruginosa* is due to its pili containing calcium and magnesium. *Pseudomonas aeruginosa* gets attached to both contact lenses and epithelial breaks due to its biofilm, a coating around the organism. (Abdullah et al., 2009).

Gram-negative corneal bacterial infections, on the other hand, are mostly rapid in onset and progress fast due to lytic enzymes like protease, lipase and elastase. These infections can lead to corneal perforation and the loss of an eye.

Cycloplegic agents such as atropine sulphate 1%, homatropine 1% or cyclopentolate 1% instilled three times a day reduce ciliary spasm and produce mydriasis, thereby relieving pain and preventing synechiae formation. (Garg et al., 1999).

Our results are in accordance with Constantinou *et al.*, 2006 and Abdullah *et al.*, 2009. They observed all these antibiotic treatments such as moxifloxacin (1.0%), ofloxacin (0.3%) tobramycin (1.33%) were effective against a wide range of ocular isolates in the treatment of severe bacterial keratitis.

Prompt diagnosis of corneal ulcers and treatment with appropriate antibiotics prevent blindness and devastating visual disability.

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