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Combination therapy on pathogenic bacteria from corneal ulcers

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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 sever, 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 antibiotic combination therapy as the initial treatment for Corneal Ulcer.

Keywords: Combination Therapy, Corneal Ulcer, Contact Lens, Pathogenic Bacteria.

1. 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) [6].
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, B.M. 1988) [2].
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 Wardrop" in 1869 in his essay on morbid anatomy of human eye. (Ninama *et al.*, 2011; Chatterjee, B.M 1988) [6, 2].
Almost any organism can invade the corneal stroma if the normal corneal defence mechanisms, i.e., lids, tear film and corneal epithelium are compromised. (Prashant Garg *et al.*, 1999) [4].
Eighty percent of bacterial corneal ulcers are caused by S*taphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas* species. The ability of an organism to adhere to the edge or base of an epithelial defect signatures its pathogenicity. certain bacterial toxins and enzymes help in the digestion and degradation of the corneal matrix. (Abdullah *et al.*, 2009) [3].
Since keratitis is not included in the five target diseases of WHO for blindness prevention, most of the data regarding keratitis is from individual publications. Bacterial keratitis is one of the most important causes of corneal opacifications, which is the second common cause of legal blindness world-wide after cataracts. The pattern of microbial keratitis varies with geographic region and according to the local climate. (Abdullah *et al.*, 2009) [3].
Progress of human beings occurs in every field as they pass on their heritage from one generation to another. Generation dies but its knowledge is passed on to the next generation which after confirming the old facts and adding its own experiences in turn passes all these to the next generation. (Ninama *et al.*, 2011) [6]
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) [3].

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 sever, requires prompt and vigorous treatment with an effective antimicrobial agents to minimize corneal scarring and vision loss. The goal of this study will to isolate the pathogenic bacteria from Corneal Ulcers and to determine the efficiency of empirical antibiotic therapy as the initial treatment for Corneal Ulcer.

2. 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.

A total of 100 samples were collected during period of one year from ophthalmology hospital, government hospital and clinical laboratories. Samples were collected in sterile container containing 0.5ml of Brain Heart Infusion Broth (BHI) as enrichment culture medium that suppors the growth of bacteria (Kaye *et al.*, 2003) [5] and then transferred immediately to laboratory for further processing.

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.

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) [1]

The Antibiotics were used: Moxifloxacin (0.5%), Ofloxacin (0.3%), Tobramycin (1.33%), Cefazolin (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).

In this study Combination Therapy was also performed by the concurrent use of Ceftazidime and Ciprofloxacin, Vancomycin and Cefazolin, Tobramycin and Cefazolin. The concurrent use of ceftazidime and amikacin or ceftazidime and ciprofloxacin as the initial treatment of keratitis and corneal ulcers suggested by Mohammadpour *et al.*, in 2011 [7].

3. 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.	*Staphylococcus aureus*	31	45.59
2.	*Pseudomonas aeruginosa*	22	32.35
3.	*Klebsiella pneumoniae*	15	22.06

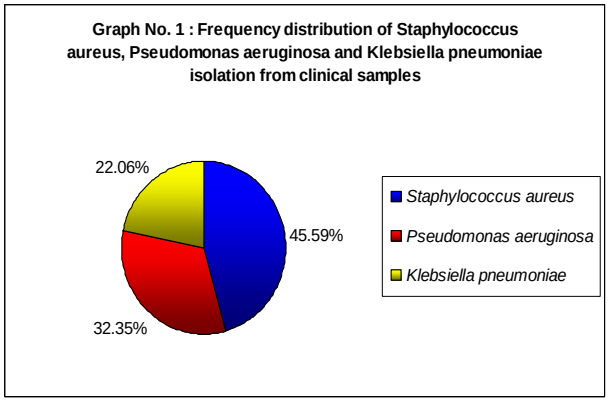


Table 2: Resistance Pattern of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* against several antibiotics

Sr. No.	Antibiotics	*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*	
		No. of Resistance	Percen- tage %	No. of Resistance	Percen-tage %	No. of Resistance	Percen-tage %
1	Ceftazidime	13	41.93	08	36.36	05	33.33
2	Cefazolin	20	64.51	16	72.72	15	100
3	Chloramphenicol	17	54.83	20	90.90	06	40.00
4	Ciprofloxacin	15	48.38	07	31.81	05	33.33
5	Gentamycin	24	77.41	18	81.81	12	80.00
6	Imipenem	19	61.29	11	50.00	09	60.00
7	Moxifloxacin	07	22.58	09	40.90	04	26.66
8	Ofloxacin	09	29.03	08	36.36	03	20.00
9	Tobramycin	11	35.48	10	45.45	06	40.00
10	Vancomycin	22	70.96	17	77.27	13	86.66

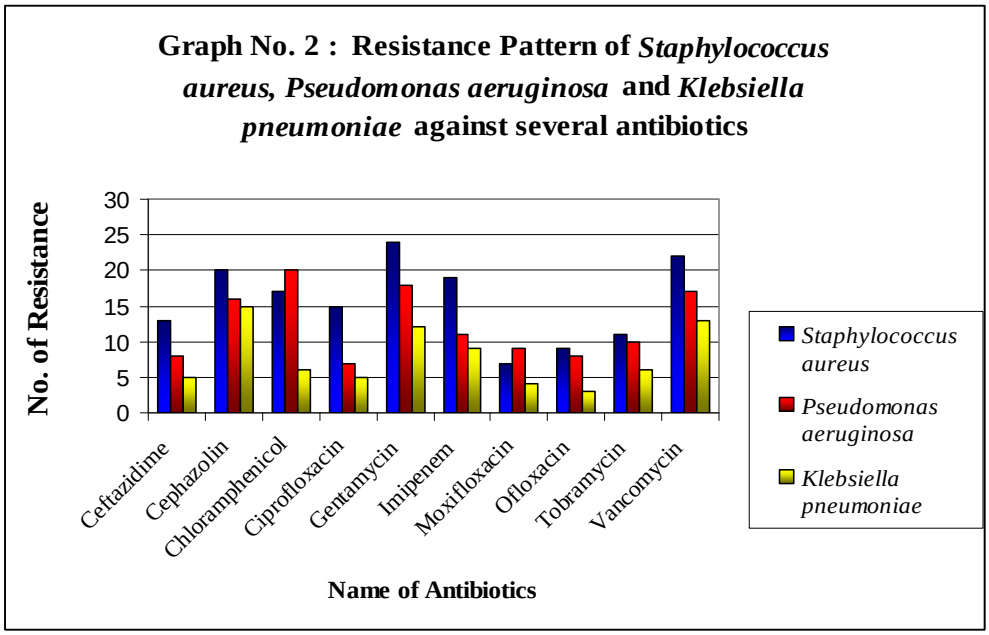
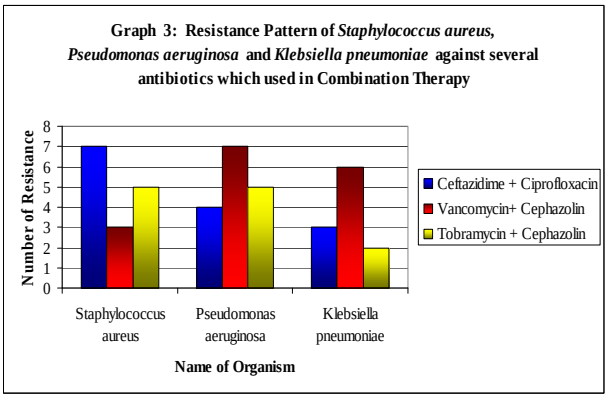


Table 3: Resistance Pattern of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* against several antibiotics which used in Combination Therapy

Sr. No.	Antibiotics	*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*	
		No. of Resistance	Percen- tage %	No. of Resistance	Percen-tage %	No. of Resistance	Percen-tage %
1	Ceftazidime + Ciprofloxacin	07	22.58	04	18.18	03	20.00
2	Vancomycin+ Cefazolin	03	09.67	07	31.81	06	40.00
3	Tobramycin + Cefazolin	05	16.12	05	22.72	02	13.33



In present study 100 samples were collected during period of one year. The patients were of both sex and age groups varying from 20 to 70 years. Out of 100 samples, bacteria were isolated from 47 samples. A total of 68 isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* isolated from the samples. Among 68 isolates 31 were *Staphylococcus aureus*, 22 were *Pseudomonas aeruginosa*, 15 were *Klebsiella pneumoniae*.

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 pyoverdin on selective media *i.e.* Cetrimide 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, Cefazolin, 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*.

Ofloxacin (0.3%) can be substituted for ciprofloxacin as a monotherapy in situations of unknown organisms or a new case or where there is no growth on first culture. Increasing resistance to the fourth generation fluoroquinolones has been reported amongst *Staphylococcus* species in the USA, and *Pseudomonas* species in India. (Kaatz *et al*., 1988) [9].

Constantinou *et al*., 2006 determine the clinical efficacy and safety of moxifloxacin (1.0%) in 77 patients with bacterial keratitis compared with 74 patients treated with ofloxacin (0.3%) or 78 patients treated with tobramycin (1.33%)/Cefazolin (5%). After corneal specimens were obtained, the assigned study medication was instilled every hour, day and night. No difference in healing rate, cure rate, or complications between fortified Cefazolin and

tobramycin, ofloxacin, or moxifloxacin was seen in this study.

Currently no single antibiotic is effective against all bacterial species causing microbial keratitis. Initial broad-spectrum therapy is recommended until the offending microorganism is identified in culture. Combination therapy with an antibiotic active against gram-positive bacteria and gram-negative bacteria. (Mohammadpour *et al.,* 2011) '[7].

In current study Combination Therapy was also performed by the concurrent use of Ceftazidime and Ciprofloxacin, Vancomycin and Cefazolin, Tobramycin and Cefazolin. Vancomycin and Cefazolin were 91% sensitive to *S. aureus.* Ceftazidime and Ciprofloxacin were 82% sensitive to *P. aeruginosa.* Tobramycin and Cefazolin were 87% sensitive to *K. pneumoniae.*

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) [3].

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) [4].

Our results are in accordance with Mohammadpour *et al.,* 2011 [7] and Abdullah *et al.,* 2009 [3]. They observed Combination therapy is the most effective regimen for the initial treatment of corneal ulcers.

4. Conclusions

This current study strongly suggests that Cefazolin in not efficacious in these bacteria. The results of the current study suggest the concurrent use of Ceftazidime and Ciprofloxacin, Vancomycin and Cefazolin, Tobramycin and Cefazolin are effective regimen for the initial treatment of corneal ulcers. Prompt diagnosis of corneal ulcers and treatment with appropriate antibiotics prevent blindness and devastating visual disability.

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