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Original Research Article

Isolation and identification of bacterial isolates causing conjunctivitis and Antibiogram study.

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Abstract

A total of about 25 samples of conjuctival samples were collected from the cataract patients of the age group above 50 years by using appropriate sterile moistened swabs and placed in sterile saline test tubes from Government Hospital, Kanchipuram. Samples were transported to the laboratory within an hour. The isolation and identification of bacterial isolates causing conjunctivitis was carried out using standard microbiological procedures. The antimicrobial assay of conjuctival isolates against standard antibiotics was carried out. The flowers of *Tabernaemontana divaricata* were collected and washed off from dust and soil particles, shade dried and grind into fine powder. The preparation of flower extracts using solvents such as aqueous, butanol, chloroform and ethanol. Antibiogram of Conjuctival isolates using *Tabernaemontana divaricata* flower extracts by AWD assay was carried out and the zone of inhibition was measured.

Keywords: conjuctival samples, bacterial isolates, *Tabernaemontana divaricata*.

Introduction

The human eye is an organ that reacts to light and has several purposes. As a sense organ, the mammalian eye allows vision. Rod and cone cells in the retina allow conscious light perception and vision including color differentiation and the perception of depth. The human eye can distinguish about 10 million colors.
Similar to the eyes of other mammals, the human eye's non-image-forming photosensitive ganglion cells in the retina receive light signals which affect adjustment of the size of the pupil, regulation and suppression of the hormone melatonin and entrainment of the body clock.

Ocular Microbiology

Ocular microbiology remains an applied science The advancements in molecular biology and the newer technologies pave way for better understanding of ocular diseases Advances in the field of infectious diseases are rapid. The developments have made major contributions in the control and probably even eradication of many types of eye infections. Blinding diseases such as trachoma has been brought under control because of development of rapid diagnostic methods and public health measures. There is dramatic decrease in classical infections of the eye, but new and emerging eye infections are on the rise.

Many opportunistic pathogenic agents are increasingly encountered in ocular infections due to widespread use of topical and systemic immune suppressive agents, increasing numbers of patients with human immune deficiency virus (HIV) infection and with organ transplants who are on immunosupressive therapy. These opportunistic pathogens also cause ocular infections due to increased use of contact lens. The dreaded infections endophthalmitis following cataract extraction and lens implantation often are caused by opportunistic pathogens.

To understand ocular microbiology and ocular diagnostic microbiology, it is essential to have the basic knowledge of anatomy of the eye and the common microbial agents associated with the ocular infections. The principles involved in mechanism of the ocular surface and parameters intraocular immunomechanisms are useful in understanding ocular microbials. Basic knowledge of pathogenesis of ocular infection and structural consequences are essential in understanding ocular microbes.

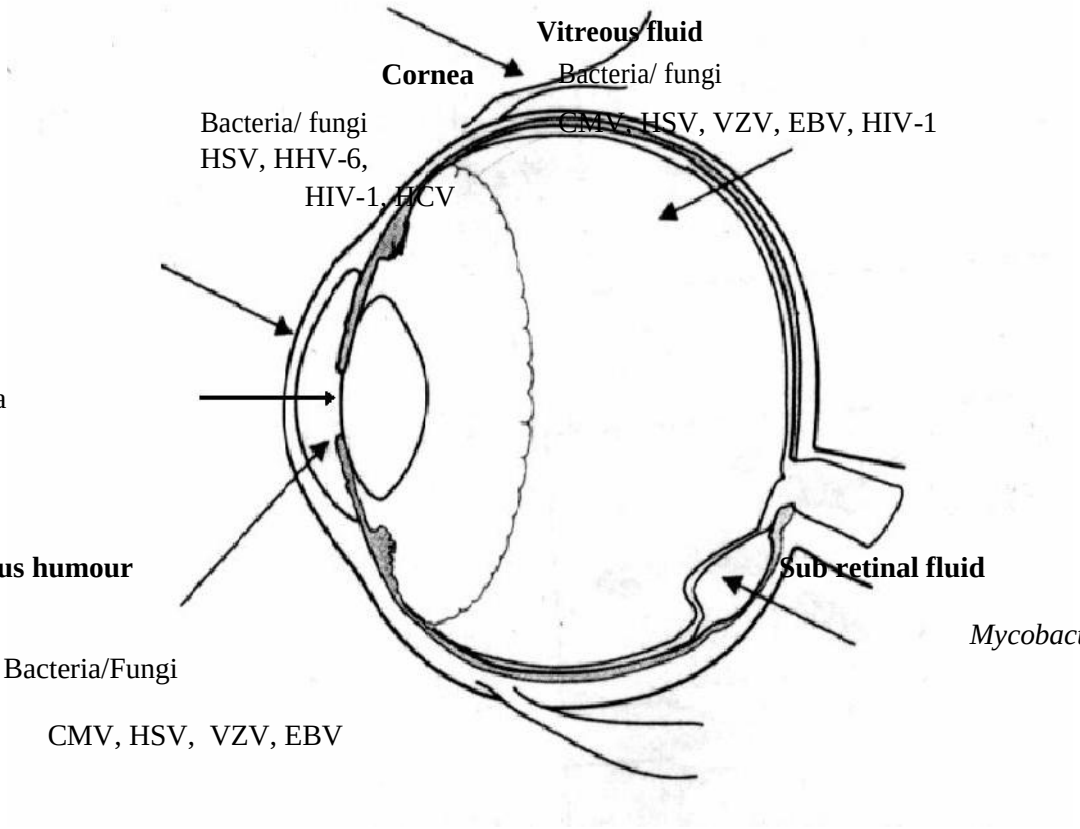
Transmission of infection in Ophthalmic practice and the methods of prevention are important public health issues. microbial agents from the environment. As in other organ systems exposed to environment, ocular surface is colonized by microbial agents which are mainly commensals. These residents induce minimal activation of inflammation and immune responses of the host. The exact microbial population of the ocular surface depends on the age of the host and geographical location and the climate.

External ocular infections are among the leading causes of ocular morbidity and blindness in developing countries. In spite of constant exposure to infective agents from environment, conjunctiva and cornea are protected by efficient defense mechanisms. Several risk factors as age, sex, immune status and socio -economic background determine the pathogenesis of infective ophthalmic diseases. Advances in microbiological techniques have made it possible not only to understand the pathogenesis of these infections but also develop better diagnostic methods. Despite dramatic decrease in classical ocular infections, newer infective diseaseare increasingly encountered.

Conjunctiva

Bacteria

Chlamydia, HSV, VZV, HPV, Adenovirus



Endophthalmitis

Endophthalmitis is an inflammation of the internal coats of the eye. It is a possible complication of all intraocular surgeries, particularly cataract surgery, with possible loss of vision and the eye itself. Infectious etiology is the most common and various bacteria and fungi have been isolated as the cause of the endophthalmitis. Other causes include penetrating trauma and retained intraocular foreign bodies.

Signs and symptoms

In cases of endophthalmitis, one usually finds a history of recent intraocular surgery or penetrating ocular trauma. In some cases of endogenous endophthalmitis—particularly in immunocompromised patients or those with diabetes—the spread of infection may have been hematogenous (via the blood-stream).

Endophthalmitis is usually accompanied by severe pain, loss of vision, and redness of the conjunctiva and the underlying episclera. Hypopyon can be present in endophthalmitis and should be looked for on examination by a slit lamp.

Causative organisms

Bacteria

Neisseria meningitidis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, other streptococcal spp., Propionibacterium acnes, Pseudomonas aeruginosa and other gram negative organisms.

Viruses

Herpes simplex virus.

Fungi

Candida spp.

Parasites

Toxoplasma gondii, Toxocara.

Complications

- Panophthalmitis — Progression to involve all the coats of the eye.
- Corneal ulcer
- Orbital cellulitis
- no light perception vision

Treatment

The patient needs urgent examination by an ophthalmologist preferably a vitreo-retina specialist who will usually decide for urgent intervention to provide intravitreal injection of potent antibiotics and also prepare for an urgent surgery (pars plana vitrectomy) as needed. Evisceration may be required to remove a severe and intractable infection resulting in blind and painful eye.

Prevention

A Cochrane Review sought to evaluate the effects of perioperative antibiotic prophylaxis for endophthalmitis following cataract surgery. Separate studies from the research showed that a periocular injection of penicillin with chloramphenicol-suphadimidine eye drops and an intracameral cefuroxime injection with topical levofloxacin resulted in a risk reduction of developing endophthalmitis following cataract surgery for subjects.

Tabernaemontana divaricata

Tabernaemontana divaricata (Apocynaceae), commonly called Pinwheel Flower, Crape Jasmine, East India Rosebay and Nero's Crown is an evergreen shrub probably native to India and now cultivated throughout South East Asia and the warmer regions of continental Asia. In zones where it is not hardy it is grown as a house/glasshouse plant for its attractive flowers and foliage. The stem exudes a milky latex when broken, whence the name Milk Flower in the Bengali language. Known as Wathusudda (meaning "garden white") in the Sinhalese language. In telugu language it's called as nandivardhanam. Other Indian names include Chandni and Taggar. The plant contains several alkaloids and, like many other Apocynaceae, is toxic and medicinal.

Aim and objectives

- Collection of ocular sample from cataract patients
- Isolation and identification of bacterial isolates causing conjuctivitis
- Antimicrobial assay of conjuctival isolates against standard antibiotics
- Collection of *Tabernaemontana divaricata* flowers

- Preparation of flower extracts using solvents such as aqueous, butanol, chloroform and ethanol.
- Antibiogram of Conjuctival isolates using *Tabernaemontana divaricata* flower extracts by AWD assay.

Materials and Methods

Collection of samples

A total of about 25 samples of conjuctival samples were collected from the cataract patients of the age group above 50 years by using appropriate sterile moistened swabs and placed in sterile saline test tubes from Government Hospital, Kanchipuram.

Transportation of sample

Samples were transported to the laboratory within an hour.

Processing of sample

Microscopic Examination

Staining Method

The collected specimen was subjected to differential staining by Gram's Staining techniques and observed for the presence of Gram positive or Gram negative organisms under oil - immersion lens of light Microscope.

Culture Media used

Basal medium
Nutrient Agar
Blood Agar
Eosin Methylene Blue Agar

Biochemical Characteristics

Catalase test

A loop full of culture was introduced into hydrogen peroxide, formation of bubbles were observed.

Oxidase test

The oxidase disc (Tetramethyl – Paraphenylene - diamine dihydrochloride) was taken and the overnight culture of the test organism was streaked on the disc using a sterile loop.
The colour change was observed.

Indole test

Tryptone broth was prepared and dispensed in the test tubes. After sterilization, the culture was inoculated and incubated at 37^0C for 24 hours. After incubation, the Kovac's reagent was added in drops and the colour change was observed.

Methyl red test

MR broth was prepared and dispensed in the test tubes.
After sterilization, the culture was inoculated and incubated at 37^0C for 24 hrs.
After incubation, Methyl red reagent was added and gently mixed.
The result was observed after 15 minutes.

Voges – Proskauer Test

VP broth was prepared and dispensed in the test tubes.
After sterilization, the culture was inoculated and incubated at 37^0 C for 24 hrs.
After incubation, 0.5 ml of VP reagent Aand 0.5 ml of VP reagent B was added and gently mixed.
The result was observed after 15 minutes.

Citrate utilization test

Slants of Simmon's Citrate agar was prepared.
After sterilization, the overnight culture was streaked in the slants and incubated at 37^0C for 24 hrs.
After incubation, the change was observed .

Triple sugar Iron Test:

The TSI agar slants were prepared.
Then, the test organism was inoculated and incubated for 24 hrs at 37^0C.
After incubation, the result was observed.

Urease test

Christensen's urea agar slants were prepared and sterilized.
The test organism was inoculated and incubated at 37^0C for 24 hrs.
After incubation, the result was observed.

Antimicrobial susceptibility of conjuctival isolates against standard antibiotics

The sterilized Mueller Hinton Agar medium was poured into a sterile Petri plate.

After solidification, a lawn culture of the organism was made and it is allowed to dry for 5 minutes.
The standard antibiotic discs were placed on to the surface of the inoculated plates (Amikacin, Rifampicin,Vancomycin, Tetracycline and Penicillin) and gently pressed in order to adhere the discs.
Then the plates were incubated at 37^0C for 18 - 24 hours.

Collection of plant flowers

The fresh flowers of the *Tabernaemontana divaricata* plant were collected. The flowers were washed with running tap water to remove the surface dust particles and blotted with clean white muslin cloth. The flowers were shade dried completely and were grind into a fine powder.

Preparation of Extracts

A known quantity of flower powder (25gm each) was taken in a separate 250 ml beaker and 100 ml of each solvent Aqueous, Butanol, Chloroform and Ethanol was added. The preparation was kept at room temperature for 48 hrs and rapidly stirred using glass rod every 4 hrs. After 48 hrs, the flower extracts were filtered through Whatmann No. 1 fitter paper to exclude the leaf powder. Then the extract was taken in separate beaker and kept in a water bath at 40-50 ^{0}C until the solvent gets evaporated. A greasy final material obtained from the flower was transferred to sterile screw capped bottle and stored under refrigerated condition till use.

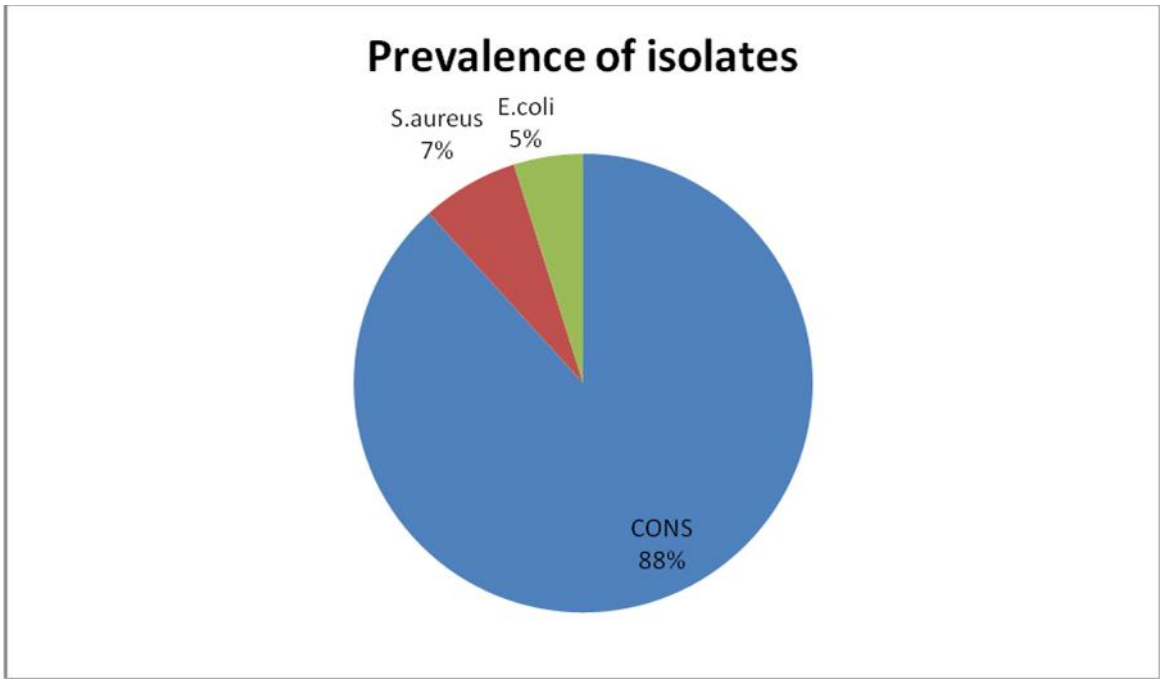
Antibiogram of Conjuctival isolates using *Tabernaemontana divaricata* flower extracts

The sterilized Muller Hinton Agar medium was poured into a sterile Petri plate. After solidification, a lawn culture of the organism was made and it is allowed to dry for 5 minutes. Using gel punch four wells were made and a known quantity of 20µl of each extract was added to the wells. Then the plates were incubated at 37^0C for 18 - 24 hours.
After incubation the zone of inhibition around the disc were measured

Results

Out of 25 samples collected, 18 were found be positive for the prevalence of (*CONS*) *Staphylococcus sps.* 6 were found to be *Staphylococcus aureus* and 1 *Escherichia coli* based on their morphology, cultural and biochemical characteristics and the results are presented in diagram – 1.

Diagram - 1 Prevalence of isolates



Identification of Conjunctival isolates

The number of isolates obtained from the conjuctival sample was given in Table – 2. and photos 1 – 7.

Table – 2 Identification of Conjunctival isolates

S.No	Ocular bacterial isolates	Number of strains	%
1.	CONS *Staphylococcus*	18	88 %
2.	*Staphylococcus aureus*	6	7 %
3.	*Escherichia coli*	1	5 %

Antimicrobial susceptibility of Conjunctival isolates against standard antibiotics

The antimicrobial susceptibility of the isolates against the standard antibiotic was given in Table – 3.

Table – 3 Antimicrobial susceptibility of Conjunctival isolates against standard antibiotics

S.No	Ocular bacterial isolates	Antibiotics used and their zone of inhibition in mm				
		Amikacin	Rifampicin	Penicillin	Vancomycin	Tetracycline
1.	CONS *Staphylococcus*	18	17	16	10	20
2.	*Staphylococcus aureus*	06	12	14	10	14
3.	*Escherichia coli*	10	12	11	14	15

Antimicrobial susceptibility of Conjunctival isolates against *Tabernaemontana divaricata* flower extracts

The antimicrobial susceptibility of Conjunctival isolates against *Tabernaemontana divaricata* flower extracts was given in Table -4 and photos 8, 9 and 10.

Table – 4 Antimicrobial susceptibility of Conjunctival isolates against *Tabernaemontana divaricata* flower extracts

S.No	Ocular bacterial isolates	*Tabernaemontana divaricata* flower extracts and their zone of inhibition in mm				
		Aqueous	Butanol	Chloroform	Ethanol	Amikacin
1.	CONS *Staphylococcus*	10	10	11	12	10
2.	*Staphylococcus aureus*	10	11	11	14	14
3.	*Escherichia coli*	14	13	11	15	14

Discussion

Endophthalmitis means bacterial or fungal infection inside the eye involving the vitreous and/or aqueous humors. Most cases are exogenous and occur after eye surgery, after penetrating ocular trauma, or as an extension of corneal infection. An increasing number of cases are occurring after intravitreal injections of anti-vascular endothelial growth factor (VEGF) medications. Endophthalmitis may also be endogenous, arising from bacteraemic or fungaemic seeding of the eye. The infected eye never serves as a source of bacteraemia or fungaemia, however. The most common pathogens in endophthalmitis vary by category. Coagulase-negative staphylococci are the most common causes of post-cataract endophthalmitis (Durand *et al*., 2013).

In this present study an attempt is made to isolate and identify the conjuctival isolates from the cataract patients and their antibiogram using standard antibiotics was carried out. The prevalence of CONS was similar to the studies conducted by Srivastava *et al*., 2014.

Other isolates were *Staphylococcus aureus* and *Escherichia coli* which were used for their studies of antibiogram against *Tabernaemontana divaricata* flower extracts carried out by Sumitha *et al*., 2015.

Tabernaemontana divaricata leaves are large, shiny and deep green in colour and the size is about 6-inches in length and 2-inches in width. The flowers are commonly known as Crape jasmine. It blooms in spring but flowers appear sporatically all year. The waxy blossoms are white five-petaled pinwheels that are borne in small clusters on the stem tips.

The flower extracts were prepared for different solvents such as Aqueous, Butanol, Chloroform and Ethanol.

They showed reasonable zone of inhibition against the *CONS Staphylococcus, Staphylococcus aureus and Escherichia coli*. The zone of inhibition for Ethanolic extract is 10mm for *CONS Staphylococcus*, 14 mm *Staphylococcus aureus* and 15 mm for *Escherichia coli*. Among the four, the ethanolic leaf extract was found to be very effective as it shows maximum zone of inhibition.

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