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Pathogenesis of microbial keratitis

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ABSTRACT

Microbial keratitis is a sight-threatening ocular infection caused by bacteria, fungi, and protist pathogens. Epithelial defects and injuries are key predisposing factors making the eye susceptible to corneal pathogens. Among bacterial pathogens, the most common agents responsible for keratitis include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Serratia* species. Fungal agents of corneal infections include both filamentous as well as yeast, including *Fusarium*, *Aspergillus*, *Phaeohyphomycetes, Curvularia, Paecilomyces*, *Scedosporium* and *Candida* species, while in protists, *Acanthamoeba* spp. are responsible for causing ocular disease. Clinical features include redness, pain, tearing, blur vision and inflammation but symptoms vary depending on the causative agent. The underlying molecular mechanisms associated with microbial pathogenesis include virulence factors as well as the host factors that aid in the progression of keratitis, resulting in damage to the ocular tissue. The treatment therefore should focus not only on the elimination of the culprit but also on the neutralization of virulence factors to minimize the damage, in addition to repairing the damaged tissue. A complete understanding of the pathogenesis of microbial keratitis will lead to the rational development of therapeutic interventions. This is a timely review of our current understanding of the advances made in this field in a comprehensible manner. Coupled with the recently available genome sequence information and high throughput genomics technology, and the availability of innovative approaches, this will stimulate interest in this field.

1. Introduction

The unique structure of the human eye along with its exposure to environment, renders it susceptible to a number of agents responsible for causing infection. Injuries and epithelial defects impair defense mechanisms and exposure to pathogenic microbes can lead to corneal inflammation or keratitis. The intact ocular surface thwarts most microorganisms but once anatomical barriers are breached, host defenses against pathogens are less than sufficient to prevent infection that can lead to eventual loss of vision. Microbial or infectious keratitis is a potentially sight-threatening ocular condition caused by bacteria, fungi, protists etc. It is the inflammation of the cornea caused by pathogenic microbes that eventually invades the corneal stroma causing inflammation, and ultimately destruction of these structures [7,210]. The most common pre-disposing factors to develop infectious keratitis include the use of contact lenses, especially overnight or extended wear lenses, inadequate disinfecting solutions, trauma, ocular surgery especially corneal surgery, chronic ocular surface disease, systemic disease like diabetes mellitus and/or extended use of topical corticosteroids [180,210]. Patients usually present with redness, tearing, rapid onset of pain and blur vision. Clinical presentation may vary depending on the causative agent responsible for causing keratitis. The condition should be treated as a medical emergency and adequate treatment should commence promptly. If appropriate antimicrobial treatment is delayed, only 50% of the eye gains good visual acuity [83]. Appropriate management and timely onset of treatment can reduce the incidence of severe visual loss restricting corneal damage. Here, we present a concise review of the bacterial, fungal and protist keratitis. Numerous microorganisms can infect the eye either by direct or indirect introduction into the eye. The most common clinically important microorganisms involved in eye infections are reviewed in this article with relation to the anatomical part of the eye involved in the disease, along with a discussion of the pathogenic mechanisms and management of the disease.

2. *Acanthamoeba* keratitis

Acanthamoeba keratitis is a rare but sight threatening corneal infection, caused by an opportunistic protist pathogen belonging to the genus *Acanthamoeba*. They are ubiquitous, free-living protists dispersed in a variety of environments including air, soil, freshwater, tap water, hospital equipments, surgical instruments, showers, ventilation ducts, air-conditioning units, chlorinated swimming pools, sewage etc. [37]; (Kilvington & White, 1994). Phenotypic switching into a cyst form enables *Acanthamoeba* to withstand adverse environmental conditions. The *Acanthamoeba* trophozoite has an amoeboid shape that contains spike-like structures known as acanthopodia and during this stage, amoebae feed and reproduce under favourable environmental conditions [111,206]. However, under extreme situations such as lack of nutrients, hyperosmolarity, desiccation, extreme pH, temperatures, and the presence of antimicrobials; the trophozoite rounds up and confines itself within a double-walled resistant cyst form that has minimal metabolic activity. The cyst stage presents a major problem in the successful treatment of *Acanthamoeba* infections as they

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are resistant to various antimicrobial agents, often leading to recurrence of the disease upon discontinuation of therapy [130]. Although exposure to *Acanthamoeba* spp. appears to be common due to its ubiquitous nature, the incidence of *Acanthamoeba* keratitis is less common. The main risk factors for *Acanthamoeba* keratitis are contact lens wear for extended periods, corneal trauma, non-sterile contact lens rinsing, swimming while wearing contact lenses and biofilm formation on contact lens [111,198]. While contact lens wear is the leading risk factor, *Acanthamoeba* can cause keratitis in non-contact lens wearers as well [36]. However, it is often overlooked in non-CLs users as a causative agent of keratitis, where it is usually associated with trauma and/or exposure to contaminated water, soil and organic matter. In addition, *Acanthamoeba* keratitis has been reported after invasive or radial keratoplasty and/or laser-assisted *in situ* keratomileusis (LASIK) where the lesions generally worsen due to a delay in the diagnosis and treatment [49,170].

Clinical manifestations of *Acanthamoeba* keratitis include excruciating pain characterized by redness, epiphora, lacrimation, eyelid diptosis, conjonctival hyperhemia, foreign body sensation and photophobia [110,130]; [3,4]. As the disease progresses, stromal involvement results in infiltration of inflammatory cells displaying a characteristic ring infiltrate. If not promptly diagnosed and treated aggressively, cornea becomes ulcerated leading to perforation, ring infiltrate, stromal abscess formation, loss of visual acuity and eventually blindness and enucleation.

Acanthamoeba was first recognized as an ocular pathogen in 1973 in the U.S.A where the first case was reported by an ocular microbiology group in Dallas [28]. However, the first published report of *Acanthamoeba* keratitis emerged in the UK in 1974 and was associated with minor eye injury [128]. The first case in a contact lens wearer was reported 10 years later in 1984 from a patient wearing soft contact lenses [165]. Since then the number of cases has been on the rise especially in recent years, mainly due to an increase in the number of contact lens users, better diagnostics and increased awareness.

2.1. Pathogenesis

2.1.1. Adhesion

The first step in the pathogenesis of *Acanthamoeba* keratitis is the ability of amoebae to bind to the corneal epithelium, a factor that determines the degree of pathogenicity of different isolates. The pathogenic cascade begins via amoebal binding to mannose glycoproteins on the corneal surface through adhesin expressed on the trophozoite membrane called the mannose-binding protein (MBP) [29,47,138]. This adherence is a crucial prerequisite for producing infection. In addition. mild trauma or corneal abrasion is required for the establishment of corneal infection. Abrasion or trauma results in increased expression of mannose glycoproteins on the corneal epithelium and hence there is increased adhesion of amoebae to the damaged cornea, compared with the healthy cornea [29]. The contact lenses, in addition to serving as a vector for the introduction of trophozoite onto the corneal surface, also up regulate mannose glycoproteins on the corneal epithelium. This results in an increased number of trophozoites binding to the contact lens-conditioned cornea compared to the normal cornea thus leading to findings that more than 80% of *Acanthamoeba* keratitis cases are associated with the use of CLs [5].

The second important element involved in adhesion is the number of acanthopodia on the amoebic surface. Pathogenic amoebae have more than 100 acanthopodia/cell compared to non-pathogenic amoebae. Therefore, non-pathogenic amoeba present very low binding levels to host cells compared to pathogenic amoebae [89,138]. Hence the number of acanthopodia is also believed to be closely related to the rate of adhesion to the corneal surface. Once attached, intracellular signaling processes trigger the pathogenic cascade involved in the pathogenesis of *Acanthamoeba* keratitis.

2.1.2. Cytopathic effect

Trophozoite binding to corneal surface is tailed by extensive desquamation of the corneal epithelium, leading to penetration of the underlying Bowman's membrane. Trophozoite-mediated cytopathic effect proceed via several mechanisms such as direct cytolysis, phagocytosis and apoptosis [2]. It has been observed that *Acanthamoeba*-mediated direct cytolysis is dependent on calcium channel activity and on cytoskeletal elements, as the inhibition of both via the calcium channel blocker, Bepridil and actin polymerization inhibitor, cytochalasin D overcomes *Acanthamoeba*-mediated cytolysis by over 98% [190]. In addition the presence of structures like food cups or amoebastomes on the surface of *Acanthamoeba* suggests that the amoeba binding to host cells leads to secondary events like phagocytosis by which the amoeba bites or engulfs host cells. It has been observed that *Acanthamoeba* phagocytose and/or engulfs corneal epithelial cells and that this activity is mediated via amoebastomes present on the surface of amoebae [89].

Apart from inducing direct cell death, several studies indicate that *Acanthamoeba* induces apoptosis of keratocytes, iris ciliary body cells, retinal pigment epithelial cells, corneal epithelial cells, neuroblastoma cells etc. [30,111,130,181]. *Acanthamoeba* induces membrane blebbing, formation of apoptotic bodies, DNA laddering, nuclear chromatin condensation of host cell, all known markers for apoptosis [3,4]; [148]. It has been observed that exposure to mannose stimulates the production of a 133-kDa protease termed mannose-induced protein (MIP133) by *Acanthamoeba* trophozoites. This production of MIP133 appears to be another vital element of the pathogenic cascade of *Acanthamoeba* keratitis. It initiates apoptosis of corneal epithelial cells in a caspase-3-dependent pathway *in vitro* and it is possible that the trophozoite uses this pathway in the desquamation of the corneal epithelial cells *in vivo* [70,71]. Furthermore, clinical isolates of *Acanthamoeba* but not soil isolates produce MIP133 and can cause disease in animals, signifying an association between MIP133 production and pathogenic potential. Hence the trophozoite-mediated cytopathic effect to destroy epithelial cells occurs by three independent mechanisms i.e., direct cytolysis, phagocytosis and apoptosis.

2.1.3. Stromal invasion

Following binding and desquamation of the corneal epithelium, the next step in *Acanthamoeba* keratitis involves the penetration and dissolution of the underlying collagenous stroma. The *Acanthamoeba* trophozoites secrete a variety of proteases with nonspecific collagenolytic activity to facilitate the invasion and degradation of stroma. These include serine proteases, cysteine proteases, metalloproteinases, elastase, collagenolytic enzyme, phospholipase A and a novel plasminogen activator [24,27,58]. Studies have shown the direct role of extracellular proteases in *Acanthamoeba* keratitis by co-incubating corneal epithelial cells with *Acanthamoeba* conditioned medium, which resulted in host cell cytotoxicity [65]. This cytotoxic effect was abolished in the presence of a serine protease inhibitor, phenylmethylsulfonyl fluoride (PMSF), suggesting a crucial role played by serine proteases in the pathogenicity of *Acanthamoeba* keratitis [38]. Furthermore, pathogenic *Acanthamoeba* exhibit higher protease activity compared to non-pathogenic amoebae [91]. Moreover, serine proteases are also able to degrade collagen, fibronectin, laminin, secretory IgA, IgG, plasminogen, BSA, fibrin, fibrinogen,

and rabbit corneal proteins [171]. These properties of proteases are important because collagen type I is the main structural protein in the corneal stroma which is important for the maintenance of its integrity. In addition sIgA, the main immunoglobulin in tears and a primary barrier against pathogenic microbes are also degraded by these proteases. Other extracellular enzymes like elastase and phospholipase A are capable of degrading connective tissues. These findings suggest that collagenolytic enzymes have a role in corneal lesions and the generation of ring-like stromal infiltrates which are characteristic features of *Acanthamoeba* keratitis [65].

Pathogenic *Acanthamoeba* can also use plasminogen activator that is constitutively present in ocular isolates, to catalyze the cleavage of host plasminogen to plasmin, which in turn activates matrix metalloproteinases (MMPs) to degrade components of extracellular matrix [13,119]. They result in ulceration of normally quiescent cornea by degrading the components of basement membrane and extracellular matrix, including type I and II collagens, laminin and fibronectin [140]. Corneal stroma, unlike other extracellular matrices is vital for the normal functioning of the eye. Its degradation affects normal vision and can lead to blindness. Studies have also shown that pathogenic isolates of *Acanthamoeba* are able to secrete ecto-ATPases which play a role in protecting the cells from effector cells of the immune system. Secondly, they play a role in repolarization of the membrane after depolarization by an active compound by acting as a proton pump. Lastly, these enzymes are thought to be related to adherence to the host cell [171].

2.1.4. Neuritis

Acanthamoeba spp. are highly motile and secrete a plethora of proteases that augment their pathogenesis by desquamating corneal epithelium, penetrating Bowman's membrane and invading the corneal stroma. This cascade of events comes to a halt and trophozoites almost are never able to penetrate the corneal endothelium and the anterior chamber of the eye [29]. However, they seem to cluster around the corneal nerves producing radial keratoneuritis [5]. Studies have demonstrated the chemotactic response of *Acanthamoeba* trophozoites towards extracts of neuronal cells and neural-crest-derived cells but not towards the corneal epithelial or stromal cells [149]. Moreover, *in vitro* studies have also demonstrated the killing of nerve cells by trophozoites via direct cytolysis and/or apoptosis [148]. These cytopathic effects contribute to nerve damage and may account for the excruciating pain experienced by the patient, coupled with this infection *in vivo*.

2.2. Treatment

Currently the treatment for *Acanthamoeba* keratitis involves topical application of cocktail of drugs, as there is no single effective drug treatment against *Acanthamoeba* keratitis. This is mainly because of the degree of virulence of the infecting isolate which is different for each individual case and therefore makes it impossible to establish a correlation between *in vitro* and *in vivo* efficacies. The best therapeutic outcomes are expected only when, it is diagnosed early, treated adequately and aggressively with a high level of patient compliance. *Acanthamoeba* trophozoites are sensitive to most available drugs; however the cysts present a major issue in the course of treatment and are the main reason for the recurrence of infection upon discontinuation of therapy. Thus, successful management most often necessitates extended therapy with a combination of well tolerated drugs with minimal toxicity to hosts cells that results in a favourable visual consequences with reduced need for surgical interventions.

The most effective topical agents currently used against *Acanthamoeba* trophozoites and cysts are the biguanides (polyhexamethylene biguanide (PHMB) 0.02–0.06% or chlorhexidine 0.02–0.2%) in combination with diamidine (propamidine isethionate 0.1% or hexamidine 0.1%) [36,90,106,211]. The former drugs are membrane-acting cationic biocides that interact with negatively charged surface proteins of *Acanthamoeba* resulting in leakage of the cellular components [146]. The latter however exert their amoebicidal effects via cationic surface-active properties inducing structural changes resulting in cell permeability and are effective DNA synthesis inhibitors [39]. *In vitro* studies revealed that cationic agents have the best and most constant amoebicidal and cysticidal activity [42,62]. If these drugs are applied early on in the development of infection, at high frequency along with neomycin and 0.15% dibromopropamidine, they show successful prognosis [106]. Hourly drops of PHMB and hexamidine day and night may be tapered off after 48 h to alleviate epithelial toxicity, to hourly drops during the day for the next 72 h. This therapy reduces viable trophozoites when they are more susceptible and prevent them from turning into fully mature cysts. This treatment is then reduced to 2-hourly application during the day for the next 3–4 weeks and then gradually tailored depending on the individual case. Treatment regimes on average last over a period of 6 months, ranging from 0.5 to 29 months [152].

In the case of inflammation along with persistent infection, corticosteroids have been implicated however, there use is controversial as they cause suppression of host immune response [106]. Recent reports also point towards the use of the azoles as an adjuvant to biguanide and diamidine therapy in resistant *Acanthamoeba* keratitis cases [11]. Therefore topical and intrastromal voriconalzole drops (1%) have been used successfully in 3 cases with resistant *Acanthamoeba* keratitis. Surgical management via epithelial debridement, corneal graft surgery (keratoplasty) and/or DALK (deep lamellar keratoplasty) may be required in some cases where the corneas are permanently scarred or where the topical and oral treatments have failed [25,74,81,144,173]. Recently, photorefractive surgery seems to be a very promising modality and have been used in four cases of early stage *Acanthamoeba* keratitis, where the patients developed large corneal abscesses in the upper third thickness of stroma [85]. Another relatively new approach is collagen cross-linking which has shown promising results in clinically [48,92,126]. It is presumed that collagen probably prevents further tissue damage and prevents reproduction of amoebae and hence the patients showed rapid reduction in ocular symptoms and ulcer size.

[122] recently reported that autophagy inhibitors have anti-amoebic effects and if used with low concentrations of PHMB (0.00125%) has very low cytopathic effect on human corneal cells and a high cytopathic effect on *Acanthamoeba* cells. In addition [9], suggested photochemotherapeutic strategy against *Acanthamoeba* infections and showed that mannose-conjugated porphyrin has the potential for targeted photodynamic therapy of *Acanthamoeba* infections. Regardless of significant advances in *Acanthamoeba* keratitis treatment over the last decade or so, prevention still seems to be the best option. In addition, contact lens wearers must be thoroughly educated on the proper hygiene to handle contact lenses to avoid this serious sight-threatening infection.

3. Mycotic keratitis

Mycotic keratitis is the fungal infection of the cornea caused by either filamentous and/or yeast-like fungi. They may account for more than 50% of all culture-positive microbial keratitis especially in tropical and sub-tropical countries [129,196,213]. A strong geograph-

ical correlation has been reported to exist between the occurrences of different types of keratomycosis. For example, the proportion of keratitis due to yeast-like fungi show a tendency to increase towards temperate climates whereas corneal ulcers caused by filamentous fungi appear to be more common towards tropical latitudes [100].

Filamentous fungi such as *Fusarium*, *Aspergillus*, *Phaeohyphomycetes, Curvularia, Paecilomyces* and *Scedosporium apiospermum* are most commonly associated with keratitis caused by filamentous fungi [196]. However *Candida albicans* and other *Candida* species are most common keratitis-causing yeast-like fungi. Former is reported to be due to ocular trauma which is usually the most important predisposing factor in healthy young males engaged in agricultural or other outdoor activities [53,129,196]. These fungi do not invade the intact cornea and penetration only occurs once the epithelium is abraded. Traumatizing agents of animal origin or vegetative matter, soil or dust particle either directly implant fungal conidia on abraded corneal epithelium for fungal invasion [53,129,196]. The use of corticosteroids, ocular surgery, ocular surface disease and contact lens wear has now also been increasingly identified as a significant risk factor. Conversely, *C. albicans* and related fungi are only linked to keratitis when there is a pre-existing ocular condition like insufficient tear secretion, defective eye closure, or some systemic illness such as diabetes mellitus or immunosuppression [195]. This form of keratomycosis may also supervene on a pre-existing epithelial defect caused by herpes keratitis or abrasion due to contaminated contact lenses.

Fungal infections of the cornea need to be promptly addressed to facilitate full recovery. In case of filamentous fungal keratitis, clinical manifestations include sudden onset of pain along with photophobia, discharge with reduced vision and opacity on the surface of the cornea suggestive of an ulcer [7]. It may involve any part of the cornea and show firm, sometimes dry elevated slough, hyphate lines extending into the normal cornea beyond the edge of the ulcers, multifocal granular or feathery grey-white satellite stromal infiltrates, immune ring, Descemet's fold and mild iritis [163,178,194,196]. Although each fungal keratitis exhibits these basic features, they may vary depending on the etiological agent. Severe, chronic filamentous keratitis somewhat resemble bacterial suppuration and may involve the entire cornea. However those due to yeast-like and related fungi may resemble bacterial keratitis with an overlying epithelial defect, discrete infiltrate and slow progression [182].

3.1. Mechanism

3.1.1. Adhesion

Interaction of pathogenic fungi with host cells is the key factor in the pathogenesis of mycotic keratitis. Adherence of microorganisms to host cells is a pre-requisite for the initiation of the infection. Hence fungal infections of the cornea begin via adhesion of fungi to the damaged cornea. Fungal pathogens display a variety of adhesins that are capable of adhering to various cell types and interact with a variety of host proteins and glycoproteins present in host cells [68]. The outer fibrillar layer of yeast and filamentous fungal cell wall is composed of mannan or mannoprotein [75,195]. The adhesive mannoproteins play an essential role in fungal binding to corneal tissues. These lectin-like proteins recognize D-mannose or mannose glycoproteins on the surface of corneal epithelial cells. Damage to the cornea results in the upregulation of mannose glycoproteins on the corneal surface [29]; it may therefore play an important role in the adhesion and pathogenesis of fungal keratitis. Ocular trauma is the most important pre-disposing factor in mycotic keratitis [53,129,196]; the absence of which prevents fungal penetration into the cornea. Thus, the correlation of ocular trauma with an increased expression of mannose glycoproteins on the corneal surface and presence of adhesive mannoproteins on the surface of fungal cell wall is likely involved in the pathophysiology of fungal keratitis.

The corneal epithelium also possess other potential fungal binding sites like laminin, fibronectin, collagen etc. which also play a role in keratitis [195]. [199] demonstrated the importance of an outer fibrillar layer of the germ tube in the process of adhesion to plastics. [16] reported that these major components of the fibrillar cell wall layer of the germ tube act as a receptor for laminin, fibrinogen and mediates its attachment to membranes. The involvement of specific interaction of *Aspergillus fumigatus* with finrinogen and its role in cell adhesion has shown that fungal conidia adhere avidly to wells coated with laminin, fibrinogen, collagen and fibronectin substrates [34]. This was further supported by Ref. [19]; who showed the ability of fibrinogen and laminin to inhibit the adherence of conidia to pulmonary epithelial cell lines. In addition sialic-acid dependent lectins have also been reported to play an important role in recognition of laminin and fibrinogen by *Aspergillus* and *Penicillium* conidia.

Furthermore, the outer layer of conidia also plays a crucial role in the early stages of infectious process. The surface of resting conidia has been reported to have proteins belonging to the hydrophobin family [143,191] which are detected in all filamentous fungi and thought to play a role in adherence [168].

3.1.2. Invasiveness and morphogenesis

The etiological agent in *Fusarium* keratitis is able to invade the cornea gaining access to the anterior chamber of the eye. There, at the pupillary area, it forms a lens-iris-fungal mass affecting the normal drainage of aqueous humor leading to an increase in the intraocular pressure causing fungal malignant glaucoma [97]. Initially, malignant glaucoma was thought to occur only in *Fusarium* keratitis, however recently [78]; reported a case of *Aspergillus*-induced malignant glaucoma where the uniform shallowing of the anterior chamber was present with raised IOP unresponsive to antiglaucoma measures. Eventually *A. flavus* was isolated from the anterior chamber of the eye.

Fungal invasiveness is directly related to the fungal load and inversely proportional to the intensity of inflammatory response [204]. Therefore, the heavier the load of the fungus, the greater the extent of invasion and the smaller the number of inflammatory cells. In early mycotic keratitis, the heavy fungal load with deep tissue penetration may overcome the inflammatory response in corneal tissue, encouraging the progression of the disease. Conversely the inflammatory response in early keratitis may be mild and therefore the fungi multiply extensively and penetrate deep into the tissues. The putative sequence of events in which it occurs still needs to be confirmed.

Phenotypic switching or morphogenesis is an important method of adaptation used by some microbes in different microenvironments to survive inside infected hosts. This permits fungi to survive in the presence of anti-fungal drugs and resist anti-microbial therapy. The same intrahyphal hyphae or hypha-in-hypha and thickened fungal cell walls are seen in corneal tissues infected with *L. theobromae* and in corneas infected with *F. solani* keratitis treated earlier with corticosteroids, as seen in the presence of antifungal drugs [94,193]. This suggests that these morphological alterations may allow fungi to evade host defenses and present a barrier against antifungal drugs [77] demonstrated the role of EFG1-regulated SAP6 gene of *C. albicans* which encodes for a unique secreted aspartyl proteinase. They concluded that proteases contribute to corneal pathogenicity in *C. albicans* keratitis and are also associated with morphogenic transformation from yeast to invasive filamentous forms.

3.1.3. Toxigenicity

Various keratitis causing fungi are known to produce mycotoxins; however their exact role in the pathogenesis of keratitis is still unknown. Several toxins produced by *Fusarium* spp. include nivalenol, T-2 toxin, deoxynivalenol, diacetoxyscirpenol and fusaric acid [157] studied the relationship of toxin production with the severity of disease and concluded that toxin production *in vitro* did not relate to the clinical presentation of severity of keratitis and the outcome of the treatment. The toxic effects of aflatoxins on the cornea of chicks has been established [86]. It was demonstrated that when aflatoxin was administered to chicks, haziness of the cornea and separation of corneal lamellae was observed in addition to infiltration by polymorphonuclear leukocytes. [101] demonstrated that *A. flavus* isolated from keratitis patients produce significantly more aflatoxin compared to those of the environmental isolates. However the reason behind this phenomenon is unclear and requires further study.

The ability of fungi to produce various enzymes could also damage tissues, facilitate invasion and eventually influence the severity and outcome of the disease. [217] examined the role of fungal proteases in the pathogenesis of mycotic keratitis. It was observed that the clinical isolate of *A. flavus*, isolated from a patient with severe keratitis, secrete variety of proteases including serine proteases, cysteine proteases, metalloproteases and concluded that fungal collagenases are the mediator of the severe corneal destruction observed in keratitis. When attempted to compare the presence of fungal proteases *in vitro* and *in vivo*, the corneal isolates of *A. flavus* and *F. solani* predominantly secreted serine proteases and little metalloproteases *in vitro*. *In vivo*, however the protease profile shifted to metalloproteases and no serine protease activity was detected [52]. Matrix metalloproteinases are thought to play a pathological role in the degradation of extracellular matrix components such as basement membrane collagen; same as those found in corneal basement membrane and stroma [18]. They have also been shown to be upregulated in ulcerative fungal keratitis in rabbit as well as in the tear film of horses with ulcerative keratitis and hence are thought to play a probable role in the pathogenesis of fungal keratitis [52,134]. However, the exact role of fungal proteases in mycotic keratitis needs further investigations.

3.2. Treatment

Fungal keratitis is a complex entity with many considerations when it comes to treatment. On the whole, treatment generally entails of chemotherapy consisting of topical and/or systemic drugs, alone or in combination with surgical treatment. However, in developing countries with limited care and economical barriers, mycotic keratitis is of major concern as it can cause visual loss in a demographic population that has limited access to care. Each antifungal has its own benefits and limitations and therefore must be selected carefully based on etiological agent and susceptibility testing. There are several classes of antifungals available like polyenes, azoles and fluorinated pyrimidines, each with their own advantages and disadvantages. Polyenes, including natamycin, nystatin and amphotericin B, disrupt fungal cell by binding to the cell wall ergosterol of both filamentous as well as yeast-like fungi. However their tissue penetrating ability is poor and therefore is mostly recommended in cases of superficial corneal infections [7,195]. Administration is every 30 min during the first 24 h and then every hour for the next 24 h and then gradually tapered off according to the response. While amphotericin B is commonly administered as a topical solution, intracameral administration is an effective alternative in reducing time to disappearance of hypopyon and final improvement [216]. Although active against both forms of fungi, it has shown variable activity against *Fusarium* species. Owing to its side effect profile and lack of coverage against *Fusarium* keratitis, it is not considered as a first line agent [7]. Natamycin on the other hand has a broad-spectrum activity against filamentous fungi and is the first line treatment against fungal keratitis and the drug of choice for *Fusarium* keratitis. The drug can only be given topically and hence can only treat superficial fungal keratitis as opposed to deep stromal fungal invasion. The presence of deep lesions may therefore require other antifungals like azoles administered via subconjunctival or intravenous routes [187].

The azoles including imidazoles and triazoles, at low concentrations inhibit ergosterol biosynthesis while, cause direct damage to cell walls at higher concentrations [188]. Fluconazole and ketoconazole show good intraocular penetration and are therefore good agents against keratitis with deep lesions [137]. They are the preferred treatment against both candida and filamentous fungi however, fluconazole has narrow coverage against filamentous organisms but ketoconazole is active against *Aspergillus*, *Candida* as well as *Curvularia* species [7,45,154]. Voriconazole is a good alternative with minimal toxicity and is not only active against *Candida* but also against filamentous fungi such as *Fusarium* spp. [99,112]. In refractory FK cases, topical voriconalzole has been used as an adjunct to natamycin along with intrastromal injections of voriconalzole with success [61]. [61] reviewed 40 case-reports and concluded that voriconalzole is a safe alternative against major ocular fungal infections but shouldn't be used as a single agent for initial treatment.

The fluorinated pyrimidines such as flucytosine etc. are converted into thymidine analog blocking fungal thymidine synthesis. They are usually administered with an azole or amphotericin B for synergistic effects and to avoid development of resistance. Subconjunctival injections have also been used in patients with severe keratitis. Regardless of the drug choice, successful therapy requires prolonged frequent drug administration and therefore adherence of patients to treatment is also an important factor affecting the outcome of treatment. In addition, liposomal preparation of anti-fungal drugs are also under trail and has demonstrated some efficacy in rabbit models [1,57]. Furthermore [156] compared the efficacy of topical clotrimazole-β-cyclodextrin (CBC), a comparatively new anti-fungal compared to amphotericin B and concluded that CBC reduces the period of treatment on average by a duration of 1 week. The authors also reported greater efficacy of CBC in cases of *Candida* keratitis.

Surgical interventions are required in case of complications of acute infectious processes as well as if the disease is refractory to medical management. Penetrating keratoplasty (PK) is the most common surgical treatment used to excise lesions of the cornea and replaced with a donor corneal graft in case of refractory or severe cases of fungal keratitis [150]. In addition, it is an effective way to treat corneal perforations as a result of fungal infections [212]. As an alternative to PK, debridment is also used where causative agent and necrotizing material is removed thereby enhancing the penetration of anti-fungal medications by the removal of epithelium. It is recommended where the necrotizing tissues are hindrance to the healing of corneal ulcers.

Alternate surgical procedure includes lamellar keratoplasty (LK) in which only diseased layers of the corneal surface are excised thereby leaving the underlying structures of the cornea intact. It is recommended when the fungal invasion is only focal [151,158]. In this way, the risk of endothelial rejection, the most severe type of corneal graft rejection, is minimized.

The challenging nature of fungal keratitis along with less than ideal outcome of current treatment regimes, various experimental ad-

vances have also been made. Corneal collagen cross-linking (CXL) is a relatively new technique currently been investigated in conjunction with photo-activated riboflavin (PAR) in refractory keratitis cases [102,137]. Investigations into the use of nanoparticle technology with terbinafine and silver have also shown promising results [189,214]. In addition, topical aqueous garlic extracts have been tested on rabbit keratitis models infected with *A. flavus* [76]. Nonetheless, fungal keratitis is a complex entity with many considerations when it comes to treatment. However, prompt identification of the etiological agent along with targeted therapy may result in favourable outcome.

4. Bacterial keratitis

The major bacterial agents of infectious keratitis include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Serratia* species [210]. The community acquired cases of bacterial keratitis are usually resolved with an empirical treatment, however if left un-attempted, may result in perforation, endophthalmitis and loss of vision [23,210]. Clinical presentation of bacterial keratitis include acute pain, redness, photophobia and corneal ulceration [179]. *Pseudomonas* ulcers are more severe at presentation than other bacterial ulcers and are often difficult to treat, resulting in worse visual outcome than other bacterial ulcers [56,183].

Bacterial keratitis accounts for approximately 90% of all microbial keratitis cases [127] with *P. aeruginosa* as the most common culprit, worldwide [40]. Corneal infection of *Pseudomonas* is most commonly associated with the use of contact lenses and was rarely reported as a problem prior to the emergence of contact lens [114,155]. The resistance of *Pseudomonas* to disinfectants, coupled with its adherence capability to plastics, facilitates its introduction into the eye where it can react with defective corneal epithelium, gaining further entrance into the corneal stroma. *S. pneumonia* on the other hand is the major cause of corneal ulcers in developing countries however, some reports emphasize on the fact that *Streptococcus* are most commonly encountered after *P. aeruginosa* and/or *S. aureus* eye infections [14,114,215]. Unlike *P. aeruginosa*, pneumococcal keratitis is not commonly associated with the use of contact lenses and the predisposing factors often include ocular trauma or surgery [31,104,114,125,160,207].

Along with *P. aeruginosa*, *S. aureus* is also a common etiological agent [79,103,114,136,209]. It is a commensal organism that can readily gain access into the eye, given the opportunity [121,174,185]. Individuals whose eyes are compromised due to various reasons including ocular surgery or trauma, contact lens use, viral infection or other eye illnesses are at high risk of developing this infection [26,32,135]. In particular, the ability of *S. aureus* to develop antibiotic resistance makes this infection among the most difficult one to treat [10]. This, along with *Serratia marcescens*, which once was considered a harmless saprophyte, has now been increasingly isolated from the corneal surfaces of keratitis patients [142]. The isolation rate of *S. marcescens* from contact lens-coupled corneal infections ranges from 5 to 28%; almost comparable to the isolation rate of *P. aeruginosa* in contact lens-related keratitis [69,167]. *S. marcescens* is a motile gram negative rod abundantly dispersed in nature and contributes to contact lens-related keratitis [141,142].

4.1. Mechanism

4.1.1. Adhesion

Bacteria initiate infection by engaging with the host cell-surface receptors via various adhesins, which mediate bacterial binding to corneal epithelial cells. Microbial adhesins not only play a role in bacterial attachment to the surface of epithelial cells but may also play an active role in subsequent interactions and infective process. They may act as toxins initiating microbial invasion and contribute to subsequent pathogenic cascade. Bacteria display several adhesins on their surface such as pili or fimbriae, which recognize specific carbohydrates or proteins on the surface of host cell. The adherence of *P. aeruginosa*, *S. pneumonia* and *S. aureus* is significantly higher to damaged corneal epithelium compared to other bacteria accounting for their frequent isolation from keratitis cases.

Studies have shown that purified pili successfully compete with cold bacteria for binding to ocular surface and have been used to protect against *P. aeruginosa* keratitis. Corneal epithelial glycoproteins act as surface receptor mediating pilus binding activity and amino sugar sialic acid was able to completely inhibit pilus binding to mouse corneal epithelial cells [63,164]. Bacterial flagella on the other hand are filamentous organelles, responsible for bacterial motility and therefore are also responsible for dissemination of infection. More than 95% of *P. aeruginosa* clinical isolates are flagellated and flagella-deficient mutants have been observed to be non-virulent [8]. In addition the anti-flagellar antibody homologous to the infecting strain protects mice from *Pseudomonas* corneal infection [164]. Furthermore, the glycocalyx may also play an important role in bacterial adhesion by producing slime aggregates resistant to phagocytosis thereby enabling them to adhere to cells [72].

S. aureus surface adhesins, collectively known as MSCRAMMS (microbial surface components recognizing adhesive matrix molecules), have also been recognized to mediate bacterial adherence to host extracellular matrix components, collagen, fibronectin, fibrinogen, laminin and elastin [80]. [161]; studied the role of MSCRAMM Cna (collagen-binding adhesin) in *S. aureus* keratitis and concluded that collagen-binding adhesin is a virulence factor for *S. aureus* keratitis and is involved in the early events of pathogenesis of *S. aureus* infection of the cornea. Similarly, [80] studied the role of *S. aureus* fibronectin-binding protein as an epithelial and/or endothelial cell adhesin/invasion protein. Their data suggested that FnBPs is a surface ligand for human corneal cells and play a key role in host-parasite interactions. It serves as an important adhesin and triggers invasion in ulcerative keratitis caused by *S. aureus*.

Likewise, *Streptococci* colonize different sites on human body by expressing multiple adhesins and hence their attachment to human tissues is mediated by a diverse group of bacterial surface proteins, MSCRAMMS [60]. Plasmin and fibronectin binding protein A (PfbA), a MSCRAMMS, is known to promote adherence and invasion of bacteria to human epithelial cells by recognizing molecules like fibronectin. In addition, pneumococcal surface adhesin A (PsaA), pneumococcal surface protein A (PspA), pneumolysin (ply), pneumococcal adherence and virulence factor A (PavA), choline-binding protein A (CbpA/PcpA), putative protease maturation protein A (PpmA), IgAI protease (IgAIp), and the streptococcal lipoprotein rotamase A (SIsA) have all been shown to be associated with pneumococcal adherence and virulence [153]. *Streptococcal* cell surface may contain fibrillar structure like pili and fibrils which may also mediate attachment to cell surface to initiate infection. The pneumococcal surface protein C (PspC) promotes adherence and uptake of pneumococci into nasopharyngeal epithelial cells (Hammerschmidt et al., 2000). Also, antibodies against PsaA which code for pneumococcal surface adhesin A (PsaA) resulted in reduced adherence to nasopharyngeal epithelial cells [162]. Therefore, these adhesins are likely involved in the initial stages of *Streptococcal* ocular infection.

Serratia marcescens on the other hand possess mannose-specific adhesins, associated with flagella. These adhesins probably interact with mannose glycoproteins expressed on the surface of corneal ep-

ithelial cells as a result of abrasion or trauma due to the use of contact lenses. [98]; identified type I fimbriae as the critical adhesin that mediate attachment of *S. marcescens* to human corneal epithelial cell surface. They also identified two AHL (*N*-acylhomoserine lactone)-regulated genes, *bsmA* and *bsmB*, to be involved in adhesion to biotic surface and the expression of these genes leads to the attachment of *S. marcescens* to HCE cells. In 2007, Shanks et al. demonstrated the role of *oxyR* and type I fimbrial genes and concluded that they are involved in cell-cell and cell-biotic surface interactions. In addition, two classes of pili, mannose-resistant and mannose-sensitive pili may also play a role in the attachment of bacteria to epithelial cells [67].

4.1.2. Bacterial invasion and cytotoxic effects

Once adhered to the epithelial surface, the pathogen invades into the corneal stroma. This invasion is facilitated via proteases, exotoxins resulting in degradation of basement membrane and extracellular matrix, causing cells to lyse. A number of exotoxins including heat-stable haemolysin, phospholipases, exotoxins play a role in invasion of bacteria into the cornea with eventual stromal necrosis [133]. Once bacterial invasion into the cornea has ensued, the infection progresses rapidly towards melting of cornea facilitated by bacterial proteases, activation of metalloproteases and stimulation of immune response resulting in further damage via release of reactive oxygen intermediates and host proteases [114,192]. Proteases contribute to the pathogenesis of keratitis by degrading basement membrane, laminin, proteoglycans, collagen, and extracellular matrix [66,201].

P. aeruginosa is capable of secreting at least 7 different proteases including elastase A and B, modified elastase, alkaline protease, protease IV, *P. aeruginosa* small protease and large exoprotease, some of which play a potential role in the pathogenesis of keratitis [113,202]. Metalloproteases especially elastase B (Las B) and alkaline protease (AP), play a considerable role in keratitis as Las B injection into the corneal stroma result in significant corneal damage [73,88]. Protease IV (PIV) on the other hand cleaves variety of host defense proteins including immunoglobulins, complement components, antimicrobial peptides and surfactants and hence contributes to the virulence of the organism [6,44]. *Pseudomonas aeruginosa* small protease (PASP) is able to cleave collagen, the chief structural component of the corneal stroma and hence it could play an important role in the obliteration of cornea [186]. Purified PASP when injected into the rabbit cornea resulted in the destruction of the epithelium and the formation of erosions that can reach into the stroma. These findings suggest that PIV provides bacteria with a defense arm against multiple host defense molecules, while PASP degrads collagen-based structural component of the eye, thereby mediating the pathophysiology of keratitis.

[120] determined the role of MucD protease of *P. aeruginosa* in keratitis in the cornea of mice. The number of bacteria and clinical score in eyes infected with *mucD* deficient strain was significantly less compared to the parent or rescued strain. In addition large number of infiltrating PMN cells were observed in *muc*D deficient eyes along with higher MIP2 levels. It was concluded that MucD protease of *P. aeruginosa* suppressed IL-1β, KC and MIP2 as well as inhibited neutrophil recruitment in the cornea and hence plays a critical role in *P. aeruginosa* keratitis by facilitating the evasion of immune response.

[87] examined the expression of three main exotoxins, ExoS, ExoT and ExoU, in clinical isolates of *P. aeruginosa* and showed that majority of strains (84%) were ExoS+ and ExoT + but lacked ExoU. However, 5 out of 7 strains that were expressing ExoU were also positive for ExoS which is usually a rare phenotype. These exotoxins are directly injected into the host cell via type III secretion system of *P. aeruginosa* where they exert their cytotoxic effects onto the host cell (Hauser, 2009). ExoU expressing strains cause rapid cell lysis whereas, ExoS strains are invasive in nature causing membrane blebbing within epithelial cells which are utilized as a site for bacterial replication and motility [46].

The capsular polysaccharide of *S. pneumoniae* which once was considered the central dogma in pneumococcal virulence is proven to be untrue in the case of keratitis; as noncapsular strains of *S. pneumoniae* are also able to cause severe keratitis in rabbit keratitis infection models [131,159]. Other than capsule, the most studied virulence factor of pneumococcus is pneumolysin, a toxin belonging to the family of bacterial cholesterol-dependent cytolysin. It causes direct cell damage by binding to the cholesterol in the host cell membrane followed by polymerization into 30–50 mers, thereby forming pores in the host cell membranes [124,197]. In addition to forming pores, it initiates immune-derived damage by activating complement system and induces inflammation [145]. Futhermore, reduced corneal virulence was demonstrated by of pneumolysin-deficient strain of *S. pneumoniae* in a rabbit model of intrastromal infection. It was shown that mutation in the complement activation domain of pneumolysin resulted in reduced polymorphonuclear leukocyte (PMN) recruitment and therefore less corneal damage and virulence compared to the wild-type strain [82].

Proteins other than pneumolysin, includes choline-binding proteins like pneumococcal surface proteins A and C (PspA and PspC), neuraminidase A (NanA) and a variety of other surface-associated proteins such as those involved in adherence, immune evasion, activation and enzymatic reactions have been identified to be involved in non-ocular models of infection Recently a metalloprotease, ZmpC, has been shown to induce ectodomain shedding of the membrane-associated mucin (MAM) from conjunctival and corneal epithelial cells leading to the loss of glycocalyx barrier function and enhanced internalization of bacterium. It was concluded that the removal of MAMs may serve to be an important virulence mechanism employed by *S. pneumoniae* [54].

The alpha-toxin is a pore-forming lytic cytotoxin produced by nearly all strains of *S. aureus*. Once it gains entry into the cytoplasmic membrane, it moves laterally until seven subunits combine in a circular arrangement [118,139,205]. The individual toxin molecules interact with cavoline-1, thereby forming pores in the membrane [23] studied the role of alpha-toxin in the corneal virulence of *S. aureus* and concluded that it plays a major role in staphylococcal keratitis, causing destruction of corneal tissues in the infected eye. Later et al., 1997 showed that even nanogram quantity of purified alpha-toxin causes extensive sloughing of the rabbit corneal epithelium, corneal edema and severe iritis. Gamma-toxin, a two component toxin from *S. aureus* is composed of an F component and an S component that are non-toxic on their own. The S component binds to the target cell followed by binding of the F component both of which then move laterally in the cell membrane joining the other F-S pairs to form a ring that penetrates the membrane resulting in cell lysis [55,184]. The pathogenic role of gamma- and alpha-toxin were determined by Ref. [35] who illustrated that the virulence of strain, Newman is mediated by both gamma- and alpha-toxin, with alpha-toxin mediating corneal epithelial erosions. The strain deficient in either toxins were considerably less virulent compared to parent or rescued strains. *S. aureus* secretes several two component toxins each of which has its own S and F components [55]. This two component toxin system of *S. aureus* is complicated by the fact that S component of one toxin can also bind to the F component of the other toxin thereby creating several unique combinations with their own specific toxicity. The role of these toxins in corneal virulence is yet to be determined. The protein produced

by *setnm-1* gene has been shown to cause extensive corneal damage; an attribute mediated by its protease activity and is now considered to be important in the virulence of *S. aureus* keratitis [21]. In addition, mutant deficient in this gene is considerably less virulent compared to its parent or rescued strain [22].

Similarly, intra-corneal injections of sub-microgram amounts of *Serratia* metalloprotease were able to elicit a rapid and extensive corneal tissue damage of rabbit corneas by causing liquefactive necrosis and descemetocele formation [95,108]. [109] showed that acute inflammation, liquefactive necrosis of rabbit cornea and descemetocele formation occurred after intra-corneal injection of *Serratia* protease. In addition, they also showed the *in vitro* solubilization of the stromal proteoglycan ground substance. Ultramicroscopic examination of damaged cornea revealed loss of ruthenium red staining of the proteoglycan ground substance and dispersal of ultra-structurally normal collagen fibrils. Therefore, the major corneal damage after the injection of *Serratia* protease is due to the solubilization and loss of ground substance of the tissue and the proteases are at least in part involved in the production of severe corneal damage caused by this bacterium. [84] identified a 56-kDa protease as one of the major factors contributing to the pathogenesis and tissue destruction caused by this bacterium. Vaccination of rabbits with purified protease preparations from *S. marcescens* exhibited significantly less corneal destruction upon corneal encounter with live bacteria, indicating that proteases are important virulence factors during the development of serratial keratitis [96]. [115] showed that mutant strains of *S. marcescens* that are deficient in the production of 56 kDa metalloprotease are considerably less cytotoxic to mammalian cells. It was further revealed that the culture filtrates of wild-type bacteria pre-treated with EDTA or 1,10-phenanthroline (inhibitor of metalloproteases) exhibited significantly reduced cytotoxicity.

Gram negative lipopolysaccharide is also an important virulence factor in infectious keratitis. It contributes to the pathogenesis of keratitis by mediating the attachment to the cornea and contact lenses, bacterial internalization and survival inside corneal epithelial cells. In addition, it confers resistance to the complement-mediated killing and stimulates neutrophil migration and infiltration into the cornea with subsequent corneal scarring and opacification. Also, exopolysaccharide formation by both gram positive and gram negative bacteria results in local immunosuppressive effects as well as interference with phagocytosis.

4.1.3. Stromal necrosis and production of ring infiltrate

Bacterial exotoxins and proteases are constitutively released during multiplication of bacteria. These toxins and enzymes persist in the cornea for a prolonged period of time causing continual stromal destruction and can deprive eye of its vision. Most exotoxins are heat-labile and have antigenic properties. The LPS, an endotoxin within the cell wall of gram negative bacteria is released resulting in the production of stromal rings. These rings consist of polymorphonuclear leucocytes within the corneal stroma, which are chemo-attracted by the alternate complement pathway.

4.2. Treatment

Due to the rapid progression of bacterial keratitis, it should be treated as a medical emergency and empirical antibiotic treatment should be promptly commenced. The objective for the initial therapy is the rapid eradication of the corneal pathogen. Basically, no single antibiotic is effective against all bacterial keratitis-causing organism and therefore an agent having broad spectrum activity covering both, gram negative and gram positive organisms is desirable. Broadly speaking, two treatment options are available; fluoroquinolones monotherapy and/or combination therapy of fortified antibiotics including cefazolin and tobramycin or gentamicin [51]. The aminoglycosides in fortified drops provides excellent coverage against gram-negative organisms and are also active against *Staphylococci* and some *Streptococci*. Cephalosporin on the other hand gives good coverage for non-penicillinase producing gram-positive bacteria. Fluoroquinolone antibiotics have a broad spectrum activity against both gram-negative and gram-positive organisms including penicillinase-producing and methicillin resistant *Staphylococci*. They inhibit DNA gyrase and topoisomerase IV; the key enzymes involved in DNA replication and transcription leading to bactericidal effect of these antibiotics [15,147,210].

The frequency of application of antibiotic drops depends on the severity of infection but usually they are administered every half an hour for the first 24–36 h [51,133]. In severe cases, an initial loading dose is achieved via a drop every 5 min for the first 30 min. The concept behind the loading dose is the vascularity of the cornea and poor penetration of the drug into the corneal stroma which requires frequent topical applications to achieve minimal inhibitory concentration. The therapy is then tapered off gradually based on the clinical response of the patient. Other antibiotics used include amikacin, vancomycin, methicillin, clotrimoxazole, and clarithromycin depending on the causative agent responsible for keratitis [51].

Owing to the rich innervation of the cornea, the disease is frequently associated with considerable pain and therefore analgesics or pain control medications are recommended, which result in improved patient comfort and effective delivery of the treatment regimen. The use of corticosteroids as anti-inflammatory agents are controversial and are best avoided until the infection is completely eradicated or at least under control [51,133]. In patients with corneal ulceration the use of non-steroidal anti-inflammatory drugs should also be avoided due to increased risk of corneal melting and perforation. Topical cycloplegics are used to relieve ciliary spasm, pain and formation of synechiae. Secondary glaucoma may result in keratitis due to increased intraocular inflammation and hence must be treated with topical antiglaucoma medications.

In the case of marked corneal ulceration, the temporary use of therapeutic soft contact lenses may facilitate stromal repair and promote re-epithelialization by protecting corneal surface from mechanical trauma. They may act as a tear film antibiotic retention device thereby facilitating penetration by prolonging contact time [20,116]. In addition, collagen corneal shields soaked in antibiotic solution have also been used as an effective adjunct and have shown to increase antibiotic penetration [132,203]. Furthermore, temporary intracanalicular collagen implants and liposomal systems have also been designed to prolong drug retention and interaction [50,166].

Therapy for bacterial keratitis is not only to eradicate the causative agent but also to prevent tissue destruction and irreversible structural alterations caused by enzymes and toxins released by the bacteria. Enzyme inhibitors like disodium edetate, acetylcysteine, Heparin have been shown to be effective experimentally [43,117,172]. Synthetic inhibitors of matrix metalloproteases have been shown to inhibit *P. aeruginosa* proteases in experimental *Pseudomonas* keratitis [12]. The corneal stroma which constitutes about 90% of corneal thickness is made up of regularly arranged collagen fibrils. Thus the collagenases from bacteria, digest the human collagen causing the cornea to melt. Moreover, collagen cross linking is a useful technique which uses riboflavin and UVA irradiation to strengthen corneal tissues thereby enhancing its rigidity [175,176,208]. The interaction between riboflavin and UVA strengthens chemical bond formation between collagen fibrils and hence increasing its resistance to enzy-

matic digestion [177]. In addition, the photoactivation of riboflavin has cidal effect on microorganisms by causing damage to microbial DNA and/or RNA [210].

In case of progressive corneal thinning or perforations, less than 2 mm cyanoacrylate tissue adhesives may be used, which may also have some inherit antibacterial activity as well [41,51,93,133]. Tissue adhesives are only suitable for small perforations, as they are toxic to corneal endothelium. In case of large perforations, therapeutic penetrating keratoplasty may be required. Corneal patch grafting may be an alternative to tissue adhesives and conjunctival flaps may help selected cases of refractory ulceration to assist healing. Severe bacterial keratitis may also result in cataract formation due to bacterial toxins, iridocyclitis and treatment toxicity [107]. Surgical intervention may be required in such cases depending on the degree of corneal scarring and opacification.

5. Concluding remarks

Microbial keratitis, is a complex entity with many considerations when it comes to its treatment. It is a major public health concern particularly in developing countries where access to care is limited and economic barriers are huge where it can become a leading cause of visual loss in a population that is young. As with all corneal infections, proper identification of the causative agent followed by appropriate targeted therapy can abate the risk of complications. A better understanding of the pathogenic cascade would no doubt lead to improved clinical treatment. However the emergence of drug-resistant strains along with the recurrence of infections emphasize on the need for more effective therapeutic modalities. Additionally, the need to identify the genetic basis of virulence factors responsible for the disease is also important, as the pathogenicity of keratitis is a sum of multiple events occurring together in time and space for the successful transmission of the pathogen to the susceptible host, overcoming its physiological barriers and eventually producing disease. Subsequently, a complete understanding of pathogenesis of the particular organism will undoubtly lead to the identification of potential therapeutic interventions.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Uncited references

[17]; [33]; [59]; [64]; [105]; [123]; [169]; [200].

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