Evaluation of Polymerase Chain Reaction and Culture for the Diagnosis of Corneal Ulcer

Rania A. Khattab[1], Salwa A. Rasmy[1], Yasser M. Ragab[1], Dalia G. Said[2], Maha M. Abdelfatah[2], Mohamed A[3]. Shemis, Dalia M. Ezzat[3] and Fatma Elzahraa S. Abdel Rahman[3]

[1]Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[2]Research Institute of Ophthalmology, Corneal unit and Microbiology Department, Cairo, Egypt
[3]Theodor Bilharz Research Institute, Giza, Egypt
Khattab500@yahoo.com lady_ok@yahoo.com

Abstract: Purpose: To compare polymerase chain reaction (PCR) to microbial culture for the detection and identification of bacterial and fungal microorganisms in microbial keratitis. **Methods:** Corneal scrapings from 150 patients clinically diagnosed as microbial keratitis, who attended the Research Institute of Ophthalmology cornea clinic were cultured, analysed by PCR and the results were compared. **Results:** Of the 150 patient samples, 104 (69.3%) were culture-positive (76 for bacteria, 19 for fungi and 9 were mixed culture); and 46 (30.7%) were culture-negative. Of these 150 patient samples, 130 (86.7%) were positive by PCR (74 bacterial, 18 fungal and 38 mixed infection); and 20 (13%) were PCR-negative. Of the 76 culture-positive for bacteria, 73 (96%) were positive by PCR; 17 (89.5%) out of 19 samples culture-positive for fungi were positive by PCR and 8 (89%) out of 9 samples culture-positive for mixed infection were PCR-positive. Of the 46 culture-negative samples, 32 (69.5%) yielded pathogen deoxyribonucleic acid (DNA) products and 14 were PCR-negative. The sensitivity of PCR in detecting bacterial, fungal, mixed culture and no growth keratitis was 94%, 86%, 88%, 79% respectively while the specificity was 90%, 82%, 95% and 83% respectively. **Conclusion:** PCR detects microbial DNA in the majority of bacterial and fungal corneal ulcers, and identifies microorganisms in a high proportion of culture-negative cases. PCR may be used as an adjunct to culture to identify microorganisms in microbial keratitis. Although being expensive, PCR remains a promising tool for faster and highly sensitive diagnosis of microbial keratitis.
[Rania A. Khattab, Salwa A. Rasmy, Yasser M. Ragab, Dalia G. Said, Maha M. Abdelfatah, Mohamed; Shemis, Dalia M. Ezzat and Fatma Elzahraa S. Abdel Rahman. **Evaluation of Polymerase Chain Reaction and Culture for the Diagnosis of Corneal Ulcer**. *Life Sci J* 2012;9(3):746-755]. (ISSN: 1097-8135). http://www.lifesciencesite.com106

Key words: diagnosis- microbial keratitis- PCR- culture.

1. Introduction

Standard microbiological techniques for diagnosing microbial keratitis rely on culturing the organisms in nutrient media. The frequency of apparent diagnostic failure (that is, no organism is isolated though an infection is clinically evident) ranges from 20% (1) to 60% (2). An additional problem is that such techniques require days to weeks for complete results, which can significantly delay appropriate treatment.

The polymerase chain reaction (PCR) is a highly sensitive and rapid technique for amplifying analytic quantities of deoxyribonucleic acid (DNA) from infinitesimal starting quantities. When applied to the detection of pathogen DNA, the technique can be used to rapidly identify the presence of specific organisms (3). The potential utility of polymerase chain reaction (PCR) based techniques for improving the diagnosis of ocular infection is well recognized (3,4), and the use of PCR for this purpose is expanding (5,6). The aim of the current study is to compare culture and microbial PCR results in a series of patients presenting with corneal ulcer and to study the sensitivity and specificity of each method in diagnosing microbial keratitis.

2. Materials

Patients

Our study included 150 (77 males and 73 females) patients with clinical evidence of microbial keratitis who attended the outpatient clinic corneal unit department of the Research Institute of Ophthalmology, Cairo, Egypt. Their ages ranged from 2 to 83 years (mean 43 years). A total of 50 patients were used as controls (30 males and 20 females), their ages ranged from 25 to 50 years (mean 35 years). Control patients had normal ocular examination with no tear film dysfunction. A detailed history was taken and a thorough slit-lamp examination was done for all patients. In patients with microbial keratitis, the size, depth and margins of the infiltrate were noted. Any epithelial defect was photographed and measured. Corneal scrapings were taken from the base and edge of the ulcers with a sterile blade, after installing local anaesthetic solution (4% xylocaine) in the eye.

Methods

Culture

The material obtained by scraping from the leading edge and the base of each ulcer was inoculated directly onto sheep blood agar, chocolate agar, and Sabouraud dextrose agar (SDA) for bacterial and fungal culture. Culture growth was read within a maximum of 5 days for bacterial growth and two weeks for fungal growth. If positive, the colony was further analysed by standard biochemical tests until a specific species was identified.

To evaluate the diagnostic value of each assay, statistical analysis was done for calculating the sensitivity, specificity, positive predictive value

(PPV) and negative predictive value (NPV) using Medcalc program.

Polymerase chain reaction

Sample collection and DNA Extraction

After culture samples had been obtained, a sterile swab was used to obtain a corneal scrape from the base and leading edge of the corneal ulcer for PCR assay. The swab was placed into a sterile micro centrifuge tube, capped and immediately transferred to -70°c for storage until processing. DNA from all samples was extracted within one month of receipt.

Briefly, DNA was extracted from each swab using QIA amp DNA Micro extraction kit from Qiagen according to manufacturer's instructions. QIA shredder from Qiagen was also used to harvest the lysate.

DNA amplification:

The primers used in this study, their sequence, product size and references are shown in table (1). Specificity of the primers was tested using DNA of various strains available in our microbiology and immunology laboratory. All the strains used as positive controls were laboratory isolates like *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Candida* spp., *Aspergillus* spp. and *Fusarium* spp..

I-Conditions for universal bacteria:

16srRNA primers (27-6 and 28-6) dissolved in 165 µl dist.H_2O to reach final conc. 50pmole/µl. (Figure 1).

PCR reaction mix (50µl):

DNA 1µl, taq 0.25µl, primers (pF 0.5 +pR 0.5), 5x buffer (GoTaq Reaction buffer) 10µl, dNTPs 4µl (2mM) and complete with dist. H_2O 33.75µl

PCR program:

Initial denaturation 96□C for 3min., denaturation 95□C for 15sec., both extension and annealing in one step 55□C for 30sec., 40 replication cycles , final extension 55□C for 10min., stop reaction at 4□ for 10min.

II- Conditions for the bacterial species analysed: (*Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas* spp.)

PCR reaction mix (50µl) for bacterial genotyping:

DNA 4µl, taq 0.5µl, 5x flexi buffer 10µl, dNTPs 1µl (10mM), Mg 7.5µl for *S.aureus* and 6.5µl for *S.epi* and *pseudomonas*, primers (0.3For +0.3Rev) complete with dist. H_2O

1-*Staphylococcus aureus*:

By using forward primer (9-6) and reverse primer (10-6), (Figure 2). (9-6 dissolved in 226µl and 10-6 dissolved in 240µl dist. H_2O)

PCR program:

Initial denaturation 95□ for 5min., denaturation at 95□c for 10 sec., annealing 60□c for 10sec., extension 72□c for 22 sec.45 cycles of replication and final extension 72□c for 5min.

2-*Staphylococcus epidermidis*:

By using GYRb FoR-9 and GyRb Rev-9, (Figure 3). (GYRb For-9 dissolved in 188µl and GYRb Rev-9 dissolved 185µl dist. H_2O).

PCR program:

Initial denaturation 95□ for 5 min., denaturation step at 94□c for 30 sec., annealing 55□c for 30 sec., extension at 72□c for 1min.,40 cycles of replication and final extension at 72□c for 2 min.

3-Pseudomonas* spp.*:

By using 21-6 and 22-6 primers**, figure (4).** (21-6 dissolved in 185µl and 22-6 dissolved in 205µl dist. H_2O)

PCR program:

Initial denaturation 95□c for 2min., denaturation 94□c for 20sec., annealing at 51□c for 20sec., extension at 72□c for 40sec., 40 cycles of replication and final extension at 72□c for 1 min.

III- Conditions for universal fungus:

ITS primers(ITS 1-9 and ITS 4-9) dissolved in 200µl dist. H_2O for final conc. 50pmole/µl, (Figure 5).

PCR reaction mix. (50µl):

DNA 4µl, taq 0.25µl, primers (pF 0.25 + pR 0.25), 5x buffer (Go Taq Reaction buffer) 10µl, dNTps 8µl (2 mM) complete with dist. H_2O 27.25 µl

PCR program:

Initial denaturation 95□c for 5min., denaturation at 95□c for 30sec., annealing 58□c for 30sec., extension at 72□c for 1min., 35 replication cycles ,final extension at 72□c for 10min., and stop the reaction at 4□c for 5min.

IV- Conditions for the fungal species analysed:

(*Aspergillus* spp*, Fusarium* spp.and *Candida* spp.)**

PCR reaction mix (25µl) for fungal genotyping:

DNA 1µl, taq (5U/µl) 0.2 µl, primers (0.125µl For. + 0.125µl Rev) (50pmole), dNTps 4µl (2 mM), 5x buffer (Go Taq reaction buffer) 5µl complete with dist. H_2O.

PCR program:

Initial denaturation 95□C for 5min., denaturation 95□C for 30sec., annealing 66□C for *Aspergillus* spp.,57□C for *Fusarium* spp. and 61□C for *Candida* spp. for 30sec., extention 72□C for 20sec.,40 repeating replication cycles, final extension 72□C for 7min**.**

1- *Aspergillus* spp. primers ASFu For-9 and Asfu Rev-9, product size 520 bp(Figure 6). (Dissolving of AsfuFor-9 by add 300µl dist.H_2O, and 255 µl to AsfuRev-9).

2-*Fusarium* spp. by FusoFor and FusoRev, product size 565 bp (Figure 7).

(Dissolving FusoFor by add 230µl injection water, and 220 µl to FusoRev)

3- *Candida* spp. by CAFOR2 -9 and CAREV3 -9,product size 402 bp (Figure 8). (Add 210 µl dist. H_2O to CaFor2-9 and 260 to CaRev3-9).

- PCR product was run on 1.5% agarose gel for medium product size (>400 bp) and 2% agarose for small products size (<250 bp), samples run with 100pb ladder
- Electrophoresis voltage range from 100:200 v, depend on the size of the gel

Small gels (50 ml) run on 100:120 V, large gels (100ml) run on ≈150 v. Microkit from Qiagen, 100bp

ladder from fermentas, Taq (5 u/µl) with its buffers and also dNTP mix from promega and primers from Bio NEER.

Table (1): Sequences of primer sets used

Microorganism	Primer Sequence	Product Size (bp)	References
Universal primer for bacteria	F 27 – 6: GGA GGA AGG TGG GGA TGA CG R 28 – 6: ATG GTG TGA CGG GCG GTG TG	241 bp	Samadi *et al*. (7)
Universal primer for fungi	F ITS 1 -9: TCC GTA GGT GAA CCT GCG G G R ITS 4 -9: TCC TCC GCT TAT TGA TAT GC	601 bp	Lindsley *et al.* (8)
S. aureus	F 9-6: CAA TGC CAC AAA CTC G R 10-6: GCT TCA GCG TAG TCT A	477 bp	Sakai *et al*. (9)
S. epidermidis	GYRB FOR. -9: CAG CAT TAG ACG TTT CAA G GYRB REv. -9: CCA ATA CCC GTA CCA AAT GC	251 bp	Yamada *et al*. (10)
Pseudomonas spp.	F 21-6: GAC GGG TGA GTA ATG C CTA R 22-6: CAC TGG TGT TCC TTC CTATA	618 bp	Theodore *et al.* (11)
Aspergillus spp.	ASFUFOR -9: CCA ATG CCC TTC GGG GCT CCT ASFUREV -9: CCT GGT TCC CCC CAC AG	520 bp	Emma *et al.* (12)
Fusarium spp.	FUSOFOR -9: CCA ATG CCC TCC GGG GCT AAC FUSOREV -9: GCA TAG GCC TGC CTG GCG	565 bp	Emma *et al.* (12)
Candida spp.	CAFOR2 -9: GGG AGG TAG TGA CAA TAA ATA AC CAREV3 -9: CGT CCC TAT TAA TCA TTA CGA T	402 bp	Emma *et al.* (12)

F= forward R= reverse

Table (1) shows the sequence of the primers used in this study, product size and references.

Universal bacteria

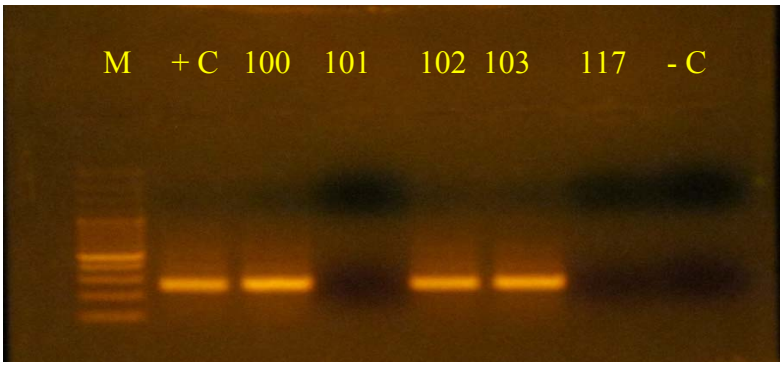

Figure (1): Agarose gel visualized in an ultraviolet transilluminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 2: positive control. Lane 8: negative control. Lanes 4, 7: negative samples. Positive PCR results are seen in lanes 3, 5, and 6. Product size 241 bp.

+C = positive control, -C = negative control, M= marker.

Staphylococcus aureus

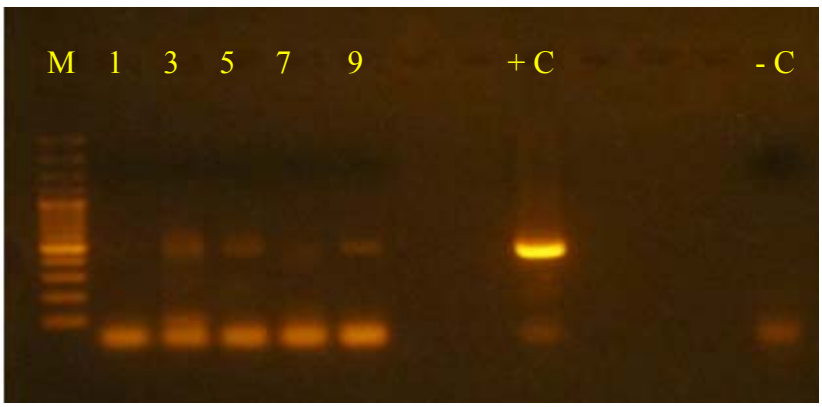

Figure (2): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 7: positive control. Lane 8: negative control. Lane 2: negative sample. Positive PCR results as seen in lanes 3, 4, 5, and 6. Product size 477 bp.

Staphylococcus epidermidis

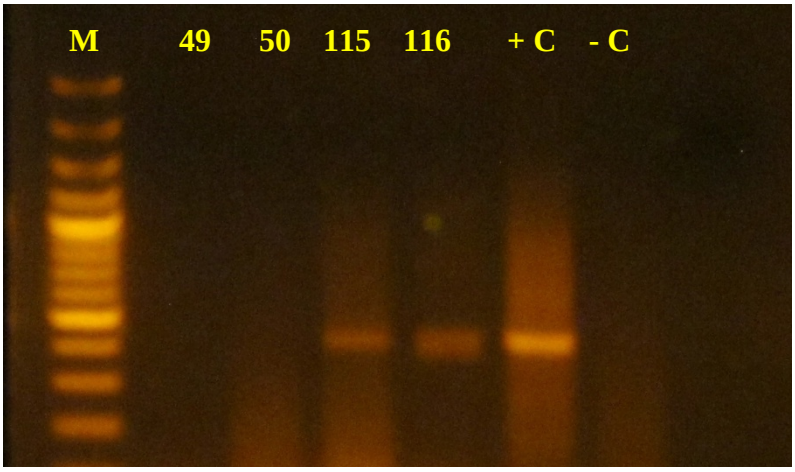

Figure (3): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 6: positive control. Lane 7: negative control. Lanes 4 and 5: positive samples. Lane 2 and 3: negative samples. Product size 251 bp.

Pseudomonas

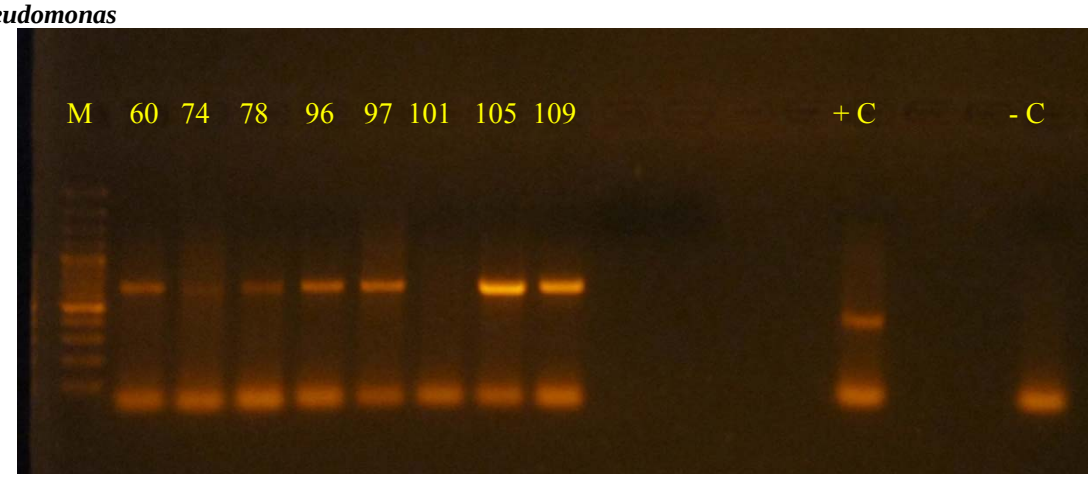

Figure (4): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 10: positive control. Lane 11: negative control. Lane 7: negative sample. Positive PCR results are seen in lanes 2, 3, 4, 5, 6, 8 and 9. Product size 618 bp.

Universal fungi

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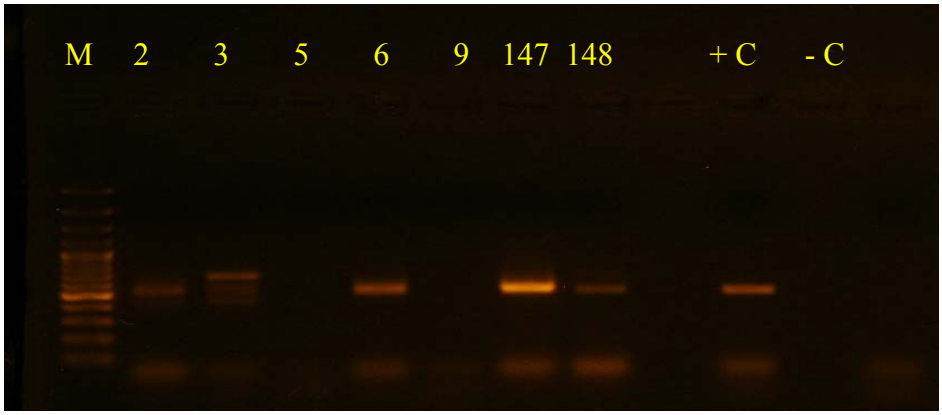

Figure (5): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 9: positive control. Lane 10: negative control. Lanes 4 and 6: negative sample. Positive PCR results are seen in lanes 2, 3, 5, 7 and 8. Product size 601 bp.

Aspergillus

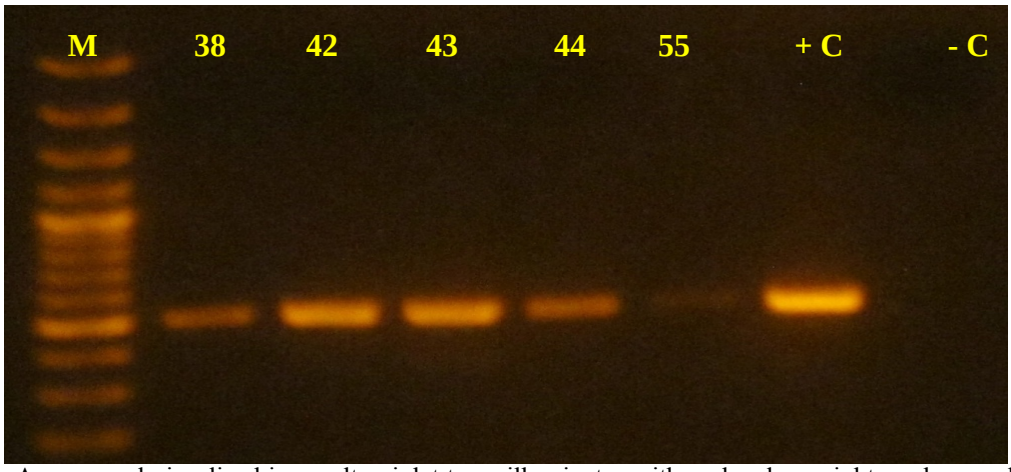

Figure (6): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 7: positive control. Lanes 8: negative control. Lanes 2, 3, 4 and 5: positive samples. lane 6: negative sample. Product size 520 bp.

Fusarium

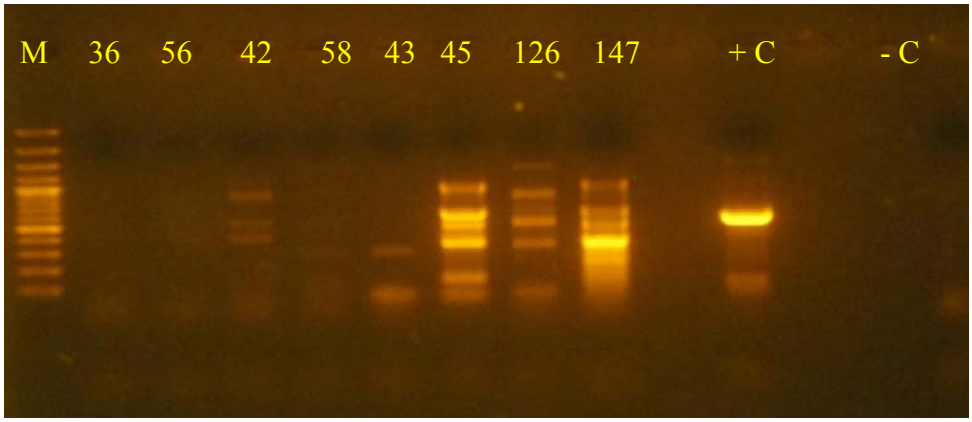

Figure (7): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 10: positive control. Lane 11: negative control. Lanes 2 and 3: negative samples. Positive PCR results are seen in lanes 4, 5, 6, 7, 8 and 9. Product size 565 bp.

Candida

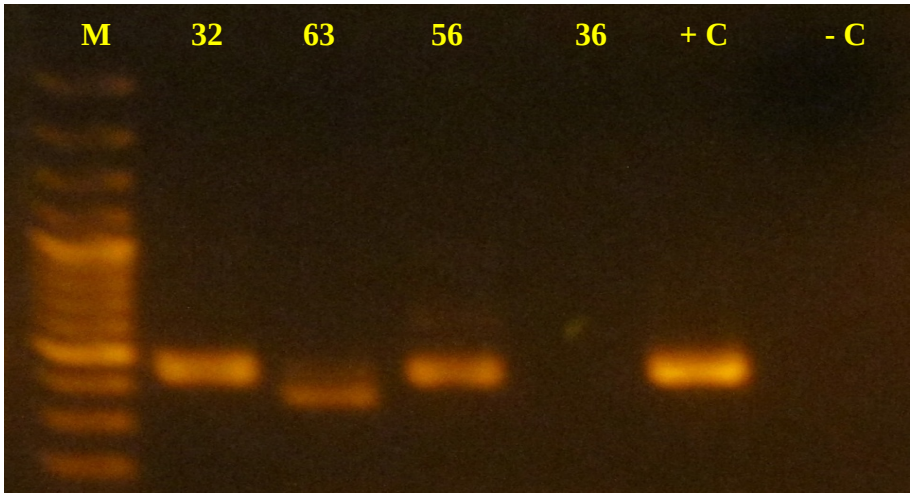

Figure (8): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1: molecular weight marker. Lane 6: positive control. Lane 7: negative control. Lanes 2, 3 and 4: positive samples. lane 5: negative sample. Product size 402 bp.

3. Results

Culture results: Of the 150 patient samples, 104 (69.3%) were culture-positive (76 bacterial, 19 fungal and 9 mixed) and 46 (30.7%) were culture-negative as shown in figure (9). Gram positive cocci (71%) were predominantly isolated from the total number of bacterial cultures and *Aspergillus* spp. (48.5%) for fungal cultures was the most common fungal isolate.

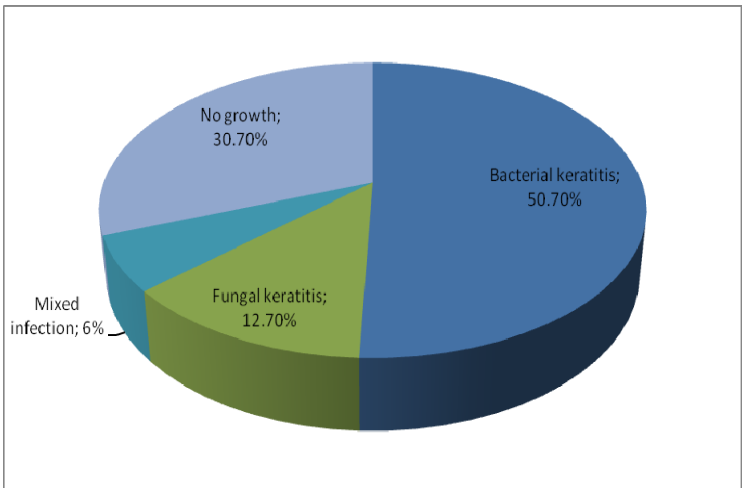

Figure (9): Culture results from 150 cases of microbial keratitis.

Polymerase Chain Reaction results:

Of the 150 samples derived from corneal ulcers, 130 (86.7%) were PCR-positive (74 bacterial, 18 fungal and 38 mixed infection) and 20 (13%) PCR-negative. Of the 76 samples culture-positive for bacteria, 73 (96%) were PCR-positive for bacteria. Of the 19 samples culture-positive for fungi, 17 (89.5%) were PCR-positive for fungi and 8 (89%) out of 9 samples culture-positive for mixed infection were PCR-positive. On the other hand, of the 46 culture-negative samples, 32 (69.5%) were PCR-positive, (one bacteria, one fungal and 30 mixed) and 14 PCR-negative. The sensitivity and specificity of PCR for the detection of microbial keratitis against the gold standard culture technique are showed in table 2 and figure 10.

Of the 74 samples positive by bacterial PCR, 73 (98.6%) were culture positive and 1 culture-negative. Seventeen (94%) out of the 18 fungal PCR positive samples were culture-positive for fungi and 1 culture-negative. Out of the 38 PCR-positive mixed infection results, 8 (21%) were also mixed culture -positive and 30 were culture-negative. Of the 20 PCR-negative samples 14 (68%) were also culture-negative, 3 culture-positive for bacteria, 2 culture-positive for fungi and 1 mixed infection culture-positive.

PCR yielded the same organism as culture in 53 samples for bacterial isolates and in 23 samples for fungal cultures (mixed infection was included). Of the 26 ulcers culture-positive for *S.aureus*, 22 (84.6%) matched PCR results, for *S. epidermidis* 23 (76.7%) out of 30 culture-positive samples were PCR-positive and for *Ps. aeruginosa* of the 10 culture-positive samples 8 (80%) were PCR-positive. Of the 4 ulcers culture-positive for *Fusarium* spp., 3 (75%) matched PCR results while for *Candida* spp., 6 (85.7%) out of the 7 culture-positive were PCR-positive and for *Aspergillus* spp. 14 (93.3%) out of the 15 culture-positive were PCR-positive. The sensitivity and specificity of PCR for the detection of bacterial and fungal pathogens are presented in table 3 and figure 11.

Table 2: Correlation between polymerase Chain Reaction and culture - based diagnosis of bacteria and fungi from corneal scrapes obtained from 150 cases with infective keratitis.

Culture results	No of cases	PCR Positive	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
Bacterial keratitis	76	73	94%	90%	85%	40%
			77% to 97%	62% to 96%	57% to 90%	25% to 44%
Fungal Keratitis	19	17	86%	82%	75%	43%
			55% to 91%	65% to 86%	58% to 79%	27% to 49%
Mixed infection keratitis	9	8	88%	95%	38%	87%
			51% to 90%	58% to 97%	23% to 51%	65% to 90%
No growth keratitis	46	32	79%	83%	44%	80%
			65% to 84%	66% to 92%	29% to 53%	54% to 83%

Table 2 shows that the sensitivity of PCR in detecting bacterial, fungal, mixed keratitis and no growth keratitis was 94%, 86%, 88%, 79% respectively while the specificity of the PCR was 90%, 82%, 95% and 83% respectively. The positive predictive value was the highest for bacterial keratitis (85%) and the lowest for mixed infection keratitis (38%), however the negative predictive value was the highest for mixed infection keratitis (87%) and the lowest for bacterial keratitis (40%).

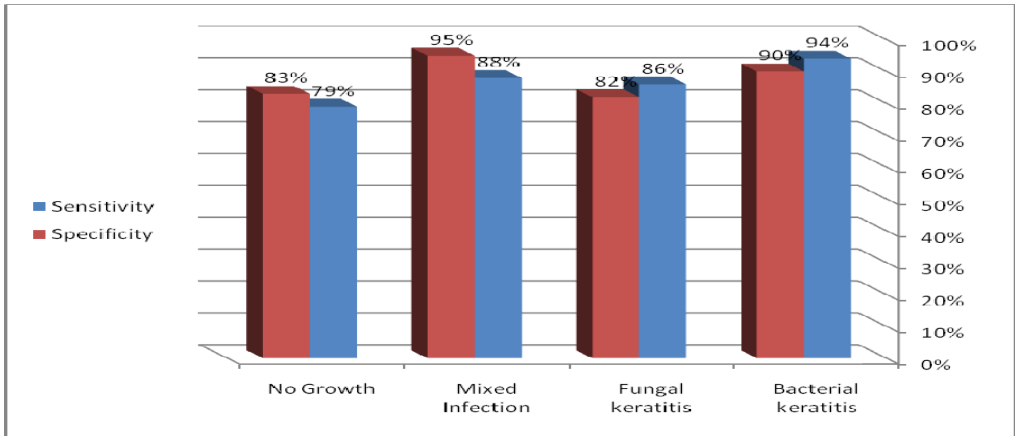

Figure (10): Comparison of sensitivity and specificity of the PCR assay for the detectionof microbial keratitis against the gold standard culture technique.

Table (3): Comparison of culture and polymerase chain reaction results for detection of pathogens in bacterial corneal ulcer

Bacterial isolates	Culture results	PCR results	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
Staphylococcus aureus	26	22	85% 68% to 90%	80% 58% to 85%	82% 62% to87%	36% 26% to 44%
Staphylococcus epidermidis	30	23	79% 58% to 84%	77% 57% to 85%	78% 67% to 81%	43% 40% to 54
Pseudomonas spp.	10	8	92% 77% to 95%	89% 73% to 92%	85% 69% to 93%	46% 33% to 55%

Table 3 shows that the sensitivity of PCR in detecting *S. aureus*, *S. epidermidis* and *Pseudomonas* was 85%, 79%, 92% respectively while the specificity was 80%, 77% and 89% respectively. The positive predictive value and the negative predictive value were nearly equivalent for the 3 organismas.

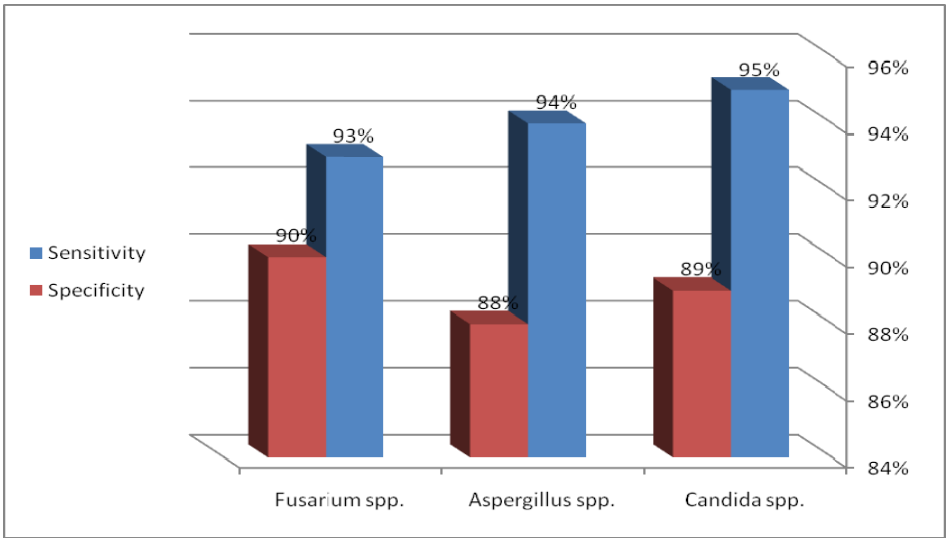

Figure (11): Comparison of sensitivity and specificity of the PCR assay for the detection of *Candida*, *Aspergillus* and *Fusarium*.

4. Discussion

Microbial culture remains the gold standard for identification of pathogens causing corneal ulcers [13]. The importance of correct identification of pathogen is increased in parts of the world where fungal keratitis is common, as choice of correct antimicrobial requires distinguishing fungal from bacterial etiology. The appearance of the ulceration is unreliable in distinguishing

fungal from bacterial ulcer [14]. Microbial culture is a relatively sensitive diagnostic test, with growth seen in about 70% of cases [15]. The PCR is a powerful technique for amplifying infinite quantities of nucleic acids for further analysis. PCR is an extremely sensitive technique able to detect single copies of pathogen DNA in complex mixtures. PCR has been successfully applied to the diagnosis of many ocular conditions [16]. The availability of DNA primer sets that effectively recognize all bacteria or all fungi suggests this technique may have utility for diagnosis of microbial keratitis. **Knox** and associates [17] studied 10 patients with culture positive microbial keratitis and 17 with culture-negative keratitis. Eight of 10 patients who were culture-positive in that study were PCR-positive. None of 17 other keratitis patients were positive for bacterial products. **Rudolph** and associates [18] studied four patients with severe infectious keratitis and either negative cultures or culture results incompatible with the clinical course, direct sequencing of PCR products revealed unusual species in several cases. **Kumar** and associates [19] have studied the use of PCR in detecting fungal pathogens in keratitis. In that study, samples from four patients with mycotic keratitis were studied, along with other samples obtained directly from fungal cultures. The authors found the PCR combined with single-stranded conformational analysis allowed rapid and precise identification of unusual mycotic pathogens [20].

In our study, microbial culture yielded an organism in 70% of cases (104 cases out of a total 150 cases). This is similar to the overall 63% bacterial and fungal culture-positive rate described in a study involved 3,298 eyes with microbial keratitis in India [21]. However contrary to their study, the majority of culture ulcers in egyptian population were bacterial (76 cases), where in India the majority of population yielded fungal culture. Of the 76 samples culture-positive for bacteria, 73 (96%) were PCR-positive and out of the 19 samples culture-positive for fungi 17 (89.5%) were PCR-positive for fungi. Thus PCR appeared to have a higher yield in bacterial ulcer than in fungal ulcer. Our results were contrary to a study by **Elma *et al.*** [22], including 108 samples, 56 were culture-positive, 25 for bacteria and 31 for fungi. Nineteen of 25 bacterial culture-positive samples were positive by PCR (76%), and 29 of 31 samples culture-positive for fungi were positive by PCR (94%). Our results showed that matching of DNA genotyping results with cultured organisms was better for fungal species than bacteria (23/26 and 53/66 respectively). This lower concordance rate with bacterial ulcer may be due to detection of normal ocular surface biota by PCR. Our study revealed 12.7 % (19 cases) culture positivity, *Aspergillus* being the most common fungus isolated by culture (48.4%). This is similar to the study by **Vengayil *et al.*** [23] in which *Aspergillus* was also the most common isolate. In our study, of the 4 ulcers culture-positive for *Fusarium* spp., 3 (75%) matched PCR results; while for *Candida* spp., 6 (85.7%) out of the 7 culture-positive were PCR-positive, and for *Aspergillus* spp. 14 (93.3%) out of the 15 culture-positive were PCR positive. The sensitivity of PCR in detecting *Candida*, *Aspergillus* and *Fusarium* was 95%, 94%, 93% respectively while the specificity was 89%, 88% and 90% respectively. The sensitivity of PCR in detecting *S. aureus*, *S. epidermidis* and *Pseudomonas* was 85%, 79%, 92% respectively while the specificity was 80%, 77% and 89% respectively. Interpretation of discrepant culture and PCR results is somehow unclear, yet in an active corneal ulcer situation it may be sensible to consider culture positive or PCR positive of highly virulent organism such as *S. aureus* or *Pseudomonas* as evidence of infection with those microbes. In a study by **Vengayil *et al.*** [23], the sensitivity of PCR for detection of mycotic keratitis was found to be 70%, the specificity 56.7%, the predictive value of the positive test was 35% and that of the negative test was 85%. However in our study, the sensitivity of PCR in detecting fungal keratitis was 86%, while the specificity of the PCR was 82%. Our positive predictive value was the highest for bacterial keratitis (85%), however the negative predictive value was the highest for mixed infection keratitis (87%). In addition, the positive predictive value for fungal keratitis was 75% but the negative predictive value was 43% opposite to what was obtained by **Vengayil *et al.*** [23]**.** In contrast, **Alexandrakis *et al*.** [24] reported a sensitivity of 89% and specificity of 88% for their PCR technique used for bacterial keratitis detection and this is in agreement with our results Also our results for fungal keratitis are near to what is obtained by **Zunaina *et al.*** [25]**,** who reported 91% sensitivity and 95% specificity in the detection of fungal aetiology in microbial keratitis by PCR. PCR reliably distinguishes bacterial from fungal pathogen [26]. Of the 46 culture-negative samples, 32 (69.5%) were PCR-positive suggestive of potential pathogens, (one bacteria, one fungal and 30 mixed) and 14 PCR-negative.

PCR results in this study seemed quite promising. However, the disparity between culture and PCR results may be explained by the fact that the culture positivity requires viable organisms, whereas a PCR-based test can detect both viable and nonviable organisms. PCR test can theoretically be positive even if only a single copy of target DNA is present. The high positivity of PCR in already treated cases in comparison to culture, reiterates the difficulty in getting a positive culture from non viable organisms in the sample.

In another study, **Ferrer *et al.*** [4] highlighted the benefit of time factor in diagnosing fungal corneal ulcer. Although their PCR assay produced results in 8 hours, culture confirmation took almost 10 days. Our study was thus very much comparable to theirs because the PCR method used by us yielded results in 4 to 8 hours, depending on the number of cycles repeated. This is a major advantage of the technique, especially when compared to culture where it took at least 5 to 7 days for a positive growth in our setup. Although various advantages have been attributed to PCR due to its rapidity and widespread applicability to bacteria and fungi, the technique has various reported complexities and drawbacks, as evidenced from our study also. Some of the limitations are logistic and some technical. Among them is the difficulty in optimization, especially in case of fungi, apart from the difficulty in differentiating between active and latent infections, viable, and nonviable cells. Moreover, the DNA sequence has to be known in advance, and the high sensitivity could lead to false-positive results.

In a well-developed modern laboratory, the gold standard of a bacterial culture should ideally be replaceable today with a reliable and reproducible PCR technique as the new gold standard. This is not to say that PCR negates the undeniable role of a bacterial culture—after all, the conventional as well as the rapid sensitivity testing, so essential for diagnosis and initiation of correct therapy (even after some time lag), are entirely dependent on culture, not on PCR [27]. PCR remains a technically complex procedure involving skilled hands, expertise, and a fair degree of experience, in addition to the learning curve and standardization in relation to individual laboratories. The cost–benefit ratio of PCR should prove efficacious in the developed world. A cost effectivity

factor would be arrived at differently by different personnel even in the same institution, but even that one patient saved would surely calculate it differently. What the future holds in store as the next viable gold standard remains to be seen. Apart from these, the unavoidable cost of the investigation at least as of today limits its widespread use [28].

Corresponding author
Rania A. Khattab
Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
Khattab500@yahoo.com lady_ok@yahoo.com

References:

1. Erie JC, Nevitt MP, Hodge DO, Ballard DJ. (1993). Incidence of ulcerative keratitis in a defined population from 1950 through 1988. Arch Ophthalmol. 111(12):1665–1671.
2. Van Gelder RN. (2001). Applications of the polymerase chain reaction to diagnosis of ophthalmic disease. Surv Ophthalmol. 46(3):248–258.
3. Whitcher JP, Srinivasan M, Upadhyay MP. (2002). Prevention of corneal ulceration in the developing world. Int Ophthalmol Clin. 42:71–77.
4. Ferrer C, Munoz G, Alio JL, Abad JL, Colomm F. (2002). Polymerase chain reaction diagnosis in fungal keratitis caused by Alternaria alternata. Am J Ophthalmol. 133(3):398–399.
5. Anand A, Madhavan HN, Neelam V, Lily TK. (2001). Use of polymerase chain reaction in the diagnosis of fungal endophthalmitis. Ophthalmology. 108(2):326–330.
6. Ghosh A, Basu S, Datta H, Chattopadhyay D. (2007). Evaluation of poly-merase chain reaction-based ribosomal DNA sequencing tech-nique for the diagnosis of mycotic keratitis. Am J Ophthalmol. 144(3):396–403.
7. Samadi, N., M. Alvandi, M.R. Fazeli, E. Azizi, H. Mehrgan and M. Naseri. (2007). PCR-based detection of low levels of *Staphylococcus aureus* contamination in pharmaceutical preparations. J. Boil. Sci., 7: 359-363.
8. Lindsley, D.L., Edington, C.W., von Halle, E.S. (1960). Sex-linked recessive lethals in Drosophila whose expression is suppressed by the Y chromosome. Genetics 45(12): 1649--1670.
9. H. Sakai, G. W. Procop, N. Kobayashi, D. Togawa, D. A. Wilson, L. Borden, V. Krebs, and T. w. Bauer. (2004). Simultaneous detection of *Staphylococcus aureus* and coagulase-negative Staphylococci in positive blood cultures by real time PCR with two fluorescence resonance energy transfer probe sets . J Clinical Microbiology. Vol 42, No.(12):5739-5744.
10. M Yamada, J Yoshida, S Hatou, T Yoshida, Y Minagawa. (2008). Mutations in the quinolone resistance determining region in *Staphylococcus epidermidis* recovered from conjunctiva and their association with susceptibility to various fluoroquinolones. *Br J Ophthalmol.* 92(6):848-851.
11. Theodore Spilker, Tom Coenye, Peter Vandamme, and John J. LiPuma. (2004). PCR-Based Assay for Differentiation of *Pseudomonas aeruginosa* from Other *Pseudomonas* Species Recovered from Cystic Fibrosis Patients Journal of Clinical Microbiology. Vol. 42, No.(5): p. 2074-2079,.
12. Emma E. M. Jaeger, Nora M. Carroll, Sarah Choudhury, Anthony A. S. Dunlop, Hamish M. A. Towler, Melville M. Matheson, Peter Adamson, Narciss Okhravi, and Susan Lightman. (2000). Rapid Detection and Identification of *Candida, Aspergillus,* and *Fusarium* Species in Ocular Samples Using Nested PCR J Clin Microbiol. 38(8): 2902–2908.
13. Morlet N, Minassian D, Butcher J. (1999). Risk factors for treatment outcome of suspected microbial keratitis. Ofloxacin Study Group. Br J Ophthalmol. 83:1027–1031.
14. Dahlgren MA, Lingappan A, Wilhelmus KR. (2007). The clinical diagnosis of microbial keratitis. Am J Ophthalmol. 143: 940–944.
15. McLeod SD, Kolahdouz-Isfahani A, Rostamian K, Flowers CW, Lee PP, McDonnell PJ. (1996). The role of smears, cultures, and antibiotic sensitivity testing in the management of suspected infectious keratitis. Ophthalmology. 103:23–28.
16. Okhravi N, Adamson P, Matheson MM, Towler HM, Lightman S. (2000). PCR-RFLP-mediated detection and speciation of bacterial species causing endophthalmitis. Invest Ophthalmol Vis Sci. 41:1438–1447.
17. Knox CM, Cevellos V, Dean D. (1998). 16S ribosomal DNA typing for identification of pathogens in patients with bacterial keratitis. J Clin Microbiol. 36:3492–3496.
18. Rudolph T, Welinder-Olsson C, Lind-Brandberg L, Stenevi U. (2004). 16S rDNA PCR analysis of infectious keratitis: a case series. Acta Ophthalmol Scand. 82:463–467.
19. Kumar M, Mishra NK, Shukla PK. (2005). Sensitive and rapid polymerase chain reaction based diagnosis of mycotic keratitis through single stranded conformation polymorphism. Am J Ophthalmol. 140:851–857.
20. Kumar M, Shukla PK. (2006). Single-stranded conformation polymorphism of large subunit of ribosomal RNA is best suited to diagnosing fungal infections and differentiating fungi at species level. Diagn Microbiol Infect Dis. 56:45–51.
21. Bharathi MJ, Ramakrishnan R, Meenakshi R, Mittal S, Shivakumar C, Srinivasan M. (2006). Microbiological diagnosis of infective keratitis: comparative evaluation of direct microscopy and culture results. Br J Ophthalmol. 90:1271–1276.
22. Elmak., Jayad. C., Muthiah S., Prajna L., Daniel W., Thomas M. L., John P. W., and Russel N. V.

(2008). Prospective Comparison of Microbial Culture and Polymerase Chain Reaction in the Diagnosis of Corneal Ulcer. Am J Ophthalmol. 146:714 –723.
23. S. Vengayil, A. Panda, G. Satpathy, N. Nayak, S. Ghose, D. Patanaik, S. Khokhar. (2008). Polymerase Chain Reaction–Guided Diagnosis of Mycotic Keratitis: A Prospective Evaluation of Its Efficacy and Limitations. India.
24. Alexandrakis G, Jalali S, Gloor P. (1998). Diagnosis of *Fusarium* keratitis in an animal model using the polymerase chain reaction. Br J Ophthalmol. 82(3):306–311.
25. Zunaina E., Wan Hazabbah W., Chan Y., Nur Haslindawaty A. *et. al*. (2008). Specific detection of fungal pathogens by 18 S rRNA gene PCR in microbial keratitis. BMC Ophthalmology, 8:7.
26. 29. Muyzer G, de Waal EC, Uitterlinden AG. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol. 59:695–700.
27. Gaudio PA, Gopinathan U, Sangwan V, Hughes TE. (2002). Polymerase chain reaction based detection of fungi in infected corneas. Br J Ophthalmol. 86(7):755–760.
28. Whitcher JP, Srinivasan M, Upadhyay MP. (2001). Corneal blindness: a global perspective. Bull W H O. 79(3):214–221.

7/2/2012