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Microbiological Analysis of Ophthalmic Solutions

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ABSTRACT

Microbial corneal contamination is the most genuine confusion of contact lens wear. A defiled contact lens arrangement and embellishments can go about as a supply for microorganisms that could conceivably agree contact lens wear and prompt sight debilitating unfavorable occasions. The rate of lens case arrangements ruining is normally more than half. The point of our exploration study to detach microorganisms from contact lens stockpiling arrangements and to describe and recognized by front line morphology, common characteristics and biochemical test. We take distinctive specimens of lens consideration stockpiling arrangement from asymptomatic patients. Culture tests on blood agar and macconkey agar for gram positive and negative microscopic organisms, SDA for contagious separation and PAS (Periodic acid–Schiff) for Acanthamoeba segregation. Furthermore, we discovered 100% lens care stockpiling arrangements debased from microorganisms. In which our outcome demonstrates that half specimens were defiled with gram positive microorganisms while half examples were tainted with gram negative microscopic organisms, and 67% Fungus were available. Acanthamoeba were not disengaged in any tried specimen, might be on the grounds that it once in a while separated invitro from tests. Subsequently it is reduced that it is critical to keep up abnormal state of contact lens cleanliness and that you tail all the cleaning systems. Genuine consideration ought to be taken by contact lens clients, to keep up abnormal state of cleanliness, appropriate changing of lens consideration arrangement after some time.

Keywords: *contactlenses, Acanthamoeba, hygiene*

INTRODUCTION

Visual diseases rate increments considerably nowadays and stick out amongst the most critical component is the expanded utilization of contact lenses. Contact lenses (CLs) are presently worn by a huge number of individuals worldwide and by around 1.65 million individuals in the UK (Roberts A, Kaye AE, *et al*, 2005). The wearing of CLs causes changes in the cornea as far as structure, turnover, and tear generation, oxygen and carbon dioxide levels. These adjustments in themselves can create issues and may likewise compound prior conditions, and results in various sorts of visual infections. A late study has found that they represent 9.1% of the referrals into the eye setback unit (Melia B, Islam T, *et al*, 2008). There is expanding proof that bacterial biofilm assume a part in an assortment of visual diseases. Bacterial development is described as a biofilm when microorganisms join to a surface and/or to each of other (Michael E. Zegans, *et al*, 2004). Microbial keratitis is a possibly blinding infection that is uncommon in typical eyes unless connected with contact lens (CL) (J.K.G. Dart, FCOphth, *et al*, 1991). Visual parasitosis in human is more common in land territories where ecological components and poor clean conditions support the parasitism in the middle of man and creatures. The main danger elements for Acanthamoeba keratitis are contact-lens wear and corneal. Despite the fact that >80% of the instances of Acanthamoeba keratitis happen in contact

lens. Clinical appearance of Acanthamoeba keratitis is outspread neuritis and serious agony that is not similar with the degree of tissue harm injury (Amal R. Nimir, Ahmed Saliem, and Ibrahim Abdel Aziz Ibrahim, *et al*, 2012). Parasitic keratitis was characterized as a corneal epithelial imperfection with basic stromal penetrate, Any atypical, infiltrative injury with badly characterized or sporadic edges, satellite or stretching injuries, and particularly in the nearness of a ring invade would raise the suspicion of contagious keratitis (Wei-Boon, Khor Tin Aung, *et al*, 2006). The range of causative agents in all microbial keratitis changes by atmosphere and inclining element. As a rule, Gram-positive microbes are all the more every now and again recuperated in calm atmosphere regions(Bourcier T, Thomas F, *et al*, 2003) whereas Gram-negative bacteria and their growth is crucial in tropical or sub-tropical atmospheres (Fong CF, Tseng CH, *et al*, 2004). Growths represent 5–40% of culture demonstrated contaminations. Pseudomonas aeruginosa is the most normally recuperated causative living being in contact lens-related sickness, trailed by Gram-positive microbes, parasites and Acanthamoeba (Galentine PG, Cohen EJ, *et al*, 1984). The lens, stockpiling case, and visual environment may offer a suitable survival corner for this ecological life form. P. aeruginosa can stick to and colonize lens materials amid wear and make due in contact lens stockpiling cases (Szczotka-Flynn LB, Pearlman E, Ghannoum M, 2010). Numerous include basic and intense to-regard microorganisms known as Staphylococcus aureus. In any case, the most extreme might be Pseudomonas aeruginosa, a quickly developing bacterial disease that can prompt an opening in your cornea. Infections likewise known not genuine eye diseases they might be transmitted from contact lenses of ailing patient, Herpes and adenoviral diseases can happen amid CL wear, Although HIV has been disconnected from visual tissues, tears and delicate CLs utilized by patients with AIDS, contact lenses might be a critical vehicle for the exchange of microorganisms from tainted contact lens answers for the cornea.

MATERIAL AND METHODS

Sample Collection: To isolate microorganisms from contact lens storage cases, we take different samples of lens care storage cases solution from asymptomatic patients. Sample was collected by swabbing on different media as according to the requirement.

Bacterial Isolation: Using a standard 5, ul bacterial loop, the lens case solution was cultured onto 5% Columbia blood agar and MacConkey agar plates and incubated in air at 30°C for 3 days. After incubation all lactose fermenting and non-lactose fermenting Gram negative bacilli were identified to the genus level using a series of manual biochemical tests." These tests included; Oxidase, motility, oxidation/fermentation, citrate, methyl red, indole, Voges-Proskauer, and growth on Macconkey agar. Catalase was assayed qualitatively using hydrogen.

Peroxide as outlined in the Manual of Clinical Microbiology.'1 Gram positive bacteria were identified by their characteristic colonial morphology and Gram stain appearance. No attempt was made to further speciate bacteria.

Fungal Isolation: A 0.5 ml aliquot of the lens case solution was cultured onto an SAC slope (Sabouraud dextrose agar + chloramphenicol 0.1% + gentamicin 0'4%) and incubated in air at 27°C. Cultures were incubated for 14 days before being discarded as negative. Positive cultures were identified by microscopic and macroscopic morphology.

Amoebal Isolation: A 1 0 ml aliquot of the contact lens case solution cultured on a Pages amoeba saline (PAS) agar plate. PAS which had previously been spread with a lawn of heat killed (65°C/30 minutes) Escherichia coli.1I The

Table 1: Morphological, Cultural & Biochemical Characteristics of the isolated gram positive bacteria

Number Of Sample	Colonial Characteristics On Ba	Cultural Characteristics	Gram R/C	Catalase	Coagulase	Growth On Msa	Identified Organism
SAMPLE#1	Gray white colonies with beta hemolysis	Cocci in chains	+ve	-ve		-	Streptococcus
SAMPLE#2	Large round yellow colonies with beta hemolysis / and gray colonies	1.Cocci in clusters/ 2.Cocci in chains	+ve	1.+ve 2. -ve	1. +ve 2. -ve	1.-ve 2.+ve	1.Staphylococcus 2.Streptococcus
SAMPLE#3	Large colonies with beta hemolysis	Scattered rods	+ve	+ve	-	-	Bacillus

Table 2: Morphological, Cultural & Biochemical Characteristics of the isolated gram negative bacteria

Number of samples	Colonial morphology	Cultural characteristic	Gram R/C	TSI	citrate	urease	oxidase	Identified organism
Sample#4	Small round non-lactose fermenting colonies	Scattered rods	-ve	K/A	+ve	-ve	+ve	Pseudomonas aeruginosa
Sample#5	Small round non-lactose fermenting colonies	Scattered rods	-ve	K/A	+ve	-ve	+ve	Pseudomonas aeruginosa
Sample#6	Large gummy lactose fermenting colonies	Rods	-ve	A/A	+ve	+ve	-ve	Klebsiella

Number of sample	Macroscopic characteristics	Microscopic characteristics	Fungus identified
Sample#4	Black colonies	Dark brown round conidia	Aspergillus niger
Sample#3	1.Black colonies 2.velvety white colony	1.dark brown conidial heads 2. sporangiospores, branched hyphae	Aspergillus niger/mucor
Sample#5	Black colonies	dark brown conidial heads	Aspergillus niger
Sample#6	Powdery / smoky green	1.Conidial heads & short conidiophores 2. septate hyphae & long conidiophores	Aspergillus fumigates/ Aspergillus flavus

plates were incubated in a humidified chamber at 30°C for up to 7 days, and examined every 48 hours. Cultured amoebae were identified as either Acanthamoeba or other free living amoebae by morphology of cyst, flagellate, and trophozoites stages.

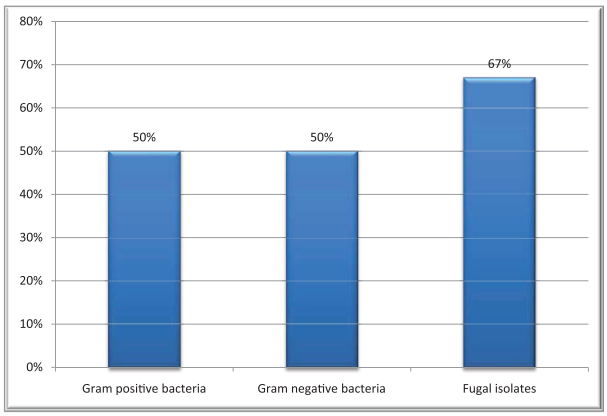

Fig. 1: Prevalence rates of microorganisms in ophthalmic solutions

RESULTS

Microbiological analysis of contact lenses showed increased level of microbial contamination. All the samples were contaminated with microbes except amoeba. Out of 6 samples, 3 were contaminated with gram positive bacteria (33.3% streptococcus, 16% staphylococcus, 16% Bacillus) while other three samples were contaminated with gram negative bacteria (33.3% *Pseudomonas aeruginosa*, 16% Klebsiella). Fungus was isolation four out of six samples (50% *Aspergillus niger*, 16% *Aspergillus flavus*, 16% *Aspergillus fumigatus*, 16% sporangium). Acanthamoeba were not isolated in any tested sample, may be because it rarely isolated invitro from samples.

DISCUSSION

Corneal disease is the most well-known vision debilitating complexity of contact lens wear. Living beings disengaged from contact lens related corneal ulcers have frequently been appeared to be indistinguishable to those confined from the contact lens case, reaching lens arrangement a conceivable load wellspring of pathogenic organisms. Regardless of the obvious adherence to prescribed cleaning and sanitizing administrations, a noteworthy level of microbial pollution of contact lens arrangements was found in this study. In our investigation a huge level of microbial defilement of contact lens stockpiling arrangement was found in this study. Large portions of the contaminants recognized were potential pathogens and in that capacity ought to have been averted by the disinfectant treatment utilized. In our examination study distinctive bacterial strains (100%) and parasitic strains (80%) detached and protozoa were not separated in our study. This study highlights that there is a requirement for development in contact lens stockpiling arrangement cleanliness. Current contact lens sterilization strategies don't have all the earmarks of being giving an alluring level of Microbial assurance. It was accounted for in the past study that most regular microbial contaminant secluded in descending prevalence were microorganisms (78%)>fungi (24%)>protozoa (20%). About all contact lens cases had blended microbial populaces. The most widely recognized bacterial contaminants detached were non-fermentative. Gram negatives took after by coliforms, other Gram negative, and Gram positive living beings (Trevor B Gray *et al.*, 1995). In our examination concentrate exceedingly pathogenic living beings were disengaged, Pseudomonas can be spread from the hands in the lens care arrangement that gets violated and is not appropriately cleaned causes ophthalmic diseases. Streptococcus was likewise confined; Streptococcal diseases are a vital reason for corneal ulcers, endophthalmitis, conjunctivitis, and dacryocystitis. Staphylococcus is additionally exceptionally pathogenic microscopic organisms causes ophthalmic diseases. There are various approaches to get Staphylococcus and Streptoccus diseases once you have a tear or damage in your cornea. Disease spread when eye comes into contact with a despoiled item, (for example, tainted water or a dirty contact lens). Bacillus is likewise pathogenic detach; the pathogencity of the gram positive spore framing bacilli for the eye was initially reported in 1980 cause's contamination of cornea additionally Bacillus.

Endophthalmitis is a profoundly unstable disease of the eye that usually brings about quick irritation and vision trouble. In parasitic detachment most normally Aspergillus species were confined from contact lens stockpiling arrangement tests. In Mycotic keratitis Two essential structures have been perceived: that because of filamentous parasites (particularly Fusarium and Aspergillus), these exceedingly pathogenic separates from our specimens may bring about serious eye diseases. Tireless microbial pollution of contact lens stockpiling arrangements is normal and is connected with microbial keratitis and clean corneal penetrates. The absolute most ideal approach to evade eye diseases is to take after appropriate lens care rules as endorsed by the eye care experts. Specifically, including a "rub and flush" stride in the lens cleaning process, minimizing contact with water while wearing contact lenses and supplanting the lens case oftentimes can lessen the danger of disease.

CONCLUSION

We conclude from our research study that the contact lens case solutions are the single most important potential reservoir for contact lens contamination leading to infection. Contact lenses and the solutions used with them are medical devices and are regulated by the Food and Drug Administration; therefore, it is extremely important that patients maintain regular appointments to ensure they are receiving clinical guidance from their eye doctor based on individual eye health needs. Clean and safe handling of contact lenses is one of the most important measures contact lens wearers can take to protect their sight. Exercising optimal care and hygiene with contact lenses can keep the eyes healthy. Our research study found that contact lens wearers who have poor hygiene habits also have increased bacterial contamination in their contact lens cases. Bacterial And fungal contamination was observed in several cases. Colonization of the lens storage case by pathogenic micro-organisms predisposes lens wearers to microbial or sterile keratitis.

Recommendations to contact lens wearers

From this and previous data, the authors suggest the following measures should result in less contact lens case and contact lens solution contamination; thereby possibly reducing the risk

of microbial keratitis, proper use of disinfectant solutions, disinfection of the contact lens case, wash hands before applying lens to the eye, homemade saline never be used, use ophthalmic solution of known high quality brand, and change it regularly.

REFERENCES

Alfonso E, Mandelbaum S, Fox MJ, Forster RK. Ulcerative keratitis associated with contact lens wear. Am J Ophthalmol 1986; 101: 429–433. | PubMed | ISI |

Anwar H, Strap JL, Costerton JW. Establishment of aging biofilms: possible mechanism of bacterial resistance to antimicrobial therapy. Antimicrob Agents Chemother 1992;36: 1347-51.

Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. Br J Ophthalmol 2003; 87: 834–839. | Article | PubMed | ISI | ChemPort |

Fong CF, Tseng CH, Hu FR, Wang IJ, Chen WL, Hou YC. Clinical characteristics of microbial keratitis in a university hospital in Taiwan. Am J Ophthalmol 2004; 137: 329–336. | Article | PubMed | ISI |

Galentine PG, Cohen EJ, Laibson PR, Adams CP, Michaud R, Arentsen JJ. Corneal ulcers associated with contact lens wear. Arch Ophthalmol 1984;102: 891–894. | Article | PubMed | ISI |

Houang E, Lam D, Fan D, Seal D. Microbial

keratitis in Hong Kong: relationship to climate, environment and contact lens disinfection. Transact R Soc Trop Med Hyg 2001; 95: 361–367. | Article |

J.K.G. Dart, FCOphth, F. Stapleton, MSc, D. Minassian, FCOphth. Volume 338, No. 8768, p650–653, 14 September 1991

Liesegang TJ , Forster RKAmerican Journal of Ophthalmology [1980, 90(1):38-47] Journal Article, Research Support, U.S. Gov't, P.H.S

Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Florida.Am J Ophthalmol 1980; 90: 38–47. | PubMed | ISI | ChemPort|

Melia B, Islam T, Madgula I, et al; Contact lens referrals to Hull Royal Infirmary Ophthalmic A&E Unit. Cont Lens Anterior Eye. 2008 Jul 1;.

Michael E. Zegans, Heidi I. Becker, Jonathan Budzik, and George O'Toole. DNA and Cell Biology. July 2004, 21(5-6): 415-420. doi:10.1089/10445490260099700.

Microbial contamination of contact lenses, lens care solutions and their accessories. Eye Contact Lens2010; 2: 116–129. | Article |

Roberts A, Kaye AE, Kaye RA, et al; Informed consent and medical devices: the case of the contact lens. Br J Ophthalmol. 2005 Jun;89(6):782-3.

Schein OD, Ormerod LD, Barraquer E, Alfonso E, Egan KM, Paton BG et al. Microbiology of contact lens-related keratitis. Cornea 1989; 8: 281–285. | Article | PubMed | ISI | ChemPort |