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Identification of Contact lens bacterial Isolates from Students of University of Benin, Nigeria, Using 16S rRNA Gene Sequence Analysis.

[1] Iyamu, E and [2] Ekhaise, F. O

[1] *Department of Optometry, University of Benin, Benin City, Edo State, Nigeria.*

[2] *Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria.*

[1,2] *Members of Applied Environmental Bioscience and Public Health Research Group.*

Corresponding author: Iyamu Eghosasere | Email: *eghosasere.iyamu@uniben.edu* | Phone: 07089260596

Abstract

Purpose: Molecular phylogenetic analyses have proven beneficial in overcoming some limitations of traditional phenotypic procedures for the detection and characterization of bacterial isolates. The aim of this study was to identify bacterial isolates from hydrogel (soft) contact lenses worn by selected students of the University of Benin, Nigeria, using 16S rRNA gene analysis.

Methods: Eleven bacterial isolates from the worn hydrogel lenses, which remained unidentified with the traditional phenotypic techniques, were transferred to the molecular biology section of the Labor Medical and Research Laboratory for possible identification by phylogenetic analysis, to have a full range of bacteria considered as normal flora of the eyes. Bacterial genomic DNA was extracted with the ZymoBIOMICS DNA Mini Kit (Zymo Research Corp., Irvin, CA, USA), amplified with polymerase chain reaction (PCR), and the amplicons were sequenced with the ABI3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were assembled and deposited in the GenBank database for alignment with the prototype strains.

Results: The bacterial isolates identified were *Burkholderia cenocepacia* GIMC4560: BCN122, *Cupriavidus pauculus* KPS 201, and *Comamonas testosteroni* M.pstv. 4.2, *Acinetobacter calcoaceticus* NBRC 13006, *Achromobacter denitrificans* SW2, *Bacillus cereus* FORC_048, *Staphylococcus saprophyticus* Marseille-P541, *Burkholderia cenocepacia* GIMC 4560: Bcn122, *Acinetobacter calcoaceticus* 7.3, and *Comamonas* sp. 6.1 and *Acinetobacter* sp. pp2a.

Conclusion: The 16S rRNA gene sequence analysis was able to identify 82% of the isolates at the strain level and 18% at the genus level..

Keywords: 16S rRNA gene sequence, contact lens bacterial isolates, hydrogel contact lenses, phenotypic methods, phylogenetic analysis, polymerase chain reaction.

Introduction

Contact lens (CL) wear has been considered a risk factor for developing contact lens-related infections of the cornea and conjunctiva. About ninety percent of the lens surface is coated with deposits from the tear film, lids and conjunctiva after eight hours of lens wear,[1] thereby creating a suitable substrate for bacteria to thrive and initiate various adverse effects such as microbial keratitis,[2] as well as contact lens-related acute red eye.[3] Adhesion and colonization of contact lenses (especially hydrogel contact lenses) by a variety of microbes particularly bacteria[2] have been implicated as a major factor in the initiation of the cascade of events characterizing sight threatening microbial keratitis (infection/inflammation of the cornea).[4] Different contact lens materials have been shown to exhibit variable characteristics to adhesion to *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are the most implicated in bacterial keratitis.[5] Hydrogel (soft) contact lenses are widely used as an alternate to spectacle lenses because of better peripheral vision, and wider field of view, for the correction of refractive ametropia and are preferred to gas-permeable or rigid contact lenses, being more comfortable and easier to adapt to when worn by a larger proportion of the population wearing contact lenses. Since invasion of lenses is inevitable, understanding the identity of bacterial isolates from worn contact lenses is essential for contact lens-related ocular diseases initiated by such organisms considered as opportunistic agents, which were hitherto members of the normal flora, so that the management of conditions arising can be precise and prompt for the full range of bacteria that constitute the normal flora from the worn CLs by individuals without ocular or systemic diseases. However, where some bacterial isolates still remain unidentified, phylogenetic techniques using 16S rRNA gene sequence analysis have to be employed for the possible identification of such isolates. Sequencing the ribosomal RNA gene is the method of choice for nucleic acid-based detection and identification of microbes, their taxonomic assignment, phylogenetic analysis, and the investigation of microbial diversity.[6] Studies have reported that the 16S rRNA gene is used as the new gold standard for classification and identification of microbes, especially bacteria.[7,8] The basic steps in this phylogenetic analysis include the assembly and alignment of datasets using available online search tools, such as the Basic local alignment search tool within the National Center for Biotechnology Information (BLAST-NCBI) website. This is followed by constructing phylogenetic trees using common computational methods like discrete data methods, such as maximum parsimony and maximum likelihood, and distance-matrix methods, including transformed Distance, the Neighbour-Related methods, or Neighbour-Joining method.[9] Phenotypic variability among strains belonging to the same species also results in some bacterial isolates presenting characteristics that are atypical

1. Bailey SC. Contact lens complications. Optometry Today. 1999; 4(1): 26 - 35.
2. Green M, Apel, A and Stapleton F. Risk factors and causative organisms I microbial keratitis. Cornea. 2008; 27: 22 - 27.
3. Holden BA, LA Hood D, Grant T, Newton-Howes J, Baleriola-Lucas C, Willcox MD and Sweeney DF. Gram-negative bacteria can induce contact lens related acute red eye (CLARE) responses. Contact Lens Association of Ophthalmology Journal. 1996; 22:47 - 52.
4. Dutta D and Willcox MDP. A laboratory Assessment of Factors that affect bacterial adhesion to contact lenses. Biology. 2013; 2(4): 1268 - 1281.
5. Iyamu E, Ekhaise, FO. (2021). Bacterial adhesion to conventional and silicone hydrogel contact lenses. Journal of Nigerian Optometric Association. 2021; 23: 25 - 35.
6. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Garza P, Peplies J, Glöckner FO. (2013). The SILVA ribosomal RNA gene datdbase project: improved data processing and web-based tools. Nucleic Acids Research. 2013; 41(D1): D590 - D596.
7. Fournier PE, Dumped, JS, Greub G, Zhang J, Wu Y, Raoult D. Gene sequence-based criteria for identification of new Rickettsia isolates and description of Rickettsia heilongjiangensis sp. Nov, Journal of Clinical Microbiology. 2003; 41: 5456 - 5465.
8. Harmsen D, Karch H. 16S rDNA for diagnosing pathogens: A living tree, ASM News. 2004; 70: 19 - 24.
9. Saituo, N. and Neo, M. (1987). The Neighbour-Joining methods: A new methods for constructing phylogenetic trees. Molecular Biology and Evolution. 1987; 4: 406 - 425.

for any candidate identification.[10] Molecular approach has been extensively used for bacterial phylogeny leading to the establishment of large public-domain databases and its application to bacterial identification, including environmental and clinical uncultured microbes, unique or unusual isolates, and collections of phenotypically identified isolates.[10] In other words, bacteria can be identified by nucleotide sequence analysis of polymerase chain reaction of 16S gene followed by comparison with known sequences of bacterial DNA database.[11] The aim of this study was to identify contact lens bacterial isolates that remained unidentified by traditional phenotypic methods using Phylogenetic analysis, so as to have a full range of bacteria isolated from healthy eyes considered as normal flora of the eyes. This would help eye care practitioners have first-hand information about the likelihood of some of them becoming opportunistic pathogens in cases of compromised epithelial structures of the cornea.

Materials and Methods

This study involved forty subjects drawn from students of the Department of Optometry, University of Benin, aged 18 to 26 years who were required to undergo tests such as non-invasive tear break-up time, tear flow rate/5 mins, slit-lamp biomicroscopy, keratometry, and anatomical parameter assessment. Informed consent was obtained from each subject after the procedures and possible outcomes were explained. The study protocol was approved by the Departmental Research and Ethics Committee in accordance with the tenets of Helsinki Declaration 2000. The lenses were sourced from Truvision Ltd, Lagos State, Nigeria, and the subjects were taught the procedure of lens insertion and removal.

The lenses were washed three times with distilled water and transferred into a Bijou bottle containing nutrient broth. The stock solution was incubated at 37°C for 24 hours to allow for bacterial growth. Of the eighty samples collected, sixty yielded growth. The stage was prepared for serial dilution, and the inoculum was transferred onto sterile Petri dishes containing nutrient agar media (NAM). Inoculated dishes were incubated at 37°C for 24 to 48 hours, and colonies were counted using Darkfield Quebec colony counter.

The majority of the CL bacterial isolates in 42 samples were identified, but eleven isolates from 18 samples were not properly identified. They were transferred to the Molecular Biology section of the laboratory for possible identification using phylogenetic analysis.

Genomic DNA was extracted from the cell pellets of all CL bacterial isolates using ZymoBIOMICSTM DNA mini kit. The protocol as described in the instruction manual was adopted. For each bacterial isolate, a single DNA fragment (about 1500bp) of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) in a thermal cycler. The universal primer sets of 16S rRNA (27 Forward-

10. Drancourt M, Billet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 16S ribosomal DNA sequence analysis of a large collection of environmental and chemical unidentifiable bacterial isolates. Journal of Clinical Microbiology. 2000; 38: 3623 - 3630.
11. Barghouthi SA. A universal method for the identification of Bacteria based on general PCR primers. Indian Journal of Microbiology. 2011; 51(4): 430 - 444.

5'-AGAGTTTGATCMTGGCTCAG-3' and 1492 Reverse-5'-CGGTTACCTTGTTACGATT-3') were used for the amplification. [12,13,14]

The PCR products were cleaned, sequenced, and injected onto ABI 3500XL Genetic analyzer for sequencing. The nucleotide sequence data of the bacterial isolates were deposited in the Public-domain database (Genbank) using BLAST algorithm available on NCBI server. 15 An accession number was assigned to each sequence deposited. The annotated information from the alignment search was further analyzed with Geneious software (Ver. 9.0.5) (Biomatters Ltd, Auckland, NZ) for phylogenies. Phylogenetic trees were then assessed by Bootrap analysis and constructed using Computational and distance-matrix methods (Neighbour-joining method) in the software.

Results

The bacterial isolates that were unidentified with the traditional/phenotypic method were identified by phylogenetic analysis to the strain and genus level respectively.
The distribution of bacterial isolates identified by phenotypic methods are presented in Table 1.

Table 1: Distribution of CL bacterial isolates identified by Phenotypic methods

Bacterial isolate.	Frequency of isolation (%)
Bacillus sp.	3 (7.14)
Coagulated negative Staphylococcus	7 (16.67)
Staphylococcus aureus	5 (11.90)
Micrococcus sp	2 (4.76)
Corynebacterium sp	1 (2.38)
Staphylococcus epidermidis	1 (2.38)
Aeromonas sp	1 (2.38)
Escherichia coli	5 (11.90)
Klebsiella sp	4 (9.52)
Proteus sp	3 (7.14)
Enterobacter sp	2 (4.76)
Proteus mirabilis	1 (2.38)
Pseudomonas aeruginosa	6 (14.29)
Serratia sp	1 (2.38)

12. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology. 1991; 173(2): 697 - 703.
13. Yadar, V, Prakash S, Srivastava S, Verma PC, Gupta V, Basu V, Rawat AK. Identification of Comamonas sp using 16S rRNA gene sequence. Bioinformatics. 2009; 3(9): 381 - 383.
14. Devereux R, Willis SG. (1995). Amplification of ribosomal RNA sequence: In: Akkerman's, A.D.L., van Elsas, J.D., De Bruijn, F.J,eds, Molecular Microbial Ecology Manual. Dorrecht, The Netherlands, Kluwer. 1995: 3.3.3: 1 - 11.

Table 2 shows the contact lens bacterial isolates identified based on alignment of their 16S rRNA gene sequence with the prototype strains in the public domain with their Accession number from the GenBank.

Table 2: Bacterial identity of isolates based on alignment of 16S rRNA gene sequence with prototype strains in GenBank with their accession numbers

Bacterial isolate GenBank	Bacterial identity	Accession number from
3C-opt-MN	*Acinetobacter calcoaceticus NBRC 13006*	AB680365
2O-opt-FH	*Achromobacter denitrificans SW2*	KX229786
2R-opt-FH	*Cupriavidus pauculus KPS201*	AM418462
1D-opt-FN	*Burkholderia cenocepacia GIMC 4560 Bcn 122*	CP020599
2B-opt-FH	*Burkholderia cenocepacia GIMC 4560 Bcn 122*	CP020599
1F-opt-FN	*Bacillus cereus FORC_048*	CP017234
3E-opt-MN	*Comamonas testosteroni M.pstv.4.2*	KM108479
1D-opt-FH	*Acinetobacter calcoaceticus 7.3*	GU385012
1A-opt-FN	*Comamonas so. 6.1*	EF426440
3T-opt-MN	*Acinetobacter sp. pp2a*	GQ360067
3Q-opt-MN	*Staphylococcus saprophyticus Marseille-P541*	LT558833

Identification of the bacterial isolate was based on the similarity sequence of its 16S rRNA gene with the prototype strain in the Public-domain database.
The distribution of the bacterial isolates phylogenetically identified is presented in Table 3.

Table 3: Distribution of Bacterial isolates identified by Phylogenetic analysis

Bacterial	Frequency of isolation (%)
Burkholderia cenocepacia GIMC 4560 Bcn 122	2 (11.11)
Staphylococcus saprophyticus Marseille-P541	4 (22.22)
Acinetobacter calcoaceticus NBRC13006	2 (11.11)
Comamonas testosteroni M.pstv.4.2	1 (5.56)
Acinetobacter sp	4 (22.22)
Achromobacter denitrificans SW2	1 (5.56)
Cupriavidus pauculus KPS201	1 (5.56)
Bacillus cereus FORC_048	1 (5.56)
Comamonas sp	1 (5.56)
Acinetobacter calcoaceticus 7.3	1 (5.56)

Phylogenetic analysis of clinical samples was based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of bacteria.

The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples (Figures 1 and 2).

Figures 1 & 2 show the Neighbour- joining trees of selected 16S rRNA gene sequences obtained from the BLAST search of some of the isolates.

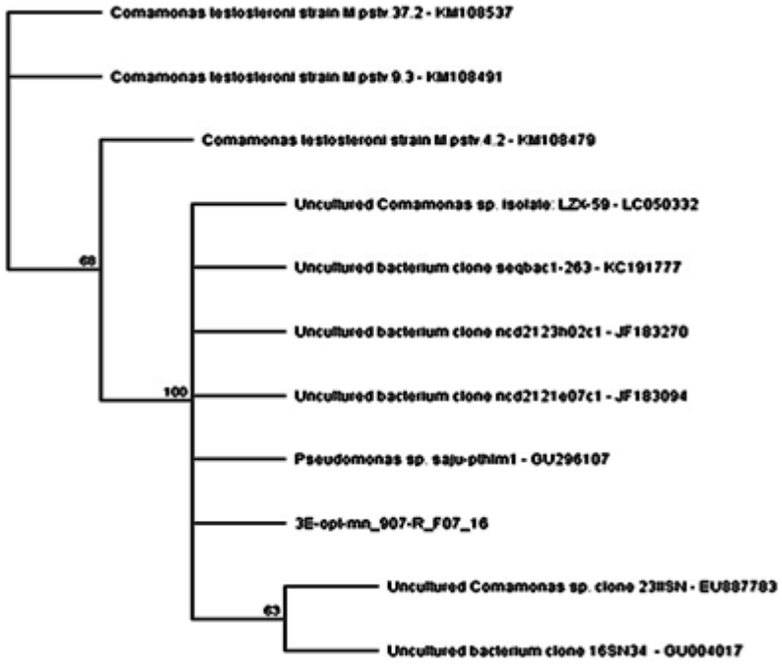

Figure 1. N-J tree of selected 16S rRNA gene sequence of the genus obtained from BLAST search of the isolate 3E-opt-MN which had sequence similarity with *Comamonas testosteroni* M.pstv.4.2 with accession number KM108479

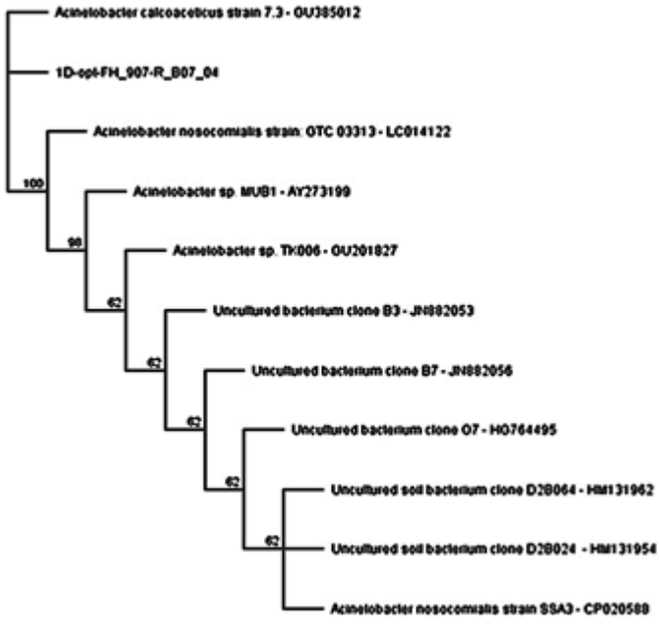

Figure 2. N-J tree of selected 16S rRNA gene sequence of the genus obtained from BLAST search of the isolate 1D-opt-FH which had sequence similarity with *Acinetobacter calcoaceticus* 7.3

Discussion

For the purpose of identifying and characterizing bacterial isolates, conventional phenotypic techniques have several drawbacks that have been partially addressed by molecular phylogenetic analyses. According to studies, 16S rRNA gene sequencing is a more objective and accurate method of identifying bacteria,[16,17] and it has been regarded as the new gold standard for bacterial identification since it is simpler to use.[7,8] The length of the 16S rRNA gene sequenced in this investigation using gel electrophoresis was 1571 base pairs (bp), which was sufficient for bioinformatics and compared favourably to the 1550 bp reported by Drancourt et al10 in their reviewed publication. Comparing 16S rRNA gene sequences typically enables genus-level discrimination between organisms and multi-level classification of strains, which comprise sets of phenotypically defined isolates as well as environmental and clinically uncultured or uncommon isolates.[10] After processing the isolates for phylogenetic approaches utilizing the 16S rRNA gene sequence analysis, those that remained unidentified by the phenotypic techniques were eventually identified. This gene sequence contains hypervariable areas that can yield species-accelerating signature sequences helpful for bacterial identification, as well as highly conserved primer binding sites for the universal primer employed in the amplification of this region during PCR.[18,19] 16S rRNA gene sequence provides genus identification of more than 90% and less in terms of species (65 to 83%), with 1 to 14% of isolates remaining unidentified after testing.[10,20,21] The challenges associated with identifying genus and species have been linked to the identification of new taxa, an inadequate number of sequences included in nucleotide databases, species with similar or identical 16S rRNA gene sequences, or issues with nomenclature resulting from several DNA groups belonging to a single species or complex.[22, 23] The Geneious software used for phylogenetic analysis uses the Bootstrap analysis to access the phylogenetic tree and construct it with the Neighbour- Joining algorithm. Phylogenetic analysis showed that our isolates are closely aligned with the prototype strains of GenBank, which contain about ninety thousand 16S rRNA gene sequences. Differences in reporting sequence similarities can be attributed to the method used to process extracted DNA, comparison of 16S rRNA sequences with prototype strains in the nucleotide database, and the software program used for phylogenetic analysis.

In this study, the 16 S rRNA gene sequence yielded a species identification rate of approximately 81 per cent, which was within the 62 to 91 per cent reported by Fontana and colleagues24 and 65 to 83 per cent reported by other studies.[10,20,21] However, two of the isolates were identified without a specific

7. Fournier PE, Dumped, JS, Greub G, Zhang J, Wu Y, Raoult D. Gene sequence-based criteria for identification of new Rickettsia isolates and description of Rickettsia heilongjiangensis sp. Nov, Journal of Clinical Microbiology. 2003; 41: 5456 - 5465.
8. Harmsen D, Karch H. 16S rDNA for diagnosing pathogens: A living tree, ASM News. 2004; 70: 19 - 24.
10. Drancourt M, Billet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 16S ribosomal DNA sequence analysis of a large collection of environmental and chemical unidentifiable bacterial isolates. Journal of Clinical Microbiology. 2000; 38: 3623 - 3630.
18. Kolbert CP, Persing DH. Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. Current Opinion in Microbiology. 1999; 2(3): 299 - 305.
19. Perenia F, Carneoro J, Matthiesen R, vanAsch B, Pinto N, Gusmao L, Amonin N. Identification of species by multiplex analysis of variable length sequence. Nucleic Acids Research. 2010; 38(22): e203Doi.10.1093/nar/gkq865.
20. Mignard S, Flandrois JP. 16S rRNA sequencing in routine bacteria identification: a 30-month experiment. Journal of microbiology Methods. 2006; 67: 574 - 581.
21. Woo PCY, Ng KHI, Lay SKP, Yip KI, Fung AMY, Leung KW, Tam DMW, Que TL, Yuen KY. Usefulness of the Microseq 500 16S ribosomal DNA-based identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. Journal of Clinical Microbiology. 2003; 41: 1996 - 2001.
22. Janda MJ, Abbott SI.16S rRNA gene sequencing for bacteria identification in the diagnostic laboratory: pluses, perils and pitfalls: A mini review. Journal of Clinical Microbiology. 2007; 45: 2761 - 2764.
23. Relman DA. The search for unrecognized pathogens. Science. 1999; 284: 1308 - 1310. Doi:10.1126/284.5418.1308.

species name (*Comamonas s*p and *Acintobacter* sp) which accounted for 18%. This may be due to Relman's assertion[23], who reported that the current databases contain insufficient numbers of entries with which species and other taxon boundaries can be defined for a wide range of microorganisms. Consequently, 16S rRNA gene sequencing has become an increasingly popular method of medical microbiology as a rapid and cheap alternative to phenotypic methods for bacterial identification.[25] Using molecular phylogenetic analysis, some of the isolates found in this investigation have been connected in some way to a few instances of eye infections. An incidence of corneal ulceration brought on by a contaminated soft contact lens has been linked to *Acinetobacter calcoaceticus*, an opportunistic pathogen[26] that is thought to be a natural part of the human gut flora.[27] Gopal *et al*.[28] described the clinical and microbiological profile of endophthalmitis, a purulent inflammatory condition of the inner cavities (i.e., aqueous and/or vitreous humor) caused by *A. calcoaceticus*. This condition may have devastating consequences for the patient's vision which may suffer greatly as a result of this illness. *Cupriavidus pauculus* is thought to be a probable ocular opportunistic pathogen that has been identified from soft contact lenses. This suggests that polluted water sources may be the source of contamination for CLs and the subsequent ocular infections that may arise. Corneal culture, contact lens wear and lens solution have been shown to be positive for *Burkholderia cenocepacia*.[29] Improper fitting of lenses and contamination of lenses or therapy were considered as the risk factors for corneal ulcer. *Bacillus cereus* is being increasingly reported to be a causative agent of serious ocular infections associated with contact lens wear.[30] In the same vein, the genetic identity of Bacillus cereus as a possible etiologic agent of contact lens associated inflammation of the cornea was reported by Pinna and colleagues.[31] Lens handling has been reported to be responsible for a great increase in the incidence of contact lens contamination. Although the ocular surface has the ability to destroy organisms through the antimicrobial agents, mostly proteins in the pre-corneal tear film, more than half of lenses are discovered to contain microbes, virtually all of which are bacteria, even after being aseptically removed from the eye.[32] Since 16S rRNA gene sequence analysis was able to identify about 82% of the bacterial isolates to species level and 18% to genus level, its overall performance

21. Woo PCY, Ng KHI, Lay SKP, Yip KI, Fung AMY, Leung KW, Tam DMW, Que TL, Yuen KY. Usefulness of the Microseq 500 16S ribosomal DNA-based identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. Journal of Clinical Microbiology. 2003; 41: 1996 - 2001.
22. Janda MJ, Abbott SI.16S rRNA gene sequencing for bacteria identification in the diagnostic laboratory: pluses, perils and pitfalls: A mini review. Journal of Clinical Microbiology. 2007; 45: 2761 - 2764.
23. Relman DA. The search for unrecognized pathogens. Science. 1999; 284: 1308 - 1310. Doi:10.1126/284.5418.1308.
25. Clarridge JE. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical and infectious diseases. Clinical Microbiology Reviews. 2004; 17(4): 840 - 862.
26. Chen TL, Siu LK, Lee YT, Chen CP, Huang LY, Wu RCC, Fung CP. Acinetobacter baylyi as a pathogen for opportunistic infection. Journal of Clinical Microbiology. 2008; 46(9): 2938 - 2944.
27. Herbs RW. Herella corneal ulcer with use of soft contact lenses. British Journal of Ophthalmology. 1972; 56: 848 - 850.
28. Gopal L, Ramaswamy AA, Madhaven HN, Battu RR, Dharma T, Shanwugam MP, Raoult MK. Endophthalmitis caused by Acinetobacter calcoaceticus: A profile. Indian Journal of Ophthalmology. 2003; 51(9): 335 - 340.
29. Ying-Cheng L, Chao-Kung L, Ko-Hua C, Wen-Ming H. Daytime orthokeratology Associated with infectious keratitis by multiple Gram-positive Bacilli: Burholderia cepacia, Pseudomonas putida and Pseudomonas aeruginosa. Eye and Contact Lens. 2006; 32(1): 19 - 20.
30. Bottone EJ. Bacillus cereus, a volatile human pathogen. Clinical Microbiology Reviews. 2000; 23: 382 - 398.
31. Pinna A, Sechi LA, Zanetti S. Bacillus cereus keratitis associated with contact lens wear. Ophthalmology. 2001; 108: 1830 - 1834.
32. Szczotka-Flynn LB, Pearlman E, Mahmoud G. Microbial contamination of contact lenses, lens care solutions and their accessories: A literature Review. Eye and Cornea. 2010; 36(2): 116 - 129.

was deemed satisfactory[10,20,21,24]. Accurate identification of bacterial isolates is essential for clinical microbiology, antimicrobial management of infectious diseases, epidemiology and disease control, especially when bacteria are involved in eye diseases. It also aids in our understanding of the entire spectrum of bacteria that are thought to be part of the normal flora of the eyes, as some of them may develop into opportunistic pathogens in cases when the cornea's epithelial structures and/or other tissues in the anterior adnexa of the eye are compromised. This can also make a difference in the understanding of the pathogenic processes and clinical outcome. This study has been able to show that phylogenetic analysis can complement the phenotypic technique to ensure that all contact lens bacterial isolates are identified to the strain and in extreme cases the genus level.

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10. Drancourt M, Billet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 16S ribosomal DNA sequence analysis of a large collection of environmental and chemical unidentifiable bacterial isolates. Journal of Clinical Microbiology. 2000; 38: 3623 - 3630.
20. Mignard S, Flandrois JP. 16S rRNA sequencing in routine bacteria identification: a 30-month experiment. Journal of microbiology Methods. 2006; 67: 574 - 581.
21. Woo PCY, Ng KHI, Lay SKP, Yip KI, Fung AMY, Leung KW, Tam DMW, Que TL, Yuen KY. Usefulness of the Microseq 500 16S ribosomal DNA-based identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. Journal of Clinical Microbiology. 2003; 41: 1996 - 2001.
24. Fontana C, Favaro M, Pellicioni M, Pistoia ES, Favalli C. Use of Microseq 16S rRNA gene-based sequencing for identification of bacterial isolates that commercial automated systems failed to identify correctly. Journal of Clinical Microbiology. 2005; 43: 615 - 619.