M. Thangaraj¹, V. T. Meenatchi², S. Padmavathy³* and N. K. Asha Devi⁴

¹Department of Computer Science, Madurai Kamaraj University, Madurai - 625021, Tamil Nadu, India; thangarajmku@yahoo.com
²Department of CA and IT, Thiagarajar College, Madurai - 625009, Tamil Nadu, India; vtmeenatchi@gmail.com
³⁴Department of Zoology and Microbiology, Thiagarajar College, Madurai - 625009, Tamil Nadu, India; padma.phd2009@gmail.com, nkashadevi@gmail.com

Abstract

**Objectives:** Even when studies report most of the Contact Lens (CLs) wearers possess improved vision, there are some potential risks with the development of microbial keratitis. This is in turn creates research issue under public health concern. **Methods/Analysis:** The methodology of the work determines the culture sensitivity of the recovered isolates from three different CLs users: Daily disposable lens, monthly disposable lens and yearly disposable lens. **Findings:** Through the machine learning tool called Waikato Environment for Knowledge Analysis (WEKA) and extensive clinical laboratory analysis, the study provides information on prevalent Contact Lens adhering bacteria involved in causing keratitis and examine microbial biofilm formation using Scanning Electron Microscopic (SEM) analysis. The sample type of the lens with the bacterial infections were then statistically analyzed, so that the knowledge mined would aid the medical practitioners in the treatment of bacterial keratitis. **Novelty/Improvement:** The present study supports the treatment of bacterial keratitis associated with Contact Lens users to reduce or to prevent the adverse effects caused by bacterial pathogens.

Keywords: Bacteria, Clinical, Contact Lens, Keratitis, Knowledge

1. Introduction

Contact Lenses are devices, made of glass, plastic material. It rectifies the vision defect¹–³. The uses of CLs range from cosmetic to functional⁴,⁵. Over 96% of CLs wearers have reported improvement in their vision.

The common problem among CLs wearers is microbial keratitis. The incorrect usage and unhygienic maintenance of CLs end in eye infections among Contact Lens wearers. Once there is an infection, the causative organisms must be identified and proper antibiotic must be provided to the patient⁶,⁷. The organisms should be periodically tested to detect the resistance trends⁸,⁹.

Results of one study suggested, that there is an increase in the quantity of bacteria in the eye during hard lens daily wear after 6 months of use¹⁰. CLs can be worn either as daily disposable, monthly disposable, extended wear or as continuous wear¹¹.

Most of the studies, examined the ability of the bacterium that adheres to CLs. The bacterial isolates, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* strains adhere in larger numbers to Contact Lenses.

Bacterial keratitis can also result in loss of vision. Though the treatment strategies are available with antibiotics, there are some drawbacks in therapy because of the indiscriminate antibiotics usage. This in turn develops resistance to most commonly used antimicrobials¹²,¹³. Therefore, detecting the resistance trends becomes challenging for the health care providers¹⁴,¹⁵.

Keeping all the above evidences and views, the research work identifies the bacterial isolates in different types of...
CLs, finds solution for the infections and also suggests with best lens type for the future use.

2. Materials and Methods

2.1 Experimental Study Population

The used CLs samples were collected from Eye Care Hospital, Dindigul. Hundred samples from each group were obtained and labelled as Daily disposable Lens: DL1 ------DL100, Monthly disposable Lens: ML1--------ML100 and Yearly disposable Lens: YL1 ---------YL100.

2.2 CLC Architecture – An Overview

A new architecture termed as Contact Lens Characterization (CLC) as shown in Figure 1 is proposed.

CLC architecture contains the following 5 main phases:

- Isolation and identification of organisms
- Categorization of organisms through machine learning
- Biochemical and physiological characterization
- Antibiotic sensitivity
- Determination through Clinical analysis

The details of the phases are as follows:

2.2.1 Isolation and Identification of Organisms

2.2.1.1 Identification of Organisms by SEM Analysis

SEM analysis employs the electron microscope as a tool, which forms an image of a specimen and examines surface topographic features. The bacterial adhesion on the Contact Lens samples was examined by studying the morphology of bacterial biofilm using Hitachi model 450.

2.2.1.2 Isolation and Identification of Organisms using Selective/Differential Media

The samples were taken as three set of groups using sterile cleaned lens cases containing fresh lens cleaning solution. Then the lens samples were washed for few minutes, to remove the dust particles and it was carefully transferred into nutrient broth, which in turn enhances the growth of all types of microorganisms. The broth containing Contact Lenses was allowed to stand for 3 to 6 hours of incubation at 37°C. After incubation, the broth containing all types of organisms was serially diluted using sterile blanks and the selected dilution was plated on fresh nutrient agar medium. The plates were incubated at 37°C for overnight. The grown colonies were sub cultured in fresh medium based on the differences in colony morphology and stored at 4°C for further characterization.

Bacterial culture was obtained by sub culturing from the stock culture in the fresh broth and the inoculum is prepared. They were then gently streaked on Blood agar, Mac Conkey agar, EMB agar, Endo agar, Mannitol Salt agar, Tech agar, Flo agar, King's B medium and Cetrimide agar. Gram's staining method was used in identifying the bacterial culture. The staining process was continued for finding pure cultures.

2.2.2 Categorization of Organisms through Machine Learning

After isolating the organisms from the CLs, the samples were categorized using WEKA tool. The CLs dataset is displayed in Attribute Relation File Format (ARFF) Viewer is shown in Table 1. The Contact Lens sample types: Y early disposable, monthly disposable and daily disposable were analyzed for identifying the bacterial isolates.

This dataset is given as input for applying classification through Random Tree algorithm. The Random Tree algorithm is a tree based classification algorithm, which derives decision rules and the rules were as shown in Figure 2.

The decision tree generated is shown in Figure 3. The algorithm achieves 81% of classification accuracy. The bacterial isolates which are classified and identified for the 3 sample types are shown in Table 2.

2.2.3 Biochemical and Physiological Characterization

The biochemical and physiological tests for bacterial identification were performed at incubation temperature of 30°C. Catalase activity was detected using 3% \( \text{H}_2\text{O}_2 \) and cytochrome oxidase presence was tested with oxidase discs. Reduction in nitrate was found in medium sup-
implemented with 0.1% KNO$_3$. Production of H$_2$S gas was tested using broth medium.

Hydrolysis of starch, gelatin, casein and urea were detected in agar plate such as starch agar, nutrient broth supplemented with 12% gelatin and milk agar respectively. Using Bergey's Manual of Systematic Bacteriology$^{19,20}$, bacterial colonies were characterized morphologically and physiologically. For bacterial identification, strains of carbon, nitrogen utilization and biochemical tests were performed according to standard methods described for bacteria$^{21}$.

### 2.2.4 Antibiotic Sensitivity

The identified isolates were grown in 5 ml Mueller-Hilton broth and incubated at 37°C for 2-8 hours. Suitable antibiotics discs such as Streptomycin, Tetracycline, Penicillin,
Gentamycin and Erythromycin were placed on the agar surface. The plates were then observed for the inhibition around discs.

2.2.5 Determination through Clinical Analysis

This phase focus on three main components: Well Diffusion Assay, Bactericidal and Bacteriostatic Activity and Minimum Inhibitory Concentration (MIC).

2.2.5.1 Well Diffusion Assay

The eye drops commercially sold for eye infections were used to determine the antagonistic activity. Agar with pH value of 7.2 ± 0.2 was poured into the plates and refrigerated for solidification. The Mueller Hinton agar plates were then uniformly inoculated using cotton swab, for the confirmation of organism growth. The wells made of cork borer was injected with 100 µl eye drop samples.

2.2.5.2 Bactericidal and Bacteriostatic Activity

To detect whether the pathogen is inhibited or killed, the area surrounding the inhibition zone is swabbed, inoculated into the nutrient medium at 37°C for 24 hours. After incubation, the broth containing tubes were observed for the turbidity. Then the broth contents were streaked in fresh nutrient agar plates. The presence of growth in the plate was inferred as an inhibitory activity (bacteriostatic), while no growth was inferred as bactericidal.

2.2.5.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Commercial eye drops which exhibited effective activity in the previous step was used for detecting the Minimum Inhibitory Concentration against the isolated organisms. The steps were followed as per the standard procedure. The test tube with lowest concentration of drug with reduction in turbidity was determined as MIC. The test sample that did not permit visible growth of the organism in broth culture was regarded as the MBC.

3. Results and Discussion

Bacterial keratitis otherwise known as corneal ulcer is the complication seen in lens wearers. When untreated, it leads to perforation and endophthalmitis. Sleeping with CLs is a major risk factor among Contact Lens wearers. Around 30,000 people were affected annually by bacterial keratitis in United States of America.

Different types of organisms based on the colony morphology were isolated by serial dilution technique and enumerated. The colonies were sub cultured in fresh medium and stored at 4°C for further characterization. From the results, it was found that all the yearly disposable lens samples contain more amount of colony forming units than other two samples. Next comes the sample of Monthly disposable lens and there is a very lower colony forming units in the daily disposable lens.

Based on the results, it was found that all the yearly disposable lens samples were provided with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus sp.*, *Serratia sp.*, and *Bacillus sp.* When considering the monthly disposable lens samples, only two samples were shown to possess with *Pseudomonas aeruginosa* and remaining samples were colonized with *Staphylococcus sp.* But, in daily disposable lens samples there was absence of other isolates like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus sp.*, *Serratia sp.* and they contain spore forming bacterium *Bacillus sp.* which may be due to the external contamination (Plate 1).

The samples were taken from three set of groups using sterile cleaned lens cases containing fresh lens cleaning solution. Then the lens samples were washed to remove the dust particles and it is carefully transferred into nutrient broth which enhances the growth of all types of microorganisms. The broth containing contact lenses is allowed to stand for 3 to 6 hrs of incubation at 37°C. Different type organisms based on colony morphology were isolated by serial dilution technique and enumerated. The colonies were sub cultured in fresh medium and stored at 4°C for further characterization (Plate 2).

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Identified Bacterial Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monthly Disposable Lens</td>
<td>- <em>Pseudomonas aeruginosa</em> - <em>Staphylococcus sp.</em></td>
</tr>
<tr>
<td>Daily Disposable Lens</td>
<td>- <em>Bacillus sp.</em></td>
</tr>
</tbody>
</table>
The isolated bacteria from three different groups of Contact Lens wearers were identified based on morphological and standard biochemical characteristics. The organisms were further confirmed by growth analysis of the organism on different selective medium. The isolated organisms from three different group lens samples were tentatively identified as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus sp.*, *Serratia sp.* and *Bacillus sp.* (Plate 3) based on Gram’s staining and biochemical characteristics.

The production of cell surface and extracellular polymeric by the bacterium had shown to play essential role during cell adhesion and biofilm formation. The SEM image results showed the bacterial adhesion on the hydrogel polymer coating of lens sample. In addition to that, it revealed the presence of rod and cocci shaped bacterium on higher magnification when comparing with the control unused Contact Lens sample (Figure 5).

Severe corneal problems are caused through bacteria. The polymer material used for construction of Contact Lenses cause bacterial adhesion and hence, the Contact Lenses made from non-ionic polymers with low water content may carry higher risks of bacterial contamination. Analysis of the bacterial trends of keratitis reveals that *Pseudomonas* spp. is the second most important cause of bacterial keratitis in India after gram positive bacteria. The present study also revealed the presence of *Pseudomonas sp.* which coincides with the earlier reports.

In vitro studies on antibacterial susceptibility tests reported by various researchers had shown an increased resistance of bacteria to commonly used antimicrobials due to the increased resistance developed by the isolates. Fluoroquinolones were used as an effective monotherapy for many patients with microbial keratitis, as they provide a good coverage for most of the gram positive and gram negative bacteria. However, reports on the emergence of resistance to fluoroquinolones have recently been published with special emphasis to *Pseudomonas spp.*

The eye drops selected for determining the antimicrobial activity were Chloramphenicol, Gentamycin, Ofloxacin, Ciproflaxacin. Based on the results obtained, the isolated bacterial samples showed an effective antagonistic mechanism even at lower concentration of test samples. Though it revealed antimicrobial potential against all the test samples, the isolates expressed sensi-
tivity results against Ciproflaxacin rather than with other drugs like Chloramphenicol, Gentamycin, Ofloxacin. Next to Ciproflaxacin, all the isolates exhibit effective inhibition zone against Ofloxacin.

Owing to the results obtained for the determination of bacteriostatic and bactericidal activity, Ciproflaxacin exhibited bactericidal activity against all the target organisms as shown in Figure 4. But the other drugs though showed antagonistic activity, they were bacteriostatic in nature against the test organisms (Table 3).

Based on the earlier results, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using Ciproflaxacin, against all the test isolates (Figure 4).

The Antimicrobial sensitive/resistant pattern of isolated bacteria in the current study from Contact Lens samples was carried out using five different commercial discs Streptomycin, Tetracycline, Penicillin, Gentamycin and Erythromycin. Based on the results, *Pseudomonas aeruginosa* and *Staphylococcus sp.* showed resistance comparing to multiple drugs used in the assay procedure. (Plate 4).

The determination of antimicrobial activity against the isolated bacterium was done using commercially available eye drops using well diffusion assay. Reported that ciprofloxacin is the best choice for treating the microbial keratitis primarily. Our studies revealed the same data on sensitive pattern with commercial eye drops.

### 4. Conclusion

From the results, it was found that all the yearly disposable lens samples contain more amount of colony forming units than other two samples. Next comes with the sample of monthly disposable lens and there is a very lower colony forming units in the daily disposable lens. Thus the objectives of the research, in isolating the bacteria in three types of lenses were clearly classified and measures were taken through various antibiotics and by

![Figure 4. Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration.](image)

![Plate 4. Sensitive and resistant pattern of bacterial isolates against standard antibiotic discs.](image)

### Table 3. Determination of bacteriostatic and bactericidal property using eye drops

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Ciproflaxacin</th>
<th>Gentamycin</th>
<th>Ofloxacin</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteriostatic</td>
<td>Bactericidal</td>
<td>Bacteriostatic</td>
<td>Bactericidal</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Micrococcus sp.</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Serratia sp.</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus sp.</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

‘-ve’ indicates no growth; ‘+ve’ indicates presence of growth
antimicrobial activity against the isolated bacterium. In summary, to reduce microbial keratitis outbreaks associated with Contact Lens wearers, we suggest the lens coated with substances with antimicrobial potential should be impregnated with hydrogel polymer. It is anticipated that the present study will provide information and also be helpful in the treatment of bacterial keratitis.

5. References


