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MICROBIAL CONTAMINATION OF CONTACT LENS CARE SYSTEM IN NEPALESE POPULATION

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Running Title: Microbial Contamination of Contact Lens..

ABSTRACT

Background: The number of contact lens users and subsequent complications have been growing steadily in Nepal but the causes have not yet been studied. So, this study was conducted to find out the bacterial, fungal and acanthamoebal contamination present in the contact lens care system which is a major factor for serious ocular infections.

Methodology: A total of 50 asymptomatic patients who used soft & Rigid Gas Permeable lenses for the correction of refractive errors were only included. The swabs of contact lens cases, conjunctivas and the lens care solutions were taken and cultured in Blood agar, Chocolate agar for bacteria, Sabourad's Dextrose agar for the fungi and Non-nutrient agar for the *Acanthamoeba* spp. The isolates were identified using standard microbiological protocols.

Results: Among the 50 contact lens wearers, 30 (60%) showed contamination in one or more components of the care system and conjunctiva. 37 (37%)of the 50 lens cases (100 wells) examined and 20 (20%) of the total 100 conjunctival swabs taken from 100 eyes of 50 patients, were contaminated. Similarly, 2 (4%) of the total 50 commercial multipurpose solutions used were contaminated. The most common microbial contaminant in the lens case was *Staphylococcus aureus* (28%) ($p>0.05$) and conjunctiva too was *Staphylococcus aureus* (12%) ($p>0.05$). One each (2%) of *P. Aeruginosa, S. aureus & E. coli* ($p>0.05$) was found in commercial multipurpose solution. Other contaminants in the lens cases were Coagulase negative staphylococcus *(S. epidermidis*), *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa & Streptococcus pneumoniae* and that in conjunctivae was Coagulase negative staphylococcus. None of the patients was found to be contaminated with *Acanthamoeba.*

Conclusions: High degree of contamination found in our study demonstrates the poor maintenance of contact lens care system in Nepal. So, proper instructions and reinforcement of contact lens care system hygiene is very essential.

Key words: bacteria; contact lens; fungi; lens case; microbial contamination.

INTRODUCTION

Contact Lens(CL) is a thin plastic wafer designed to fit or rest on the cornea or sclera, ordinarily used to correct refractive errors. It is made up of different materials like Poly-Methyl Methacrylate (PMMA), Hydroxy-ethyl Methacrylate (HEMA), Silicone acrylate, Fluoro-silicone Acrylate etc.

Contact Lenses can be used for different purposes like optical, therapeutic, prosthesis & cosmesis.

The Contact Lens first originated in Europe & the introduction of Soft Contact Lens into general clinical use was brought in the 1960s. In the past decade, the number of CL wearers have grown steadily[1].The FDA has estimated that 97% of CL wearers use lenses for cosmetic reasons.[2]

CL practice in Nepal is significantly different from that of other countries & is somewhat sophisticated because it has recently started & also because of the unavailability of various lens types & lack of research.

As newer technology and materials are developed, more patients are seeking good services from the practitioners despite the increase in the contact lens related ocular complications with these facilities.

Bacteria have been associated with adverse corneal events such as microbial keratitis[3], CLARE (Contact Lens Associated Red Eye)[4,5,6] & Contact Lens Associated Peripheral ulcers(CLPU)[7,8,9].Gram negative bacteria, particularly Pseudomonas aeruginosa & Haemophilus influenzae are associated with microbial keratitis [3]& CLARE [4,5] respectively & gram positive bacteria such as

Staph. Aureus & Streptococcus Pneumonia are associated with CLPU.[8,10] During asymptomatic lens wear, the eye & lens are colonized by low numbers of predominantly gram positive bacteria such as coagulase negative *Staphylococcus spp., Propionibacterium spp*. & *Corynebacterium spp*.[11,12,13,14] These bacterial types form the normal ocular microbiota & are rarely associated with disease.[15] a variety of gram negative bacillus & anaerobic organisms [16,17] have been cultured from the conjunctival sac although in less frequency than the normal conjunctival flora.

The corneal ulcer is the most serious frequent complication of wearing contact lens. About 25 to 30 % of corneal ulcers are associated with CL wear. [18,19,20,21] Most of the studies indicate a higher incidence of infectious keratitis in patients using extended wear lenses than in those wearing daily wear lenses.[22,23] Factors that may be important in the development of corneal ulcer(microbial keratitis) include the type of lens material, wearing schedule, adherence of microorganisms to the lens & microbial contamination of the care system.

Contact lens care system denotes the system for care of contact lenses, which requires a combination of cleaning, rinsing, disinfecting, soaking, wetting & storing and its goal is to prevent the undesirable complications[24] including contaminating organisms.

Contact lens care system should maintain all types of contact lenses in a perfectly clean & sterile condition for optimal physical, physiological & optical performances. It should simple, easy to use, economical and safe for eye.

[25]Contact lens care system comprises lens storage cases, lens solutions & lens disinfecting processes.

The contact lens solutions include wetting, cleaning, soaking solutions; enzyme cleaners, saline solution, disinfecting solution, and others. Multipurpose solution, the most widely used solutions nowadays are an all inclusive type of solution.

The most widely used methods of contact lens disinfection are:

- Heat disinfection
- Chemical disinfection

Prevention of contact lens infection is difficult to determine without knowing the source of contamination of lens and its care system. [24] The sources of the contamination in contact lenses and its care system include:

i. Poor patient hygiene
ii. Contaminated contact lens solution
iii. Infected water used to clean the lenses
iv. From the normal flora of the skin or infected skin of eyelid
v. Infection from the lacrimal sac
vi. From the surrounding air.
vii. From the oil, cosmetics used
viii. From the storage case
ix. Infected water while swimming or taking bath
x. Aging of the lens making sterilization more difficult
xi. Failure to reach sterilization temperatures

xii. Trial lenses and the solutions used by clinicians.

Microbial contamination of the care system may represent the source of the infecting organisms. Studies of patients with CL associated corneal ulcers have demonstrated contamination of the care systems.[26,27,28,29,30]

In addition to causing ocular infection, microbial contamination of the care system may lead to the production of toxins that affect the eye. *Pseudomonas aeruginosa* & its endotoxin have been implicated in the development of corneal ring infiltrates in Soft Contact Lens wearers.[31,32]

Despite low rate of contact lens induced complications they are an important health concern because a very large population is at risk as the population of CL wearers is growing steadily in Nepal. Therefore, this cross-sectional study was carried out to determine the type and source of contamination in the contact lens care system of different contact lens wearers in Nepal. Comparison of the contamination with duration of contact lens wear and finding out its gender-wise distribution were also our other objectives.

MATERIALS & METHODS

Study design

This was a cross-sectional study of contamination in contact lens care system which included lens cases & solutions. Conjunctival contamination were also seen for further comparisons and analysis.

Inclusion criteria

All the patients who had worn CL for more than 3 months for correction of refractive errors only and attended the contact lens clinic of BPKoirala Lions Center for Ophthalmic Studies (BPKLCOS), Institute of Medicine, Tribhuvan University. Through the period of 1st July 2005 to 30th June 2006 and patients who were able to give informed verbal consent or whose guardian would consent to participate in the study. They should also have the contact lens storage cases and the solutions they were using at the moment with them. We chose 3 months as a cut off point as the contact lens wearer would be a good user by that time and will have established a definite regimen of contact lens wear and hygiene.

Exclusion criteria

Patients who had worn CL for less than 3 months and/or showed ocular symptoms & evidence of any anterior segment pathology of infective nature were excuded from the study.

Collection of Data and Specimens

Interview

At the time of examination, participants who enrolled in the study were questioned about Type of lens wear, Period of lens wear, Wearing schedule, Type of disinfecting system, Methods of handling & caring lenses, cases & solution etc. All the answers were recorded in the proforma maintained for the study. Any patient reporting poor compliance with lens care regimen was warned of the risks of poor lens care technique and was instructed about correct procedures. Any patient whose cultures had yielded ocular pathogens was also warned about contamination at their next visit, and the need for compliance with good contact lens care routines emphasized.

Collection of specimens

Specimens were collected by means of conjunctival swabs, swabs from CL cases/containers & a few drops of Care solutions.

The Conjunctival swabs for potential pathogens were taken from the inferior cul-de-sac by using a sterile cotton swab moistened with sterile saline. The swabs were taken from both eyes separately & kept on separate tubes.

Contact lens cases/container were opened without touching the interior of the case and a sterile cotton swab/a sterile nichrome wire loop was used to take the solution from inside the case. If no solution was present inside the case, a sterile cotton swab premoistened with sterile saline was used. Separate cotton swabs for right & left containers of contact lens cases were used. The bottle of care

solution was shaken vigorously and a few drops of solution were collected in sterile clean bottles.[34]

A complete proforma was maintained for recording the data & results.

Culturing of specimens

All the specimens were processed immediately by inoculating them into different culture media. A separate was swab was used for each culture medium.

The bacterial cultures were performed on Blood agar & chocolate agar. Subcultures of the some specimens were done on special media for futher identification. The Swab from right conjunctiva was inoculated in horizontal streaks & left conjunctival swab in vertical streaks, each on one half of the same agar plate. Similarly, swabs from container of both right and left side of CL cases were also streaked in the similar pattern in agar plates.

A loopful of CL care solution was inoculated on the medium & streaks were marked over the medium. The agar plate was then marked as R& L to mark the respective sites. The agar plates were then incubated at 37 ° Celsius for 24 hrs. Bacterial growth was observed following overnight incubation & colonies were detected.[33]

The fungal cultures were performed on Sabouraud's dextrose agar media. The swabs were inoculated in the similar pattern as in bacterial culture. The fungal cultures were then incubated at 25 deg Celsius. If no growth was detected within 3 weeks, it was considered to be negative. [33]

The acanthamoebal culture was done by firstly overlaying the Non-nutrient agar with autoclaved (killed) E. coli suspension, which was spread evenly on all agar surfaces and was left for drying for some minutes. The swabs were inoculated in a similar pattern as in bacterial culture. The plates were then incubated with lid facing downwards at 37 degree Centrigrade by sealing them with tape. The plates were examined daily using a light microscope or other ordinary microscope at 10x or 40x and recorded as negative if no trophozoites or cysts were apparent by 3 weeks.[33]

Identification of the bacterial contaminants:

Sub-cultures were performed in different media when required. The positive bacterial cultures obtained after overnight incubation at 37 °C were identified by Gram's staining & biochemical tests following standard techniques.

Data Management and analysis:

All the collected data were filled up in a specifically developed research proforma and the association of specific microorganism as a contaminant to others were determined using Chi-square ($\chi 2$) test with the help of computer data analysis software (SPSS 11.0). Significance was set at $p<0.05$ for all tests.

Due to the lack of any Ethics Committee at the institution where this study was conducted at that period, we were not able to take any ethical approval but care

was taken to ensure all the involved human subjects were treated best possible ethically.

RESULTS

During the study period, thirty-five females and fifteen males were recruited.their samples were prospectively analysed for the determination of contamination.More than one microbe was isolated from few subjects.

The mean age of patients was 21.68 ± 3.53 yrs (range 14 to 28). The maximum numbers of patients were in the age group >20 – 25 yrs.

Out of total 50 patients, 46(92%) were wearing conventional daily wear soft contact lenses. All the soft lenses were made up of poly-hydroxyethylmethacrylate (PHEMA), with a hydration of 45%. Four of them (8%) were in Rigid Gas Permeable(RGP) lenses made up of silicon acrylate.

The range for the duration of contact lens use by the patients was from 3 months to 3 yrs. Most of the patients had used their lenses for 12 to 24 months (i.e.44%) while 6% of the people had used the lenses for >2yrs.

Insert Fig. 1 here.

Majority of SCL wearers were found with 8-10 hrs per day use, while RGP wearers 12-15 hrs per day.

None of the patients were found with the history of sleep on contact lens. However, 22% of the patients gave the history of swimming/bathing while putting lens.

All of the lens users used commercial MPS for cleaning and disinfecting of their lenses. Among the users, 56% used MPS containing Polyhexamethylene

Biguanide (PHMB), 24% used MPS containing Ethylenediaminetetraacetic acid (EDTA), 8% used MPS containing Disodium edetate and Polyaminopropyl biguanide, 2% used MPS containing Polyquad & Aldox, while 2% used MPS containing other preservatives. Similarly, there were also 8% of RGP users on MPS containing Sorbic acid and Disodium edetate. Most of the patients had used their solution for 1-2 months (32%) while 20 % had used their solution for >3 months. None of them were found using intensive cleaners. Few patients also used Hydrogen peroxide based disinfection system in addition to routine MPS disinfection system.

Insert Fig. 2 here

All the patients were found using CL cases made up of polystyrene. Majority of the patients (36%) had used their CL cases for 1-2 yrs while 10% had used their cases for >2 yrs. 24 % of the patients cleaned their CL cases once a month while 22% cleaned once a week. 52% of the patients used plain tap water for cleaning their cases, while 24% used soap and water & 24% boiled their lenses for about 5-10 minutes for cleaning & disinfection of their cases.

Among the total wearers, 30(60%) had microbial contamination while 20(40%) were found free of contamination.

Among the soft contact lens wearers, the containers were predominantly contaminated accounting 39.13% where as in RGP lens wearers, the conjunctiva was found to be contaminated more accounting 37.5%.

In Overall, the major contamination was in CL containers 37% followed by conjunctiva 18%.

Table 1: Pattern of contamination in different types of lens wearers

Sites of contamination	SCL	RGP	Total
	No. of contamination/ No. tested	No of contamination/ No. tested	No of contamination/ No. tested
Case(wells)	36/92(39.13%)	1/8(12.5%)	37/100 (37%))
Conjunctiva	15/92(16.30%)	3/8(37.5%)	18/100 (18%)
Solution	2/46(4.34%)	0/4	2/50 (4%)

Staphylococcus aureus was the most common microbial contaminant found in 43 wearers (64.18%) followed by *Staphylococcus epidermidis* (14.93%). *Streptococcus pneumoniae* (Pneumococcus) was the least observed contaminant 1.5%.

Insert Fig 3 here.

Out of 100 cases, 37 (37%) were contaminated with bacterial contaminants. More than one microbe was isolated from the comtaminated lens cases. The most

common bacterial contaminant in the contact lens case was *Staphylococcus aureus* (28%) followed by *S. epidermidis* (4%), *E. coli* (6%) and *P. aeruginosa* (3%). Statistically no significant difference was found (p>0.05). the longer a person used a contact lens case, higher was the contamination seen. (fig. 5)

Insert Fig 4 here

Among wearers, 18% were contaminated with bacterial contaminants. More than one microbe was isolated from the contaminated conjunctiva. The most common bacterial contaminant in the conjunctiva was *S. aureus* (12%) followed by *S. epidermidis* (6%). Statistically no significant difference was found (p>0.05)

Out of 50 commercial MPS in use, 3 (6%) were contaminated with bacterial contaminants. More than one microbe (*P. aeruginosa, S. aureus, E.coli*) was isolated from the contaminated solutions. All of them were found in single number (2%).Statistically no significant difference was found (p>0.05).

Insert Fig. 5 here.

Although the female wearers (70%) were predominant, the contamination rate (60%) was similar in both the gender.

Insert Fig 6 here

DISCUSSION

Among the fifty samples taken from the contact lens wearers, all were subjected for microbiological examinations via a safe mode so as to prevent further contamination. The result revealed a high frequency of microbial contamination (60%) in the CL care system. A total of 37% containers were contaminated. Containers of soft contact lens wearers were contaminated more (39.13%) than that of RGP wearers (12.5%).

Most people had used their lenses for the period of 1-2 yrs (44%). Patient using CL for >2 yr had high contamination. Majority of SCL wearers used their lenses for around 8-10 hrs per day, while RGP wearers used their lenses for 12-15 hrs per day. This has also helped to lessen the severity of contamination among the wearers. A few patients who used soft contact lenses for more than 10 hrs per day showed higher contamination.

Several studies [6,9,13,15,28] also reveal the risk of contamination on prolong wear of lenses. Prolong wear of CL poses a metabolic stress to the normal eye including hypoxic edema, microscopic epithelial breaks, and increased accumulation of microorganisms leading to more contamination & finally causing corneal infections. However, none of the patients had used lenses while sleeping or short naps.

Most common contaminating bacteria in the containers were *Staphyloccus aureus* (28%); *Staphylococcus epidermidis* (4%). However, in a similar study conducted by Donzis P. B. et al.[33] showed relatively high contamination rate of *S.*

epidermidis (22%) in containers. As *S. epidermidis* is a part of the normal skin flora, it is more likely to present in containers while handling the lens. But in our study, *S. aureus* was found more because conjunctival flora was dominated by this organism in our wearers which might be due to their unhygienic practices. Donzis & others suggest that contamination of the care system may influence the conjunctival flora at least on a short term basis but have found no long term shifts in the conjunctival flora.

The presence of virulent pathogen like *Pseudomonas aeruginosa* (a Gram negative rod) was found in 3% containers. Its presence was less than quoted in the study by Donzis et al. [33](23.7 % in lens cases) & Velasco & Bermudez [34] (7.94 %). According to several studies Pseudomonas are noted to account for 2/3 to 3/4th of CL associated corneal ulcers. It is not significantly observed in our cases as they followed the appropriate care system. The reason for infection among others could be due to use of plain tap water to clean the containers.

Other Gram negative rods isolated from lens cases were *E. Coli* (6%) & *Kleibsella* (2%). They are normal human gastrointestinal tract flora. The source of contamination may be the dirty hands of lens wearers as well as tap water used by these patients to clean their containers.

All the organisms isolated from the CL cases were equally responsible for contamination of CL case as the contamination was not statistically significant ($p>0.05$) for any one organism.No fungal contamination was found in CL cases.

No acanthamoeba contamination was found in CL cases. It could be related to less numbers involved in swim and bath using contact lens.

Among the users, 4% of contamination was found in MPS used by the wearers. This doesn't tally with Donzis P.B.et al [33] who had found 13 % contamination in their commercial care solutions and Velasco et al [34] study who found 63.09% contamination. Contamination in one of the MPS was by *Pseudomonas aeruginosa* & the other by *S. aureus* & *E. coli* which could be as a result of prolong use (6 months). No fungal & acanthamoebal contamination was found in MPS.

Potential pathogens were isolated from the 18 conjunctivas of 12 patients (18%), however, it was found less in the Donzis et al (9%) study which has not included *S. epidermidis*. When *S. epidermidis* is excluded from our study, it still accounts for more contamination (12%) than that of Donzis et al study. In our study, *S. aureus* (12) & *S. epidermidis* (6) were found from total 18 contaminated conjunctivas. *S. aureus* was found more which might be due to carrying of bacteria from contaminated CL cases while lenses are handled. Both the contaminants were equally responsible for the contamination of conjunctiva of CL wearers ($p>0.05$). No fungal & acanthamoebal contamination was found in conjunctiva.

Female wearers predominated over males by two times; however, contamination rate was same (60 %) for both. This study showed multifactor causes for the

large contaminations in contact lens wearers. The different confounding factors could be:

- unhygienic condition of wearers (dirty hand, finger nails)
- improper cleaning & disinfection of CL cases (52% using plain tap water for cleaning their cases)
- inadequate cleaning & disinfection of CL cases (24 % of the patients cleaning their CL cases once a month only)
- Longer duration of use of same CL storage cases (36% using their CL cases for 1-2 yrs while 10% using their cases for >2 yrs.)
- reuse of same solution of cases for storing CL for many days instead of changing daily
- Use of cosmetics like eyeliners, mascara, kajal etc. by female patients.
- Use of lenses while bathing/swimming, working in dirty environment also contribute as an additional factors, which could aid the auto- inoculation of bacteria on to the CL & its care system.

Some of the sample collected showed no microbial growth, which might be because some of them had just undergone antibiotic therapy before testing, some had disinfected their lenses & containers before taking samples while others had taken good care of their CL & its care system.

CONCLUSION

A large number (60%) of patients showed overall microbial contamination of their CL care system and conjunctiva.The commonest contamination rate was observed in containers 37% followed by conjunctiva 18%.Bacteria were the only microbial contaminants observed. Fungi or Acanthamoeba were not found. *S. aureus* was the most commonly isolated bacterial contaminant in containers (28%) and conjunctiva (12%). Microorganisms isolated in CL containers was highest followed by conjunctiva and care solutions. Both genders showed equal contamination rates (60%).High risk of contamination was observed as the same pair of lens and its care products were used for longer than prescribed duration. These high rates revealed the likelihood of bacterial infections including corneal ulcers.

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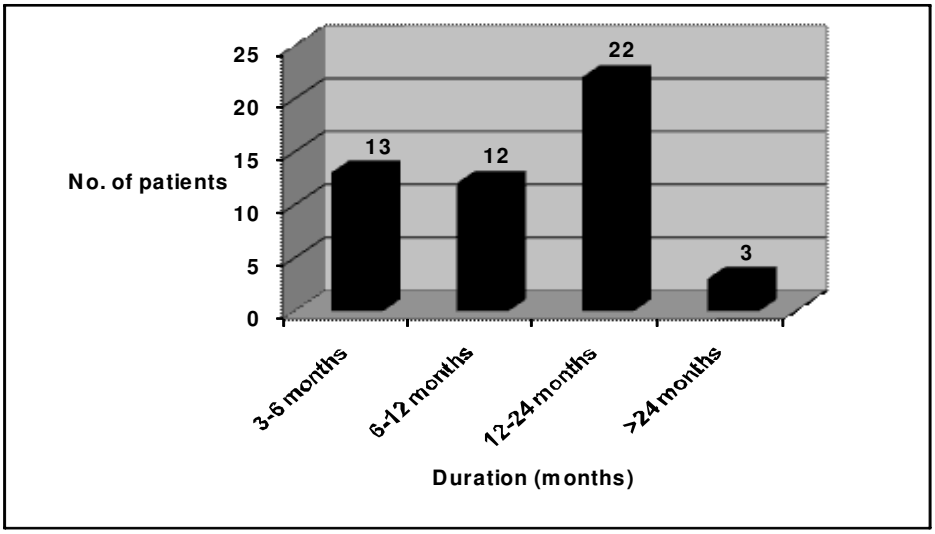
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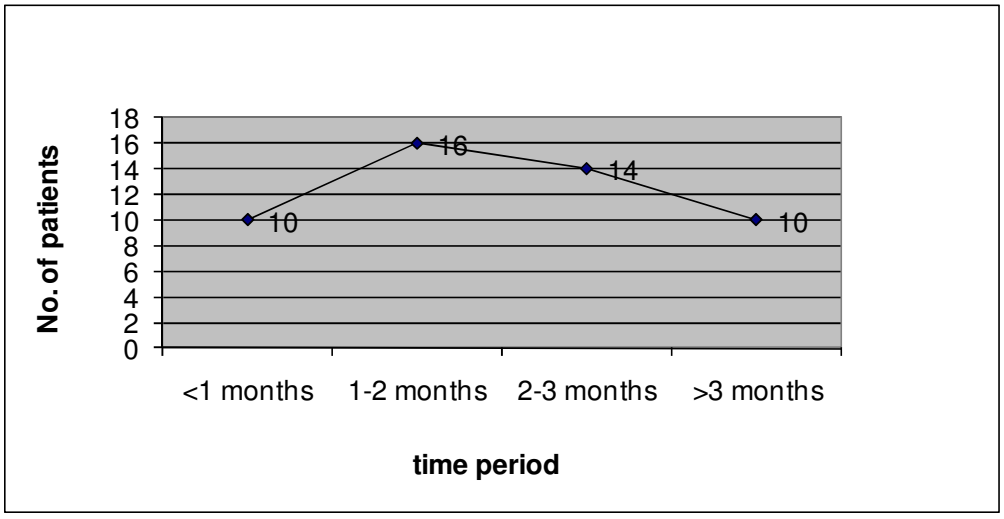
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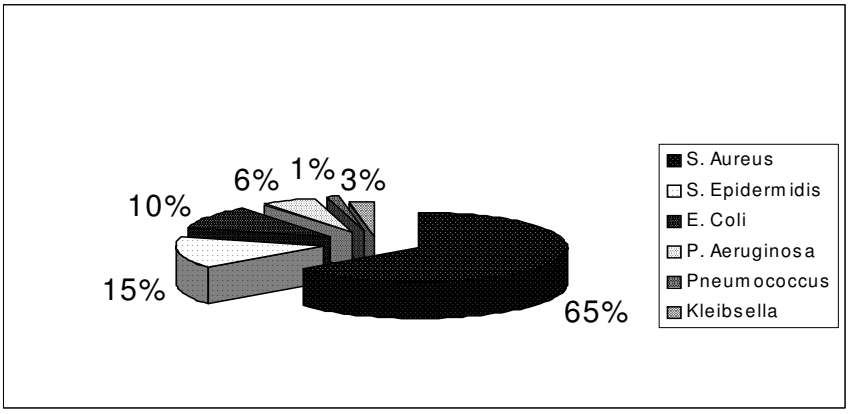
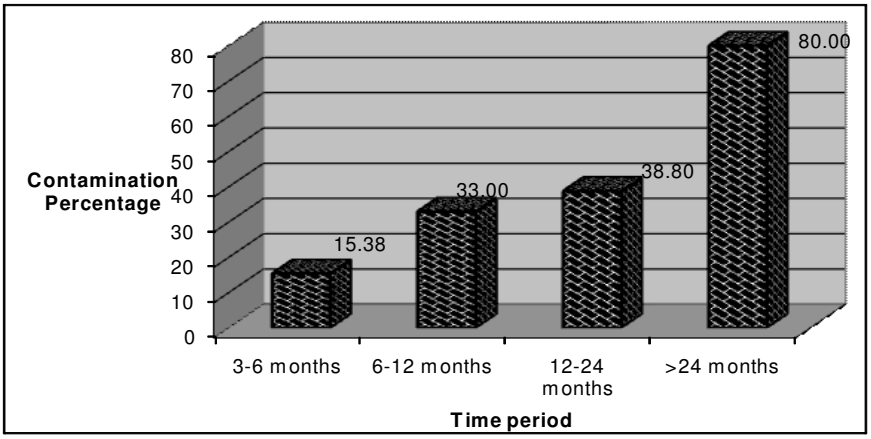
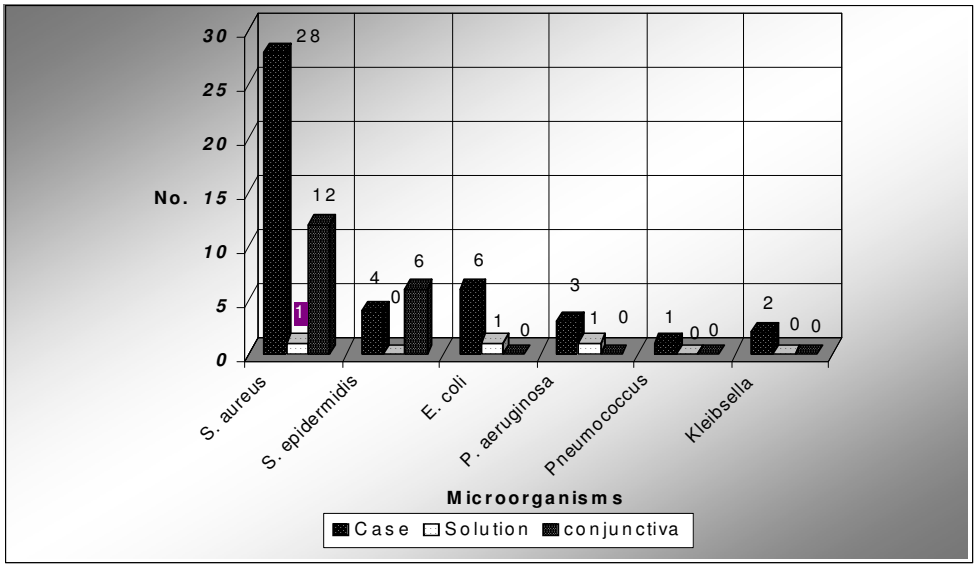
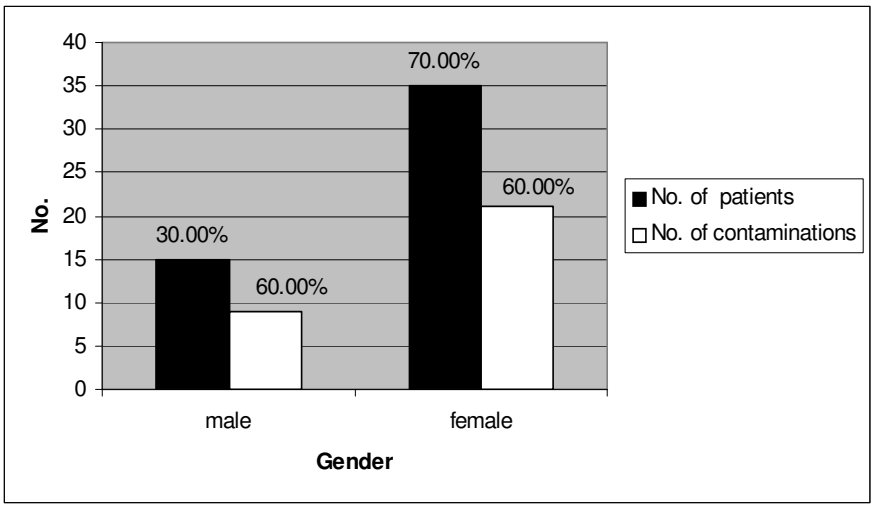
>3 months
time period


6%
1%
3%
10%
15%
65%
S. Aureus
S. Epidermidis
E. Coli
P. Aeruginosa
Pneumococcus
Kleibsella


Contamination Percentage
80
70
60
50
40
30
20
10
0
15.38
33.00
38.80
80.00
3-6 months
6-12 months
12-24 months
>24 months
Time period


30
25
20
No. 15
10
5
0
28
1
12
4
0
6
6
1
0
3
1
0
1
0
0
2
0
0
S. aureus
S. epidermidis
E. coli
P. aeruginosa
Pneumococcus
Kleibsella
Microorganisms
Case
Solution
conjunctiva


40
35
30
25
20
15
10
5
0
No.
70.00%
60.00%
30.00%
60.00%
male
female
Gender
No. of patients
No. of contaminations