Investigation of Microbial Contamination of In-Use Contact Lens Solutions Correlated to Habits and Practices

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Dedication

الحمد لله الذي أتَمّ عليّ هذا.

أهدي حبّي إلى **عمّان،** المدينة السّاحرة التي شَهِدت على نجاحاتي وعثراتي، و منحتني دروساً مُختلفةً كفُصولها الأربعة.

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List of Abbreviations

BK	Bacterfial Keratitis
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical & Laboratory Standards Institute
CoNS	Coagulase Negative Staphylococci
CoPS	Coagulase Positive Staphylococci
DNase	Deoxyribonuclease
FDA	Food and Drug Administration
Gm+ve	Gram Positive
Gm-ve	Gram Negative
IVC	Insight Vision Centre
LCLC	Left Contact Lens Case
M.O	Microorganism
MAC	MacConkey's Agar
McF	McFarland
MHA	Mueller-Hinton Agar
MK	Microbial Keratitis
MRCoNS	Methicillin Resistance Coagulase Negative
MRSA	Methicillin Resistance *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin Sensitive *Staphylococcus aureus*
NA	Nutrient Agar
OD	Optical Density
PAPB	Polyaminopropyl Biguanide
PHMB	Polyhexamethylene Biguanide
RCLC	Right Contact Lens Case
SB	Solution Bottle
SD	Standard Deviations
SDA	Sabouraud Dextrose Agar
TSA	Trypticase Soy Agar
TSB	Trypticase Soy Broth
U.S.	United States

Appendix

Investigation of Microbial Contamination of In-Use Contact Lens Solutions Correlated to Habits and Practices

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Abstract

Contact lenses (CLs) are medical or cosmetic device, comfortable and more convenient alternative to eyeglasses. When microbes are inoculated onto CLs will result in eye infection because defense against microbial invasion in the anterior chamber of the eyes is weak due to the very poor blood supply. The present study, investigates microbial contamination of CL cases, solutions and rims of solution bottles and assess habits and practices of thirty CL wearers toward lens care to understand factors associated with CL units contamination through a structured questionnaire. Characteristics of grown colonies were determined through morphological and biochemical testing. Five (16.6%) CL units were found not contaminated. Eye redness after CLs wearing was almost statistically significant sign associated with CL units contamination (p=0.088). Sixty four isolates were obtained; the most prevalent bacteria were *Pseudomonas spp.*(25%), *Staphylococcus spp*. (21.9%), *Serratia spp.* (14.1%), and *Acinetobacter spp*. (6.2%). Two types of CL solutions (A and B) exceeded required level of bacterial reduction (3 log) and reached up to 5 log reduction. With regard to biofilms formation, after 4hr,

tested solutions were able to reduce biofilm by more than 50% of all tested bacterial biofilms, regardless of the cleanness/dirtiness status. Using water to wash hands and CL cases has been incriminated for increasing contamination. Impurities in CL cases lead to reduced effectiveness of lens solutions. Value-added awareness of CL wearer should be improved by regular visit to eye care professional.

1. Introduction

Contact lenses (CLs) are cosmetic or medical devices regulated by the United States (U.S.) Food and Drug Administration (FDA), as a convenient and comfortable alternative to eyeglasses for many people (Bailey, 1987). Soft contact lenses were first introduced to the U.S. in 1971 (Epstein, 2007). Currently, ninety percent of contact lens wearers use soft contact lenses (Collier et al., 2014, Cope et al., 2017). Nowadays, CLs are one of the most biomedical appliances. Centers for Disease Control (CDC, 2020), has estimated that 45 million people in the U.S. wear contact lenses, two third of them are female with the average age worldwide of 31 years old. Nevertheless, worldwide estimates of contact lens wear are more difficult to score, but it is estimated to be in excess of 150 million wearers (Moreddu et al., 2019).

Wearing lenses may cause changes in the cornea which in turn create problems and exacerbate pre-existing conditions. Problems are related to the type of lenses used, the frequency with which the lenses are changed, the cleaning systems of lenses, and wearer-related factors (Bourne, 2001).

The most common complications caused due to long term wearing CLs include: minor problems such as discomfort, eyes may become dry and irritable, burning eyes when putting in lenses, allergy and physiological problems (Weissman, 2006). These problems might be associated with the lens itself such as: poor fit, poor care, lens damage and lens dryness. Fortunately, most problems associated with contact lenses are not serious and will resolve if the lens is removed for a period of time (Suchecki et al., 2003).

Major problems, although less common, but they are more dangerous for vision which include: Conjunctiva problems (Siddique et al., 2007) particularly, allergic

conjunctivitis (Arita et al., 2012) and corneal problem mainly, Microbial Keratitis (MK) which is the most severe and the most common complication of CLs use and may result in impaired vision (Lemp, 2003). This problem may occur due to dry eyes. The eyes may become worsen by different factors such as smoking, dust, air-conditioned rooms, and medication. Besides, wearing CL while sleeping have led to increased prevalence and severity of all complications especially the risk of MK (Weissman, 2006).

Microbial keratitis may occur as a result of adhesion of microbial cells onto CL surface (Willcox and Holden, 2001). These microorganisms (M.O) include mainly, bacteria (Houang et al., 2001) which is responsible for 90% of all keratitis cases (Eltis, 2011); fungi (Yildiz, Abdalla et al. 2010) and protozoa principally *Acanthamoeba* (Anger and Lally, 2008). If MK is not diagnosed and treated at once, vision loss and blindness may result. Centers for Disease Control in U.S estimates that MK influences 5 to 10 out of every 10,000 CL wearers (Eltis, 2011); and accounts for 1 million clinic visits annually (Collier et al., 2014). One out of 2,500 of CL wearers are susceptible to MK yearly with daily use of CLs (Weissman, 2006).

Hygiene of CLs and their cases is necessary for keeping safe CLs wear, they should be kept clean with a correct care; otherwise eye infections could result. Despite using disinfecting agent, CL cases are the most allegeable CLs item to be contaminated. Thereby, leading to a continuous bacterial survival (McLaughlin-Borlace et al., 1998). Microbes may approach lenses from the wearer's fingers and eyelid edges while inserting lenses; from CL cases, which in turn introduced to disinfectant solution resulting in decreased preservative efficacy of CL solutions. Thereby, this solution will act as a good substrate for these microbes (Fleiszig and Evans, 2010). Adhesion of M.O to CL will introduce them to the cornea; leading to the development of MK or

noninfectious keratitis as infiltrative keratitis (Suchecki et al., 2003); Contact lens peripheral ulcer (Silbert, 2007).

Therefore, CLs act as a vector for commensal (Resident) and transient potential microorganisms to adhere to and transfer to the ocular surface resulting in inflammation or infection (Devonshire et al., 1993, Wu et al., 2010, Yung et al., 2007; Dantam, et al 2016).

Several studies were carried out on CL cases, solutions and lenses to identify contaminating M.O which was traceable to users' dirty hands, or the tap water used to rinse the lens storage cases, and/or air contamination during drying of the cases. Coagulase positive *Staphylococci* (CoPS), coagulase negative staphylococci (CoNs), *Pseudomonas aeruginosa , Streptococcus spp., Escherichia coli and Klebsiella spp* and *Serratia spp*. were the most common species identified (Yung et al., 2007, Rahim et al., 2008, Emina and Idu, 2011, Wu et al., 2011, Mohamed et al., 2017). *Staphylococcus* is an aerobic Gram-positive (Gm+ve) commensal bacterium carried by 50–60% of normal population on the hands, face, nose, and skin as, and can readily find access to the eye. Therefore, *Staphylococcal* ocular infection is almost certainly due to hand-to-eye transfer (Jalbert et al., 2000). *Pseudomonas* is opportunistic pathogen whose nutritional requirement is very low and is commonly found in many environments, including water and is able to survive in dilute solutions of disinfectants (Willcox, 2007). *P. aeruginosa* keratitis associated with contact lens wear and is difficult to treat because it can display multiple resistant to antibiotics (Chalita et al., 2004). Significance of methicillin resistant CoPS or CoNS in ocular infections was claimed in many studies (Al-Hammadi et al., 2006, Melton et al., 2010).

Moreover, Biofilm formation is another complication as Wu et al. (2011) proved that biofilms were formed by *Staphylococcus aureus* and *P. aeruginosa* on two unused CL cases.

A study by Nzeako and Al-Sumri (2011) indicated that contact lens disinfecting solutions with the same formulations, but manufactured by different companies, possessed different disinfecting potentials. Lakkis and Fleiszig (2001) indicated that disinfection solutions seemed to be selective for contamination with cytotoxic strains of *P. aeruginosa*. Nevertheless, Dantam et al. (2014) study demonstrated that microbial contamination of storage cases varies with the use of different formulations of CL care solutions.

Aims of the study:

This study aims to investigate the microbial contamination of CL cases, solutions, and rims of the bottles and correlate those findings to the awareness of CL wearers in handling their CLs that will be achieved by:

1. Investigate through questionnaire, habits and practices in handling CLs.
2. Isolate and identify microorganisms contaminating contact lenses, cases and bottles mouth rims of disinfecting solutions used by CL wearers.
3. Identify recovered bacteria to species level by studying their cultural, morphological and biochemical characteristics.
4. Determine antibiotic susceptibility of most prevalent species
5. Evaluate the disinfecting potential of two types lens solutions available in Jordan.
6. Assessing biofilm formation and reduction experimentally

2. Literature review:

2.1. Contact Lenses

Contact Lenses are thin, light weight, almost invisible discs that serve as an excellent option for people who need vision correction and give the same corrective purpose as glasses. They are designed as bowl shaped simulating the cornea structure. Lenses rest on the cornea or the sclera or both and appearing as an anterior of eye (Mandell, 1965). There are two types of CLs in terms of components: (1) Soft CLs are of made of hydrogel or silicone which allows oxygen crossing through CLs to the cornea, and (2) Hard CLs are made of polymethylmethacrylate, that is movable material allow oxygen within tears to flow under CLs (Dart, 1997 and Park et al., 2018). For more than a century, CLs have been used for refractive errors correction; beautifying and they have really achieved clinical spread in the last decades. Surface tension of tear film assists contact lenses to stay on the cornea (Weissman, 2006, Talu et al., 2011).

Different types of contact lenses have been developed through advances in technology. Basically, three wearing types were industrialized, these are: daily disposable wear, daily wear and extended wear. Disposable wear should be used once and thrown away daily after wearing. The daily wear is used through insertion and removal for a specific several days. Extended wear is the continuous wear overnight; this type is associated with corneal infection or inflammation (Keay et al., 2007, Stapleton et al., 2008).

Stapleton et al. (2017) conducted a study to verify risk factors causing keratitis in daily disposable CL wearers. Contact lens wearers through completing a questionnaire describing wear history and hygiene. Results show that although daily CL wear is related with a less risk of MK compared to extended CL wear, increased

exposure in daily wear, poor hand hygiene, poor CL practice care and smoking were also risk factors for MK.

Hazards and threats of wearing contact lenses have been described by Insight Vision Center (IVC, 2017) who has listed eight risks and side effects that may face CLs of wearers, these include:

1. Prevent of oxygen reach to cornea
2. Dry eyes
3. Irritation when combined with medication.
4. Diminished corneal reflex
5. Corneal abrasion or ulcer
6. Eyes redness or conjunctivitis
7. Ptosis: Eyelids start trickling and the affected individuals are unable to open their eyes fully.

2.2. Hygienic Practices

Eye infections may happen to everyone but infection can be more severe for people wearing CLs. Microorganisms are able to build up on the lenses when not following proper methods of handling, wearing or storing lenses. Therefore, doctors, pharmacists or eye care practitioners should teach CL wearers, how to care and handle CLs step by step. Beforehand and prior to CLs wearing, washing hands is the starting step, cleaning CLs with a suitable solution by rubbing and rinsing its surface or by only rinsing/ immersion after each use and leaving CL soaked for a sufficient period of time in the solution for disinfection. Commitment to these steps or procedures should be always through discussion with the doctors, pharmacists or eye care practitioners (Weissman, 2006).

However, CLs wearer who is not committed to these procedures may be at greater risk for keratitis. A study in Malaysia was carried out by Bhandari and Hung (2012) to find out correlation between habits of CL wearers and certain factors of lens care. Questionnaire answered by 100 CL wearers about them hygienic practices. The non-compliance resulted poor CL cases (46%), inadequate cleaning of lens before storing (38%) and wearers did not remember the times they were advised to get for an aftercare (24%). It was concluded that CL care may affect in success of CL use and wearers satisfaction, the lack of awareness relating aftercare leads to increasing risks of complications associated with CL wear.

Gans (2015) summarized important steps directed to CL wearers for keen and smart hygiene habits. These include:

1. Hands should be washed thoroughly and dried before touching or handling CLs.
2. Daily changing of CLs solution to keep it fresh and new solution should never be added to use old solution.
3. Storing CL cases should be always in a clean condition and should be replaced every three months.
4. Never use water, saliva, tong or even puffing to clean lenses. As these are the main and key routs of getting eye infection.
5. Preservative-free or contact lens-compatible eye drops are necessary to lubricate eyes.
6. It is important to remove CLs before sleeping, bathing or swimming.
7. Medical examination is required at least once a year.
8. Contact lenses should not be worn when feeling not right.

2.3. Microorganisms Implicated in Microbial Keratitis

Contact lenses are foreign body in the eye and wearing them continuously may lead to small corneal abrasion, and because the lenses decrease the amount of oxygen reaches the corneas, microbial contamination will result (Gans, 2015). A report from CDC (2014) indicated that each year, Americans make about a million visit to doctors with complaints of CLs related eye infections. Prevalence of bacterial, fungal and parasitic flora in asymptomatic disposable and extended contact lens wearers, lens cases and solutions were evaluated and determined worldwide (Gray et al., 1995, Rahim et al., 2008, Emina and Idu, 2011, and Mohamed et al., 2017,).

Prevalence of microbial keratitis has increased in the 1970s after the introduction of CLs. The most common pathogens related to bacterial keratitis (BK) is *P. aeruginosa* followed by *S. aureus* (Eltis, 2011, Lin et al., 2016).

In a study on various in-use commercial and noncommercial solutions carried out by Mayo et al. (1987), they found that eight patients developed BK are infected with *S. marcescens* as the pathogenic factor contaminating the solution. Most isolates were non-pigmented. Other isolated microorganisms included *Enterobacter cloacae*, *Klebsiella ozaenae* and *Pseudomonas spp.* were related to the traditional saline solutions especially *P. aeruginosa* which occurred in 55% of non-preserved solution, it survived due to the presence of organic matter or impurities.

In a study conducted by Yung et al. (2007) on CL cases, solutions and lenses used by a group of students wearing CLs. They found that: 34%, 11% and 9% were contaminated respectively. Coagulase positive *Staphylococci*, coagulase negative *Staphylococci* and *Serratia spp*. were the most common species identified. Washing

CLs by disinfectant solution was shown to be effective in reducing CLs contamination by pathogenic M.O.

To determine the potential risks of MK among soft CL wearer, Rahim et al. (2008) obtained samples from CLs, CL cases and conjunctiva from 100 wearers. Each sample was inoculated into broth, incubated at 37°C for 24 hours, and then samples were cultured on a number of selective and differential media. The CLs, CL cases and conjunctiva were found 65%, 89% and 32% contaminated respectively. The most frequent contaminant was *S. epidermidis* (39.8%) followed by *P. aeruginosa* (34.9%). The major reason for infection was contributed to contamination of the care system.

Another study carried out in Lagos State, Nigeria aimed to identify the prevalence of M.O in extended and disposable CL wearers. Fifty-two out of 74 extended (70.27%) and 50 out of 82 disposable CL (60.98%) were found contaminated. Bacterial species identified in extended and disposable CL wear were: *E. coli* (15.49% and 14.29%), *Klebsiella spp.* (12.69% and 12.99%) *Streptococcus spp*. (4.23% and 3.9%), respectively. Percentage recovery of Amoebae was 6.49% from disposable and 4.23 % from extended wear. The study confirmed that prevalence of M.O in CL poses threat to the wearers (Emina and Idu, 2011).

Nzeako and Al-Sumri, (2011) reported forty percent of CL cases or original bottles solutions showed contamination by various types of M.O, these are: *P. aeruginosa* (23.5%); *Penicillium spp*. (13%); *Candida spp*. (9.2%); coagulase negative *staphylococci* (9.2%); *S. marcescens* (6.1%); *Bacillus*, 5.1%; *Aspergillus flavus*, (5.1%); *Serratia liquefaciens*, *Pseudomonas fluorescens, Enterobacter cloacae* and Aspergillus niger, (4.1%) each; *Chryseomonas luteola* and *Chryseomonas indologenes*, (3.1%) each; *Stenotrophomonas maltophilia, Serratia odorifera*, (2.0%) each; *Enterobacter*

P. aerogenes and *Klebsiella pneumoniae,* (1%) each. According to previous studies, Gram Negative (Gm-ve) bacteria are the major pathogenic M.O causing BK in CL wearers.

2.3.1. Pseudomonas spp.

Pseudomonas species is the most common Gm-ve isolated from CLs (Al-Yousuf, 2009, Fleiszig and Evans, 2010, Wu et al., 2015, Lin et al., 2016). This may be due to its ability to adhere strongly to CLs and CL cases compared with other M.O (Henriques et al., 2005). Also, it can form biofilms on their surfaces which facilitates its persistence (Janakiraman et al., 2009, Szczotka-Flynn et al., 2010). *Pseudomonas* species has complex genetic structure which increases its capability to survive in a wide variety of natural environments (Sankaridurg, 2004). *P. aeruginosa* is the most causative agent of corneal ulceration (Pokra et al., 2016) and keratitis caused by *Pseudomonas* species is characterized by supportive stromal infiltrates with tissue necrosis and excessive muco-purulent discharge (Stern, 1990). Besides, it is associated with antibiotic resistance which increases the hazard of keratitis (Willcox, 2011).

P. aeruginosa isolates from cosmetic CLs show resistance to disinfectant solutions, especially to solution contain polyaminopropyl biguanide (Shen et al., 2019).

In addition, experiments have demonstrated that *P. aeruginosa* has the capacity to become more virulent with time when adhere to corneal epithelial cells. Thus, extended wear could provide more time for bacteria to adapt to the environment and be more virulent (Maltseva et al., 2007).

2.3.2. Serratia spp.

The next most common Gm-ve bacteria isolated from CLs is *S. marcescens* (Das et al., 2007, Szczotka-Flynn et al., 2010) which is facultative aerobic rod, motile, within

the family Enterobacteriaceae. It has survival potentials to grow in extreme conditions (Hejazi and Falkiner, 1997). Some strains produce a dark red to pale pink pigment called prodigiosin (Sehdev and Donnenberg, 1999). On the other hand, a non-pigmented strain related to nosocomial infections which is proven to be fatal was isolated (Hejazi and Falkiner, 1997, Zhou et al., 2016). Its ability to produce gelatinase and alkaline protease play role in the pathogenesis of CL-related keratitis (Pinna et al., 2011). Most CL solutions are ineffective against *S. marcescens* within the minimum recommended time (Hume et al., 2007, Pinna et al., 2011). Also, it is resistant to solutions containing Polyquaternium-1 which should lead to lysis of the spheroplasts of *S. marcescens* (Codling et al., 2003, Hume et al., 2007). Clinical isolates of *Serratia* often show resistance to antibiotics (Varaprasathan et al., 2004, Das et al., 2007). *S. liquefaciens* is another species isolated from CL cases and solutions (Sankaridurg et al., 1996, Wu et al., 2015). Although *S. liquefaciens* was a rarely reported in ophthalmology, Ranganatha et al. (2018) reported a case of orbital cellulitis secondary that progressed to severe MK.

2.3.3. Staphylococcus spp.

Gram positive bacteria commonly related with BK in CL wearers, CoPS and CoNS are responsible for 45% of all BK (Giraldez et al., 2010, Ahn et al., 2011). *S. aureus* was the most common isolated Gm+ve bacteria (Eltis, 2011, Otri et al., 2013, Lin et al., 2016). Contact lenses solution efficacy may be insufficient against clinical isolates *S. aureus* (Mohammadinia et al., 2012). Frequency of methicillin resistant *S. aureus* (MRSA) strains resistant to ciprofloxacin, the first line therapy for MK, ranges from 30-97% (Marangon et al., 2004). The other *Staphylococcus* species isolated from CLs are *S. epidermidis* (Rahim et al., 2008); members of this species are designated as CoNS. They are commensals within healthy human skin flora, with low pathogenicity but recently are found to be responsible for many wound infections (Moran et al., 2017).

Coagulase negative *Staphylococci* have the property of strong adherence to CLs which is considered a major risk factor for corneal problems (Fleiszig et al., 1996, Kodjikian et al., 2003, Fleiszig and Evans, 2010). Some CoNS clinical isolates are methicillin-resistant *S. epidermidis* (Guo et al., 2019) and were isolated as well as MRSA from conjunctivitis by Al-Hammadi et al. (2006) and Melton et al. (2010). *S. saprophyticus* is another CoNS found in the female genital tract, perineum and gastrointestinal animal as normal flora (Widerström et al., 2012, Ehlers and Merrill, 2018). It is one of the common causes of urinary tract infection (UTI) in women (Kuroda et al., 2005). However, although being CoNS but its mannitol fermenting and may look like *S. aureus* (Shittu et al., 2006). The study of El-Ganiny et al. (2017) reported percentage frequency of *S. saprophyticus* isolated from eye conjunctivitis and CL cases is (8.1%), among which weak biofilm producers are reported.

2.3.4. Acinetobacter spp.

Acinetobacter spp. is a Gm-ve, encapsulated coccobacilli (Kurcik-Trajkovska, 2009), it may cause ophthalmitis or keratitis associated with CL use (Bergogne-Berezin and Towner, 1996, Peleg et al., 2008, Almasaudi, 2018). Kuzman et al., (2014) found *Acinetobacter spp.* in CL cases (13%) and in CL case covers (17%).

2.3.5. Shigella spp.

Shigella is a Gm-ve facultative anaerobic member in the family Enterobacteriaceae. Low incidence of this bacteria is reported among CLs isolates (Wiley et al., 2012).

2.3.6. *Shewanella* spp.

Gram negative oxidase positive bacteria, identified from marine environment. It is a normal flora on the surface of fish, and has the ability to grow in 6.5% NaCl

(Khashe and Janda, 1998). Moon et al. (2019) have reported a case of infectious keratitis caused by *Shewanella* species. Another case of keratitis caused by *Shewanella* spp. was reported after fishing and confirmed by Bravenec et al. (2019). These two cases indicate *Shewanella* species involve in human infection.

2.4. Contact Lenses Solution

Contact lenses require an efficient care system for surface cleaning and sterilization. Disinfecting care solutions were improved over the years to become more efficient, it contains combinations of cleaning, disinfecting, moisturizing, and preventing of tear agents (Szczotka-Flynn et al., 2010). Although solutions contain disinfectants; bacterial biofilm if formed is undoubtedly resistant to their antimicrobial activity (Wu et al., 2010). Bacterial resistance to preservatives indicates significance of CL wearers commitment to cleaning and disinfection practices (Mayo et al., 1987).

In Dantam et al. (2014) study, CL wearers were assigned to use CLs daily, these lenses are kept in new cases. One of four types of CL care solutions was used by each group for 2 weeks. Contamination was detected in 80% of CL cases using any type of solution. CL cases with disinfectant solution containing 0.001% polyquaternium-1 and 0.0006% myristamidopropyl dimethylamine compared to those maintained in 0.00013% polyaminopropyl biguanide (PAPB) and 0.0001% polyquaternium solution. Significantly greater levels of contamination were identified in CL cases with solutions containing 3% Hydrogen peroxide and 0.79% NaCl, compared to solution containing 0.0003% Polyquaternium-1 and 0.00016% Alexidine.

In another aspect, Nzeako and Al-Sumri (2011) study on students using different types of disinfecting solutions, reported that 65% of users solutions containing

Polyhexamethylene biguanide (Polyhexanide, PHMB) revealed microbial growth and 5% of users solutions containing PAPB also showed microbial growth.

Mohammadinia et al. (2012) evaluate three multipurpose CLs disinfecting solutions against clinical and standard strains of *P. aeregenosa* and *S. aureus*. Active ingredients of the three CL solutions tested were PAPB, and PHMB in two different concentration 0.0001%, and 0.0002%. The efficacy of both PHMB concentrations was acceptable in reducing clinical isolates. Polyaminopropyl biguanide exerted minimum efficacy in reducing clinical isolate of *S. aureus*, and did not reduce clinical isolate of *P. aeregenosa.*

2.5. Biofilm Formation

Biofilm is defined as a bacterial cells community that irreversibly attach to each other, onto firm surfaces, or tissues and it is comprised of matrix of polymeric substances that producing it (Donlan et al., 2002). Presence of biofilms was not associated with infection until the 1970s, when Høiby et al. (1973) observed mucoid strains of *P. aeregenosa* with patients suffering from chronic cystic fibrosis. After that, several infectious diseases were correlated to bacterial biofilms. Microorganisms producing biofilms are highly resistant to antimicrobial agents and colonize several types of medical devices (Costerton et al., 1999). Both types of CLs (soft and hard) and CL cases are susceptible to readily bacterial adhesion and biofilm formation (Miller and Ahearn, 1987, Dart, 1997). The primary source is M.O contaminating CLs and its disinfectant solutions in CL cases, McLaughlin-Borlace et al. (1998) found that bacterial biofilms were present in 17 of 20 of CL cases while in 11 of 20 CL surfaces. Bacteria that have demonstrated for adherence of biofilm on CLs and cases were:

P. aeregenosa, *Serratia spp*., *S. aureus*, *S. epidermidis*, and *E. coli* (Donlan et al., 2002, Pinna, et al., 2011, El-Ganiny, et al., 2017).

Wu et al. (2011) reported that biofilms by *S. aureus* and *P. aeruginosa* were formed on two unused CL cases despite each CL case was subjected to one of four cleaning systems: rinse and air-dry," "rub, rinse and air-dry," "tissue wipe and air-dry," and "rub, rinse, tissue-wipe, and air-dry" by using two different disinfecting solutions. The residual biofilm was quantified using viable counts. The most effective ways to remove biofilms were "Rub, rinse, tissue-wipe, and air-dry" this removing 4 to 6 log Colony Forming Unit (CFU) of bacteria what is greater than these removed by the manufacturers' guidelines way (rinse and air-dry), 1 to 2 log CFU reduction.

Artini et al. (2015) tested the ability of three different types of CL solutions to inhibit bacterial biofilms. Three different types of CL solutions contain oxychlorite, PAPB, and polyquad and aldox as disinfectant agents respectively. All CL solutions are able to inhibit biofilm formed by *S. marcescens* and *Staphylococcus spp.* after 4 hrs, and reduce *Pseudomonas* biofilm formation

3. Methodology:

3.1. Samples

Samples were collected through the period from October 2019 to March 2020. Participants were students in Isra University randomly selected from different colleges. All practical experiments were processed and completed in microbiology laboratory which is equipped with devices, instruments, culture media, chemicals, glassware and materials necessary for researchers and postgraduate students at the Faculty of Pharmacy / Isra University.

All participants signed the informed consent without any obligation. The informed consent explains study objective, procedures applied to the samples and enough information to make an informed decision. Participant's inquiries were answered and clarified. Contact lens wearers included in the study aged >18 years and currently wearing CLs. All of them use either long-lasting lenses or daily use lenses. They were not taking any antibiotic nor eye medications (Mohamed et al., 2017) and no one was suffering from any eye disease, inflammation or infection at the time of sampling. Samples were taken from right and left CL cases (CL storage cases), disinfectant solution bottles, and mouth rims of solution bottles.

3.2. Questionnaire

Questions were constructed after searching through various articles (Liesegang, 1997, Yung et al., 2007, Bhandari and Hung, 2012, Stapleton et al., 2017). The part of questionnaire considering CLs wearing recommendations was determined according to the American Optometric Association (AOA). Participants answered questionnaire after handling their samples. The questionnaire included various questions forms some of which are closed-ended and others are open-ended. The questionnaire included

demographic data, eye-related health, personal hygienic habits, and CLs hygienic habits (Appendix 1).

3.3. Sampling

Four samples were taken from each contact lens unit of each participant. These included the solutions from each: (1) Right and (2) Left Contact Lens Case (RCLC and LCLC respectively), (3) Disinfectant solution from its original bottle, and (4) Swabbing mouth rim of disinfectant solution bottle.

Under sterile conditions, 0.5 ml of solutions from each RCLC, LCLC, and SB and swabs were suspended in 4.5 ml Trypticase Soy Broth (TSB, biolab) containing 3% Tween 80 (Polysorbate 80) to neutralize the effect of disinfectant (Kelsey, 1974, Denyer et al., 2008) then incubated at 35 °C to 1-2 hrs to allow stressed microbial cells to recover. Two-fold dilutions of each sample (100 µL,50 µL) were spread onto duplicate Trypticase Soy Agar (TSA, biolab) plates using sterile L-shaped solid glass rod (Dipped into spirit then flamed). Plates were incubated at 35 °C for 24-48 hrs.

Besides, loopfuls from each TSB inoculums of the four sources were inoculated onto the following selective and differential media. These are: MacConkey's (MAC) agar (Scharlau), Mannitol Salt Agar (MSA, biolab) and Sabouraud dextrose agar (SDA biolab). Then incubated at 35 °C for 24-48 hr. SDA plates were incubated for at least 1 week to ascertain presence or absence of growth. Isolated colonies grown on TSA were counted with the aid of Colony Counter (WTW, Keimzählgerät BZG 28) to calculate CFU originally present in each solution (Denyer et al., 2008). Colonies were purified on Nutrient Agar (NA, Scharlau) plates then Gram stained. Pure colonies were also cultured on nutrient agar slants and stored in the fridge to be used in identification studies. Colonies grown on the selective and differential media were also characterized

and identified preliminary according to their growth reaction in these media (Subhash, 2012). All autoclaving operations were performed using AUTOCLAVI DA PAVIMENTO ATV80 device.

3.4. Identification of Isolates to the Generic Level

3.4.1. Cultural Characteristics

Characteristics of grown bacterial colonies were determined by examining colonial morphology on TSA. These include size, edge, elevation, consistency and pigmentation (Subhash, 2012).

3.4.2. Staining

Preliminary identification of isolates to the genus level was made by examination of growth characteristics of colonies on differential media and by Gram stain for bacteria and simple stain for yeast according to Barrow and Feltham (1993).

3.4.3. Biochemical Identification

The following biochemical tests are performed to confirm identification of colonies to the generic level. These include: ability to produce catalase, oxidase, and deoxyribonuclease (DNase), whether performing mixed acids pathway (Methyl red test), utilization of citrate as a carbon source, utilization of Triple Sugar Iron (TSI, HIMEDIA®) agar with the production (or not) of hydrogen sulfide (Subhash, 2012) and production of coagulase enzyme (To differentiate between CoNS and CoPS) to enables conversion of fibrinogen to fibrin (Rakotovao-Ravahatra et al., 2019). *Serratia* Differential Medium (HIMEDIA®, Twin Pack, M1288) was used to differentiate between *S. marcescens*, and *S. liquefaciens* species depended on ability to ferment L-arabinose and decarboxylate ornithine (Faddin, 1985). HiCrome TM Acinetobacter Agar Base (HIMEDIA, M1938) was used to detect *Acinetobacter* spp.

3.4.4. Rapid Identification Technique

Identification of isolates to the species level was achieved using Raped identification (Kit technique). RapID™ ONE System (Thermo Scientific™) is a panel of kits incorporating arrangements of eighteen biochemical tests (Yung et al., 2007). These tests are: Urea, Arginine, Ornithine, Lysine, Aliphatic thiol, Fatty acid ester, Sugar aldehyde, Sorbitol, ρ-Nitrophenyl-β,D-glucuronide, σ-Nitrophenyl-β,D-galactoside, ρ-Nitrophenyl-β,D-glucoside, ρ-Nitrophenyl-β,D-xyloside, ρ-Nitrophenyl-n-acetyl-β,D-glucosaminide, Malonate, Proline-β-naphthylamide, ɣ-Glutamyl-β-naphthylamide, Pyrrolidonyl-β-naphthylamide, and Adonitol Tryptophane.

3.5. Antibiotic Susceptibility

3.5.1. Agar Dilution Method

Isolates of *Staphylococcus* were examined for their susceptibility or resistance to methicillin using the antibiotic oxacillin. Isolates were subcultured on NA for 18-24 hrs then few colonies were transferred to TSB and standardized to yield a turbidity equal to 0.5 McFarland (McF) standard as measured by N4S UV-Vis spectrophotometer (Cockerill et al., 2013). Two media were prepared: MSA and Mueller-Hinton Agar (MHA, HIMEDIA®) containing 4% NaCl. After sterilization, they were left to cool down to around 50°C. Oxacillin (6 µg/ml) was incorporated into each medium, slowly mixed then dispensed into sterile petri dishes (Pillai et al., 2012). Loopful from prepared TSB were inoculated onto the prepared plates of MSA and MH agar (with 4% NaCl) supplemented with 6 µg/ml Oxacillin then incubated for 48 hrs at 33-35°C (Thornsberry and McDougal, 1983, Cockerill et al., 2013). Appearance of growth on either media was recorded as resistance to methicillin.

3.5.2. Disc Diffusion Method

The most prevalent Gram negative and Gram positive bacteria from contaminated CL units were examined for their susceptibility to various antibiotics adopting disc diffusion test according to Clinical and Laboratory Standards Institute (CLSI) 2013 (Patel et al., 2013). Pure 18-24 hr. old colonies grown on TSA were subculture on TSB to make suspensions equivalent to. 0.5 McFarland (as above). Sterile cotton swabs were used to inoculate and spread bacterial suspensions evenly on MHA. Plates were allowed to dry for 5 - 10 minutes then antibiotic disc dispenser (Oxoid) was used to place antibiotic discs at equal distances on MHA plates then incubated for 18-24 hrs at 35°C.

Gram negative bacteria were tested against the following antibiotics (Oxoid): ciprofloxacin 5 µg, ceftazidime 30 µg, cefotaxime 10 µg, cefepime 30 µg, Gentamicin 10 µg, levofloxacin 5 µg, tetracycline 30 µg, and ceftriaxone 30 µg (Sohail et al., 2016, Carvalheira et al., 2017, Kanafani and Kanj, 2018, Chopra and Roberts, 2001, Traub, 2000, Stock et al., 2003, Simsek, 2019)

Gram positive were tested against: penicillin 10 units, amoxicillin-calavulanic acid 30 µg and , erythromycin 15 µg (Patel et al., 2013). Zones of inhibition were measured by millimeter and results were recorded as sensitive and resistant (intermediate were included with the resistant ones); after interpretation with standard tables from the CLSI criteria (Patel et al., 2013). The test was repeated twice, and the Standard Deviation (SD) of zone diameter was calculated.

3.6. Survival Study

For evaluation disinfection efficacy of CL solutions; survival of most prevalent bacteria was examined (Mowrey-McKee et al., 2012). Two CL care solutions

commonly used by participants, coded A and B bought from Jordanian pharmacies were evaluated. Both solutions were within expiration dating and were tested according to manufacturer's labeled recommendation for disinfection time. Ingredients and recommended disinfection time for each solution are shown in (Table1). Three species of the most frequently isolated bacteria from CL units were tested, these are: *S. marcescens*, *S. liquefaciens*, *Acinetobacter spp*., MRSA and Methecillin Sensitive *Staphylococcus aureus* (MSSA). Isolates were grown for 18-24 hr. on TSA at 35°C. Cultures were suspended with sterile 0.9% NaCl to obtain a concentration of 1.0×10^7-1.0×10^8 CFU/ml (Mohammadinia et al., 2012).

The experiment was conducted under clean and dirty condition to mimic real situation. To provide clean conditions, aliquots of 0.5 ml bacterial suspensions were added to 4.5 ml of each CLs solution. To provide dirty conditions, the above steps were repeated but with addition of 0.3% yeast (Denyer et al., 2011, Campana et al., 2018). The tubes of test solutions were mixed thoroughly for even dispersion with the bacterial suspension. Positive control samples were prepared by addition of 0.5 ml of each bacterial suspension to tubes containing 4.5 ml of 0.9% NaCl. Negative control was prepared by addition 0.5 ml of each CL solution to tubes containing 4.5 ml of 0.9% NaCl (Mohammadinia et al., 2012, Budiman et al., 2017). All tubes were allowed to stand at room temperature for 4 hr. From each test tube, 0.5 ml was withdrawn and diluted with 0.4 ml TSB containing Tween 80 (1%) and left at least for10-15 minute at room temperature to neutralize residual solution (Mohammadinia et al., 2012, Ranya et al., 2013). Aliquots of 10µl and 100µl were plated on TSA and incubated at 35°C for 24-48hr.

Table (1): Ingredients and recommended disinfection times of the two tested contact lens solutions.

Solution code	Disinfectant agent	Other ingredients	Recommended time
A	Polyhexamethylene biguanide (Polyhexanide)	Potassium Chloride, Disodium Edetate Poloxamer, Sodium Hyaluronate, HPMC, Sodium Phosphate Buffer.	at least 4 hr.
B	Polyaminopropyl biguanide	Hydroxyalkylphosphonate, boric acid, Ethylenediaminetetraacetic acid disodium, poloxamine, sodium borate and sodium chloride.	at least 4 hr.

According to Laxmi et al. (2018), the number of surviving tested bacteria was counted, and the logarithmic reduction in growth by each CLs solution and the control (10^5) was calculated as following:

$$\log \text{reduction} = \log 10\ (\text{Control CFU/ml}) - \log 10\ (\text{Final CFU/ml})$$

3.7. Biofilm Formation and Inhibition Assay

To test ability of most prevalent bacteria to adhere to the inner surfaces of plastic tubes. Colonies were transferred into plastic tubes containing 5 ml of TSB and incubated for 24 hrs. The plastic tubes were emptied and stained with crystal violet for 15 min then the stain was decanted and tubes were left to dry. Biofilm formation was reported positive when inner sides of tubes were stained (Christensen et al., 1985). The ability of CL solutions to reduce biofilm production was tested. One colony of each isolate was transferred to TSB and incubated to 24 hrs. Then aliquots 100 µl of TSB inoculum were placed in wells of 96-microtitre plates filled with 100 µl of each CLs solution. Also, one drop of 0.3% yeast was added in other wells to provide dirty conditions. Control samples contained 100µl of TSB inoculum with 100µl of pure TSB.

Plates were incubated for two periods: 4 and 24 hr. at 35°C. After each period, the content of plates was poured out and the wells were washed with running tap water. Wells were stained with crystal violet for 15 min then excess crystal violet was washed away with running tap water and dissolve the stain by ethanol then allowed to air dry (Artini et al., 2015). Optical Density (OD) of each well was measured at 600 nm by AccuReader M965 Microplate Reader (Metertech Inc.) for staining biofilm (McBirney et al., 2016).

Inhibition of biofilms was calculated as following:

$$\text{Inhibation Biofilm \%} = \frac{\text{OD of Control} - \text{OD of Solution}}{\text{OD of Control}} \times 100$$

3.8. Statistical Analysis

Microsoft Excel was used for data storage and graphs generation. All statistical analyses were conducted using SPSS software version 25 for Windows. *p* value of equals or less than 0.05 was set as the significance level. Participants characteristics were reported by using means and SD for continuous variables while frequencies with percentages were used for categorical variables. A dichotomous variable that represent the isolate status was generated and was used to compare contaminated and non-contaminated samples using Chi square test ($\chi 2$).

Ethical Approval:

Approval to conduct the study was obtained from the Ethical committee at Isra University (Ph/ 9/2019).

4. Results:

4.1. Demographic Data

Demographics characteristics are illustrated in Table (2). A total of thirty CL wearers participated in the present study, all of them were females; age ranged between 19 - 36 years ($\bar{x}$ = 23.5 years, SD ± 3.2 years). Each participant completed a questionnaire correlated to their habits and practices through wearing contact lenses. The majority of participants are undergraduate university students (86.7%), attending health-related colleges (63.3%) and studied a microbiology course (60%). All post graduate students are studying in health colleges. The overwhelming majority of participants are non-smokers (86.7%). Sixteen participants (53.3%) use CLs for cosmetic reasons while the remaining use CLs for medical reasons. Additionally, long-lasting CLs were by far the most popular type used (86.7%). Almost all participants (96.7%) wearing CLs for at least one year. Microbial contamination was detected at least in one item of CL units of 25 participants.

Table (2): Demographics data of contact lense wearers

Demographic Data	Response	N=30 n (%)	Microbial Contamination n =25 (%)	*p*-value
Health education*	Yes	19 (63.3%)	16 (84.2%)	0.865
	No	11 (36.7%)	9 (81.8%)	
Educational level	Undergraduates	26 (86.7%)	22 (84.6%)	0.631
	Post graduates	4 (13.3%)	3 (75.0%)	
Studied microbiology	Yes	18 (60%)	15 (83.3%)	1.000
	No	12 (40%)	10 (83.3%)	
CLs experience	≥ One year	29 (96.7%)	24 (82.8%)	0.649
	< Year	1 (3.3%)	1 (100%)	
Reason for wearing CLs	Cosmetic	16 (53.3%)	13 (81.3%)	0.743
	Medical	14 (46.7%)	12 (85.7%)	
Type of CLs	Long-lasting	26 (86.7 %)	21 (80.8%)	0.337
	Daily use (Disposable)	4 (13.3 %)	4 (100%)	
Smoking status	Yes	4 (13.3%)	4 (100%)	0.337
	No	26 (86.7%)	21 (80.8%)	
Frequency of wearing CLs	Daily	11 (36.7%)	8 (72.7%)	0.378
	Weekly	5 (16.7%)	5 (100%)	
	Monthly	14 (46.6%)	12 (85.7%)	

Periods of wearing CLs (hours)	1-4	6 (20%)	5 (83.3%)	0.071
	5-8	14 (46.7%)	11 (78.6%)	
	9-12	9 (30%)	9 (100%)	
	> 12	1 (3.3%)	-	

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4.2. Eye-related Health Status

Table (3) demonstrates eye-related health status. Almost two-thirds (63.3%) of participants denied having any pervious eye-related medical conditions/diseases. Out of eleven CL wearers who had a previous eye-related conditions/disease (36.7%), nine received medical examination. Diagnoses included: infection (n=4), inflammation (n=3) and dryness of eyes (n=2), out of these diagnostic cases, microbial contamination was detected at least in one item of CL units belonging to 2, 3 and 2 participants respectively. Reported conjunctivitis and keratitis were treated by antibiotics. None of participants reported active eye infections at the time of the study.

Eye redness after wearing CLs is almost significant sign associated with microbial contamination of CL units. All CL wearers, who continuously or intermittently suffered from eyelid boils, have microbial contamination in their CL units.

Table (3): Previous eye-related medical problems

Eye complications	Response	N=30 n (%)	Microbial contamination n =25 (%)	*P*-value
Eye medical condition / disease	Yes	11 (36.7%)	9 (81.8%)	0.865
	No	19 (63.3%)	16 (84.2)	
Eye redness after CL wearing	Always/Often	5 (16.6%)	5 (100%)	0.088
	Sometimes	18 (60%)	16 (88.9%)	
	Rarely/Never	7 (23.3%)	4 (57.1%)	
Eyelid boils	Always/Often	2 (6.7%)	2 (100%)	0.401
	Sometimes	5 (16.7%)	5 (100%)	
	Rarely/Never	23 (76.7%)	18 (78.3%)	

4.3. Personal Hygienic Habits of Contact Lens Wearers

Most CL wearers (60%) have received instructions of CLs wearing and caring presented by health professional (e.g. pharmacists, ophthalmologist, or optometrist).

Participants were asked to evaluate their knowledge about instructions: eleven (36.7%) believed that their information regarding lens care is excellent, while 12 (40%) and 7 (23.3%) categorized their information as very good and good, respectively (Table 4).

Table (4): Participants awareness of instructions related to wearing contact lenses

Participants attitude toward instructions of CLs wearing and caring	**Response**	**N=30 n (%)**	**Microbial Contamination n=25 (%)**	***P*-value**
Instructions presented by health professional	Yes	18 (60%)	16 (88.9%)	0.317
	No	12 (40%)	9 (75%)	
Self- evaluation	Excellent	11 (36.7%)	8 (72.7%)	0.135
	Very good	12 (40%)	12 (100%)	
	Good	7 (23.3%)	5 (71.4%)	
	Poor	-	-	
Commitment to instructions	Always/ Often	26 (86.7%)	22 (84.6%)	0.631
	Sometimes	4 (13.3%)	3 (75%)	
	Rarely/Never	-	-	

Table (5) demonstrates that most (86.7%) CL wearers do not require assistance for wearing CLs. All wearers wash their hands and dry them prior to CLs application apart from four (13.3%) who do not dry their hands after washing. Microbial contamination appeared in CL units of all wearers, who wash their hands only with water, and water or soap (alternatively). The majority (83%) avoided washing their faces with tap water while wearing CLs. Only one participant reported bathing/swimming while wearing CLs and another one shared his contact lenses with other person. Contact lens wearers who scarcely avoid touching their nails with CLs have 83.3% contamination in CL units.

Table (5): Personal habits of wearers during wearing contact lenses.

Personal habits	Response	N=30 n (%)	Microbial Contamination n=25 (%)	*P*-value
Personal use of CLs	Yes	29 (96.7%)	24 (82.8%)	0.649
	No	1 (3.3%)	1 (100%)	
Assistance required to wear CLs	Always/ Often	2 (6.7%)	1 (20%)	0.362
	Sometimes	2 (6.7%)	2 (100%)	
	Rarely/ Never	26 (86.7%)	22 (84.6%)	
Hand washing before wearing	Always/ Often	30 (100.0%)	25 (83.3%)	-
	Sometimes	-	-	
	Rarely/ Never	-	-	
Hand washing by:	Soap	22 (73.3%)	17 (77.3%)	0.336
	Water	4 (13.3%)	4 (100%)	
	Soap or water (alternative)	4 (13.3%)	4 (100%)	
Drying washed hand	Always/ Often	26 (86.7%)	21 (80.8%)	0.337
	Sometimes	4 (13.3%)	4 (100%)	
	Rarely/ Never	-	-	
Avoiding touching CLs with fingernails	Always/ Often	8 (26.7%)	7 (87.5%)	0.861
	Sometimes	4 (13.3%)	3 (75%)	
	Rarely/ Never	18 (60.0%)	15 (83.3%)	
Sleeping while wearing CLs	Always/ Often	-	-	-
	Sometimes	-	-	
	Rarely/ Never	30 (100.0%)	25 (83.3%)	
Bathing or swimming while wearing CLs	Always/ Often	1 (3.3%)	-	0.023
	Sometimes	-	-	
	Rarely/ Never	29 (96.7%)	25 (86.2)	
Washing face while wearing CLs	Always/ Often	1 (3.3%)	-	0.061
	Sometimes	4 (13.3%)	3 (75%)	
	Rarely/ Never	25 (83.3%)	22 (88%)	
Avoiding smoking places while wearing CLs	Always/ Often	12 (40.0%)	10 (83.3%)	0.698
	Sometimes	8 (26.7%)	6 (75%)	
	Rarely/ Never	10 (33.3%)	9 (90%)	

4.4. Hygienic Habits Associated with Contact Lens Units

Few numbers of participants (10.0%) use a special plastic forceps to apply CLs. More than one half of them (56.7%) rinse their CLs with CLs solution, while only one third rub CLs while rinsing them. Rubbing lens with CL solution is a significant sign

associated with microbial contamination of CL units. Eight participants (27.6%) reported using tap water instead of the recommended solution to store contact lenses at some points. A summary of contact lenses wearers' hygienic habits toward contact lenses and solutions are shown in Table (6). It should be noted that percentage of participants using water or using water and CL solution alternatively for washing CL cases always was (38.9%) and (16.7%), respectively.

Table (6): Hygienic habits of contact lens wearers' toward contact lenses and solutions

Hygienic Habits	Responses	N= 30 n (%)	Microbial Contamination n =25 (%)	*P*-value
Using forceps for wearing CLs	Yes	3 (10%)	2 (66.7%)	0.414
	No	27 (90%)	23 (85.2%)	
Rinsing lens with CL solution	Always/ Often	17 (56.7%)	14 (82.4%)	0.603
	Sometimes	9 (30%)	7 (77.8%)	
	Rarely/ Never	4 (13.3%)	4 (100%)	
Rubbing lens with CL solution	Always/ Often	10 (33.3%)	8 (80%)	0.021
	Sometimes	6 (20 %)	3 (50%)	
	Rarely/ Never	14 (46.7%)	14 (100%)	
Using water for CLs storage	Always/ Often	4 (13.8%)	4 (100%)	0.316
	Sometimes	4 (13.8%)	4 (100%)	
	Rarely/ Never	21 (72.4%)	16 (76.2%)	
Duration of adding solution to CL cases	Daily	17 (56.7%)	14 (82.4%)	0.494
	Weakly	8 (26.7%)	6 (75%)	
	Monthly	5 (16.7%)	5 (100%)	
Frequency of washing CL cases	Always/ Often	18 (60%)	16 (88.9%)	0.157
	Sometimes	4 (13.3%)	4 (100%)	
	Rarely/ Never	8 (26.7%)	5 (62.5%)	
Washing CL cases by:	Solution	12 (40%)	10 (83.3%)	0.845
	Water	11 (36.7%)	9 (81.8%)	
	Both	3 (10%)	3 (100%)	
	Not washing	4 (13.3%)	3 (75%)	
CLs cases replacement	Always/ Often	15 (50%)	12 (80%)	0.852
	Sometimes	6 (20%)	5 (83.3%)	
	Rarely/ Never	9 (30%)	8 (88.9%)	
Addition of residual old solution to the new one	Always/ Often	4 (13.3%)	3 (75%)	0.494
	Sometimes	1 (3.3%)	1 (100%)	
	Rarely/ Never	25 (83.3%)	21 (84%)	

Duration of wearing CLs and solutions usage are reported from the first date of commencement of use. Duration of CL solutions usage is a significant sign associated with microbial contamination of CL units. More than one half (53.3%) had their contact

lenses for more than one year. Only (50%) adhered to the manufacturer recommendations for less than 3 months for proper using of CLs' solution. Table (7) summarizes periods of using solutions and lenses. It should be noted that 45.5% of wearers using solutions for less than one month and used water when solution was not available.

Table (7): Duration of used disinfectant solutions and lenses

CLs units	Duration Periods (Month)	N=30 n (%)	Microbial Contamination n =25 (%)	*P*-value
CLs Solution	<1	11 (36.7%)	10 (90.9%)	0.020
	1-3	4 (13.3%)	1 (25%)	
	4-6	2 (6.7%)	2 (100%)	
	7-12	4 (13.3%)	4 (100%)	
	>12	9 (30.0%)	8 (88.9%)	
Contact lenses	<1	3 (10.0%)	2 (66.7%)	0.105
	1-3	3 (10.0%)	3 (100%)	
	4-6	7 (23.3%)	7 (100%)	
	7-12	1 (3.3%)	-	
	>12	16 (53.3%)	13 (81.3%)	

Contact lens wearers used two types of multipurpose solutions either included with the CLs when purchasing (53.3%) or using commercially available in pharmacies (46.7%). Polyhexanide was the disinfectant agent in all solutions supplied with the CLs, though with different concentrations. The trade names of solutions available in pharmacies were: solution A (PAPB as disinfectant agent) in 23.3%, and solution B (PHMB as disinfectant agent) in 20%, and solution C (Polyquaternium and Myristamidopropyl dimethylamine as disinfectant agent) in 3.3%. Table (8) shows microbial contamination in CL units associated of with solutions types.

Table (8): Disinfectant agents of contact lens solutions used by wearers.

Type of solution	Disinfectant agent	N=30	Microbial Contamination n =25 (%)	*P*-value
Solution supplied with the CL	Polyhexamethylene biguanide	16 (53.3%)	13 (81.3%)	0.964
A	Polyhexamethylene biguanide	7 (23.3%)	6 (85.7%)	
B	Polyaminopropyl biguanide	6 (20%)	5 (83.3%)	
C	Polyquaternium and Myristamidopropyl dimethylamine	1 (3.3%)	1 (100%)	

4.5. Frequency of microbial contamination

Twenty-five (83.3%) of 30 CL units examined, revealed contamination in at least one item. None of the disinfecting solution from bottle was found contaminated. Incidence of microbial contamination in RCLCs, LCLCs and rims of solution bottles was 21 (70%), 17 (56.7%) and 6 (20%) respectively. Microorganisms contaminating rims of solution bottles were also present in the immersed solution of either RCLC or LCLC or both. Only one (3.3%) bottle rim was contaminated without its CL case. Cases of CLs were found contaminated with one or more microorganisms (Table 9).

Table (9): Microbial contamination of immersion solutions in contact lens cases and rims of solution bottles.

Microbial contamination	Right cases n = 21 (%)	Left cases n= 17 (%)	Rims n= 6 (%)
Type of contamination: Monomicrobial	12 (57.1%)	11 (64.7%)	6 (100%)
Polymicrobial	9 (42.9%)	6 (35.3%)	-
Colony Forming Unit: < 30 CFU/ml	6 (28.6%)	6 (35.3%)	-
>30-300 CFU/ml	7 (33.3%)	2 (11.8%)	
> 300 CFU/ml	8 (38.1%)	9 (52.9%)	

4.6. Identification of microbial contamination

All colonies grown on MAC, MSA, SDA, and TSA were primarily characterized and identified. Sixty-four isolates were obtained from contaminated samples. These included: 60 (93.8%) bacteria and 4 (6.3%) yeasts. Forty-six (71.9%) isolates of bacteria were Gm-ve and the remaining 14 isolates (21.9%) were Gm+ve. Table (10) demonstrates distribution frequency of M.O. in each item of CL units.

Table (10): Frequency distribution of microorganisms in contact lens cases and rims of solution bottles.

No. of Isolates	Right case n=33 (%)	Left case n = 25(%)	Rim n = 6 (%)	Total n = 64 (%)
Gram-negative bacteria	25 (75.8%)	19 (76%)	2 (33.3%)	46 (71.9%)
Gram-positive bacteria	5 (15.2%)	6 (24%)	3 (50%)	14 (21.9%)
Yeast	3 (9.1%)	-	1 (16.7%)	(6.3%)

4.6.1. Identification of Gram-negative bacteria

Forty-six Gm-ve bacteria were isolated from MAC agar; five (10.9%) non-lactose fermenting isolates were lost during curfew period before identification. Besides, two lactose fermenting colonies (4.3%) grown on MAC agar gave up to short-rods, oxidase negative bacteria were not identified by RapID One System (Fig. 1).

Figure (1): Unidentified lactose fermenting colony grown on McConkeys agar isolated from CL case

In addition to one unidentified Gm-ve appeared under microscope like filaments (Fig. 2), it ferments mannitol when grown on MSA, oxidase negative, and catalase positive.

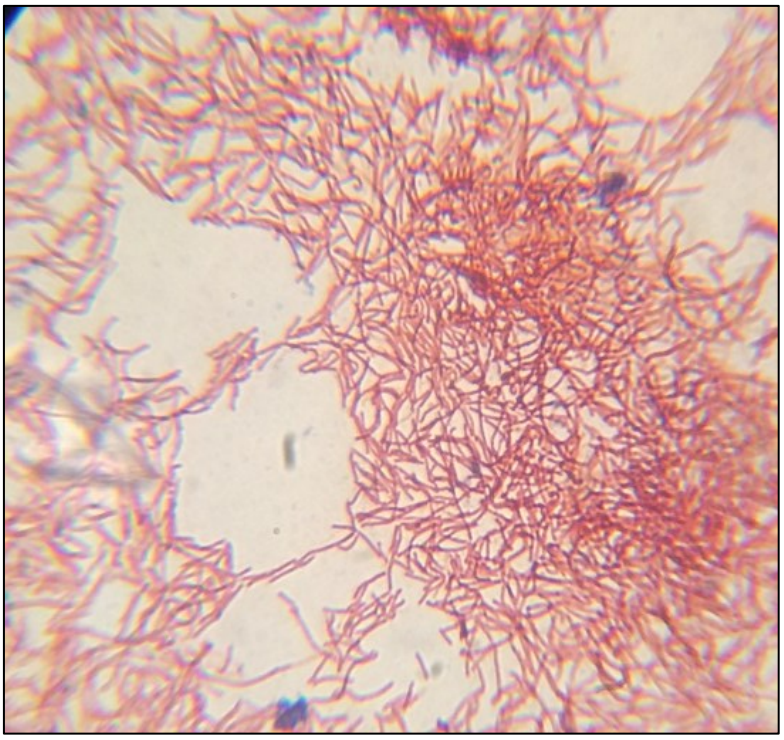

Figure (2): Gram stain of filamentous bacteria isolated from CL case (X1000)

Identification of the remaining thirty eight isolates to the genus level is shown in Table (11).

Table (11): Frequency of identified Gram-negative bacteria isolated from CL units

Organisms	N= 46 (%)
Pseudomonas spp.:	**21 (45.7%):**
Pseudomonas aeruginosa	5
Shewanella putrefaciens	3
Burkholderia pseudomallei	2
*Pseudomonas spp**	11
***Serratia spp.*:**	**9 (19.6%):**
S. marcescens	8
S. liquefaciens	1
***Acinetobacter spp.*:**	**4 (8.7%):**
A. calcoaceticus	2

*Acinetobacter spp.**	2
Shigella spp.	2 (4.3%)
Salmonella spp	1 (2.2%)
Escherichia spp.	1 (2.2%)
Filamentous bacteria*	1 (2.2%)
Short-rod shaped*	2 (4.3%)
Non-Lactose fermenters**	5 (10.9%)

*Require further identification tests
** Lost during curfew.

Twenty-one colonies producing oxidase and catalase but did not ferment carbohydrates in TSI agar, these were identified as *Pseudomonas spp*. Five of them were identified preliminary as *P. aeruginosa* for producing blue-green color (pyocyanin pigment) when grown on NA (Fig. 3).

Figure (3): *P. aeruginosa* isolated from CL case producing green coloration on nutrient agar

Two isolates produced black color in TSI agar which is indicative of ferrous sulfide (H_2S_2) production (Fig. 4) and was identified as *Shewanella putrefaciens* (Previously named *Pseudomonas putrefaciens*).

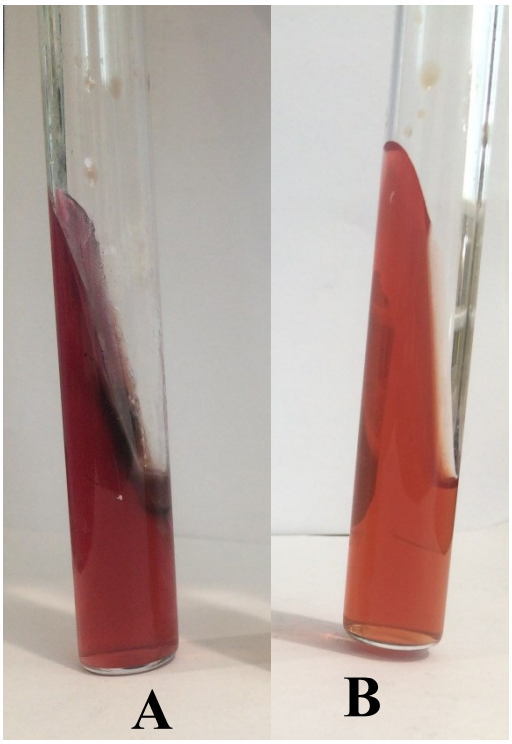

Figure (4): Triple sugar iron agar, (A) *S. putrefaciens* isolated form rim of solution bottle; (B) Control tube

By Gram staining cells of one isolate appeared as short rods with bipolar staining like safety pin (Fig. 5), it was preliminary identified as *Burkholderia pseudomallei* (Previously named *P. pseudomallei*). Further tests are required for definite species identification of *Pseudomonas spp.*

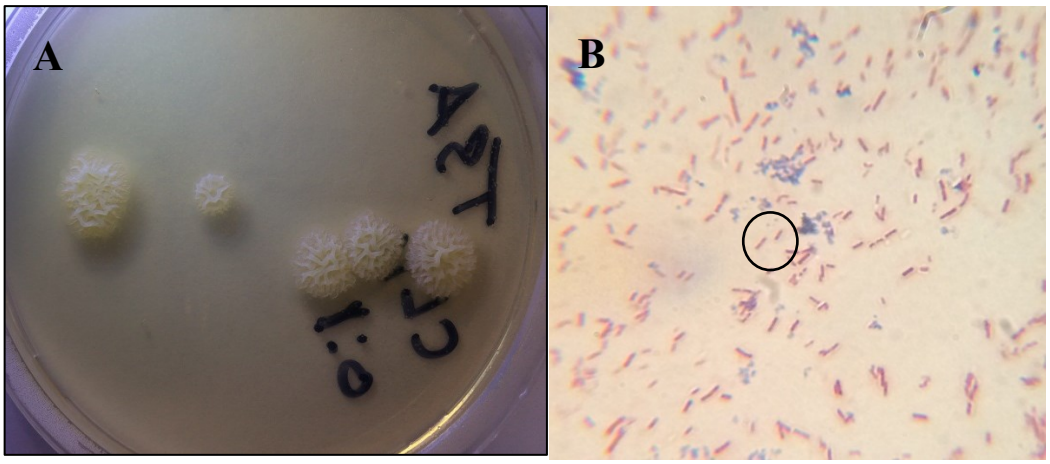

Figure (5): *B. pseudomallei* (A) Colonies of on Trypticase soy agar; (B) Cells showing bipolar staining

Nine oxidase negative Gm-ve rods, producing DNase and Acid/Acid in TSI agar were primarily identified as *Serratia spp*. RapID One System indicate their identification as *S. marcescens*. By growing these nine isolates in tubes containing *Serratia* differential semisolid medium, eight isolates turned the medium to purple confirming the identification as *S. marcescens* while the 9th isolate formed purple band at the top of the medium and the butt was greenish yellow; thereby, identified as *S. liquefaciens* (Fig. 6).

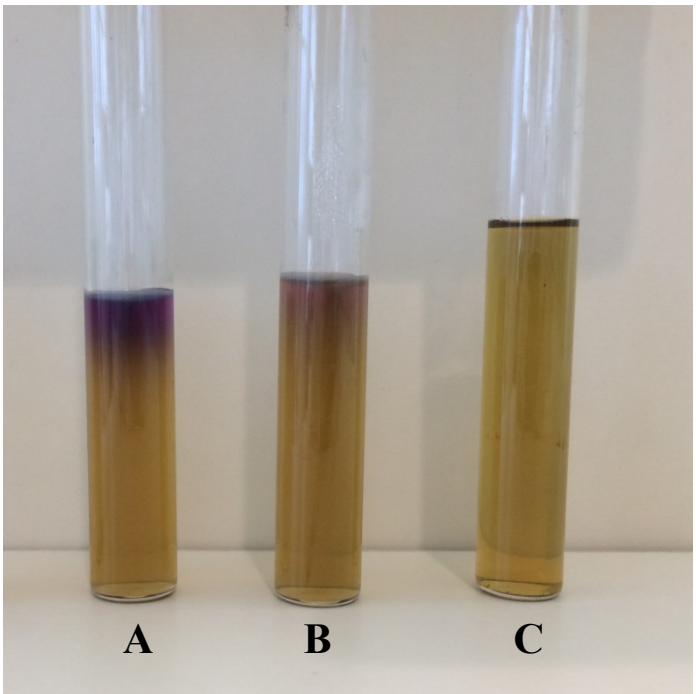

Figure (6): *Serratia* Differential Medium, (A) *S. liquefaciens*; (B) *S. marcescens*; (C) Control tube

Only two of the four isolates of *Acinetobacter spp*. were identified at species level as *A. calcoaceticus* by RapID ONE system.

One lactose fermenting isolate on MAC agar was preliminary identified as *E. coli* and identification was confirmed by the production of green metallic sheen after growing the isolate on EMB agar. *Shigella spp.* and *Salmonella spp* Identification was confirmed by RapID ONE system.

4.6.2. Identification of Gram-positive bacteria

Fourteen isolates were Gm+ve bacteria cluster shaped cocci, six of them ferment mannitol when grown on MSA, oxidase negative and produced catalase and coagulase They were identified as CoPS. The six CoPS produced DNase, which is indicative for identification as *S. aureus*. The 7th mannitol fermenting isolate did not produce coagulase.The other seven cluster shaped cocci were oxidase negative producing catalase but not coagulase, were identified as CoNS (Table 12).

Table (12): Frequency of CoPS and CoNS isolated from from CL units

Mannitol Fermentation	CoPS	CoNS
Fermenters	**6 (100%)**	**1 (12.5%)**
Non- fermenters	**-**	**7 (87.5%)**

4.6.3. Yeasts

Four Yeast- like isolates were detected on TSA and SDA as orang-pink colonies. The fourth one was cream-white colony on TSA. By simple staining, both show budding cells under the microscope (Fig. 7)

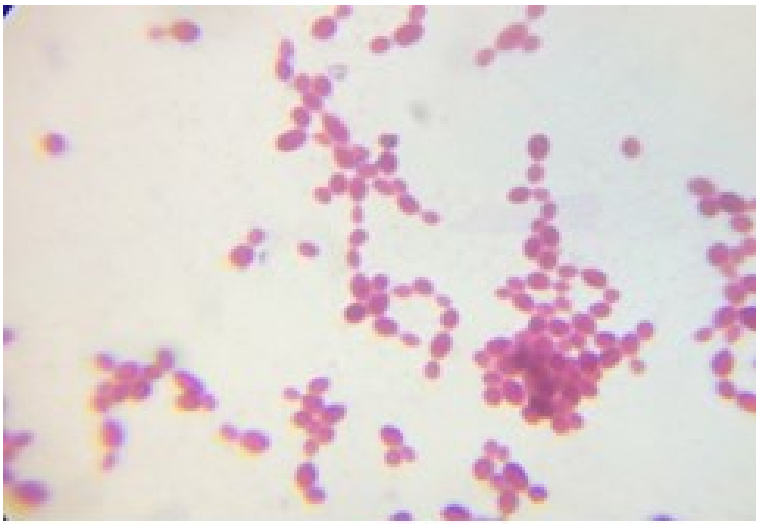

Figure (7): Simple stain of yeast under microscope isolated from CL case (X1000)

Frequency distribution of identified M.O. in CL cases and rims of solution bottles is shown in Table (13). Figure (8) illustrates distribution of identified M.O.

Table (13): Frequency distribution of microorganisms isolated from indicated contact lenses units.

Organisms	Right cases n= 33 (%)	Left cases n= 25 (%)	Rim n= 6 (%)	Total N=64
Pseudomonas spp.	6 (18.2%)	5 (20%)	-	11 (17.2%)
S. marcescens	3 (9.1%)	5 (20%)	-	8 (12.5%)
CoNS	3 (9.1%)	2 (8%)	3 (50%)	8 (12.5%)
S. aureus	2 (6.1%)	4 (16%)	-	6 (9.4%)
Pseudomonas aeruginosa	1 (3%)	3 (12%)	1 (16.7%)	5 (7.8%)
Shewanella putrefaciens	2 (6.1%)	-	1 (16.7%)	3 (4.7%)
Burkholderia pseudomallei	1 (3%)	1 (4%)	-	2 (3.1%)
Acinetobacter spp.	1 (3%)	1 (4%)	-	2 (3.1%)
A.calcoaceticus	2 (6.1%)	-	-	2 (3.1%)
Shigella spp.	2 (6.1%)	-	-	2 (3.1%)
S. liquefaciens	1 (3%)	-	-	1 (1.6%)
Salmonella spp	-	1 (4%)	-	1 (1.6%)
E. coli	1 (3%)	-	-	1 (1.6%)
Filamentous bacteria*	1 (3%)	-	-	1 (1.6%)
Short-rod shaped*	1 (3%)	1 (4%)	-	2 (3.1%)
Yeast	3 (9.1%)	-	1(16.7%)	4 (6.3%)
Non-Lactose fermenting**	3 (9.1%)	2 (8%)	-	5 (7.8%)

*Require further identification tests
** Lost isoates durning curfew

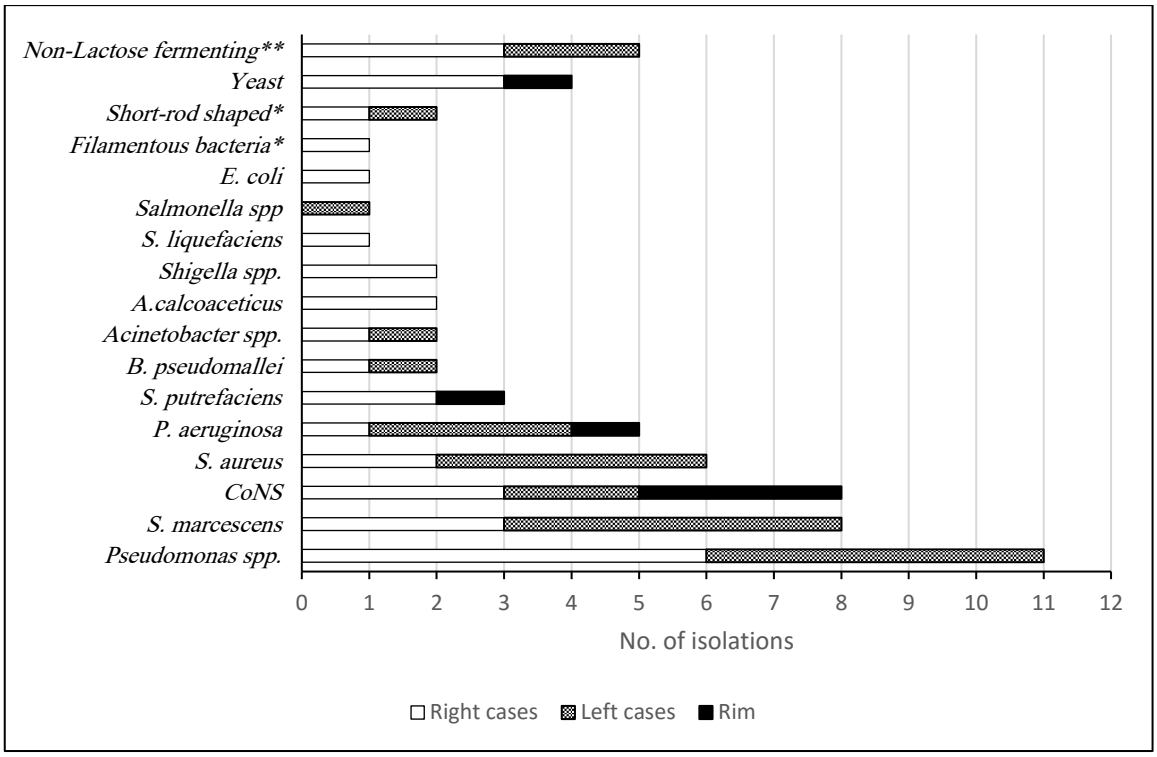

Figure (8): Frequency of microorganisms isolated from indicated contact lenses units
**Require further identification tests
** Lost isoates durning curfew

4.7. Antibiotic Susceptibility

4.7.1. Agar Dilution Method

This method was applied for *Staphylococcus spp*. only, whether CoPS or CoNS to examine their resistance to methicillin. Two (25%) CoNS isolates, one from CL case and the other from the rim of one bottle of the same CLs unit. Both slowed resistance to 6 µg/ml oxacillin, hence termed methicillin resistance (MRCoNS). Two *S. aureus* from different CL cases also showed resistance to 6 µg/ml oxacillin, thus termed MRSA. (Table 14).

Table (14): Methicillin Resistant *Staphylococcus spp.*

Antibiotic	Susceptibility	*S. aureus* N= 6	CoNS N= 8
Oxacillin 6 µg/ml	Resistant	2 (33.3%)	2 (25%)
	Sensitive	4 (66.7%)	6 (75%)

It should be noted that cells of MRSA appeared, under the microscope, larger in size than cells from colonies grown in medium without oxacillin (Fig. 9).

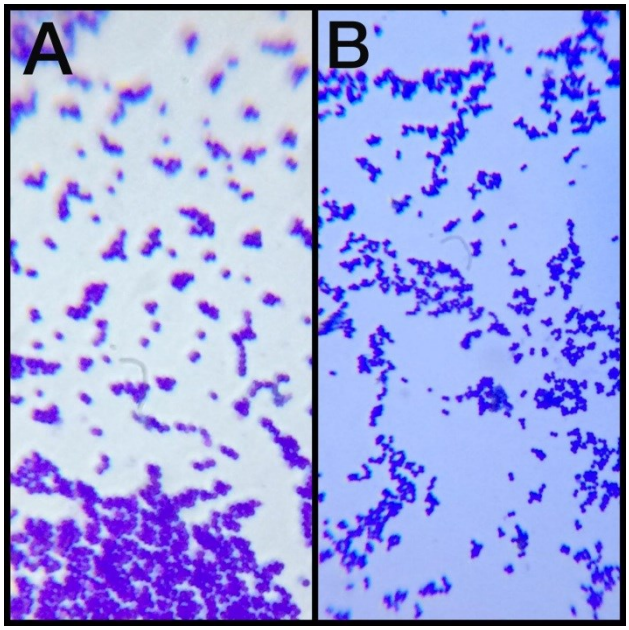

Figure (9): Gram stained *S. aureus* cells under microscope (A) MRSA cells from a colony grown on agar with oxacillin (B) Cells from a colony grown on nutrient agar (X1000)

4.7.2. Disc Diffusion Method

Table (15) shows antibiotic susceptibility of two isolates from of *S. marcescens*, and one isolate of each *S. liquefaciens* and *Acinetobacter spp*. toward eight antibiotics. Isolates of *S. marcescens* and *S. liquefaciens* isolate behaved similarly, they were resistant to six antibiotics, and these are: ceftazidime, cefotaxime, gentamicin, ceftriaxone, tetracycline, and cefepime. *Acinetobacter* isolate shows resistance to four antibiotics and was sensitive to ceftazidime, gentamicin and tetracycline.

Table (15): Antibiotic susceptibility of *Acinetobacter spp*., and *Serratia spp.* isolates.

Antibiotic (µg)	Zone Diameter Criteria (mm)			*Acinetobacter* Diameter (mm)	*S. marcescens* Diameter (mm)	*S. marcescens* Diameter (mm)	*S. liquefaciens* Diameter (mm)
	S	I	R				
Ciprofloxacin (5)	≥ 21	16–20	≤ 15	20 *	29.5 (±0.7)	27 *	30.5 (±0.7)
Ceftazidime (30)	≥ 18	15–17	≤ 14	24 (±1.4)	10 *	10 *	12 *
Cefotaxime (10)	≥ 23	15–22	≤ 14	20 *	16 (±1.4)	15 (±1.4)	17 (±1.4)

Gentamicin (10)	≥ 15	13–14	≤ 12	25 (±1.4)	12.5 (±0.7)	12 *	14 *
Levofloxacin (5)	≥ 17	14–16	≤ 13	27 (±1.4)	23.5 (±0.7)	24 *	26 *
Ceftriaxone (30)	≥ 21	14–20	≤ 13	20 *	N/A	N/A	N/A
	≥ 23	20–22	≤ 19	N/A	22.5 (±3.5)	20 *	20 *
Tetracycline (30)	≥ 15	12-14	≤ 11	20 *	-	4 *	4.3 (±0.4)
Cefepime (30)	≥ 18	15–17	≤ 14	26 *	13.5 (±2.1)	17 (±1.4)	-

*Standard Deviation = 0.
S: Sensitive, I: Intermediate, R: Resistance
N/A: Not applicable.

Table (16) shows susceptibility of MRSA and MSSA isolates toward three antibiotics. Both are resistant to penicillin and sensitive to amoxicillin-clavulanic acid and vary toward erythromycin.

Table (16): Antibiotic susceptibility of MRSA and MSSA isolates

Antibiotic	Zone Diameter Criteria (mm)			MRSA	MSSA
	S	I	R		
Pencillin (10 units)	≥ 29	-	≤ 28	28 *	28.5 (±2.1)
Amoxicillin-clavulanic acid (30 µg)	≥ 20	-	≤ 19	22 (±1.4)	19.5 (±0.7)
Erythromycin (15 µg)	≥ 23	14–22	≤ 13	2 (±2.8)	20 *

*Standard Deviation = 0.
S: Sensitive, I: Intermediate, R: Resistance

4.8. Survival Study

Log reduction at the minimum recommended disinfection time for the two solutions: A and B against *S. marcescens*, *S. liquefaciens*, *Acinetobacter spp*., MRSA and MSSA in clean and dirty conditions is illustrated in Tables 16 and 17 respectively. Generally, reduction in bacteria growth is evident in clean as compared to dirty condition.

Table (17): Efficacy of solution A in log reduction of tested bacteria under clean and dirty conditions, after 4hr.

Test bacteria	Clean	Dirty
Acinetobacter	5.625312	5.535294
S. marcescens	5.585461	5.553883
S. liquefaciens	5.609594	5.574031
MRSA	5.580925	5.536558
MSSA	5.556303	5.444045

Table (18): Efficacy of solution B in log reduction of tested bacteria under clean and dirty conditions, after 4hr.

Test bacteria	Clean	Dirty
Acinetobacter	5.6148972	5.342423
S. marcescens	5.5888317	5.519828
S. liquefaciens	5.5599066	5.394452
MRSA	5.6148972	5.541579
MSSA	5.553883	5.318063

Maximum efficacy was against *S. liquefaciens* and *Acinetobacter spp*. in both clean and dirty conditions (Fig. 10 and 11).

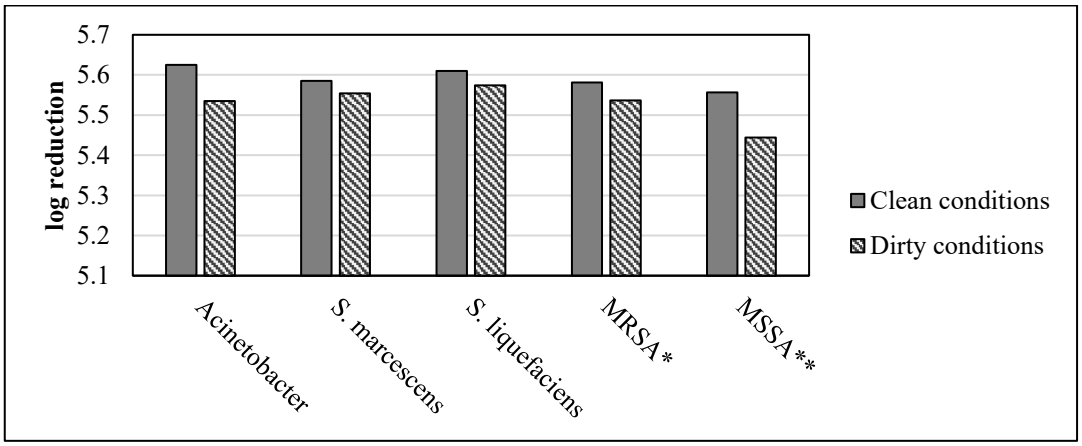

Figure 10: Log reduction of tested bacteria solution A under clean and dirty conditions, after 4 hr.

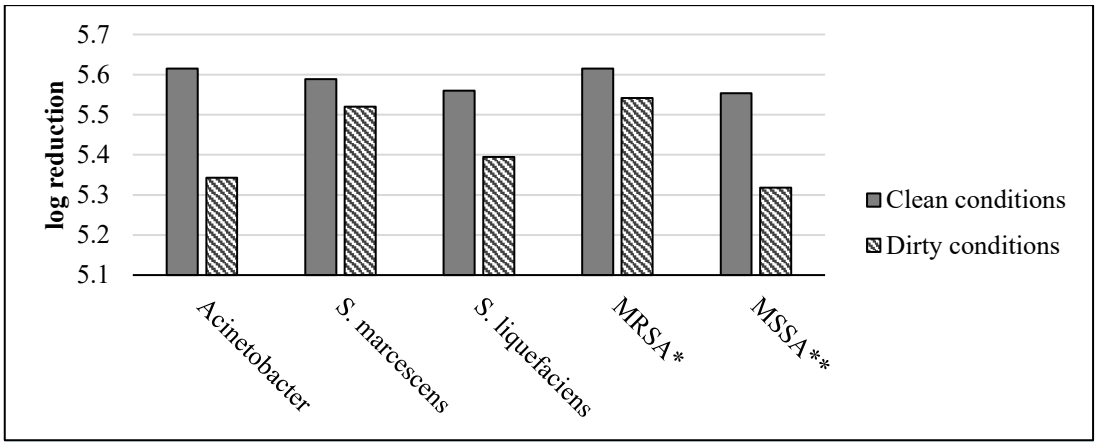

Figure 11: Log reduction of tested bacteria in solution B under clean and dirty conditions, after 4 hr.

4.9. Biofilm Formation and Inhibition

Adhesion to plastic surface by *S. marcescens*, *S. liquefaciens*, *Acinetobacter* spp., MRSA, and MSSA was reported positive by staining the inner sides of the tubes. Optical density was measured twice, after 4 and 24 hr. of application CLs solutions (Table 19 and 20).

Table (19): Optical density and inhibation biofilm using solution A against isolates for two time periods under clean and dirty conditions.

Test bacteria	OD of Control	OD of Clean conditions	Inhibition biofilms (%)	OD of Dirty conditions	Inhibition biofilms (%)	Period
Acinetobacter spp.	0.177	0.052	70.6%	0.053	70.1%	After 4hr
S. marcescens	0.275	0.055	80%	0.054	80.4%	
S. liquefaciens	0.37	0.051	86.2%	0.055	85.1%	
MRSA	0.645	0.061	90.5%	0.055	91.5%	
MSSA	0.192	0.065	66.1%	0.056	70.8%	
Acinetobacter spp.	0.1	0.055	45%	0.058	42%	After 24hr
S. marcescens	0.38	0.057	85%	0.059	84.5%	
S. liquefaciens	0.38	0.061	83.9%	0.058	84.7%	
MRSA	0.95	0.364	61.7%	0.37	61.%	
MSSA	0.1	0.052	48%	0.052	48%	

Table (20): Optical density and and inhibation biofilm using solution B aganist isolates for two time periods under clean and dirty conditions.

Test bacteria	OD of Control	OD of Clean conditions	Inhibition biofilms (%)	OD of Dirty conditions	Inhibition biofilms (%)	Period
Acinetobacter spp.	0.177	0.052	70.6%	0.057	67.8%	After 4hr
S. marcescens	0.275	0.129	53.1%	0.11	60%	
S. liquefaciens	0.37	0.147	60.3%	0.151	59.2%	
MRSA	0.645	0.053	91.8%	0.064	90.1%	
MSSA	0.192	0.053	72.4%	0.069	64.1%	
Acinetobacter spp.	0.1	0.05	50%	0.067	33%	After 24hr
S. marcescens	0.38	0.06	84.2%	0.098	74.2%	
S. liquefaciens	0.38	0.06	84.2%	0.101	73.4%	
MRSA	0.95	0.35	63.2%	0.367	61.4%	
MSSA	0.1	0.06	40%	0.068	32%	

Figure (12) and (13) clarify the percentage of biofilms inhibition as compared to control for each bacterium. After 4hr, solutions A and B were able to reduce biofilms formation by more than 50% of all tested bacterial biofilms, regardless of the cleanness/dirtiness status. Biofilms produced by *S. marcescens* and *S. liquefaciens* using solution A archived significantly higher inhibition rates when compared to solution B (Fig 12). MRSA biofilm inhibition percentage was by far the most affected by both solutions after 4 hr (Fig 12).

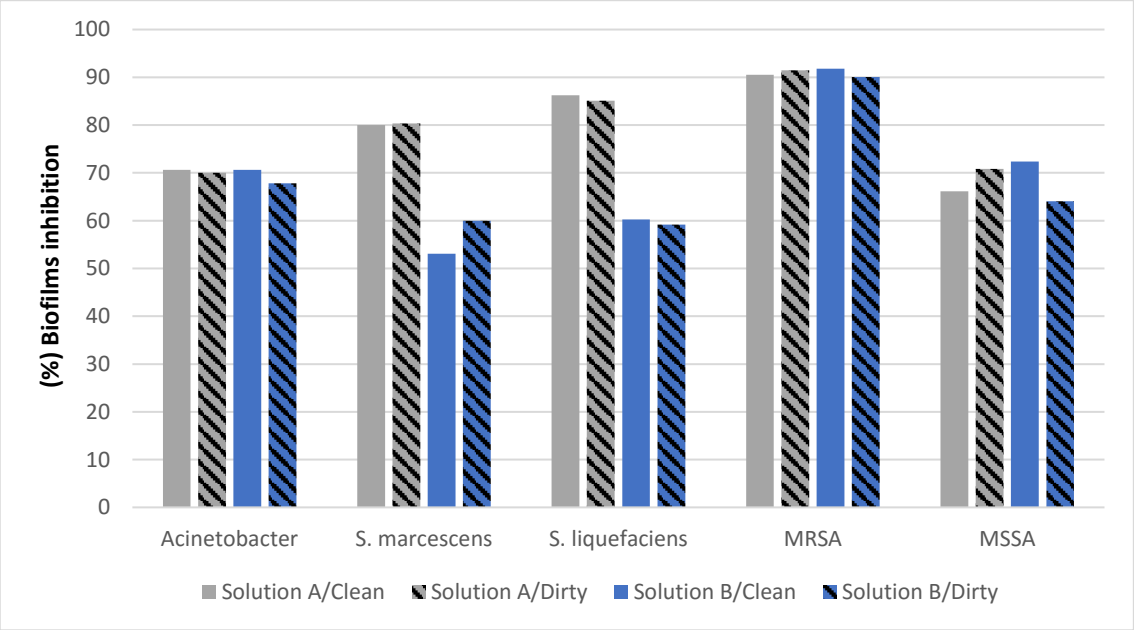

Figure (12): Efficacy of solution A and B in inhibition biofilm formation after 4 hrs.

In contrast, after 24hr, only *S.marcesence, S.liquefaciens,* and MRSIA were able to achieve inhibition rates exceeding 50%, regardless of the cleanness/dirtiness status. Generally, the biofilm reduction abilities of solution A and B were comparably similar across all types of bacterium. Compared to results achieved after 4hr (Fig 12), the percentage of biofilm inhibition of *Acinetobacter*, MRSA and MSSA were comparably lower after 24hr (Fig 13).

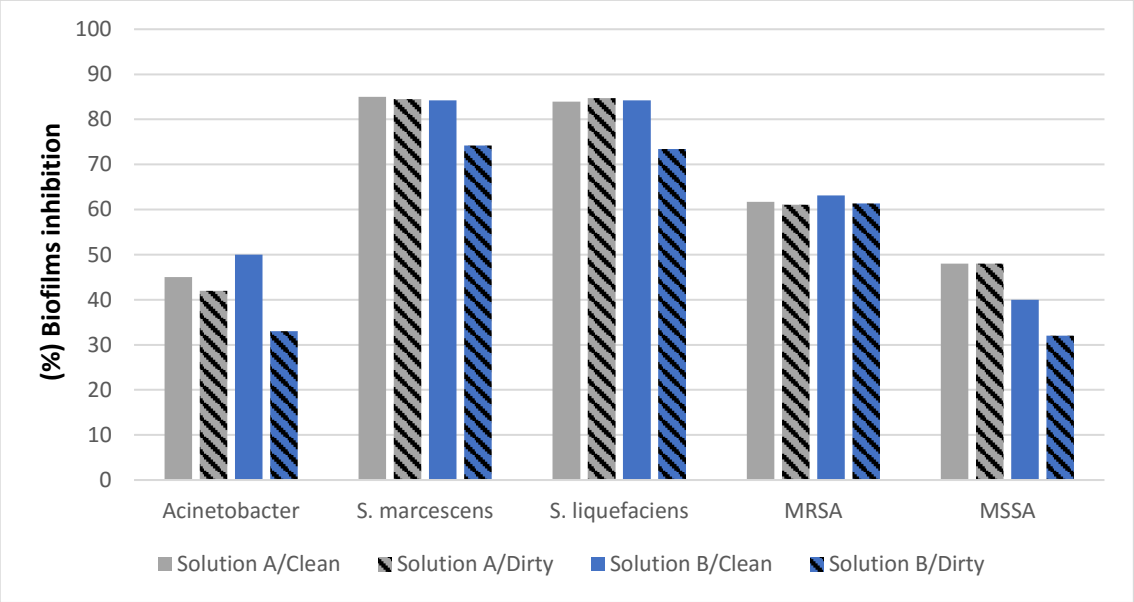

Figure (13): Efficacy of solution A and B in inibition biofilm, after 24 hrs.

5. Discussion:

The present study has shown that 80% of participants, who wear CLs for periods ranging from 5 to 12 hours daily, have contaminated CL units, particularly, those wearing CLs for periods exceeding 9 hours (Table 2). This is in accordance with the study conducted among Indian college students which showed that 70% of those who reported problems related to use of CLs (e.g., foreign body sensation, dry eyes, and watering eyes) wear CLs 8 to 16 hours daily (Unnikrishnan and Hussain, 2009).

In Table (3), most CL wearers, who suffered from eye redness after wearing CLs, had microbial contamination in their CL units which is in accordance with McVeigh et al. (2017).

Although (60%) of CL wearers have received instructions for handling CLs from health professional, 88.9% of their CL units were found exposed to contamination (Table 4). This may be due to the quality of information received or poor commitment of lens wearers to these instructions. According to Lievens et al. (2017), contact lens wearers education and compliance with hygiene habits are some of challenges facing care providers, where experience of wearers is not necessarily sufficient for commitment and protection from risks.

Bakkar and Alzghoul (2020) concluded that level of commitment is high for four habits, these are: personal use of CL, not sleeping while wearing, hand washing before wearing and not using water to clean lenses. In addition, level of commitment was medium to low towards the following habits: bathing or swimming while wearing, using CLs and solutions longer than expired date, and rinsing CL cases and aftercare visits. These almost agree to participants' awareness in the present study (Tables 5 and 6).

Although all CL wearers wash their hands before wearing, microbial contamination was higher in CL units of wearers washing their hands alternatively with water or soap compared to those who use soap always. Barlow et al., (1994) indicated that using of antibacterial liquid soap greatly inhibit microbial contamination in CLs compared to ordinary soap or using water only or those not washing their hands before wearing CLs.

Water or water mixed with solution to wash CL cases was used by some participants in the present study which is in accordance with Zimmerman et al. (2017), study who reported that many CL wearers use tap water to wash CLs and CL cases. Microbial contamination may result from using water. Tap water may contain dangerous M.O. Minogue et al. (2015) detected many bacterial species such as *P. aeruginosa, Burkholderia cepacia,* and *S. marcescens* in distribution systems of water. However, even distilled water if used to wash CL cases, CLs solution remains the most effective for cleaning. Nevertheless, Wu et al. (2011) used CL solutions to wash lens cases by different ways and used distilled water as an alternative control for the solution. Regardless of the washing method and type of solution, they found that all solutions exert a significant decrease in biofilm compared to water (P= 0.05).

According to instructions of use labeled on the bottles of CL solutions, the solution should not be used for more than three months after opening. In the present study, there were statistically significant differences between duration periods for using solution and microbial contamination in CL units ($p = 0.020$). CL solutions, of the overwhelming majority of wearers used solutions for more than three months were found to have microbial contamination (Table 7). However, contamination was reported in 90% of CL units wearers using solutions for less than one month, which may be due to the fact that almost one half (45.5%) of them use water when solution was not

available. Dantam et al., (2016) have reported a significant difference ($p = 0.013$) between Gm-ve bacteria contaminated of CL cases with different CL solutions types.

Commensal skin microorganisms present normally on lid margins and conjunctivae in addition to potential transient pathogens that may be found on the ocular surface will contaminate CLs in vivo. When solutions in lens cases, adhere to the lens, it may easily become contaminated and become a source of microbes that cause infection of the cornea and inflammatory reaction (Szczotka-Flynn et al., 2010).

In present study, more than one half CL wearers use their contact lenses for more than one year and have microbial contamination, which is largely inconsistent with the FDA (2019) recommendations. However, Kim et al., (2017) did not find microbial contamination in all expired or nearly expired CLs but there was a statistically significant modification in CLs shape, thickness, and diameter. This change may lead to variations in CLs function

Swabs were taken from rims of solution bottles because rims are the first point that disinfectant solution pass through before reaching CLs, also it may touch solution of CL cases while immersing CLs. This was confirmed by the recovery of the same type of M.O in the rim of bottles as well as its CL cases. However, absence of contamination in solution bottles indicates that microbes present in the CL cases are because of some wearer's personal habits and not solution bottle. Nevertheless, Nzeako and Al-Sumri (2011) found that CL disinfectant solution was contaminated by the same organisms in CL cases, so solution in bottle was contaminated before CLs immersion in its cases.

In the preliminary experiments, organisms were not able to grow due to the presence of amounts of residual disinfectant. Because concentration exponent of the active agent is low (Biguanide = 1.5-1.6), hence, dilution will not be effective for

neutralizing residual disinfectant (Denyer et al., 2011). Therefore, addition of the neutralizer Tween 80 was adopted according to Kelsey (1974), 3% Tween 80 diluted in TSB was added to inactivate residual disinfectant.

Bacterial isolates reported in the present study included pathogens and normal flora of gastrointestinal tract, skin, and environment which may cause CLs related MK. Incidence of contamination is greatly affected by the manner of handling each item of CL unit, improper hygienic practices and failure of some preservative systems were implicated in the development of the contamination. When lens wearers use bared fingers during immersion of lenses or removal from the disinfecting solutions; faecal bacteria including *Enterobacter*, *Serratia* and *Klebsiella* species may stuck in lens case and subsequently can be transferred to the disinfectants. *Serratia* and *Pseudomonas* species are known for their resistant to some disinfecting solutions (Willcox, 2011).

Right CL cases were the most frequently contaminated, and up to three different microbes were recovered in some of them (Table 9). Despite disinfecting agent, contact lens cases may not be completely free of contaminants, because some factors may lead constant bacterial survival. For example, CL disinfection solutions seemed selective for contamination with cytotoxic strains as *P. aeruginosa* (Lakkis and Fleiszig, 2001). Biofilm formation is another factor, it can form in a contact lens case, which protect bacteria and prevent disinfectant solution to reach it (McLaughlin-Borlace et al., 1998). Eltis (2011) and Mohammadinia et al. (2012) reported that 90% of the causative agent of microbial keratitis is *P. aeruginosa*, followed by *S. aureus.*

S. marcescens poses a high risk for CL wearers as it is the most common cause of microbial keratitis (Wu et al., 2015); it has the ability to produce proteases to destroy corneal cells, and over activation of host immune system during MK (Willcox, 2007). *S.*

marcescens isolated from CL cases in the present study were non-pigmented which is related to nosocomial infections (Zhou et al., 2016). In the present study, two *S. marcescens* and one *S. liquefaciens* behave similarly toward antibiotics, they were found resistant to ceftazidime, cefotaxime, gentamicin, ceftriaxone, tetracycline, and cefepime (Table 15). Simsek (2019) reported that clinical isolates of *Serratia* have a high resistant rate to ceftriaxone and ceftazidime, while rate of resistance to cefotaxime and gentamicin was very low. Also, in Boston hospital, 97% of *Serratia spp.* were found resistant to tetracyclines (Chopra and Roberts, 2001).

Two isolates of *Acinetobacter* identified by Acinetobacter agar, but species were not identified due to the lack of media-specific supplementation. One *Acinetobacter* isolated from CL cases was resistant to ciprofloxacin, cefotaxime, ceftriaxone, and cefepime (Table 15). Sohail et al. (2016) revealed that *Acinetobacter* clinical isolates were mostly resistant to cefotaxime, ceftazidime and least resistant was shown against gentamicin. It seems that susceptibility of *Acinetobacter* depends on its source (Askari, et al 2020).

One of *Staphylococcus* isolated from CL cases ferments mannitol but did not coagulase rabbit plasma. Shittu et al., (2006) indicated that further tests preferably molecular are required for such isolates to differentiate them from *S. aureus.* Kateete et al. (2010) reported that efficacy of coagulase test may be improved by mannitol fermentation on MSA, and production of DNase for the identification of *S. aureus*.

Agar dilution method with oxacillin is more reliable than disc diffusion for detecting MR. Oxacillin is out performing methicillin in maintaining its effectiveness during storage; besides, methicillin is considered commercially missing. Strains that are

oxacillin and methicillin resistant are described as methicillin resistant for historical interest (CDC, 2019).

In present study, resistance to methicillin was reported when *S. aureus* isolates grow on MSA supplemented with 6 µg /ml of oxacillin, while it was considered resistant if grow with 2 µg /ml of oxacillin concentration (Askarian et al., 2009). Mueller-Hinton agar was reinforced by 4% NaCl to make the media more hypertonic which is recommended to improve MRSA growth, in addition to incubation below 35°C. Both media gave similar recovery frequency of methicillin resistant *Staphylococci*.

Cells of MRSA isolated from CL units grown on agar with oxacillin was observed under light microscope to have slightly larger size. This was explained by García et al. (2017) who compared between MRSA and MSSA cell wall and septum thickening under electron microscopy to detect resistance changes in morphology of membrane. They concluded that methicillin resistance was associated with an increase in cell wall and division septum thickness. However, increase in size may be due to methicillin resistance and/or for survived in a growth condition with high salt content in the presence of mannitol.

In the present study, MRSA isolate was resistant to all antibiotics tested except amoxicillin-clavulanic acid, this may be caused by clavulanic acid present (Table 16). However, according to Côté et al. (2019) study, MRSA isolated from hospitalized patients, most of MRSA were resistant to penicillin, amoxicillin-clavulanic acid, and erythromycin.

In present study, *S. putrefaciens* was isolated from CL cases and rim of solution bottle. *S.putrefaciens* is rarely reported from CLs, Nevertheless, Bôas et al. (2018) have

isolated *S. putrefaciens* from contact lens cases of workers at Hospital de Base in São José do Rio Preto and from people who did not visit hospitals.

S.putrefaciens from infected ear, hospital beddings and floor was isolated by Al-Hadithi and Attia (2015), they demonstrated its ability to form biofilms and described the ability of the antiseptic to reduce biofilm formation.

Shigella was isolated from different CL cases of different wearers. *Shigella* is rarely reported to be associated with CLs. Wiley et al. (2012) reported isolation of *Shigella* from CLs.

According to International Standards Organization (ISO) 14729 guidelines that determined standard in industry of active CLs disinfecting solutions against microorganisms, CLs solution is considered effective, if it reduces viability of initial concentration of bacteria species by at least 3 log which is (99.9%) of bacteria concentration at recommended exposure time (Rosenthal et al., 2002).

Although there were differences between the efficacies of the two solutions, both exceeded the required 3.0 log reduction in growth of isolates recovered from CL units (Figure 10 and 11). Lever and Borazjani (2001) tested efficacy solution contain PAPB, they found it was exceeding the minimum acceptable criteria after one hour which is one quarter of labeled minimum disinfection time with reference strain.

The two solutions were shown to be more effective in clean conditions than in dirty conditions. Polyhexanide works by electrostatic interaction, it has positive charge that binds to phosphate negative charge of phospholipids at bacteria cell wall, protective out layer and cell membrane were splintered, then cytoplasm leak causing cell death (Kaehn, 2010). This means that the disinfectant is reacting with the organic matter instead of bacteria or react with impurities adhered to bacterial cell surface then prevent

PHMB or/and PAPB from binding to bacterial cell wall. This explains importance of good cleaning practices where impurities reduce disinfectant efficacy; thus, providing more suitable environment for microbes.

According to Laxmi Narayana et al. (2018) study, two solutions: containing PAPB and PHMB reached the 3 log and 5 log reduction criteria, respectively. Also, they observed that effectiveness of CL solutions varies against different bacterial species such as *S. aureus* and *S. marcescens*.

After 4hr, biofilms produced by *S. marcescens* and *S. liquefaciens* using solution A archived significantly higher inhibition rates when compared to solution B (Fig 12). Artini et al. (2015) concluded that CL solutions able to inhibit biofilm formed by *S. marcescens* and *S. aureus* after 4 hrs.

Noteworthy, after 4 hours, MRSA biofilm reduction was the most affected by both CL solutions used in present study (Fig.12). Kamaruzzaman et al. (2017) suggest that PHMB is effective against *S. aureus* and can damage biofilm structures between 28 to 37% of biofilm produced by *S. aureus*.

Strengths:

1. The study design included two parts: A questionnaire-based on attitude, practice and hygienic habits connected to CLs handling and a laboratory-based CLs microbiological profiling.

2. This study has implemented a variety of cultural, morphological and biochemical characterization tests which significantly increase the accuracy of microbial identification.

3. The unique aspect of this study that no literatures are available on swabbing rims of solution bottles which provided valuable information on this topic.

Limitation

The study faced a number of limitations in methodology such as:

1. Small sample size
2. Only female participants were available
3. Direct eye swab was not included
4. Molecular investigation is not available to improve identification of M.O.

In addition, a number of logistic hurdles was experienced, mainly a direct consequence of COVID-19 pandemic. On March 17, 2020, the Jordanian government imposed 24-hours, nationwide curfew to limit the spread of Corona virus. Thus, reaching university's facilities to check on the ongoing research experiments was impossible. As a result, a number of microbial cultures were ruined and perished due to power outage that affected the microbiology research lab's onsite refrigerator, therefore. it was not possible to identify a number of bacteria that were lost. Standard strains were lost, so it was not possible to compare with results of isolates from CL units.

6. Conclusions and Recommendations

The present study has identified important findings about CLs hygiene of students in university, namely:

1. Prevalence of microbial contamination in CL units among 30 wearers was high (83.3%)
2. Long hours of wearing CLs and eye redness after CLs wearing were almost statistically significant in existence of microbial contamination.
3. Microbial contamination was higher in CL wearers using water for washing hands before wearing and storage of CLs or those using solutions for more than three months.
4. *Pseudomonas spp*. was the most frequent Gm-ve bacteria isolated followed by *S. marcescens*, CoNS species and *S. aureus*.
5. MRSA and MRCoNS were isolated from CL units.
6. Solution A and B are the most frequent CL solutions among CL wearers. They exceeded the ISO 14729 acceptable criteria in log reduction of bacterial growth.
7. Dirty condition has marked effect in reducing CLs solution efficacy.
8. After 4hr, solutions A and B were able to reduce biofilms formation by more than 50% of all tested bacterial biofilms, regardless of the cleanness/dirtiness status.

Recommendations

1. Care practice including well hand washing with soap and water and drying them before both CL wearing and removing.

2. Contact lenses and CL cases should be rubbed by disinfectant solution and avoid using water.

3. Daily disposable CLs are very convenient, and less susceptible to infection complication than other lens types.

4. Contact lenses should not be used for longer than prescribed to use.

5. New solution should not be added to the old one or vice versa. Solution in CL case should be changed at recommended time because it has already become dirty due to the CL and less effective.

6. CL cases must also be replaced every three months at least, even if it looks hygienic; as increased period (time) of wearing leads to increased risk of contamination.

7. Importance of visiting clinics and examination of eye safety periodically should not be neglected.

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Appendix

Research Questionnaire:

Participant's number _____

- Name _____ (Optional)
- Nationality_____
- College _____

(Mandatory)

Gender

- Female
- Male

Have you ever studied microbiology at undergraduate?

- Yes
- No

What is the main reason for wearing contact lenses?

- Medical (Vision Correction)
- Cosmetic

What type of contact lenses does you use?

- Daily use
- Long-lasting use

Have you ever worn contact lenses for daily use for more than a day?

- Always
- Often
- Sometimes
- Rarely
- Never

How long have you used your current lens? (Participate in the study)

- Less than month
- 2-3 months
- 4-6 months
- 7-12 months
- More than 12 months

What is the brand name of contact lenses that you participate in this study?

_____

How long have you started using contact lenses for the first time?

- Less 3 months
- 3-6 months
- 7-12 months
- More than 12 months

When you first started using contact lenses, did your doctor, pharmacist, or health practitioner explain to you the basic recommendations for using and maintaining contact lenses?

- Yes
- No

How do you assess your knowledge of the recommendations for maintaining contact lenses and the steps to take care of them when wearing them and removing them?

- Excellent
- Very good
- Good
- Weak
- I do not now

Through your knowledge about the recommendations for maintaining contact lenses and the steps to take care of them, do you think that you adhere to the recommendations when wearing and removing glasses?

- Always
- Often
- Sometimes
- Rarely
- Never

Whether you wear cosmetic or medical lenses, what is your average need to wear contact lenses normally?

- Daily
- Weekly
- Monthly

If you wear contact lenses daily, what is the average number of times you wear and remove contact lenses in approximately one day?

- Once
- Twice
- More than twice

What is the average number of hours during which you wear contact lenses every time?

- 1-4 hours
- 5-8 hours
- 8 hours
- More than 12 hours

Do you consider removing contact lenses when sleeping?

- Always
- Often
- Sometimes
- Rarely
- Never

Do you remove contact lenses before bathing or swimming?

- Always
- Often
- Sometimes
- Rarely
- Never

Do you consider not washing your face with water while wearing contact lenses?

- Always
- Often
- Sometimes
- Rarely
- Never

Have you ever been a smoker of any type of smoking?

- Yes
- No

Are you a smoker at the moment? (Ignore the question if you answered question 18 “no”)

- Yes
- No

How many cigarettes do you smuggle daily?_____

Are any of your family members or smokers sharing your home?

- Yes
- No

Do you avoid smoking or smoke-prone areas while wearing contact lenses?

- Always
- Often
- Sometimes
- Rarely
- Never

Have you previously shared your contact lenses with another person?

- Yes
- No

When you wear contact lenses, do you wear them without your help?

- Always

- Often
- Sometimes
- Rarely
- Never

Before you start wearing or contacting your contact lenses, do you wash your hands?

- Always
- Often
- Sometimes
- Rarely
- Never

If you wash your hands before contacting contact lenses, what do you often use to wash hands?

- Water
- Soap
- Soap or water

If you wash your hands before contacting and wearing contact lenses, do you dry them?

- Always
- Often
- Sometimes
- Rarely
- Never

If your fingernails are not striped and you want to wear or remove contact lenses, do you take care that your nails do not come in contact with the lens?

- Always
- Often
- Sometimes
- Rarely
- Never

Do you use tongs or any other tool to wear lenses instead of holding them with fingers?

- Yes
- No

Do you wash contact lenses before or after wearing them?

- Always
- Often
- Sometimes
- Rarely
- Never

If you wash your contact lenses, would you do this by rubbing them with one finger on the palm of the palm with the solution? (Ignore this question if your answer to question 30 is "never")

- Always
- Often
- Sometimes
- Rarely
- Never

If you wash it another way, mention it:

_____

_____

What are you used to moisturize and store contact lenses?

- Contact lenses solution
- Water

Have you ever used water to moisten contact lenses when a multi-use solution is not available? (Disregard the question if you answer question 33 "water")

- Always
- Often
- Sometimes
- Rarely
- Never

What type of preservative solution do you use to moisturize contact lenses?

- Contact lenses solution from pharmacy
- Contact lenses solution with lenses

What is the brand name of your solution?

_____

_____

How long have you used the current solution? (Study participant)

- Less than month
- 2-3 months
- 4-6 months
- 7-12 months
- More than 12 months

On average, how often do you add solution to contact lenses to moisturize them?

- Daily
- Weekly
- Monthly

Have you previously added your remaining old solution to the new solution box?

- Always
- Often
- Sometimes

- Rarely
- Never

Have you ever packed the contact lens solution in a smaller bottle to make it easier to hold and carry around?

- Always
- Often
- Sometimes
- Rarely
- Never

Do you wash contact lens storage case?

- Always
- Often
- Sometimes
- Rarely
- Never

What do you use when you wash your contact lens storage case? (Skip this question if your answer to question 41 is "never")

- Water
- Solution
- Water and solution

Would you replace contact lens storage case with a new storage case?

- Always
- Often
- Sometimes
- Rarely
- Never

Have you ever experienced irritation or redness in the eye while wearing contact lenses?

- Always
- Often
- Sometimes
- Rarely
- Never

Have you ever had a beggar eye (boil in the eyelid) or an infection in the eyelid after wearing contact lenses?

- Always
- Often
- Sometimes
- Rarely
- Never

Have you ever had any disease or infection in the eye?

- Yes
- No

What are the symptoms that you suffer from in the eye? (Disregard this question if you answered question 46)

_____

_____

Have these symptoms been diagnosed by any doctor, pharmacist or health practitioner?

- Yes
- No

What is the diagnosis your doctor has told you? (Ignore this question if you answered question 48)

_____

_____

What has the doctor prescribed you a treatment for?

_____

_____

If you have any comments that you would like to clarify, include them here:

_____

_____

_____

_____

Abstract in Arabic

العدسات اللاصقة عبارة عن جهاز تجميلي أو طبي بديل أكثر راحة وملاءمة من النظارات. ولضعف دفاع الغرفة الأمامية للعين تجاه الغزو الميكروبي بسبب قلة تدفق الدم فإن تشوب العدسات اللاصقة بالميكروبات يؤدي إلى حصول عدوى في العين.

تبحث هذه الدراسة في التشوب الميكروبي الحاصل في حافظات العدسات، وقارورة المحاليل وفوّهات قارورة المحاليل، وتقيّم من خلال استبيان لعادات وممارسات ثلاثين شخصًا من مرتدي العدسات نحو العناية بها لفهم العوامل المرتبطة بتشوب وحدات العدسات المختلفة. تم تشخيص المستعمرات المعزولة من الاوساط الزعية الانتقائية من خلال الاختبارات الشكلية والكيمو حيوية. وجد ان خمس (16.6) من وحدات العدسات غير مشوبة. كان احمرار العين بعد ارتداء العدسات له علامة ذات دلالة إحصائية تقريبًا مرتبطة بتلوث وحدات العدسات اللاصقة (P= 0.088). تم الحصول على 64 عزلة من وحدات العدسات اللاصقة المشوبة. وكانت أكثر أنواع الجراثيم شيوعًا هي (*Pseudomonas spp* %25) و (*Staphylococcus* spp.%21.9) و (*Serratia spp.*%14.1) و (*Acinetobacter spp.* %6.2).

كما تم الحصول على أربعة عزلات خمائر. تجاوزت قدرة اثنين من محاليل العدسات اللاصقة في تقليل اعداد الجراثيم باعلى من الحد المطلوب (3 لوغارتم) الى 5 لوغارتم. بعد 4 ساعات، كان المحلولان A و B قادرين على تقليل تكوين الأغشية الحيوية بأكثر من 50٪ لجميع الكائنات المختبرة، بغض النظر عن الحالة النظافة / المتسخة.

يؤدي استخدام الماء لغسل اليدين وحافظات العدسات الى زيادة في نسبة التلوث فضلا عن التشوب الميكروبي وبدورهما يؤديان إلى تقليل فعالية محلول العدسات. يجب رفع مستوى الوعي عند مرتدي العدسات اللاصقة والارتقاء بمعلوماتهم في التعامل الصحيح والسليم معها من خلال الزيارة المنتظمة الى أخصائي العيون.