ANTIMICROBIAL EFFICACY OF CONTACT LENS SOLUTIONS AGAINST *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS*

BY

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ประสิทธิภาพของน้ำยาล้างคอนแทคเลนส์ในการยับยั้ง *PSEUDOMONAS AERUGINOSA* และ *STAPHYLOCOCCUS AUREUS*

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โดย

เมธาวี หวงพลานันท์

วิทยานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตาม
หลักสูตรวิทยาศาสตรมหาบัณฑิต สาขาวิชาวิทยาศาสตร์ชีวการแพทย์
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กิตติกรรมประกาศ

วิทยานิพนธ์ฉบับนี้ สำเร็จลุล่วงไปได้ด้วยดี ด้วยความอนุเคราะห์จาก อาจารย์ที่ปรึกษาวิทยานิพนธ์ ผศ.ร.ต.ท.หญิง ดร.อัจฉราวรรณ ทองมี อาจารย์ประจำมหาวิทยาลัยรังสิต ซึ่งได้ประสิทธิ์ประสาทวิชาความรู้ และให้การสนับสนุนในทุกด้านด้วยความเอาใจใส่ รวมทั้งให้ข้อแนะนำ การตรวจทานเอกสาร และแก้ไขข้อบกพร่องต่างๆ ทุกขั้นตอนจนทำให้การศึกษาวิจัยและการจัดทำวิทยานิพนธ์ฉบับนี้เสร็จสมบูรณ์ จึงใคร่ขอกราบขอบพระคุณท่านที่เมตตาให้ความช่วยเหลือเป็นอย่างดีที่สุด

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MEDHAVI HOUNGPARANUN: ANTIMICROBIAL EFFICACY OF CONTACT LENS SOLUTIONS AGAINST *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS.* THESIS ADVISOR: ASST. PROF. POL. LT. ACHARAWAN THONGMEE, Ph.D., 38 p.

Contact lenses are used as an alternative to eyeglasses for correction of eyesight problems such as myopia, hyperopia and astigmatism. However, contact lenses wearers, especially extended wearers, are at risk for bacterial keratitis. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most common pathogens causing bacterial keratitis. To minimize the risk of eye infections, contact lens care solutions were used in order to decrease the amount of potential pathogens during contact lens storage.

The purpose of this study was to evaluate the antimicrobial activity of multipurpose contact lens care solutions available in Thailand against the common causes of bacterial keratitis, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Four multipurpose contact lens care solutions, designed as A, B, C and D, were determined for antimicrobial activities against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC25930 and *Pseudomonas aeruginosa* ATCC2785 by agar-well diffusion method. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were also determined. In addition, the contact time between test bacteria and each contact lens care solution was determined for the effective duration time that can reduce 99.9% (3logs) of test bacteria.

The results showed that all test contact lens care solutions did not show inhibition zone when tested with methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin-sensitive *S. aureus* (MSSA) ATCC25930 and *Pseudomonas*

aeruginosa ATCC2785 by agar-well diffusion method diffusion. However, the test contact lens care solutions at the concentration ranging between 12.5-25% of the initial concentration inhibited the growth of all test bacteria. This study also revealed that contact lens care solution D has more killing effect than contact lens care solution A, B and C since it can reduce the number of test bacteria more than 99.9% (3logs) in 2 hours. The different in killing effect might be due to the difference in active ingredients in each solutions. Therefore, the information from this study may be helpful for formulation of contact lens care solutions with more potent antibacterial activity.

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คำสำคัญ : น้ำยาล้างคอนแทคเลนส์

เมธาวี หวงพลานันท์: ประสิทธิภาพของน้ำยาล้างคอนแทคเลนส์ในการยับยั้ง *PSEUDOMONAS AERUGINOSA* และ *STAPPHYLOCOCCUS AUREUS* (ANTIMICROBIAL EFFICACY OF CONTACT LENS SOLUTIONS AGAINST *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS*) อาจารย์ที่ปรึกษา: ผศ.ร.ต.ท.หญิง ดร. อัจฉราวรรณ ทองมี, 38 หน้า.

ในปัจจุบันมีการนำคอนแทคเลนส์มาใช้เพื่อแก้ปัญหาสายตา ได้แก่ สายตาสั้น สายตายาว และสายตาเอียง แต่การใช้คอนแทคเลนส์โดยเฉพาะผู้ที่ใช้คอนแทคเลนส์เป็นเวลานานมีความเสี่ยงการติดเชื้อที่กระจกตา ซึ่งแบคทีเรียที่เป็นสาเหตุสำคัญก่อให้เกิดการติดเชื้อที่กระจกตาได้แก่ *Pseudomonas aeruginosa* and *Staphylococcus aureus* ดังนั้นจึงมีการใช้น้ำยาล้างคอนแทคเลนส์ทำความสะอาดและแช่คอนแทคเลนส์ เพื่อลดจำนวนแบคทีเรียก่อโรคที่อยู่ที่คอนแทคเลนส์

วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาประสิทธิภาพของน้ำยาล้างคอนแทคเลนส์ที่จำหน่ายในประเทศไทย ในการต้านการเจริญของ *Pseudomonas aeruginosa* and *Staphylococcus aureus* ซึ่งเป็นแบคทีเรียที่เป็นสาเหตุสำคัญก่อให้เกิดการติดเชื้อที่กระจกตา น้ำยาล้างคอนแทคเลนส์ 4 ชนิด กำหนดชื่อเป็นน้ำยา A, B, C และ D ถูกนำมาทดสอบประสิทธิภาพการต้านเชื้อ methicillin- resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin- sensitive *Staphylococcus aureus* (MSSA) ATCC25930 และ *Pseudomonas aeruginosa* ATCC2785 ด้วยวิธี Agar-well diffusion และ ตรวจวิเคราะห์ความเข้มข้นที่น้อยที่สุดของน้ำยาในการต้านการเจริญของเชื้อและฆ่าเชื้อ (Minimal Inhibitory Concentration, MIC and Minimal Bactericidal Concentration, MBC) รวมทั้งศึกษาระยะเวลาที่แบคทีเรียทดสอบสัมผัสน้ำยาแล้วทำให้แบคทีเรียลดลง 99.9% (Time kill assay)

จากการวิจัยพบว่าน้ำยาทั้ง 4 ชนิด ไม่แสดงฤทธิ์การต้านเชื้อทดสอบด้วยวิธี Agar-well diffusion method แต่จากการทดสอบหา Minimal inhibitory concentration (MIC) โดยใช้ Broth microdilution assay พบว่าน้ำยาล้างคอนแทคเลนส์ชนิด A มีฤทธิ์ยับยั้งเชื้อ methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin-sensitive *S. aureus* (MSSA)

ลายมือชื่อนักศึกษา..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ATCC25930 และ Pseudomonas aeruginosa ATCC2785 ที่ความเข้มข้นระหว่าง 12.5-25% และจากการทำ Time kill assay พบว่าน้ำยาล้างคอนแทคเลนส์ D มีประสิทธิภาพยับยั้งเชื้อทดสอบมากกว่าน้ำยาล้างคอนแทคเลนส์ A, B และ C ซึ่งประสิทธิภาพของน้ำยาแต่ละชนิดที่แตกต่างกันนี้อาจเนื่องจากมีสารต่างๆที่เป็นองค์ประกอบที่แตกต่างกัน ดังนั้นงานวิจัยนี้อาจนำมาใช้เป็นแนวทางในการออกแบบน้ำยาล้างคอนแทคเลนส์ที่มีประสิทธิภาพยับยั้งเชื้อ

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INTRODUCTION

1.1 Background

Contact lenses can be used as alternative to eyeglasses for improving visions by correcting refractive errors (Mohammadinia et al., 2012). Contact lenses bend light evenly in every directions, therefore, they are typically used for correction of eyesight problems such as Myopia, Hyperopia and Astigmatism. One of commonly used contact lenses is soft contact lens. Soft contact lenses are made of plastic materials that can incorporate water which makes lens soft and flexible. However, contact lenses can cause a variety of problems in contact lens wearers, e.g., dry eyes, allergic eye disease, distortion of the cornea, blood vessels growing in the cornea due to a lack of oxygen, scratches on the cornea and cornea infection which can lead to blindness if the infection is severe. It has been shown that contact lenses wearers, especially extended wearers risk for corneal ulcers and bacterial keratitis (Mohammadinia et al., 2012).

The U.S. Centers for Disease Control estimated that 0.05-0.1% of contact lens wearers has microbial keratitis. In addition, bacterial keratitis represent 90% of all microbial keratitis cases, with *Pseudomonas aeruginosa* being the most common pathogen, followed by *Staphylococcus aureus* (Bassyouni, Kamel, Abdelfattah, & Mostafa, 2016; Eltis, 2011) stated that *Pseudomonas aeruginosa* and *Staphylococcus aureus* are major causes of contact lens related bacterial keratitis especially in the extended contact lens wearers (Budiman, Fauzi, Sulistiyaningsih, & Sriwidodo, 2017). *Pseudomonas aeruginosa* is a gram negative bacterium that is commonly found in environment including water. It is an opportunistic pathogen and survives to dilute disinfectant solutions. In addition, *P. aeruginosa* keratitis associated with contact lens

wearers is difficult to treat because *P. aeruginosa* resist many antibiotics. *Staphylococcus aureus* is a gram positive bacterium that can readily access to eyes. Generally, Staphylococcal ocular infection is most likely due to hand-to-eye transfer. It has been showed that *S. aureus* is the most common bacteria that cause of contact lens induced peripheral ulceration. Untreated or severe bacterial keratitis lead to perforation and endophthalmitis (Bassyouni et al., 2016). To minimize the risk of contact lens infections, contact lens care solutions were used for soaking contact lens in order to decrease the amount of potential pathogens during contact lens storage.

Contact lenses care solutions are used to clean, disinfect and store contact lenses. Therefore, they compose of surfactant, disinfectant, antifungal and antibacterial agents. In addition, they help to remove dirt and protein deposits from the surface of contact lens. One type of contact lens care solution is the multipurpose contact lens care solution which is used for cleaning, rinsing, disinfecting and storing of contact lens. The disinfectant ability of the contact lens care solution is important since it is the major condition for decreasing the amount of infectious keratitis.

Since there are many different brands of contact lens care solution with different ingredients in the market. Therefore, the purpose of this study is to evaluate the antimicrobial activity of the multipurpose contact lens d care solutions available in Thailand against the common causes of bacterial keratitis, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In addition, the contact time between pathogens and the contact lens care solution are determined since it is necessary to determine the effective duration time for each contact lens care solution. The information from this study will be helpful for formulation of contact lens care solutions with more potent antibacterial activity. In addition, the suitable exposure time of contact lens care solutions and the contact lens or disinfection time will be obtained from this study.

1.2 Objectives of research

1.2.1 To evaluate the antimicrobial activity of four commercial contact lens care solutions against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

1.2.2 To determine the duration of contact time between the contact lens solutions and culture of *P. aeruginosa* and *S. aureus* that can reduce 99.9% of the test bacteria.

1.3 Research hypothesis

Different ingredients of the contact lens care solutions will show difference in the antibacterial efficacy of the contact lens care solutions against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Therefore, the commercial contact lens care solutions with different ingredients may show different antibacterial activity.

1.4 Scope of the research

In vitro antimicrobial activity of four commercial contact lens care solutions against *Pseudomonas aeruginosa* and *Staphylococcus aureus* will be investigated. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these care solutions will also be determined. In addition, the time-kill assay will be performed to investigate the duration of contact time between the contact lens solutions and culture of *P. aeruginosa* and *S. aureus* that can reduce 99.9% of the test bacteria.

CHAPTER 2

LITERATURE REVIEWS

2.1 Contact lens

It has been estimated that there are approximately 140 million contact lens wearers worldwide (Yamasaki, Saito, Ota, & Kilvington, 2018). The first commercial contact lens, PMMA lens, was created in the early 1950s (Cho, & Boost, 2013). The contact lens and contact lens care solutions were classified as active implantable medical devices (AIMD) or in vitro medical devices (Zaki, Pardo, & Carracedo, 2019).

Contact lens are optical medical devices used to correct refractive errors such as myopia, hyperopia and astigmatism. Contact lens is a lens placed on the cornea to improve vision. They alter the direction of light rays and focus light properly onto the retina. For nearsighted, light rays focus too early within the eye so they form a focus point in front of the retina instead of directly on it. Contact lenses correct nearsightedness by diverging light rays, which reduces the eye's focusing power. This moves the eye's focus point backward onto the retina where it belongs. For farsighted, the eye does not have adequate focusing power so light rays fail to form a focus point by the time they reach the retina. Contact lens correct farsightedness by converging light rays, which increases the eye's focusing power. This moves the eye's focus point forward onto the retina. Contact lens powers are expressed in diopters (D). Lens powers that correct nearsightedness start with a minus sign (–), and lens powers that correct farsightedness start with a plus sign (+) all about visions.

There are many types of contact lens such as corrective contact lens, cosmetic contact lens, therapeutic contact lens and rigid contact lens. Corrective contact lens are designed to improve vision by correcting refractive error. While cosmetic contact lenses

are designed to change the appearance of the eye and may also correct refractive power. Therapeutic contact lenses are often used for treatment of non-refractive disorders of the eye. Rigid contact lens can correct for astigmatism and corneal irregularities, Keratoconus.

Contact lens is a good option for many people as an alternative to eyeglasses. However, the use of contact lenses also brings a higher risk of infections. The causes of infections may include sleeping while wearing contact lenses, not cleaning the lenses or lens case properly and sharing lenses, or wearing contact lenses during water activities.

2.2 Eye infections in contact lens wearers

Many people who wear contact lenses may develop eye infections from some bacterial colonies and from bacterial biofilms inside lens storage cases. Lens biomaterials act as a place for microbial adherence and subsequent transfer to ocular surface. The major risk factors for eye inflammation and eye infections are lens deposits, hypoxia, change in pH and oxygen, carbon-dioxide concentration, corneal surface disruption and cytotoxicity of care solutions (Liaqat, Saleem, Tahir, Arshad, M., & Arshad, N., 2019).

Contact lens wearers have a high risk for keratitis, an infection of the cornea, the clear outer covering of the eyes. It's also called corneal ulcers. Microbial keratitis is a devastating ocular infection and an important cause of visual impairment that is frequently associated with contact lens wear (Siddiqui, Lakhundi, & Khan, 2015). In addition, pinkeye or conjunctivitis also occur in contact lens wearers. These infections come from bacteria adhered in the thin membrane covering the white part of the eyes and the inside of the eyelids (Siddiqui et al., 2015).

Bacteria are the predominant causative agents in contact lens associated microbial keratitis (Lam et al., 2002). Most common pathogens causing bacterial keratitis are *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Eryilmaz,

Kaskatepe, Kiymaci, Simsek, & Erol, 2018). These bacteria can cause vision loss and blindness if not diagnosed and treated promptly (Lin, Kim, Chen, Kowalski, & Nizet, 2016). However, the range of organisms associated with contact lens microbial keratitis varies depend on regions. Gram negative bacteria keratitis are more common in tropical climates (Lam et al., 2002). *Pseudomonas aeruginosa* can accelerate colonization of contact lens surfaces in the presence of dying neutrophils in vitro and formed biofilm (Hinojosa, Patel, Zhu, & Robertson, 2017). A significant increase of ocular infections caused by *P. aeruginosa* also have been associated with antibiotic resistant strains. Pseudomonas keratitis is characterized by rapid, superlative stromal infiltrates with tissue necrosis and excessive mucopurulent discharge (Willcox, 2011). *Staphylococcus aureus* is one of the most common causative agents of bacterial keratitis. The incidence of Staphylococcal keratitis has been increased by methicillin-resistant *S. aureus* (MRSA) and coagulase-negative staphylococci. In addition, contact lens related bacterial keratitis associated with drug resistant strains can increase morbidity and treatment cost and have a poor prognosis (Willcox, 2011).

2.2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative, rod-shaped bacterium that is an opportunistic pathogen and cause infections in many organs. This bacterium has genetic variation and resists many antibiotics. *P. aeruginosa* is a ubiquitous environmental bacterium that can survive in a variety of nutritional environments. It is found on many parts of human body such as skin. In addition, *P. aeruginosa* can be found in environment such as in soil and water. *P. aeruginosa* can form biofilm during colonization. In addition, *P. aeruginosa* activates several pathways of the immune system during bacterial keratitis, and the activation often involves receptors on the corneal epithelial cells called toll-like receptors (TLRs). These TLRs recognize lipopolysaccharide or flagella from *P. aeruginosa* and activate the epithelial cells to produce inflammatory mediators such as cytokines and chemokines. These cytokines recruit white blood cells, predominantly neutrophils to the infection site so that they can phagocytose and kill bacteria. However, continued recruitment and presence of these neutrophils and other white blood cells in the corneal tissue leads to destruction of

corneal cells and tissue components. This can lead to scarring and vision loss (Willcox, 2007). Factors that play important roles in the adhesion process of bacteria to contact lenses include surface hydrophobicity, host receptor interaction, and binding molecules present on the bacterial cells. Bacterial adherence to the epithelial surface occurs due to molecular interactions between bacterial surface proteins and protein receptors on the cell surfaces. Surface hydrophobicity of the contact lens has been found to enhance bacterial adhesion (Ajayi, 2012).

2.2.2 *Staphylococcus aureus*

Staphylococcus aureus is a facultative anaerobic gram-positive cocci, is commonly found in and on the human body. *S. aureus* is carried by 50-60% of the normal population on hands, face, nose and skin. It processes catalase and nitrate reductase enzymes. *S. aureus* can also produce exotoxin that binds to antibodies and activates inflammation. Resistant strains of *S. aureus* include methicillin-resistant *S. aureus* (MRSA), Vancomycin-intermediate *S. aureus* (VISA) and Vancomycin-resistant *S. aureus* (VRSA), causing more serious infections in immunocompromised patients. *S. aureus* is one of common bacteria causing contact lens induced peripheral ulceration (Jalbert, Willcox, & Sweeney, 2000).

Contact lens can transfer microorganisms to ocular surface whereas potential pathogens found on eyelids or ocular surface can also contaminate contact lens. Contact lens infections occurred in contact lens wearers are often associated with improper hygiene practice. Thus proper care of contact lens is very important for preventing infections of the eyes (Eryilmaz et al., 2018). Therefore, reusable contact lenses require properly daily wash with suitable contact lens care solutions for disinfection and removal of deposits from the contact lens surface when they are not worn. Therefore, the Contact Lens Association of Ophthalmologists recommends cleaning and disinfecting contact lenses on a daily basis, as infection is the greatest risk to contact lens wearers.

2.3 Contact lens care solution

Contact lens care solutions help to remove dirt and protein deposits from the surface of the lens. In addition, they are used to clean, disinfect and store contact lenses especially soft lens. The effective removal of denatured proteins is important for contact lens care since protein can bind to lenses and made them opaque which reduce visual acuity (Raja, Manimaran, & Balasubramanian, 2015). Thus the contact lens care solution should be able to both clean and disinfect contact lens (Iguban, Nañagas, & Mesa-Rodriguez, 2013). Commercially available contact lens care solutions have different ingredients that can affect to eye discomfort and lens disinfection (Raja et al., 2015). The discomfort is associated the deposits and also with problem associated with solution uptake into the lens following disinfection. Soft contact lenses have been shown to uptake constituents of contact lens care solutions and leach out into the eye during the wearing time (Cho, & Boost, 2013). However, many contact lens care solution formulas are usually optimized to provide preservation and disinfection in qualities. Multipurpose contact lens care solution is one type of contact lens care solutions that is commonly used for the care of soft contact lens. They comprise of agents for rinsing, disinfection and storage of contact lens and are also composed of preservatives, buffer system, and other agents to aid contact lens comfort and clean (Kilvington, Powell, Lam, & Lonnen, 2011).

Lyndon Jones and Michelle Senchyna described that the contact lens solution should have the following characteristics as shown in table 1.1 (Jones, & Senchyna, 2007)

Table 2.1 Characteristics of the ideal contact lens care solution

Effective disinfection against a wide variety of pathogenic organisms
Non-toxic to ocular tissues
Compatible with all contact lens materials
Simple to use
Rapid disinfection capability
Condition lens surface to enhance wettability and in eye comfort
Minimize deposition of tear film components
Inexpensive to purchase

Source: Jones & Senchyna, 2007

The primary role of the antimicrobial agent of contact lens care solution is to provide a lens that is suitably disinfected so it is safe to be inserted into the eye, typically following overnight soaking. The agent of choice must be effective against a wide variety of pathogens, and not against ocular tissues (Jones, & Senchyna, 2007). The mechanisms of action of antimicrobial agents are varies depend on types of agents including disruption of cell membranes, inhibition of key enzymes, inhibit cell wall synthesis, inhibit nucleic acid synthesis, and involve microbial metabolisms. Available antimicrobial agents in contact lens care solution include hydrogen peroxide, boric acid, polyhexamethylene biguanide, polyquaternium-1, alexidine and amidoamine (Jones, & Senchyna, 2007). Biguanides or polyquaternium-1 disrupts microbial membranes leading to microbial death while hydrogen peroxide (H_2O_2) which is a strong oxidizing agent act on lipids, protein and DNA leading to cell death.

However, certain antimicrobial agents in the contact lens care solution such as quaternary ammoniums and polyhexamethylene biguanides (PHMB) may exhibit negative effects on the corneal epithelium (Oh, McCanna, Subbaraman, & Jones, 2018). During wearing contact lens, contact lens materials can adsorb these components and release them onto the corneal surface, potentially eliciting cytotoxic and inflammatory response. These effects may contribute to wearer discomfort and subsequent in tolerance to contact lens wear. Therefore, contact lens care solution user should be aware of increasing the risk of corneal infiltrative and inflammatory events (Oh et al., 2018).

The other important ingredient in contact lens care solution is surfactant. The surfactants in contact lens care solutions have two functions. First, surfactants are used as detergents, removing loose debris and deposits by combining these substances to form micelles which are more easily suspended in the liquid. The micelles are then removed during the rinsing procedure. Therefore, some contact lens care solutions can be used for cleaning debris buildup on the contact lens surface without scratching it. Removal of protein deposits is a particular concern as it can cause complications such as contact lens-induced papillary conjunctivitis. The second function of surfactants relates to their ability to enhance the wettability of hydrophobic substrates (Jones, & Senchyna, 2007). The common surfactants found in contact lens care solutions are poloxamines and poloxamers.

Chelating agents is also an ingredient in some contact lens care solutions. They act synergistically with other agents to improve disinfection efficacy or to aid in removal of tear film components, typically proteins. The common chelating agents are Ethylene diamine tetraacetic acid (EDTA) and sodium citrate. Ethylenediamine tetraacetic acid (EDTA) is a cationic chelating agent that binds free metals and enhances antimicrobial activity of disinfectant since it involves the sequestration of ions such as calcium and magnesium that normally compete with positively charged preservative molecules for active sites on microbial cell walls. Sodium citrate, is a sequestering agent that aids in the removal of protein (Jones, & Senchyna, 2007)

CHAPTER 3

MATERIALS AND METHODS

3.1 Bacterial Strains

Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin-sensitive *S. aureus* (MSSA) ATCC25930 strains and *Pseudomonas aeruginosa* ATCC27853 were kindly provided by the culture collections of the Microbiology Unit, Department of Medical Sciences, Faculty of Science, Rangsit University.

3.2 Contact lens care solutions

Four contact lens care solutions assigned as A, B, C and D were purchased from drug store. The ingredients of test contact lens care solutions was shown in table 3.1.

3.3 Sterility test of the contact lens care solutions

One hundred microliter of each contact lens care solution was spread on a Tryptic soy agar (TSA) plate. The plate was then incubated at 37°C for 24 hours and colonies were counted and expressed as colony forming unit/ mL (CFU/ mL). Each solution was repeated in triplicates.

3.4 Screening of antibacterial activity of contact lens care solutions

3.4.1 Preparation of inoculum

Methicillin susceptible *Staphylococcus aureus* (ATCC 25923), Methicillin resistant *Staphylococcus aureus* (ATCC 43300), and *Pseudomonas aeruginosa* (ATCC27853) were grown on a TSA plate at 37°C for 18-24 hours. One or two isolated colonies of each bacterium were cultured in 5 mL of Tryptic soy broth (TSB) at 37°C for 18-24 hours and then adjusted to match the McFarland standard No. 0.5.

3.4.2 Agar well diffusion method

Antimicrobial activity of contact lens care solutions was determined by agar well diffusion method according to Clinical and Laboratory Standard Institute (CLSI) protocol guideline (CLSI, M02-A11, 2012). A suspension of any tested bacteria containing about 10^8 CFU/mL was spread on TSA by three-way swab technique using sterile cotton swabs. TSA plate was pouched by cork borer (Ø 6 mm) and 50 µl of contact lens care solutions were added in each well. Standard antibiotic discs such as oxacillin and ciprofloxacin were also used as references. The plates were then incubated at 37°C for 24 hours and the diameter of the inhibition zone was measured. Triplicates of each plate have been done.

3.5 Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

3.5.1 Preparation of inoculum

Methicillin susceptible *Staphylococcus aureus* (ATCC 25923), Methicillin resistant *Staphylococcus aureus* (ATCC 43300), and *Pseudomonas aeruginosa* (ATCC27853) were grown on a TSA plate at 37°C for 18-24 hours. One or two isolate colonies were cultured in 5 mL of TSB at 37°C for 18-24 hours and then adjusted to match McFarland standard No. 0.5. The working inoculum was obtained by diluting to 1:100 in TSB to give a concentration of 10^6 CFU/mL.

3.5.2 Minimal inhibitory concentration (MIC)

The Minimal inhibitory concentration (MIC) of contact lens care solution was determined by the broth microdilution method as recommended by CLSI (CLSI, M7-A7, 2006). Each contact lens care solution was two-fold serial diluted in 100 µL of TSB into 96 well plates. The one-hundred µL of bacterial suspension were added in each dilution and then the plate was incubated at 37°C for 18-24 hours. Each dilution was repeated in duplicates. The MIC was taken as the minimum concentration of the test reagent that inhibited the growth of bacteria.

3.5.3 Minimal bactericidal concentration (MBC)

The Minimal bactericidal concentration (MBC) was defined as the lowest concentration of the reagent to kill bacteria. All the dilutions that did not show any turbidity of the bacterial suspension in the MIC studies were determined for MBC. The suspension (10 µL) was dropped onto TSA and incubated at 37˚C for 18-24 hours. The lowest concentration that showed no growth on TSA was taken as MBC.

3.6 Kinetics of kill testing

3.6.1 Preparation of inoculum

Methicillin susceptible *Staphylococcus aureus* (ATCC 25923), Methicillin resistant *Staphylococcus aureus* (ATCC 43300), and *Pseudomonas aeruginosa* (ATCC27853) were grown on a TSA plate at 37°C for 18-24 hours. One or two isolated colonies were cultured in 5 mL of TSB at 37°C for 18-24 hours and then adjusted to match McFarland standard No. 0.5.

3.6.2 Kinetics of killing testing

One hundred microliter of the inoculum was added to 9.9 ml of each contact lens care solution. Kinetics of kill testing was conducted by exposing 10^6 cells of bacteria to the test agent for various periods of time. At the end of the exposure time (i.e. 0, 2, 4 and 6 hours), the "treated cells" were serially ten-fold diluted with 0.9% sterile saline solution and 100 µL of each dilution was plated on TSA plates. The plates were incubated at 37˚C for 18-24 hours and colonies were counted and expressed as surviving colony forming unit/ mL (CFU/ mL). Each test solution was repeated in triplicates.

"Growth control" was done parallel to "treated cells" to insure that each strain was still alive at the time of the exposure to the test agents. In "growth control" 100 µL of the inoculum was added to 9.9 ml of sterile saline solution instead of contact lens care solution. At the end of the exposure time, the suspension was serially ten-fold diluted with 0.9% sterile saline solution and 100 µL of each dilution was plated on TSA plates. The plates were incubated at 37˚C for 18-24 hours and colony forming unit/mL (CFU/mL) was determined.

3.7 Calculations and statistical analysis

Determination of the logarithmic reduction of the growth in each contact lens care solution and the growth control was calculated by the following equations;

$$\log \text{reduction} = \log_{10} (\text{initial CFU/mL}) - \log_{10} (\text{final CFU/mL})$$

$$\% \log \text{reduction} = \frac{\log_{10} (\text{initial CFU/mL}) - \log_{10} (\text{final CFU/mL})}{\log_{10} (\text{initial CFU/mL})} \times 100$$

Log reductions among each contact lens care solution were compared using an analysis of variance (ANOVA) with a level significance of 0.05.

Table 3.1 The ingredients and recommended contact time of test contact lens care solutions

Contact lens care solution	Ingredients		Contact time (hour)
A	Boric acid Sodium borate Sodium chloride Poloxamine Hypromellose	Edelate disodium Citric acid Polyethylene-glycon 4000 Polyaminopropyl bi-guanide	Over night
B	Sodium chloride Potassium chloride Disodium edelate Poloxamer	Hypromellose Disodium hydrogen phosphate Sodium Dihydrogen phosphate Polyhexanide 0.0001% (w/ v)	6
C	Boric acid Sodium borate EDTA	Poloxamer 407- HPMC Polyhexamethylene biguanide	6
D	Sodium citrate Sodium chloride Sodium borate Propylene glycol Tearglyde™	Tetronic® 1304 Nonanoyl ethylenediaminetriacetic acid Polyquad® (Polyquaternium-1) 0.001% Aldox® (myristamidopropyl dimethylamine) 0.0005%	6

CHAPTER 4

RESULTS

4.1 Sterility test of the contact lens care solutions

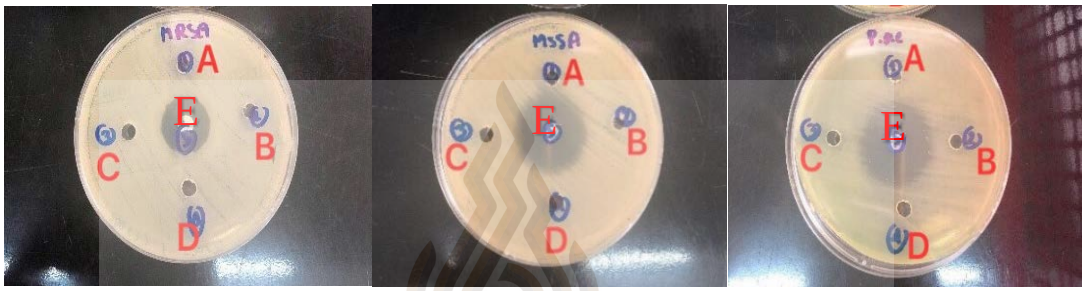
Sterility of the contact lens care solutions was investigated in order to confirm that there is no bacteria in these solutions. Therefore, only tested bacteria, i.e. methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin-sensitive *S. aureus* (MSSA) ATCC25930 and *Pseudomonas aeruginosa* ATCC2785 were tested for antibacterial activity of the contact lens care solutions. In the sterility test one hundred microliters of each contact lens care solution were plated on a Tryptic soy agar (TSA) plate and then the plates were incubated at 37°C for 24 hours. The results revealed that no bacterial colony was found on those agar plates after incubation period. Therefore, there was no bacteria in test contact lens care solutions.

4.2 Screening of antibacterial activity of contact lens care solutions

The antibacterial activities of contact lens care solutions were tested against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, methicillin-sensitive *S. aureus* (MSSA) ATCC25930 and *Pseudomonas aeruginosa* ATCC2785 by agar well diffusion method. The results of this study showed that no contact lens care solution possessed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA) and *Pseudomonas aeruginosa* since there was no inhibition zone as shown in Figure 4.1.

The MIC and MBC of contact lens care solutions against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA) and *Pseudomonas aeruginosa* were shown in Table 4.1. The contact lens care solutions B, C and D did not show inhibitory effect against MSSA and *P. aeruginosa*. The MIC of

the contact lens care solution A against *P. aeruginosa* is lower than the MIC of this solution against MSSA and MRSA. The contact lens care solutions B, C and D did not show inhibitory activity against MSSA and *P. aeruginosa*. In addition, all test contact lens care solutions did not show bactericidal activity against MRSA, MSSA and *P. aeruginosa*.



(a) (b) (c)

Figure 4.1 Antibacterial activities of contact lens care solutions (A, B, C and D) and positive control (E) against MRSA (a), MSSA (b) and *Pseudomonas aeruginosa* (c) by agar well diffusion method

Table 4.1 MIC and MBC of contact lens care solutions against *P. aeruginosa,* MRSA and MSSA

Contact lens care solutions	MIC (% v/v)			MBC (%v/v)		
	P.aeruginosa	MRSA	MSSA	*P.aeruginosa*	MRSA	MSSA
A	12.5	25	25	not kill	not kill	not kill
B	not inhibit	50	not inhibit	N/A	not kill	N/A
C	not inhibit	50	not inhibit	N/A	not kill	N/A
D	not inhibit	50	not inhibit	N/A	not kill	N/A

N/A: not applicable

4.3 Kinetics of kill testing

The results of kinetics of killing test showed in Table 4.2 -4.4 and Figure 4.2 - 4.7. The numbers of test bacteria after an exposure to contact lens care solutions for various contact times were demonstrated in Table 4.2. Log reduction values and % log reduction for MRSA, MSSA and *P.aeruginosa* after disinfection with contact lens care solutions were shown in Table 4.3 and Table 4.4, respectively. This study revealed that contact lens care solution D at concentration of 100% has more killing effect than contact lens care solution A, B and C since it can reduce all test bacteria more than 3 logs in 2 hours. Contact lens care solution B, C and D killed all test bacteria more than 3 logs in 2 hours whereas contact lens care solution A took more than 2 hours to achieve the same kill. In addition, Contact lens care solution D showed good bactericidal activity towards *S.aureus* compared with other solutions while contact lens care solution B showed good bactericidal activity towards *P.aeruginosa* compared with other solutions. Contact lens care solution A showed less bactericidal activity towards *S.aureus* and *P.aeruginosa* compared with other solutions. However, it showed 4logs reduction on MRSA and 7logs reduction on *P. aeruginosa* after an exposure for 6 hours.

The bacterial cell number (log CFU/mL)of *S. aureus* (MRSA), *S.aureus* (MRSA) and *P. aeruginosa* after an exposure to contact lens care solutions for 2, 4 and 6 hours were showed in Figure 4.2, 4.4 and 4.6, respectively. In addition, % log reduction of *S. aureus* (MRSA), *S. aureus* (MRSA) and *P. aeruginosa* after an exposure to contact lens care solutions for 6 hours were showed in Figure 4.3, 4.5 and 4.7, respectively. The contact lens care solution D has more killing effect against *S. aureus* (MRSA) than contact lens care solution A, B and C since it can reduce all test bacteria more than 7 logs in 2 hours. However, contact lens care solution B, C and D completely killed *S. aureus* (MRSA) after an exposure time of 6 hours. All test contact lens care solutions have bactericidal activity against *S. aureus* (MSSA) and *P. aeruginosa* after an exposure time of 6 hours.

Table 4.2 The bacterial cell number (CFU/mL) of test bacteria after an exposure to contact lens care solutions for various contact times.

Bacteria	Contact time (hours)	Bacterial cell number (CFU/mL)				
		Control	Solution A	Solution B	Solution C	Solution D
MRSA	0	2.8×10^{7}	2.8×10^{7}	2.8×10^{7}	2.8×10^{7}	2.8×10^{7}
	2	3.9×10^{5}	1.7×10^{5}	3.5×10^{2}	6.0×10^{2}	0
	4	8.7×10^{5}	3.3×10^{4}	4.2×10^{2}	0	0
	6	9.6×10^{5}	9.1×10^{2}	0	0	0
MSSA	0	1.5×10^{7}	1.5×10^{7}	1.5×10^{7}	1.5×10^{7}	1.5×10^{7}
	2	9.6×10^{4}	2.5×10^{3}	1.3×10^{3}	0	0
	4	3.5×10^{3}	1.4×10^{2}	0	0	0
	6	6.3×10^{3}	0	0	0	0
P. aeruginosa	0	1.9×10^{7}	1.9×10^{7}	1.9×10^{7}	1.9×10^{7}	1.9×10^{7}
	2	1.7×10^{7}	5.7×10^{4}	0	5.3×10^{3}	6.0×10^{2}
	4	1.4×10^{7}	10	0	24	0
	6	6.1×10^{6}	0	0	0	0

Table 4.3 Log reduction values of MRSA, MSSA and *P.aeruginosa* after an exposure to contact lens care solutions for various contact times.

Bacteria	Contact time (hours)	Log reduction values				
		control	Solution A	Solution B	Solution C	Solution D
MRSA	2	1.86	2.22	4.90	4.67	7.45
	4	1.51	2.93	4.82	7.45	7.45
	6	1.49	4.49	7.45	7.45	7.45
MSSA	2	2.19	3.78	4.06	7.18	7.18
	4	3.63	5.03	7.18	7.18	7.18
	6	3.38	7.18	7.18	7.18	7.18
P. aeruginosa	2	0.05	2.52	7.28	3.56	4.50
	4	0.13	6.28	7.28	5.90	7.28
	6	0.49	7.28	7.28	7.28	7.28

Table 4.4 % Log reduction of MRSA, MSSA and *P.aeruginosa* after an exposure to contact lens care solutions for various contact times.

Bacteria	Contact time (hours)	% Log reduction				
		control	Solution A	Solution B	Solution C	Solution D
MRSA	2	24.92	29.77	65.84	62.70	100.00
	4	20.25	39.32	64.78	100.00	100.00
	6	20.05	60.27	100.00	100.00	100.00
MSSA	2	30.57	52.65	56.61	100.00	100.00
	4	50.61	70.09	100.00	100.00	100.00
	6	47.06	100.00	100.00	100.00	100.00
P. aeruginosa	2	0.67	34.66	100.00	48.84	61.84
	4	1.83	86.26	100.00	81.04	100.00
	6	6.79	100.00	100.00	100.00	100.00

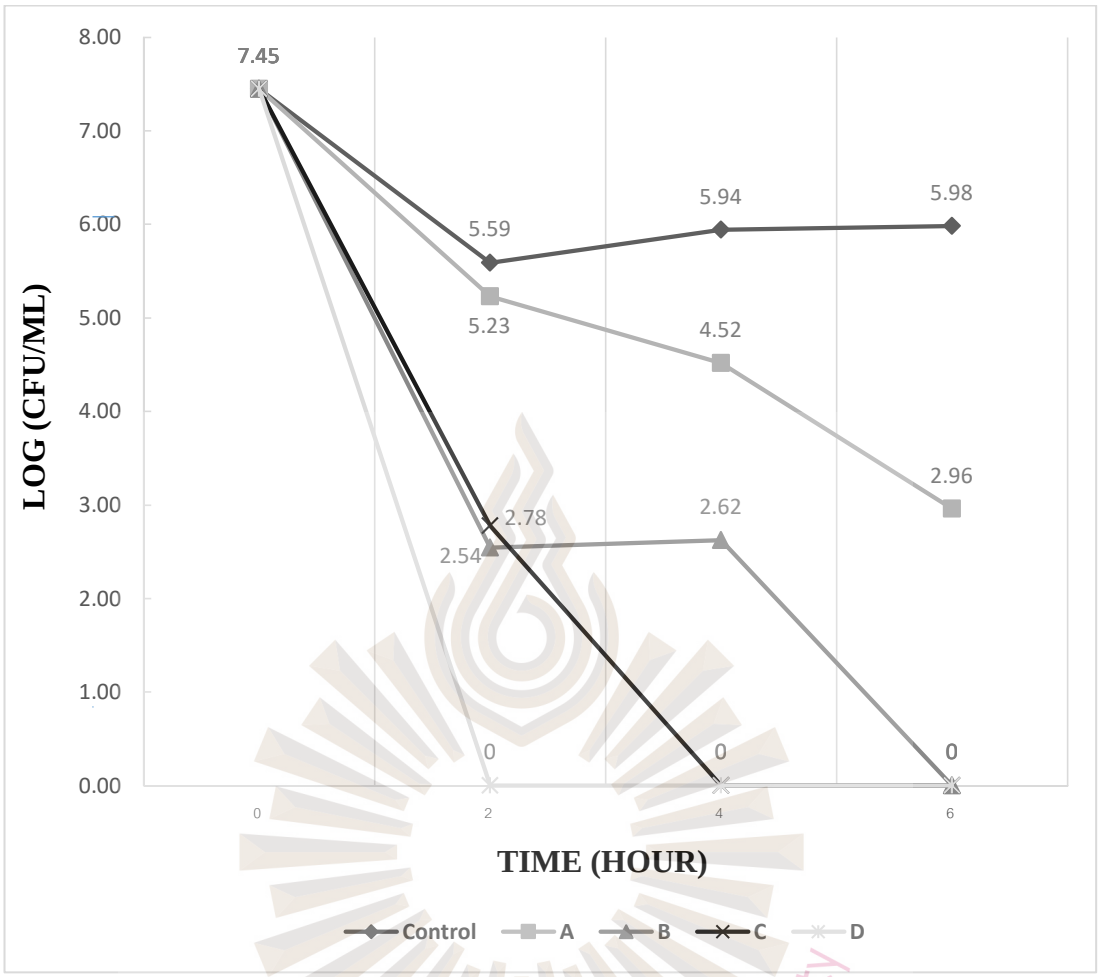


Figure 4.2 The bacterial cell number (log CFU/mL) of *S. aureus* (MRSA) after an exposure to contact lens care solutions for 2, 4 and 6 hours.

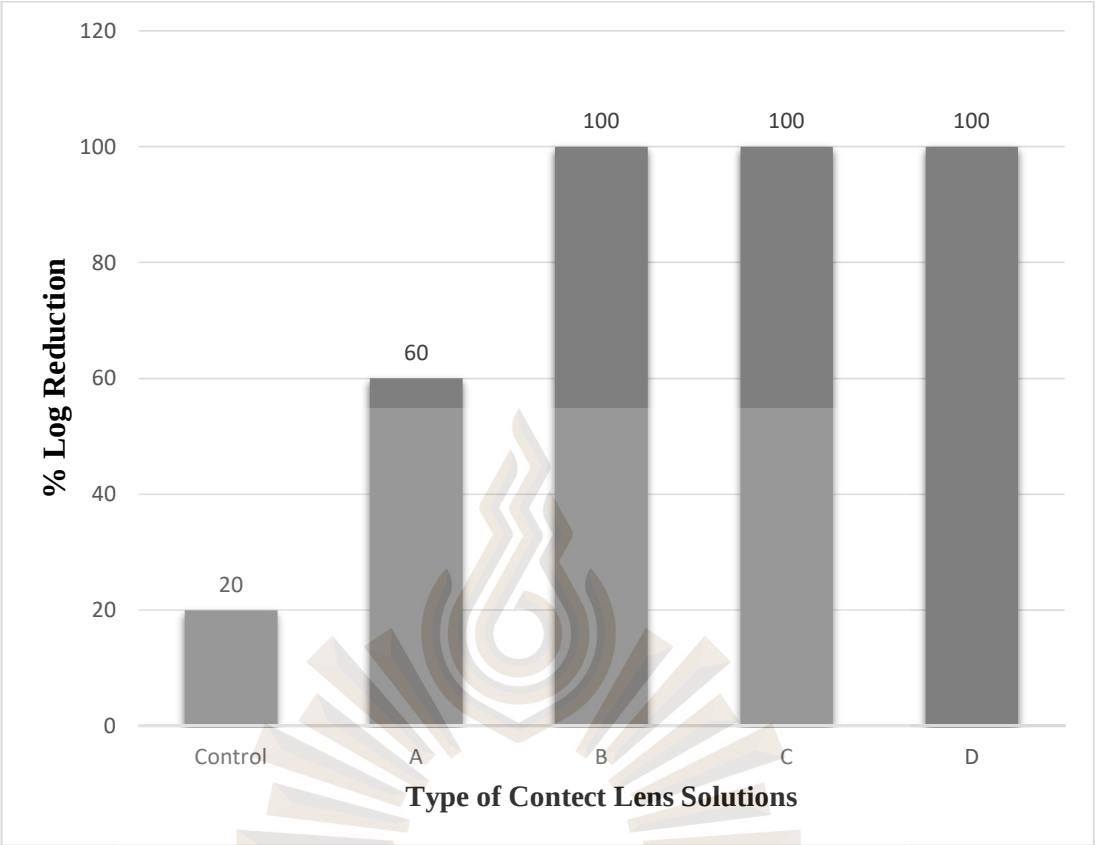


Figure 4.3 %Log reductions for *S. aureus* (MRSA) following incubation with contact lens care solution A, B, C and D for 6 hours

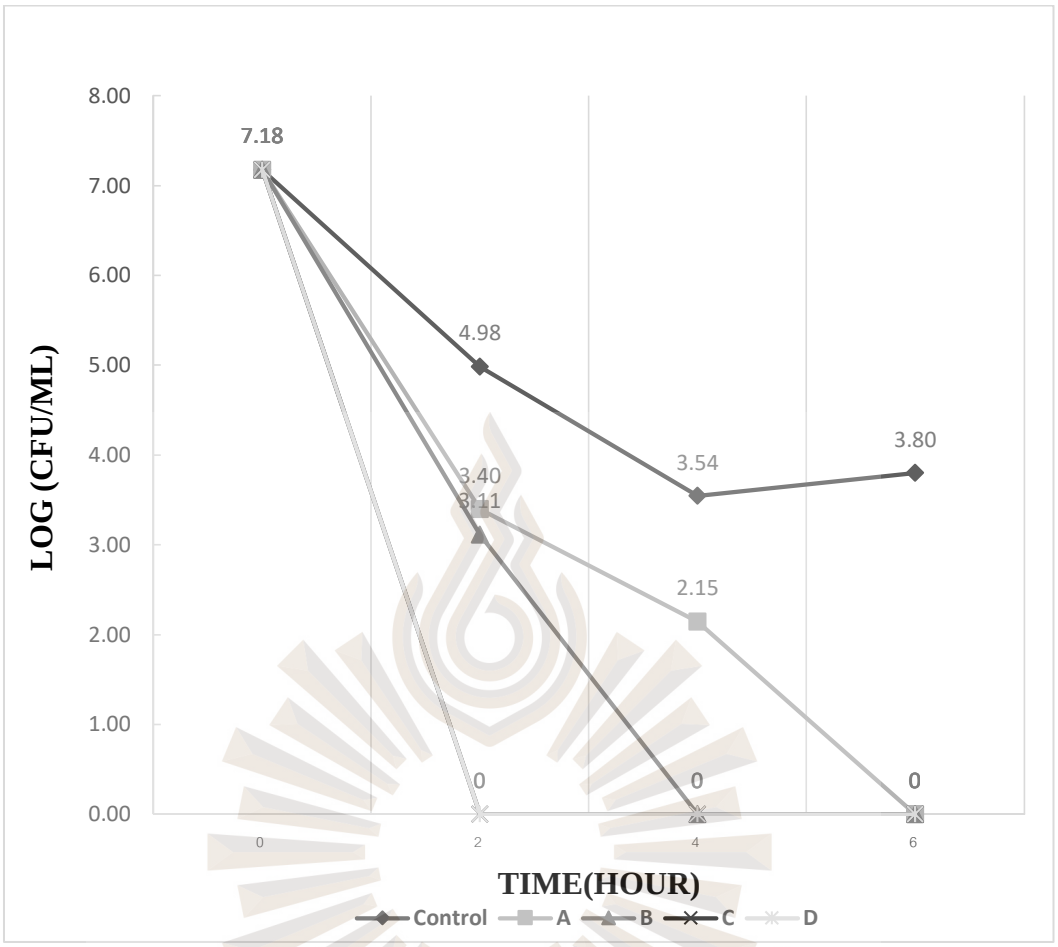


Figure 4.4 The bacterial cell number (log CFU/mL) of *S. aureus* (MSSA) after an exposure to contact lens care solutions for 2, 4 and 6 hours

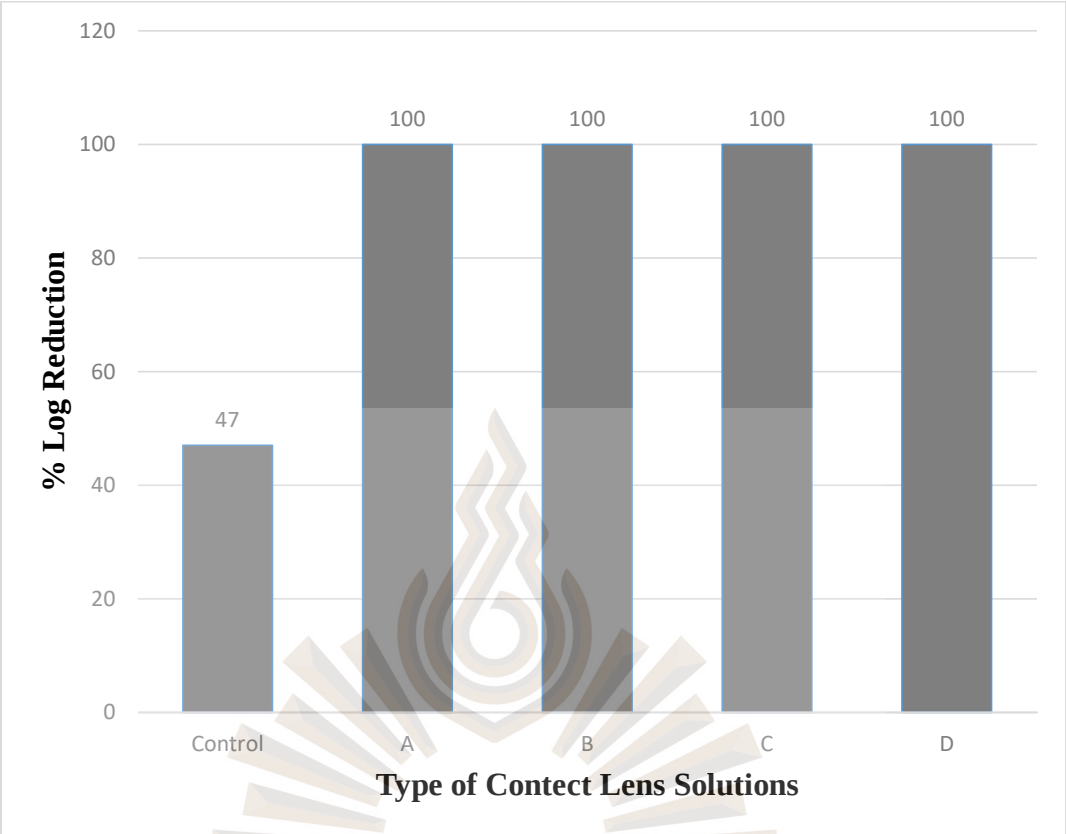


Figure 4.5 %Log reductions for *S. aureus* (MSSA) following incubation with contact lens care solution A, B, C and D for 6 hours

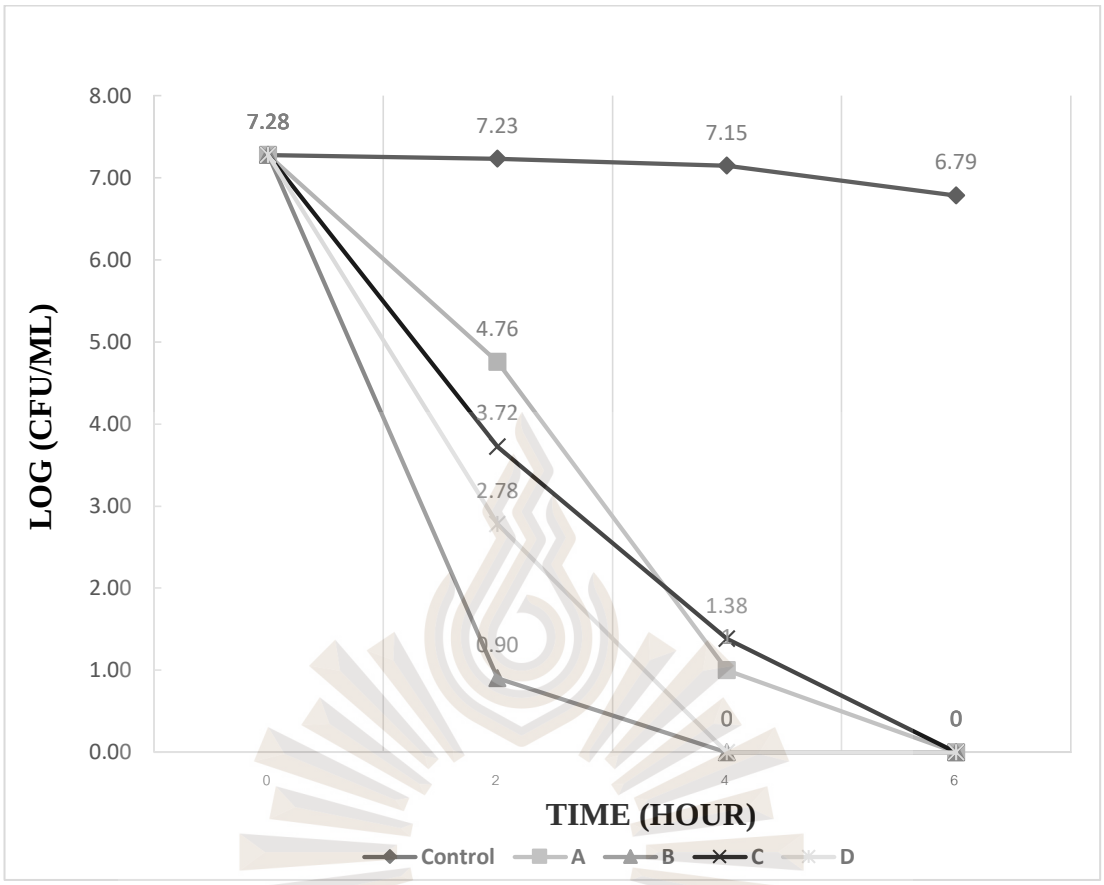


Figure 4.6 The bacterial cell number (log CFU/mL) of *P.aeruginosa* after an exposure to contact lens care solutions for 2, 4 and 6 hours.

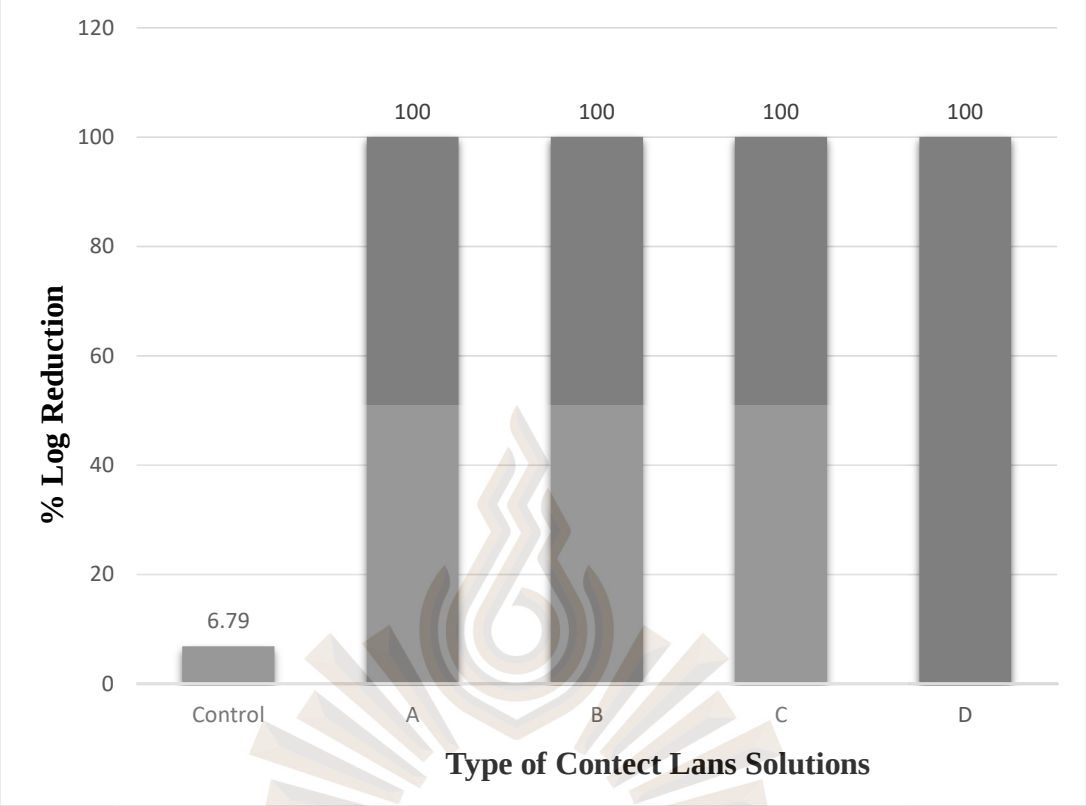


Figure 4.7 %Log reductions for *P. aeruginosa* following incubation with contact lens care solution A, B, C and D for 6 hours

CHAPTER 5

DISCUSSIONS AND CONCLUSIONS

Worldwide, millions of people use contact lenses as an alternative to eyeglasses. However, it has been shown contact lenses wear, especially extended wear, is a major risk for microbial keratitis and corneal ulcers (Preechawat, Ratananikom, Lerdvitayasakul, & Kunovisarut, 2007). Contact lens-related microbial keratitis is an important health concern because it causes poor visual outcome and blindness (Stapleton, Keay, Cole, & Jalbert, 2007; Stapleton et al., 2008). Many reports showed that contact lens related microbial keratitis is most commonly caused by bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Willcox, 2007; Wu, Thakur, Stapletonb, & Willcox, 2000).

Pseudomonas keratitis associated with contact lens wear is difficult to treat because *P. aeruginosa* resist many antibiotics. In addition, extended wear of soft contact lenses increases the adherence of *P. aeruginosa* to the epithelial cells of cornea because of biofilm produced by this bacterium (Fleiszig, Efron, & Pier, 1992) *S. aureus* is a commensal bacterium reside on the hands, face, nose, and skin that can access to the eye due to hand-to-eye transfer. It has been shown that *S. aureus* is the most common bacterial cause of contact-lens-induced peripheral ulceration (Jalbert et al., 2000)

Contact lens care solutions are used for cleaning, disinfecting and storing contact lenses. Contact lens care solutions help to decrease amount of microorganisms, remove dirt and protein deposits from the surface of the lens. Therefore, antibacterial activity of contact lens care solutions is one of the solution characteristics that should be concerned since it is the major property for decreasing the amount of infectious keratitis associated with contact lens.

The aim of this study is to evaluate the antibacterial activity of the four contact lens care solutions available in Thailand when inoculated with the standard ATCC strains of *P. aeruginosa* and *S. aureus*. Four contact lens care solutions assigned as A, B, C and D were verified for inhibitory activity against *P. aeruginosa* and *S. aureus* by the agar-well diffusion method. The result revealed that all test contact lens care solutions did not show antibacterial activity against *P. aeruginosa* and *S. aureus*. In this study, minimal inhibitory concentration (MIC) of the contact lens care solutions against *P. aeruginosa* and *S. aureus* were also investigated by broth microdilution assay. The result showed that all test contact lens care solutions at the concentration of 12.5-25% of the initial concentration inhibit the growth of methicillin-resistant ***Staphylococcus aureus*** (MRSA) ATCC43300, methicillin-sensitive *S. aureus* (MSSA) ATCC25930 and *Pseudomonas aeruginosa* ATCC2785. Antibacterial activity of contact lens care solutions tested by agar well diffusion was incompatible to the results from broth microdilution. This incoherent result may due to the different amount of reagent and amount of bacteria used for the assay. Since in agar well diffusion assay only 50 µL of reagent was tested with 108 cells/mL of bacteria while in microdilution assay 50 µL of reagent was tested with 106 cells/mL of bacteria. In addition, lack of inhibition zone in agar well diffusion assay could be as a result of incapability of the active chemical constituents to diffuse freely in these conditions.

Kinetics of kill studies showed that all test contact lens care solutions at concentration of 100% have killing effect since they reduced the number of bacteria more than 3 logs in 6 hours. According to the guidelines for International Standards Organization. ISO 14729 Ophthalmic optics criteria, an active contact lens disinfecting solution must be able to reduce the viability of starting concentration of bacterial species by 3 log (99.9%) and fungal species by 1 log (90%) at minimum disinfecting time as specified according to the manufacturer's label (Rosenthal, Sutton, & Schlech, 2002). Therefore, all contact lens care solutions used in this study meet this requirement. This study also revealed that contact lens care solution D has more killing effect than contact lens care solution A, B and C since it can reduce the number of test bacteria more than 3 logs in 2 hours. Contact lens care solution B and C killed MRSA and MSSA at least

3 logs in 2 hours whereas contact lens care solution A took more than 2 hours to achieve the same kill. The study showed that contact lens care solution D showed good bactericidal activity against *S.aureus* compared with other solutions while contact lens care solution B showed good bactericidal activity against *P.aeruginosa* compared with other solutions. In addition, contact lens care solution A showed less bactericidal activity towards *S.aureus* and *P.aeruginosa* compared with other solutions. The differences in killing effect of these contact lens care solutions could be due to the different active ingredients in each contact lens care solution.

Contact lens care solution A has Boric acid, Sodium borate and Polyamino-propyl bi-guanide as active ingredients while contact lens care solution B has poly-hexanide 0.0001% W/V. Contact lens care solution C has polyhexamethylene biguanide (PHMB), EDTA and contact lens care solution D has Sodium citrate, Sodium borate, Polyquaternium- 1, Myristamidopropyl dimethylamine and Ethylene diamine triacetic acid as active ingredients. The dominant active substances in the contact lens care solutions C is polyhexamethylene biguanide which initiates an attack right at the bacterial surface through to the cytoplasm and cytoplasmic membrane. The effects are higher on Gram negative bacterium where an action on the membrane acid leads to an increase in fluidity and permeability, causing the release of lipopolysaccharide (Yasuda, Ohmizo, & Katsu, 2003). In addition, EDTA which is a chelating agent in contact lens care solutions C and D acts synergistically with other agents to enhance antimicrobial activity of disinfectant since it involves the sequestration of ions that normally compete with positively charged preservative molecules for active sites on microbial cell walls. (Jones & Senchyna, 2007). The other active substance in the contact lens care solutions D is Quaternary ammonium compound which also has detergent property and is easily incorporated into epithelial cell membranes and directly damage the cell membrane of bacteria by disrupting their lipid component causing cell lysis.

In conclusion the present study revealed that the contact lens care solution containing polyquaternium/Polyquad (0.001%), and myristamidopropyl-dimethyl-amine/Aldox (0.0005%) has good disinfecting property against *S. aureus* and *Pseudomonas aeruginosa* but contact lens care solution containing polyaminopropyl

biguanide has less antibacterial activity. In addition, the antimicrobial activity of different solutions varies with respect to time of incubation. The solution with better disinfecting action and sufficient hygiene measures is recommended for everyday use for cleaning by contact lens users.

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